3-(2-Alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones and their antimicrobial activity

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3-(2-Alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones were synthesized from 6-bromo-3-hydroxymethyl-2-benzothiazolinone and 2-alkylthio-6-aminobenzothiazoles (alkyl = methyl to n-nonyl, isopropyl, allyl, cyclopentyl, and benzyl). Majority of the tested compounds exhibit antifungal, antialgal, and antiprotozoal activity.

3-(2-Алкилтио-6-бензотиазолиламинометил)-6-бром-2-бензотиазолиноны были синтезированы из 6-бром-3-гидроксиметил-2-бензотиазолинона и 2-алкилтио-6-аминобензотиазолов (алкил = метил по н-нонил, изопропил, аллил, циклопентил, и бензил). Большинство испытанных соединений проявляли антифунгальную, антиалгальную и антипротозоальную активности.

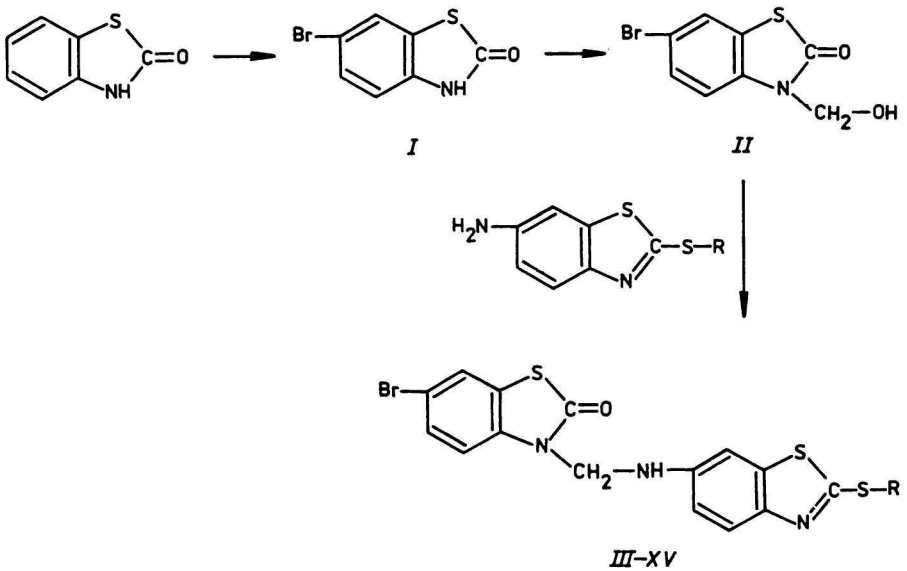
By the Mannich synthesis 3-substituted 2-benzothiazolinones (some of them exhibiting antiviral activity [1]) were prepared from 2-benzothiazolinone and commercially available amines (ethyl-, diethyl-, allyl-, and benzylamines, aniline, 4-toluidine, pyrrolidine, morpholine, and some alkoxy substituted aliphatic amines).

With the aim to prepare new, biologically highly effective Mannich compounds, in the present work 6-bromo-2-benzothiazolinone as H-active component and antimycobacterially [2] and antifungally [3] highly effective 2-alkylthio-6-aminobenzothiazoles (alkyl = methyl to n-nonyl, isopropyl, allyl, cyclopentyl, and benzyl) as amines were used. It is known that aromatic primary amines give in the Mannich reaction monoderivatives [1, 4, 5]. The same is valid for 2-alkylthio-6-aminobenzothiazoles.

Starting 6-bromo-2-benzothiazolinone (*I*) can be prepared by the direct bromination of 2-benzothiazolinone or 2-chlorobenzothiazole with elemental bromine, by diazotization and Sandmeyer reaction from 6-amino-2-benzothiazolinone [6], or by alkaline hydrolysis of 2,6-dibromobenzothiazole [7]. We found that bromination of 2-benzothiazolinone with *N*-bromosuccinimide is

easier and safer method for the preparation of *I*. After one crystallization the product (proved by elemental analysis) with sharper melting point was obtained than it is described in literature [6].

