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Antifungal Activity of 2-(4-Chlorophenyl)- -1,2-Benzisoselenazol-3(2H)-One, the Analog of Ebselen

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Abstract. The antimicrobial activity of 8 selenoorganic compounds (3-benzisoselenazoles, 1-benzisoselenazolone oxide and 4-disaryl diselenides) was investigated. It was found that selenoorganic compounds from benzisoselenazolone group suppress growth of some fungi and bacteria. The growth of *Saccharomyces cerevisiae* Σ127-8b and *Candida albicans* 258 strains was strongly inhibited by the 2-(4-chlorophenyl)-1,2-benzisoselenazol-3(2H)-one. Also Ebselen and 2-acetyl-1,2-benzisoselenazol-3(2H)-one caused strong inhibition of *Saccharomyces cerevisiae* Σ127-8b growth but a lower effect was observed in assays with *Candida sp.* strains. Benzisoselenazolones were also found to have antibacterial activities. They significantly reduced the growth of Gram-negative *Escherichia coli* K-12 ROW and Gram-positive *Staphylococcus aureus* 209P (Oxford) bacteria strains.

Key words: selenoorganic compounds; *Saccharomyces cerevisiae*; *Candida albicans*; *Staphylococcus aureus*; *Escherichia coli*.

Introduction

Biological activity of selenoorganic compounds has been extensively studied during the last decade. Originally they were described as antiinflammatory, antioxidant or glutatione – peroxidase – like agents and as inhibitors of prostaglandin and leukotriene synthesis, as well as nitric oxide synthase inhibitors[29, 30]. These compounds have also been found to be inducers of interferon γ and tumor necrosis factor in human peripheral blood leukocytes[11, 12, 13]. It was also demonstrated that selenoorganic compounds have immunotropic activities. They affected humoral and cellular immunological responses in mice and chickens[3, 4]. One of the most interesting preparates with low toxicity – Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is a potential antiinflammatory drug of the new generation[25] and may find application in therapy of rheumatoid arthritis, psoriasis and inflammatory bowel disease[24, 26, 27, 31, 33, 36].

In this paper antifungal activity of selected selenoorganic compounds, and additionally their influence on Gram-negative and Gram-positive bacteria as control microorganisms, is reported.

Materials and Methods

Compounds. The selenoorganic compounds were synthesized according to procedures reported earlier[20, 21, 22, 32]. Chemical structure of Ebselen – AE 6 (2-phenyl-1,2-benzisoselenazol-3(2H)-one), AE 3 (2-dode-

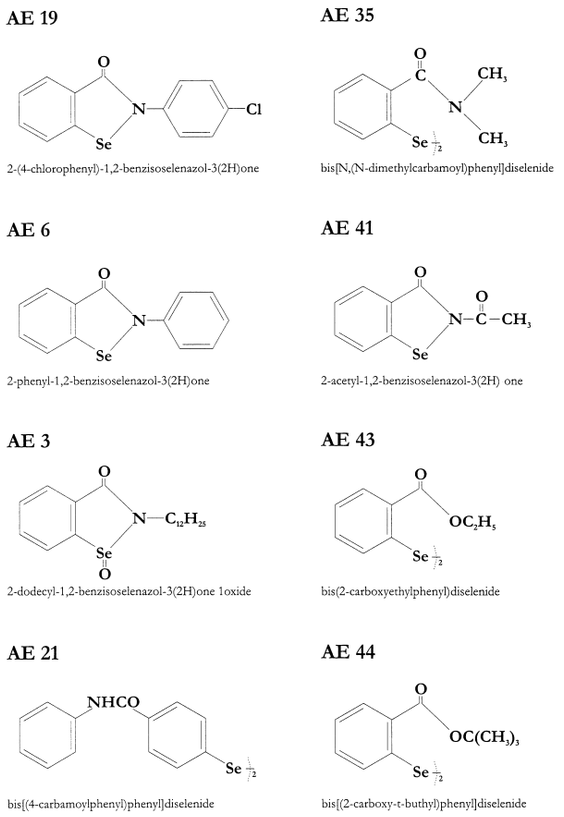

Fig. 1. Chemical structures of the selenoorganic compounds

cyl-1,2-benzisoselenazol-3(2H)-one 1 oxide), AE 19 (2-(4-chlorophenyl)-1,2-benzisoselenazol-3(2H)-one), AE 21 (bis[(4-carbamoylphenyl)phenyl]diselenide), AE 35 (bis[N,(N-dimethylcarbamoyl)phenyl] diselenide, AE 41 (2-acetyl-1,2-benzisoselenazol-3(2H)-one), AE 43 (bis-(2-carboxyethylphenyl)diselenide), AE 44 (bis[(2carboxy-t-buthyl)phenyl]diselenide), are shown in Fig. 1. These compounds (used in experiments as a stock solution) were dissolved in dimethyl sulfoxide (DMSO) in concentration of 1000 μg/ml and frozen. Before experiments each compound (initially dissolved in DMSO) was further diluted in tested medium in diminishing concentrations: 50, 25, 12.5, 6.25, 3.12 and 1.56 μg/ml and used for experiments.

