COMPUTER AIDED BENZOTHIAZOLE DERIVATIVES. SYNTHESIS, STRUCTURE AND BIOLOGICAL STUDY OF NEW PUSH-PULL CONJUGATED BENZOTHIAZOLIUM SALTS

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Abstract. As the conjugation is assumed to enhance the biological activity of push-pull type benzothiazolium salts, new compounds with extended conjugated bridge between benzothiazolium and phenyl ring were designed and synthesized. The compounds have been tested against the model microorganism *Euglena gracilis* as well as 6 microorganisms including Gram-positive and Gram-negative bacteria , a yeast and a mould. In accordance with predictions, the prepared compounds showed enhanced activity against *Euglena* and Gram-positive bacteria and some of them also interesting fungicidal and fungistatic activity.

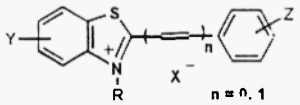
Introduction

Recently we have designed, synthesized and studied benzothiazolium derivatives with enhanced activity against the model microorganism *Euglena gracilis* (1-3). The structural features connected with the biological activity of studied benzothiazolium compounds have been suggested as follows:

1) the phenyl ring bearing an electron-donating substituent (*sec* amino group in *para* position is preferred) bridged to the C-2 carbon of the benzothiazole;

2) the bridge that makes possible the conjugated connection between the benzothiazolium and the phenyl ring;

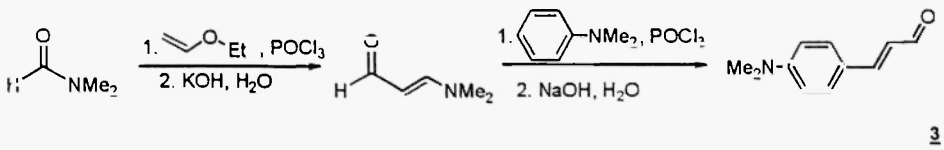
3) the allyl, propargyl or methyl group bonded to the heterocyclic nitrogen.



The next step in the modification of the structure under study is the extension of the conjugated bridge between the heterocycle and the phenyl ring. In this paper we would like to present the synthesis and the study of new benzothiazole derivatives with a buta-1,3-dienyl or a hexa-1,3,5-trienyl bridge between the benzothiazole and the phenyl ring (structure above with n=2 and 3). Nine new compounds were prepared and biologically tested.

Results and Discussion

Target compounds were prepared according to the procedures shown in the Schemes 1-4. The starting aldehydes 3 and 6 were obtained either by Vilsmeier formylation of ethylvinylether (4) and subsequent aromatic electrophilic substitution on the N,N-dialkylaniline (5) (Scheme 1) , or by nucleophilic addition of the protected acetaldehyde 5 on the carbonyl carbon to result in an extension of conjugated system (6) (Scheme 3).



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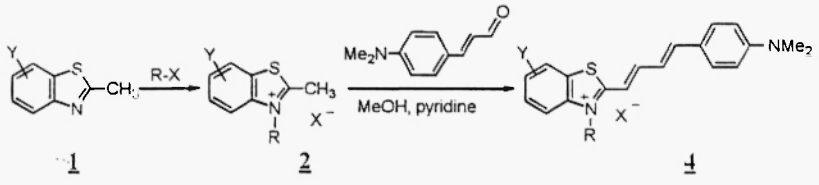
Scheme 1

3-Alkyl-2-methyl benzothiazolium salts 2 were prepared by alkylation of 2-methyl benzothiazoles 1 (Scheme 2). The decisive factor for obtaining good yields of quarternary salts is the temperature. While the yield of 3-allyl-2-methylbenzothiazolium bromide did not exceed 65% when reactants were heated to 70 °C (b.p. of allylbromide) after 30 h. in nitromethane , heating of 2-methylbenzothiazole with excess of allylbromide to 120 °C in autoclave after 10 h. afforded the crude product in 95% yield. Using an autoclave in the case of alkylation with propargylbromide can be dangerous because of the explosiveness of the neat propargylbromide (7). Refluxing of 2-methylbenzothiazole in freshly distilled iodomethane gave the desired salt in good yield.