The experiments proved that it is better to carry out the preparation of 3-(2-alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones *III—XV* in two steps, *i.e.* to prepare from 6-bromo-2-benzothiazolinone (*I*) and formaldehyde 6-bromo-3-hydroxymethyl-2-benzothiazolinone (*II*), which smoothly reacts in the second step with 2-alkylthio-6-aminobenzothiazoles (Scheme 1). The advantage of the described method consists in keeping very strict stoichiometric ratios and avoiding the possible side reaction of formaldehyde with the amino group of 2-alkylthio-6-aminobenzothiazole (in one-pot synthesis).



Scheme 1

Compounds *III—XV* were prepared in 48—87 % yields (Table 1). They exhibit very good antiyeast activity (in μmol dm^{-3}) against *Candida albicans*: benzyl (*XV*) (ED_{50} = 78, ED_{100} = 210), n-hexyl (*XI*) (ED_{50} = 40, ED_{100} = 470), n-octyl (*XIII*) and methyl (*III*) derivatives being the most effective. For 2-mercaptobenzothiazole (2-MBT) used as a standard ED_{50} = 360, ED_{100} = 470. Some compounds exhibit even better activity against *Saccharomyces cerevisiae* (lower value of ED_{100}). The most effective were (in decreasing order of activity): n-pentyl (*IX*) (ED_{50} = 18, ED_{100} = 32), n-hexyl (*XI*) (ED_{50} = 15, ED_{100} = 54),

Table 1

Characterization of the prepared compounds *III*—*XV*

Compound	R	Formula	M_r	w_i(calc.)/% / w_i(found)/%					Yield	M.p.
				C	H	Br	N	S	%	°C
III	CH_3	$C_{16}H_{12}BrN_3OS_3$	438.38	43.83 43.78	2.75 2.73	18.22 18.15	9.58 9.62	21.94 22.00	80.0	174—176
IV	C_2H_5	$C_{17}H_{14}BrN_3OS_3$	452.41	45.12 44.99	3.11 2.95	17.66 17.68	9.28 9.32	21.26 20.80	77.7	165—167
V	$(CH_2)_2CH_3$	$C_{18}H_{16}BrN_3OS_3$	466.43	46.34 46.23	3.45 3.43	17.13 17.22	9.00 8.90	20.62 20.19	66.0	169—172
VI	$CH(CH_3)_2$	$C_{18}H_{16}BrN_3OS_3$	466.43	46.34 46.77	3.45 3.50	17.13 16.68	9.00 9.03	20.62 20.08	52.0	168—170
VII	$CH_2CH{=}CH_2$	$C_{18}H_{14}BrN_3OS_3$	464.42	46.54 46.53	3.03 2.95	17.20 16.80	9.04 8.98	20.71 20.52	87.0	180—183
VIII	$(CH_2)_3CH_3$	$C_{19}H_{18}BrN_3OS_3$	480.46	47.49 47.67	3.77 3.74	16.63 16.70	8.74 8.75	20.02 20.10	77.0	167—169
IX	$(CH_2)_4CH_3$	$C_{20}H_{20}BrN_3OS_3$	494.49	48.57 48.71	4.07 3.99	16.16 15.62	8.49 8.48	19.45 19.03	81.0	145—148
X	$CH(CH_2)_4$ cyclo	$C_{20}H_{18}BrN_3OS_3$	492.48	48.77 49.37	3.68 3.67	16.22 15.98	8.53 8.53	19.52 19.50	48.0	149—152
XI	$(CH_2)_5CH_3$	$C_{21}H_{22}BrN_3OS_3$	508.51	49.59 49.70	4.35 4.40	15.71 15.36	8.26 8.23	18.91 18.74	82.0	135—137
XII	$(CH_2)_6CH_3$	$C_{22}H_{24}BrN_3OS_3$	522.54	50.56 50.65	4.62 4.64	15.29 15.01	8.04 7.92	18.41 17.98	80.0	132—135
XIII	$(CH_2)_7CH_3$	$C_{23}H_{26}BrN_3OS_3$	536.56	51.45 51.04	4.88 4.91	14.89 14.71	7.83 7.74	17.92 17.54	52.0	130—132
XIV	$(CH_2)_8CH_3$	$C_{24}H_{28}BrN_3OS_3$	550.59	52.35 52.04	5.12 5.15	14.51 14.73	7.63 7.47	17.47 17.13	50.0	129—131
XV	$CH_2C_6H_5$	$C_{22}H_{16}BrN_3OS_3$	514.47	51.35 51.65	3.13 3.07	15.53 14.96	8.16 8.15	18.69 18.48	80.0	152—155