Microorganisms. Antifungal activity of selenoorganic compounds was tested on selected pathogenic, clinical origin, yeast-like strains of *Candida sp*. *(Candida albicans* 95, 254, 258, *C. parapsilosis*, *C. pseudotropicalis* 206, *C. guilliermondii* and *C. krusei*) and nonpathogenic yeast strain *Saccharomyces cerevisiae* Σ127-8b. These strains were obtained from collection of the Institute of Microbiology, Wrocław University. They were stored on Sabouraud agar slants at 4°C.

Antibacterial activity was evaluated on reference strains *Staphylococcus aureus* 209P (Oxford) and *Escherichia coli* ROW from the collection of Institute of Microbiology, Wrocław University. They were stored in semisolid agar medium at room temperature.

Methods. The preliminary experiments were performed on overnight cultures of bacteria and fungal strains. The yeast strains were grown on liquid and solid Sabouraud medium. The bacteria were grown in broth medium and solid agar medium[2, 28].

The samples of overnight precultures of the investigated strains were inoculated into fresh broth (for bacteria) and Sabouraud liquid medium (for fungi) to get 10^7 and 10^5 cells per 1 ml, respectively. Next the standard broth technique to determine minimal inhibitory concentration (MIC) value for tested selenoorganic compounds according to McGinnis[19] was applied. These cultures were cultivated at 28°C (fungi) and at 37°C (bacteria). Survival of the microorganisms was determined at selected time (0, 4, 24 h). Viable counts in each interval were determined by colony scoring on solid agar medium for bacteria and on Sabouraud solid medium for fungi after 2 day incubation at 28°C and 37°C, respectively. As controls we used culture media without selenoorganic compounds.

Additionally, an antifungal activity of chosen compounds, AE 6 and AE 19, was tested on *S. cerevisiae* Σ127-8b suspended in 0.9% physiological saline.

Results

To determine an antifungal activity of selenoorganic compounds we have used assays performed on nonpathogenic *S. cerevisiae* Σ127-8b and pathogenic *C. albicans* 258 strains. Eight selenoorganic preparations were administered to the medium, where the fungal strains were growing.

We have found that 4 drugs under investigations inhibited the growth of *S. cerevisiae* Σ127-8b. The inhibition was differentiated and depended on concentration of selenic compounds in medium. Preparate AE 19 used in concentrations 50.0, 25.0, 12.5 and 6.25 μg/ml, significantly decreased the number of colonies of *S. cerevisiae*Σ127-8b (Fig. 2). MIC for AE 19 in Sabouraud medium was determined between 6.25–12.5 μg/ml. Ebselen (AE 6) inhibited the growth of *S. cerevisiae* Σ127-8b when was used in concentration 50.0, 25.0 and 12.5 μg/ml (Fig. 3). MIC for this compound was determined between 12.5–25.0 μg/ml. The antifungal activity of compound AE 3 was lower (Fig. 4). The same activity, as preparate AE 19, exhibited preparate

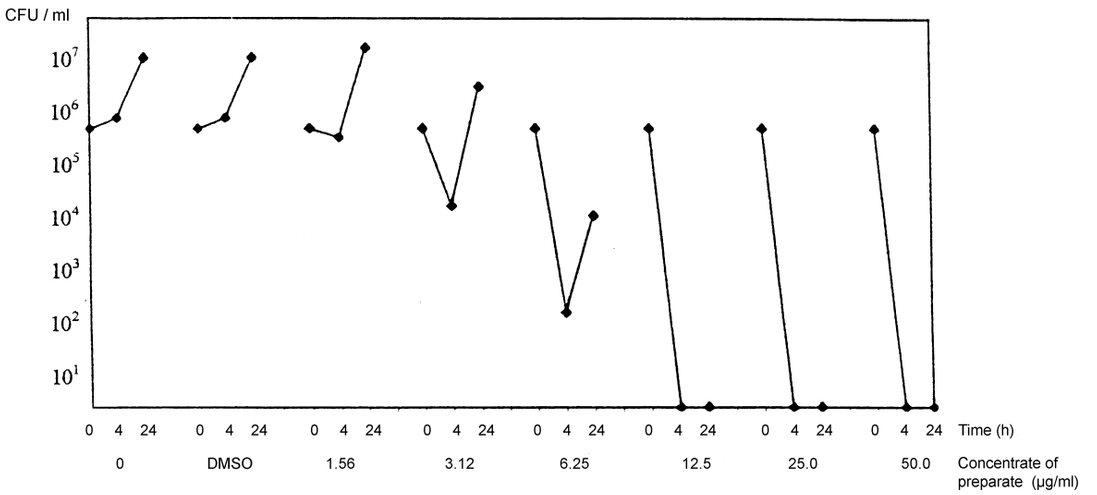

Fig. 2. Effect of the compound AE 19 on *S. cerevisiae* Σ127-8b in Sabouraud medium

