Antimicrobially active 1-(2-alkylthio-6-benzothiazolylaminomethyl)-5-(3-X^1-4-X^2-phenyl)-1,2,3,4-tetrazoles

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1-(2-Alkylthio-6-benzothiazolylaminomethyl)-5-(3-X^1-4-X^2-phenyl)-1,2,3,4-tetrazoles (X^1 = H, Cl; X^2 = H, Cl, NO$_2$) were synthesized by the Mannich reaction. The title compounds exhibit antifungal and antialgal activity.

Путем реакции Манниха был синтезирован ряд 1-(2-алкилтио-6-бензотиазолиламинометил)-5-(3-X^1-4-X^2-фенил)-1,2,3,4-тетразолов (X^1 = H, Cl; X^2 = H, Cl, NO$_2$). Названные соединения проявляют антифунгальную и антиалгальную активности.

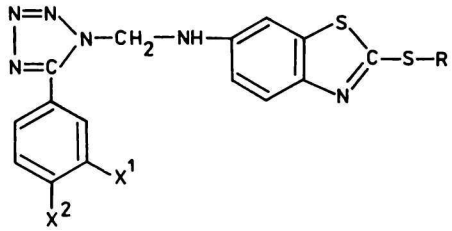
1-(2-Alkylthio-6-benzothiazolylaminomethyl)-5-(3-X^1-4-X^2-phenyl)-1,2,3,4-tetrazoles (X^1 = H, Cl; X^2 = H, Cl, NO$_2$) are biologically active compounds with a wide spectrum of activity. Papers concerning the study of their antimycobacterial, antiviral [1], antihelminthic [2], anorectic [3], and antitumour [4] activity have already been published.

The aim of the present work was to extend the range of the above-mentioned compound by further 3,4-dichlorophenyl derivatives and to perform more extensive microbiological study of the whole series.

New 1-(2-alkylthio-6-benzothiazolylaminomethyl)-5-(3,4-dichlorophenyl)-1,2,3,4-tetrazoles *XII*, *XIV—XVIII* were prepared by the Mannich synthesis from 2-alkylthio-6-aminobenzothiazoles [5], 30 % aqueous solution of formaldehyde, and 5-(3,4-dichlorophenyl)-1,2,3,4-tetrazole [6], similarly as ethyl [1] and isobutyl [2] derivatives.

Majority of the tested compounds exhibit very good antiyeast activity (in μmol dm^{-3}). The most effective compounds against *Candida albicans* were 1-(2-benzylthio-6-benzothiazolylaminomethyl)-5-phenyl-1,2,3,4-tetrazole (*VIII*) (ED_{50} = 20, ED_{100} = 87) and newly synthesized 1-(2-alkylthio-6-benzothiazolylaminomethyl)-5-(3,4-dichlorophenyl)-1,2,3,4-tetrazoles (in rank of decreasing activity): n-pentyl (*XVIII*) (ED_{50} = 15, ED_{100} = 100), n-butyl (*XVI*) (ED_{50} = 22,

ED_{100} = 100), *sec*-butyl (*XVII*), n-propyl (*XIV*), and isopropyl (*XV*) derivatives. For 2-mercaptobenzothiazole (2-MBT) ED_{50} = 360, ED_{100} = 1000. Very low activity is exhibited by nitro derivatives *X*, *XI* and the derivative with unsubstituted phenyl and C_9 alkyl group (*VII*).



I–XVIII

	R	X^1	X^2		R	X^1	X^2
I	C_2H_5	H	H	*X*	C_2H_5	H	NO_2
II	$(CH_2)_2CH_3$	H	H	*XI*	$CH_2CH(CH_3)_2$	H	NO_2
III	$CH_2CH(CH_3)_2$	H	H	*XII*	CH_3	Cl	Cl
IV	$(CH_2)_4CH_3$	H	H	*XIII*	C_2H_5	Cl	Cl
V	$(CH_2)_2CH(CH_3)_2$	H	H	*XIV*	$(CH_2)_2CH_3$	Cl	Cl
VI	$(CH_2)_7CH_3$	H	H	*XV*	$CH(CH_3)_2$	Cl	Cl
VII	$(CH_2)_8CH_3$	H	H	*XVI*	$(CH_2)_3CH_3$	Cl	Cl
VIII	$CH_2C_6H_5$	H	H	*XVII*	$CH(CH_3)C_2H_5$	Cl	Cl
IX	$CH_2CH(CH_3)_2$	Cl	H	*XVIII*	$(CH_2)_4CH_3$	Cl	Cl

Dichloro-substituted and unsubstituted phenyl derivatives *XVIII* (ED_{50} = = 23, ED_{100} = 51), *XVI* (ED_{50} = 87, ED_{100} = 190), *IV*, *XVII*, and *I* alternate in decreasing order of the activity against *Saccharomyces cerevisiae*. For 2-MBT ED_{50} = 520, ED_{100} = 1000. According to the given results newly synthesized compounds *XVIII*, *XVI*, and *XVII* can be considered as the most active anti-yeast compounds.