benzyl (*XV*) (ED_{50} = 100, ED_{100} = 200), n-heptyl (*XII*) and cyclopentyl (*X*) derivatives. For 2-MBT ED_{50} = 520 and $ED_{100} \geq 1000$.

The activity of the prepared compounds against filamentous microscopic fungi *Aspergillus niger* is lower, methyl, ethyl, and n-propyl derivatives *III*—*V* being the relatively most active (MIC = 1000 after 14 d). For 2-MBT MIC = = 1000 after 4 d as well as after 14 d.

n-Propyl derivative *V* was the most effective against *Penicillium cyclopium* and *Rhizopus oryzae* (MIC = 200 after 14 d). For 2-MBT MIC = 1000 after 6 or 14 d.

Alternaria alternata were the most sensitive filamentous microscopic fungi to the tested compounds — ethyl (*IV*), n-pentyl (*IX*), and n-heptyl (*XII*) derivatives being the most effective (MIC = 40 and 200 after 7, resp. 14 d for all three derivatives). For 2-MBT MIC = 200 and 1000 after 7, resp. 14 d.

Derivatives with linear (C_3—C_6) alkyl chain exhibit the best antialgal activity against *Chlorella vulgaris*. For n-propyl (*V*), allyl (*VII*), n-butyl (*VIII*), and n-hexyl (*XI*) derivatives after 21 d MIC was lower than 40. n-Pentyl and n-heptyl derivatives had not been tested. For 2-MBT MIC = 200 both after 10 or 21 d.

n-Hexyl derivative *XI* exhibits the best antiprotozoal activity against *Euglena gracilis* (MIC = 200 after 7, resp. 10 d). For 2-MBT MIC = 1000 after 7, resp. 10 d. From the point of the combined activity against different microorganisms derivative *XI* can be evaluated the most positively.

Starting compounds *I* and *II* had not been effective against *Candida albicans* and *Saccharomyces cerevisiae* even in concentrations (in $\mu mol\ dm^{-3}$) 1000. Compound *I* was effective against filamentous microscopic fungi in concentration 1000 and compound *II* was active only against *Alternaria alternata* in concentration 200 on the 7th day and 1000 on the 14th day. Against *Chlorella vulgaris* compound *I* was effective after 10 as well as 21 d in concentration 1000 and compound *II* in concentration 200. MIC against *Euglena gracilis* was in the case of compound *I* after both 7 or 10 d > 1000 and for compound *II* 1000.

Comparison of antimicrobial activity of *III*—*XV* with the activity of their starting compounds *I* and *II* has shown that the substitution of *I* in the position 3 is a necessary condition for the activity, which was proved to a small degree already in the case of 3-hydroxymethyl derivative (*II*). The contribution of the other component, *i.e.* 2-alkylthio-6-aminobenzothiazole to the activity of the title compounds would be the subject of our next work.

Now it is obvious that antimicrobial activity of the tested compounds varies with the presence of different alkyl groups R in position 2 of the heterocycle. This dependence is fairly different for different microorganisms.

Experimental

Melting points of the synthesized compounds were determined on a Kofler block. Physical constants, analytical data, and yields of compounds *III*—*XV* are given in Table 1.

Liquid synthetic medium containing vitamins was used for 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 the graphical-mathematical methods. These values relate to the sixth day (*Candida albicans*) or the fourth day (*Saccharomyces cerevisiae*) of the cultivation, when the controls reached maximal growth [8].

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

Chodat medium (20 cm^3) was used for determination of the antialgal activity. Cultivation at 20 °C lasted 21 d, with permanent lighting. Consequent concentration of the tested compounds in medium and the reference sample was 1000, 200, 40, and 8 μmol dm^{-3}. In the appropriate time the increase of the green algae was evaluated visually [9].