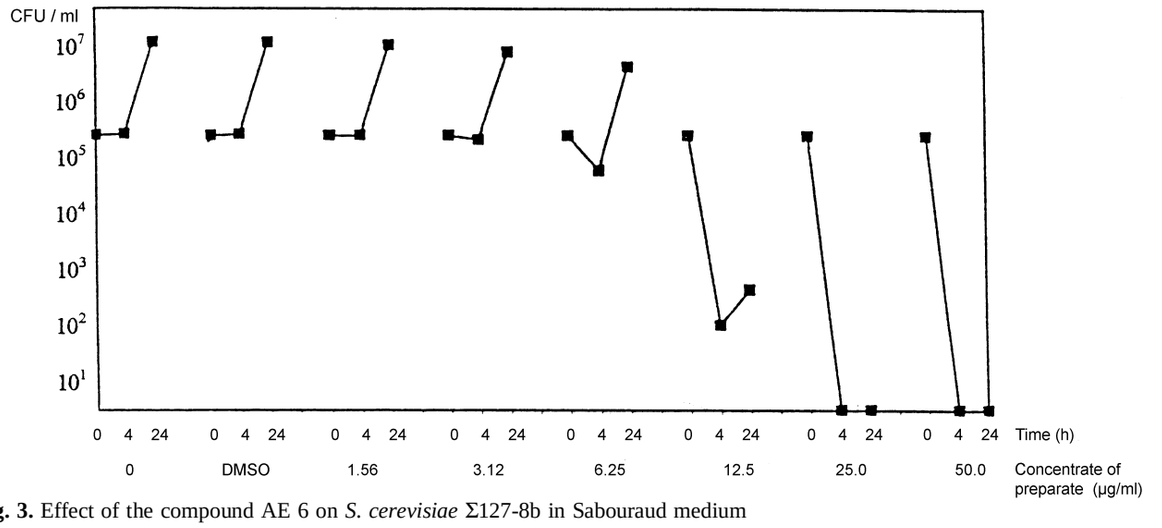

Fig. 3. Effect of the compound AE 6 on *S. cerevisiae* Σ127-8b in Sabouraud medium

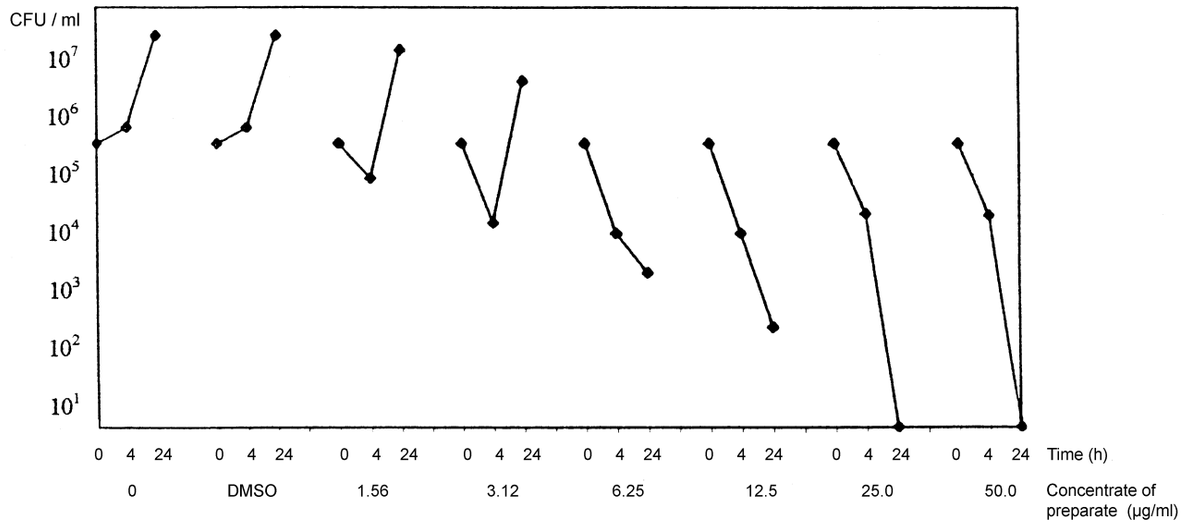

Fig. 4. Effect of the compound AE 3 on *S. cerevisiae* Σ127-8b in Sabouraud medium