Tested compounds did not reveal antifungal activity against filamentous microscopic fungi *Aspergillus niger*, *Penicillium cyclopium*, and *Rhizopus oryzae*. However, some derivatives were effective against *Alternaria alternata* (the most sensitive one from the four filamentous fungi). The most effective were compounds *XVII* (MIC = 40 after 10 d) and *I* (MIC = 40 and 200 after 7, resp. 10 d). For 2-MBT MIC = 200 and 1000 after 7, resp. 10 d. From the compounds with unsubstituted phenyl, benzyl (*VIII*) and C_2—C_4 alkyl (*I*—*III*) derivatives were efficient. From dichlorophenyl analogues only derivatives with C_1 alkyl (*XII*) and C_4 alkyl (*XVI*, *XVII*) groups were effective (*sec*-butyl deriva-

tive *XVII* being by one order of magnitude more efficient than n-butyl derivative *XVI*).

The tested compounds did not exhibit significant antiprotozoal activity against *Euglena gracilis*. On the contrary, all tested compounds showed antialgal activity against *Chlorella vulgaris*, 1-(2-isobutylthio-6-benzothiazolylaminomethyl)-5-(4-nitrophenyl)-1,2,3,4-tetrazole (*XI*) being the most effective (MIC < 40 after 21 d), followed by n-octyl (*VI*) and n-nonyl (*VII*) derivatives (MIC = 40 after 21 d, in both cases).

Presented results show that 1-(2-alkylthio-6-benzothiazolylaminomethyl)-5-(3-X^1-4-X^2-phenyl)-1,2,3,4-tetrazoles are a perspective group of compounds for further study of biological activity.

Experimental

Physical constants, analytical data, and yields of newly synthesized compounds are given in Table 1. Synthesis and physical constants of other tested compounds are published in our previous papers [1, 2].

Liquid synthetic medium containing vitamins was used for the determination of antiyeast activity, with static cultivation at 28 °C. Compounds dissolved in dimethyl sulfoxide were added to the medium first, followed by the inoculum. Yeast multiplication was followed turbidimetrically and from the constructed growth curves ED_{50} and ED_{100} were obtained by means of graphical-mathematical method. These values relate to the sixth day (*Candida albicans*) and the fourth day (*Saccharomyces cerevisiae*) of cultivation, when controls reached the maximal growth.

For determination of the activity of the compounds against filamentous fungi, liquid modified Czapek—Dox medium with tested compounds dissolved in dimethyl sulfoxide was used. Consequent concentration of the compounds in medium was 1000, 200, 40 and 8 μmol dm^{-3}. Cultivation was static at 28 °C. Growth was evaluated visually [7].

Chodat medium (20 cm^3) was used for determination of the antialgal activity. Cultivation at 20 °C lasted 21 d with permanent lighting. Dimethyl sulfoxide was used as a solvent and its concentration in the cultivation medium did not exceed 1 %. Consequent concentration of the tested compounds and the reference sample was 1000, 200, and 40 μmol dm^{-3}. In the appropriate time the increase of the green algae was evaluated visually [8].

1-(2-Alkylthio-6-benzothiazolylaminomethyl)-5-(3,4-dichlorophenyl)-1,2,3,4-tetrazoles XII, XIV—XVIII

The suspension of 2-alkylthio-6-aminobenzothiazole (0.01 mol) and 5-(3,4-dichlorophenyl)-1,2,3,4-tetrazole (2.15 g; 0.01 mol) in ethanol (20—60 cm^3) was stirred and

Table 1

Characterization of the prepared compounds *XII*, *XIV*–*XVIII*

Compound	Alkyl	Formula	M_r	w_i(calc.)/% w_i(found)/%					Yield	M.p.
				C	H	Cl	N	S	%	°C
XII	CH_3	$C_{16}H_{12}Cl_2N_6S_2$	423.35	45.39	2.85	16.74	19.85	15.14	85.0	157—159
				45.60	2.90	16.92	19.84	15.06		
XIV	$(CH_2)_2CH_3$	$C_{18}H_{16}Cl_2N_6S_2$	451.38	47.89	3.57	15.70	18.61	14.20	68.8	131—132
				47.96	3.21	15.77	18.47	14.09		
XV	$CH(CH_3)_2$	$C_{18}H_{16}Cl_2N_6S_2$	451.38	47.89	3.57	15.70	18.61	14.20	70.9	138—140
				47.65	3.44	15.74	18.18	14.34		
XVI	$(CH_2)_3CH_3$	$C_{19}H_{18}Cl_2N_6S_2$	465.41	49.03	3.89	15.23	18.05	13.77	70.0	123—125
				48.69	3.70	15.07	17.96	13.67		
XVII	$CH(CH_3)C_2H_5$	$C_{19}H_{18}Cl_2N_6S_2$	465.41	49.03	3.89	15.23	18.05	13.77	78.0	128—130
				48.58	3.80	15.29	17.92	13.59		
XVIII	$(CH_2)_4CH_3$	$C_{20}H_{20}Cl_2N_6S_2$	479.44	50.10	4.20	14.78	17.52	13.37	70.8	122—123
				50.06	3.84	14.74	17.54	13.35		

heated to 35—40 °C. At that temperature 30 % aqueous solution of formaldehyde (2 cm^3; 0.02 mol) was added to the reaction mixture. After few minutes white product precipitated. After 20 min of stirring, the product was sucked and washed by ethanol, dropwise (7—15 cm^3). Products did not need further purification.

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