Antiprotozoal activity was determined on the liquid Mego's modified medium with added compounds at 20 °C and permanent lighting during 10 d. Again, in the appropriate time the whole growth was evaluated visually [10].

6-Bromo-2-benzothiazolinone (I)

To the homogeneous mixture of pulverized 2-benzothiazolinone (15.1 g; 0.1 mol) and *N*-bromosuccinimide (17.8 g; 0.1 mol) chloroform (50 cm^3) was added and the reaction mixture was heated to reflux. After the solid was dissolved, the mixture was heated for 5 min. During that period solid product started to precipitate. After cooling the reaction mixture to room temperature, crude 6-bromo-2-benzothiazolinone was sucked and washed with tetrachloromethane. After drying, the product was boiled in 200 cm^3 of water and immediately sucked and washed with cold water. Product with m.p. = 225—230 °C was obtained in 73.4 % yield (17.1 g).

The sample for elemental analysis was crystallized from ethanol (using active carbon). Melting point of pure compound *I* was 229.5—231 °C. Literature gives m.p. = = 226—228 °C [6].

6-Bromo-3-hydroxymethyl-2-benzothiazolinone (II)

Suspension of *I* (23.0 g; 0.1 mol) in ethanol (200 cm^3) was heated to reflux and formaldehyde (20 cm^3; 0.2 mol) was added through the condenser in small portions. Two

minutes after the solution became clear, white crystalline product started to precipitate. The reaction mixture was then put aside for 24 h at room temperature. The product was sucked off and washed with small portions of ethanol (50 cm^3 — total volume). Yield = 24.0 g (92.3 %), m.p. = 186—189 °C. For $C_8H_6BrNO_2S$ (M_r = 260.10) w_i(calc.): 36.94 % C, 2.32 % H, 30.72 % Br, 5.38 % N, 12.32 % S; w_i(found): 36.80 % C, 2.18 % H, 31.07 % Br, 5.38 % N, 12.19 % S.

3-(2-Alkylthio-6-benzothiazolylaminomethyl)-6-bromo-2-benzothiazolinones III—XV

2-Alkylthio-6-aminobenzothiazole (0.01 mol) and *II* (2.6 g; 0.01 mol) were dissolved in the necessary amount of methanol (20—50 cm^3) on steam bath. The reaction mixture was then stirred without further heating. After cooling to room temperature the solid product was sucked off and washed dropwise with ethanol (10—20 cm^3).

The compounds were purified for analysis by crystallization from the appropriate amount of acetone necessary for dissolving and decolourization by active carbon. Water was added dropwise to the hot filtrate until the first permanent turbidity. The crystalline product was washed with ethanol or petroleum ether.

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References

1. Sutoris, V., Šušoliaková, M., Holbová, E., and Rada, B., *Chem. Zvesti 34*, 700 (1980).
2. Sidóová, E., Odlerová, Ž., Volná, F., and Blöckinger, G., *Chem. Zvesti 33*, 830 (1979).
3. Kuchta, T., Straková, H., Sidóová, E., and Neslušanová, E., *Acta Fac. Rerum Nat. Univ. Comenianae (Microbiologia) 17*, in press.
4. Holbová, E., Sutoris, V., and Blöckinger, G., *Chem. Zvesti 30*, 195 (1976).
5. Holbová, E., Sidóová, E., and Odlerová, Ž., *Chem. Zvesti 30*, 709 (1976).
6. Colonna, M., *Publ. Inst. Chim. Univ. Bologna 1943*, 3; *Chem. Abstr. 41*, 754g (1947).
7. Elderfield, R. C. and Short, F. W., *J. Org. Chem. 18*, 1092 (1953).
8. Zemanová, M., Metwali, M. R., and Gvozdjaková, A., *Acta Fac. Rerum Nat. Univ. Comenianae (Microbiologia) 15 16*, in press.
9. Zemanová, M., *Acta Fac. Rerum Nat. Univ. Comenianae (Microbiologia) 3*, 91 (1973).
10. Foltínová, P., Ebringer, L., and Sutoris, V., *Folia Microbiol.* (Prague) *25*, 412 (1980).

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