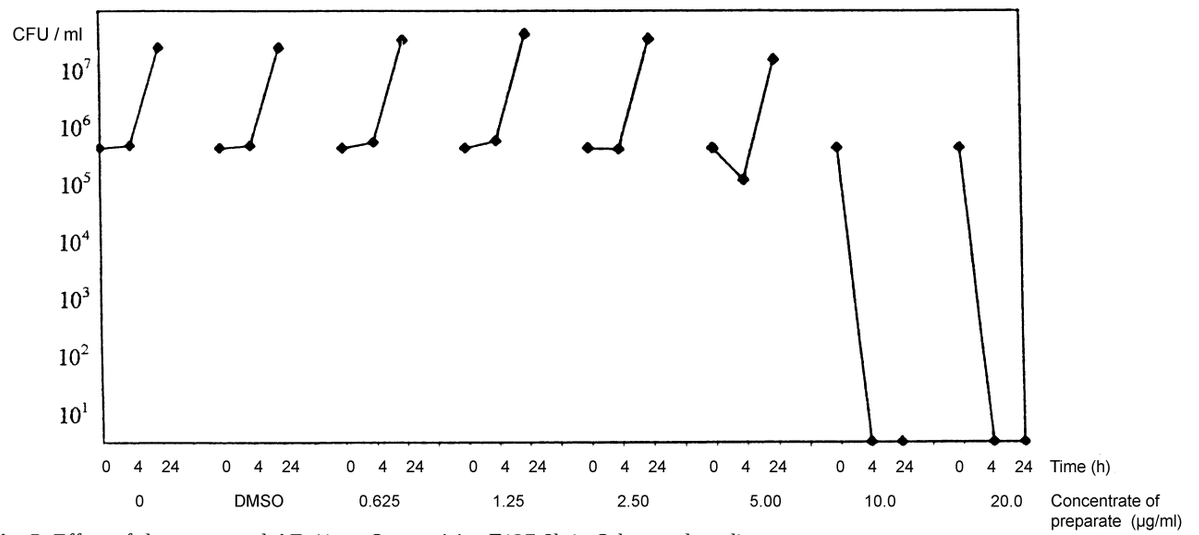

Fig. 5. Effect of the compound AE 41 on *S. cerevisiae* Σ127-8b in Sabouraud medium

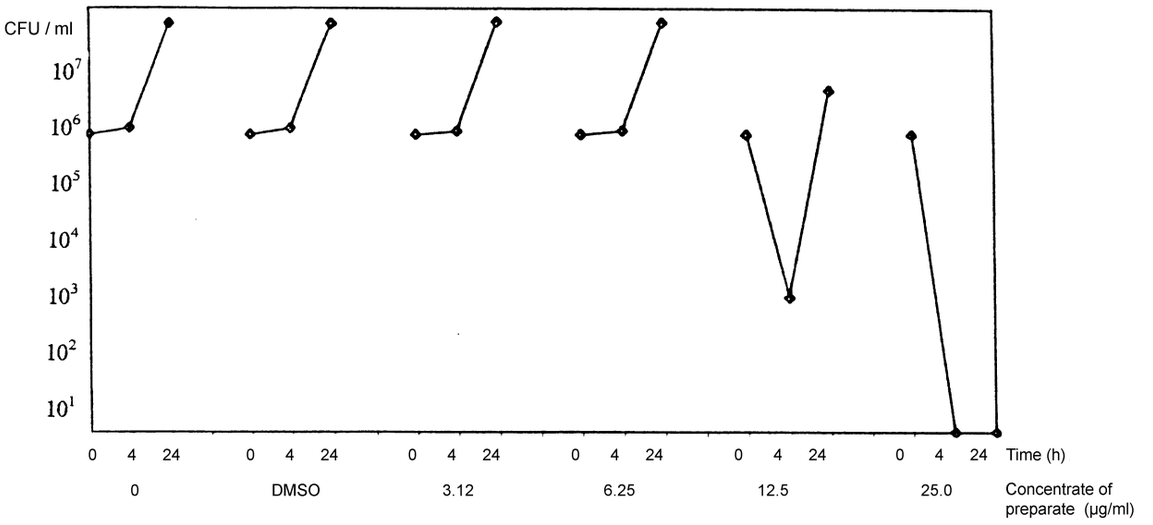

Fig. 6. Effect of the compound AE 19 on *C. albicans* 258 in Sabouraud medium

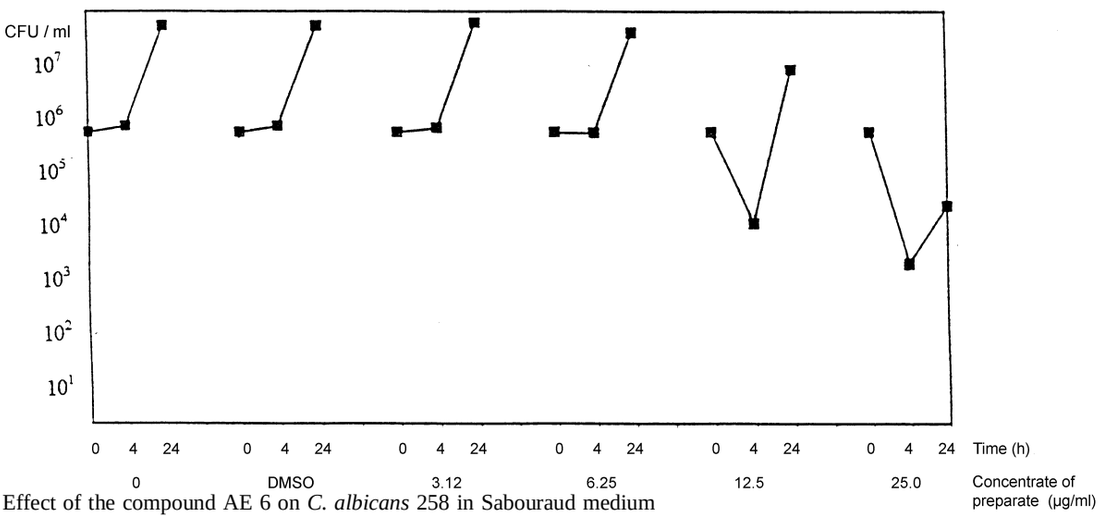

Fig. 7. Effect of the compound AE 6 on *C. albicans* 258 in Sabouraud medium

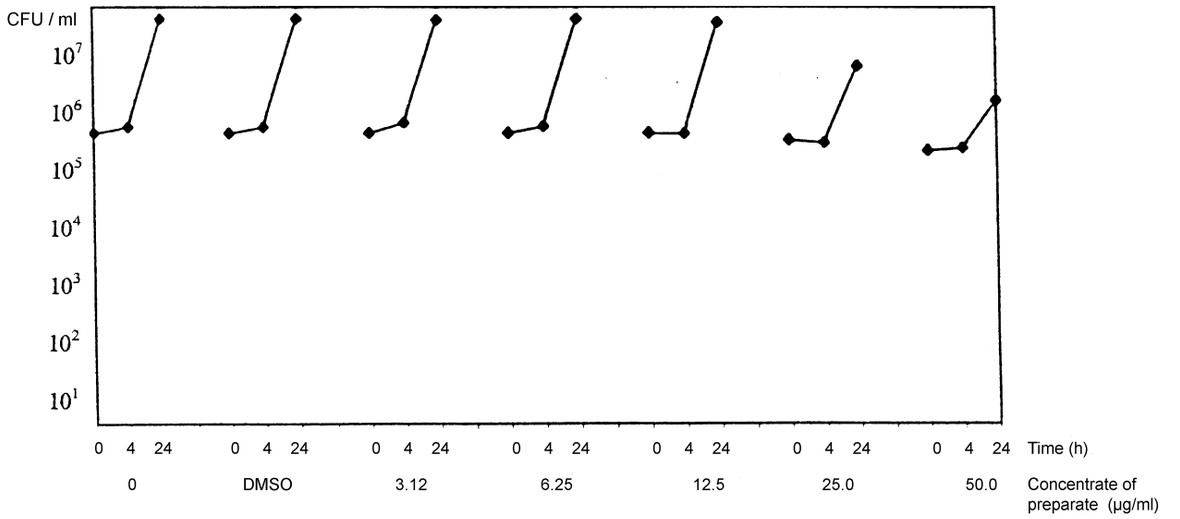

Fig. 8. Effect of the compound AE 3 on *C. albicans* 258 in Sabouraud medium

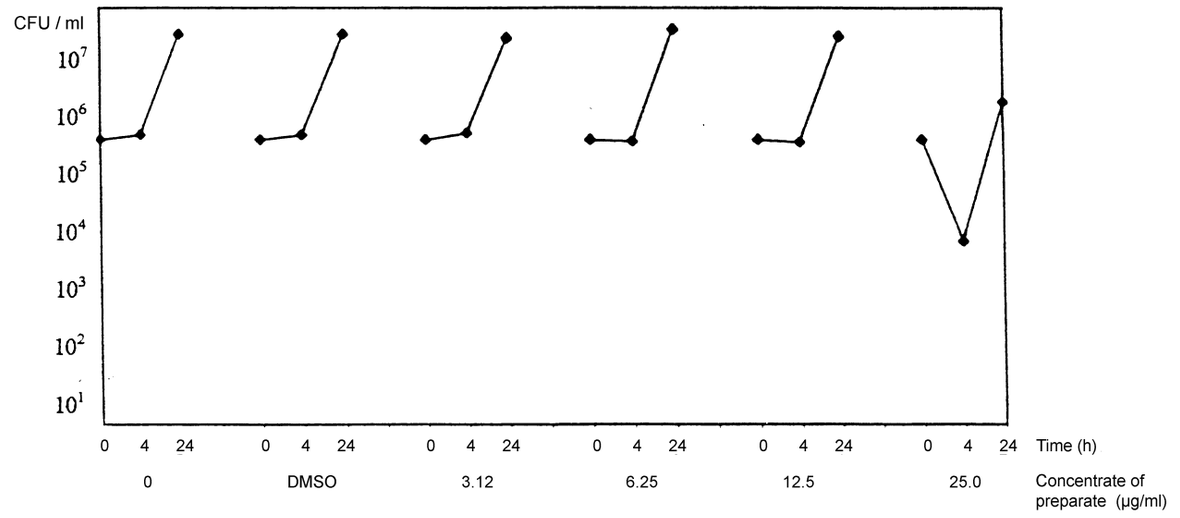

Fig. 9. Effect of the compound AE 41 on *C. albicans* 258 in Sabouraud medium

AE 41 (Fig. 5). Preparates AE 21, AE 35, AE 43 and AE 44 had no influence on that fungi strain growth.

The effect of AE 6 and AE 19 selenic compounds on *S. cerevisiae* Σ127-8b suspended in physiological saline, was also investigated. It was found that after 24 h of incubation both preparates caused the decrease in cell number of *S. cerevisiae* strain (Table 1). In this condition MIC for both compounds is similar (2.5–5.0 μg/ml). These results showed that MIC for tested preparates depends on condition of cultivation.

Clinical, pathogenic strain *C. albicans* 258 was affected by selenoorganic compouds in different kind. The preparate AE 19 caused a strong reduction in colony forming units of *C. albicans* 258 (Fig. 6). A lower effect had Ebselen (Fig. 7). MIC for AE 19 was found as 25 μg/ml and for AE 6 and AE 41 above 25 μg/ml. AE 3 had no effect (Fig. 8). A slight activity exhibited preparate AE 41 (Fig. 9). Preparates AE 21, AE 35, AE 43 and AE 44 had no antifungal effect on *C. albicans* 258.

Antifungal activity of AE 6 and AE 19 was investigated in experiments performed on selected strains of *Candida sp.* It occurred that both preparates affected all tested fungi. In some cases (*C. albicans* 258, *C. parapsilosis*, *C. guilliermondii* and *C. krusei*) compound AE 19 applied in concentration 12.5 μg/ml was more efficient as compared with Ebselen (Table 2).

Finally, we used Gram-negative and Gram-positive bacteria as control microorganisms and investigated the effect of selenoorganic compounds on *E. coli* K-12 ROW and *S. aureus* 209P (Oxford).

Table 1. Surviving of *Saccharomyces cerevisiae* Σ127-8b in physiological saline under the influence of selenoorganic compounds

Concentration (μg/ml)	Compounds			
	AE 19		AE 6	
	time of incubation (h)			
	0	24	0	24
	CFU*/ml			
0[a]	$2.7 \cdot 10^5$	$2.1 \cdot 10^5$	$2.7 \cdot 10^5$	$2.1 \cdot 10^5$
0[b]		$2.0 \cdot 10^5$		$2.0 \cdot 10^5$
0.625		$1.4 \cdot 10^5$		$2.0 \cdot 10^5$
1.25		$9.8 \cdot 10^5$		$1.4 \cdot 10^5$
2.50		$3.7 \cdot 10^5$		$1.1 \cdot 10^5$
5.0		0		0

* Colony forming units, [a] control, [b] control + DMSO.

Compounds AE 19 and AE 6 in concentration 12.5 and 25.0 μg/ml significantly depress the growth of *E. coli* K-12 ROW. AE 19 was slightly, efficient (MIC 12.5 μg/ml) (Table 3). Compounds AE 41, AE 3, AE 21, AE 35, AE 43 and AE 44 had no effect on multiplication of this bacterium strain.

Similar results we obtained in experiments performed on *S. aureus*. Compounds AE 19 and Ebselen in concentration 12.5 and 25.0 μg/ml suppress the growth these bacteria to zero. In concentration 6.25 μg/ml AE 19 decreased the number of *S. aureus* cells stronger than AE 6. AE 41 used in 12.5 and 25.0 μg/ml induced the inhibition of bacteria growth and AE 3 accomplished it when applied in concentration 6.25, 12.5 and 25.0 μg/ml (Table 4). Preparates AE 21, AE 35, AE 43 and AE 44 had no antimicrobial effect on *S. aureus* 209P (Oxford). MICs for 4 active preparates were: AE 19 – 6.25–12.5; AE 6 – 12.5, AE 41 and AE 3 – 12.5–25 μg/ml.

Discussion

It is well established that selenium is an essential food additive for animals and men although it may be

Table 2. The effect of selenoorganic compounds on pathogenic *Candida sp.* strains in Sabouraud medium

Strains	Concentration (μg/ml)	Compounds			
		AE 19		AE 6	
		time of incubation (h)			
		0	24	0	24
		CFU*/ml			
C. albicans 258	0[a]	$3.9 \cdot 10^5$	$5.0 \cdot 10^7$	$3.9 \cdot 10^5$	$5.0 \cdot 10^7$
	0[b]		$5.3 \cdot 10^7$		$5.3 \cdot 10^7$
	12.5		$2.7 \cdot 10^6$		$6.8 \cdot 10^6$
	25.0		0		$1.4 \cdot 10^4$
C. albicans 95	0[a]	$1.9 \cdot 10^5$	$3.1 \cdot 10^7$	$1.9 \cdot 10^5$	$3.1 \cdot 10^7$
	0[b]		$2.1 \cdot 10^7$		$2.1 \cdot 10^7$
	12.5		$4.4 \cdot 10^6$		$1.3 \cdot 10^7$
	25.0		0		0
C. albicans 234	0[a]	$2.3 \cdot 10^5$	$5.9 \cdot 10^7$	$2.3 \cdot 10^5$	$5.9 \cdot 10^7$
	0[b]		$4.0 \cdot 10^7$		$4.0 \cdot 10^7$
	12.5		$3.8 \cdot 10^3$		$3.9 \cdot 10^5$
	25.0		0		0
C. parapsilosis 210	0[a]	$5.2 \cdot 10^5$	$2.0 \cdot 10^6$	$5.2 \cdot 10^5$	$2.0 \cdot 10^6$
	0[b]		$2.0 \cdot 10^6$		$2.0 \cdot 10^6$
	12.5		$4.4 \cdot 10^4$		$2.3 \cdot 10^5$
	25.0		$1.0 \cdot 10^3$		$1.4 \cdot 10^3$
C. pseudotropicalis 206	0[a]	$6.0 \cdot 10^5$	$4.9 \cdot 10^7$	$6.0 \cdot 10^5$	$4.9 \cdot 10^7$
	0[b]		$4.9 \cdot 10^7$		$4.9 \cdot 10^7$
	12.5		$1.3 \cdot 10^6$		$1.1 \cdot 10^5$
	25.0		$1.3 \cdot 10^3$		$1.9 \cdot 10^3$
C. quilliermondii 23	0[a]	$9.2 \cdot 10^5$	$8.8 \cdot 10^7$	$9.2 \cdot 10^5$	$8.8 \cdot 10^7$
	0[b]		$8.0 \cdot 10^7$		$8.0 \cdot 10^7$
	12.5		$2.4 \cdot 10^2$		$8.4 \cdot 10^3$
	25.0		0		0
C. krusei 13	0[a]	$3.4 \cdot 10^5$	$1.3 \cdot 10^7$	$3.4 \cdot 10^7$	$1.3 \cdot 10^7$
	0[b]		$1.3 \cdot 10^7$		$1.3 \cdot 10^7$
	12.5		$4.4 \cdot 10^2$		$6.0 \cdot 10^4$
	25.0		0		$2.0 \cdot 10^1$

* Colony forming units, [a] control, [b] control + DMSO.

Table 3. The effect of selenoorganic compounds on *Escherichia coli* K-12 ROW in broth medium

Concentration (μg/ml)	Compounds											
	AE 19				AE 6				AE 41			
	time of incubation (h)											
	0	2	4	24	0	2	4	24	0	2	4	24
	CFU*/ml											
0[a]	$8.5 \cdot 10^6$	$3.8 \cdot 10^7$	$2.4 \cdot 10^8$	$2.4 \cdot 10^9$	$8.5 \cdot 10^6$	$3.8 \cdot 10^7$	$2.4 \cdot 10^8$	$2.4 \cdot 10^9$	$8.5 \cdot 10^6$	$3.8 \cdot 10^7$	$2.5 \cdot 10^8$	$2.4 \cdot 10^9$
0[b]		$3.8 \cdot 10^7$	$2.4 \cdot 10^8$	$2.4 \cdot 10^9$		$3.8 \cdot 10^7$	$2.4 \cdot 10^8$	$2.4 \cdot 10^9$		$3.8 \cdot 10^7$	$2.5 \cdot 10^8$	$2.4 \cdot 10^9$
3.125		$3.2 \cdot 10^7$	$2.4 \cdot 10^8$	$2.5 \cdot 10^9$		$4.3 \cdot 10^7$	$2.4 \cdot 10^8$	$2.5 \cdot 10^9$		$3.5 \cdot 10^7$	$2.7 \cdot 10^8$	$2.6 \cdot 10^9$
6.25		$2.0 \cdot 10^6$	$6.0 \cdot 10^6$	$2.5 \cdot 10^9$		$4.3 \cdot 10^7$	$7.4 \cdot 10^7$	$2.0 \cdot 10^9$		$3.0 \cdot 10^7$	$2.8 \cdot 10^8$	$2.7 \cdot 10^9$
12.5		0	0	0		0	0	$6.6 \cdot 10^2$		$3.1 \cdot 10^7$	$1.5 \cdot 10^8$	$2.7 \cdot 10^9$
25.0		0	0	0		0	0	0		$1.4 \cdot 10^4$	$5.0 \cdot 10^4$	$2.5 \cdot 10^9$

* Colony forming units, [a] control, [b] control + DMSO.

Table 4. The effect of selenoorganic compounds on *Staphylococcus aureus* 209 P Oxford in broth medium

Concentration (μg/ml)	Compounds							
	AE 19		AE 6		AE 3		AE 41	
	time of incubation (h)							
	0	24	0	24	0	24	0	24
	CFU*/ml							
0[a]	$1.8 \cdot 10^7$	$1.5 \cdot 10^9$	$1.8 \cdot 10^7$	$1.5 \cdot 10^9$	$1.8 \cdot 10^7$	$1.5 \cdot 10^9$	$1.8 \cdot 10^7$	$1.5 \cdot 10^9$
0[b]		$1.5 \cdot 10^9$		$1.5 \cdot 10^9$		$1.5 \cdot 10^9$		$1.5 \cdot 10^9$
3.125		$1.0 \cdot 10^8$		$3.8 \cdot 10^8$		$1.6 \cdot 10^8$		$1.0 \cdot 10^9$
6.25		$9.2 \cdot 10^5$		$2.0 \cdot 10^7$		$2.0 \cdot 10^4$		$9.5 \cdot 10^7$
12.50		0		0		$4.0 \cdot 10^1$		$1.0 \cdot 10^3$
25.00		0		0		0		0

* Colony forming units, [a] control, [b] control + DMSO.

highly toxic when used in excessive doses[37]. On the other hand, it is known that the toxicity of many selenoorganic structures is remarkably low. It was discovered that the selenoorganic compounds have many activities which appear to be connected with the presence of Se. It has to be taken into consideration that Se for years has been known for its association with humoral and cellular immunity[3-9, 14, 15]. Deficiency of selenium has been observed in HIV-infected and AIDS patients what suggested that this can contribute to the pathology AIDS as a constituent of glutatione peroxidase[34]. These observations indicate that Se may play important role in viral biochemistry[7, 22]. To determine antimicrobial activity of selenic drugs under study one should use assays with different fungi and their products[1, 2, 16, 18, 23, 35].

The main finding of present study is that some of the selenoorganic compounds have the antifungal activity.

We have shown in this report that among the tested compounds benzisoselenazolones exhibited significant antifungal activity. To this group belong N-substituted benzisoselenazolones, such as Ebselen (AE 6) and two its analogues – AE 19 and AE 41. The representative of the second group having a selenoxide function – compound AE 3, was not active as an antifungal factor against pathogenic yeast-like strains of *Candida sp.* Disaryl diselenides having carbamoil or carboxyalkil function (AE 21, 35, 43 and 44) had no influence on fungi growth.

Because of limited number of the compounds tested it is difficult to discuss the relationship between structure of drug and biological response. Nevertheless, the antimicrobial activity of the compounds having benzisoselenazolone moiety (AE 6, 19, 41) is substantially higher than activity of other selenoorganic compounds tested. It seems to be possible that selenium – nitrogen bond, present in the heterocyclic ring is a crucial factor for activity, such as in case of other selenium and nitrogen-containing heterocycles (1, 2, 3 selenodiazoles and 1, 2, 5 selenodiazoles) as reported by LALEZARI et al.[17] and HUNT and PITTILLO[9].

Our preliminary results may be useful to find fungistatic of fungicidal drugs. Antimicrobial activity of benzisoselenazolones, especially AE 19 and their low toxicity make these compounds interesting as potential therapeutic agents.

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