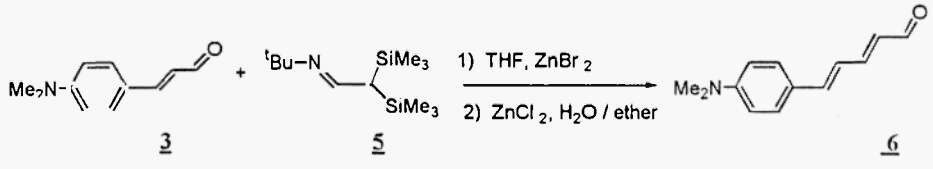


1 2 4

4	Y	R	X
4a	H	Me	I
4b	H	allyl	Br
4c	H	propargyl	Br
4d	5-CH_3	allyl	Br
4e	5-CH_3	propargyl	Br
4f	6-OCH_3	allyl	Br

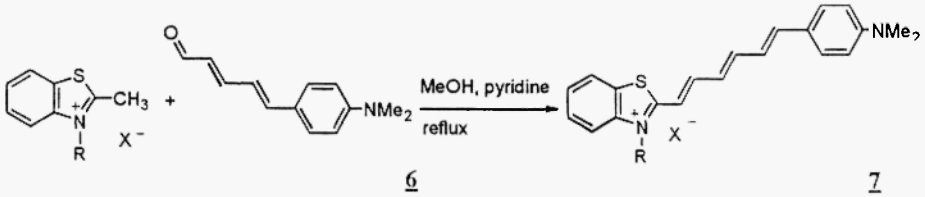
Scheme 2

Condensations of 3-alkyl-2-methylbenzothiazolium salts 2 (8, 9) with *para*-substituted cinnamaldehydes 3 and 5-phenylpenta-2,4-dienal 6 proceeded in refluxing methanol under pyridine catalyse (Schemes 2,4). Yields of prepared 3-alkyl-2-[4-(4-dimethylaminophenyl)buta-1,3-dien-1-yl]benzothiazolium salts 4 and 3-alkyl-2-[6-(4-dimethyl aminophenyl)hexa-1,3,5-trien-1-yl]benzothiazolium salts 7 were determined after the crystalization of the reaction mixture in ethanol. All the compounds were characterized by physical constants, elemental analysis, UV-VIS (methanol, concentration 5.10^{-5} $mol.l^{-1}$) and ^1H NMR spectral data (Table 2 and 3).



3 5 6

Scheme 3



6 7

7	R	X
7a	Me	I
7b	allyl	Br
7c	propargyl	Br

Scheme 4

Table 1. Biological activities of the compounds 4a-e, 7a-c

comp.	*Euglena*	*S.aureus*	*B.subtilis*	*E.coli*	*Ps.aerugin.*	*C.albic.*	*Micros.*
4a	6.437	10	50	250	>250	50	50/10 *
4b	6.600	2	10	250	>250	50	2/0.5
4c	6.821	2	10	250	>250	50	2/0.5
4d	7.011	2	10	250	>250	20	2/0.5
4e	6.939	2	10	250	>250	20	2/0.5
4f	6.457	2	10	>250	>250	50	10/2
7a	6.976	2	10	50	>250	50	5/2.5
7b	6.986	0.5	2	50	>250	10	5/1
7c	6.978	2	10	50	>250	10	5/2.5

* fungicidal / fungistatic activities

Table 1 presents the results of biological tests against the unicellular flagelate *Euglena gracilis* (log ($1000/ED_{50}$) in 10^{-3} $mol.l^{-1}$) as well as antimicrobial MIC values in mg/ml against Gram-positive (*Staphylococcus aureus* CCM 2394, *Bacillus subtilis* CCM 18/64) and Gram-negative bacteria (*Escherichia coli* 326/71), a yeast (*Candida albicans* Pn-10) and a mould (*Microsporum gypseum*). In accordance with the prediction, the tested compounds show high activity against *Euglena gracilis*. Compounds 4d, 4e and 7a-c are the most active.The activity against Gram-positive bacteria is also high and compound 7b is the best one. Compounds 4b-e show interesting fungicidal and fungistatic activity against *Microsporum gypseum*.

Experimental

Synthesis

Melting points were determined on a Kofler block and are uncorrected. Elemental analysis were determined on CarloErba 1016 instrument. ^1H NMR spectra were recorded with a Variant Gemini 2000 , 300MHz instrument in DMSO-d6 using TMS as an internal standard. UV-VIS spectral data were recorded on spectrophotometer HP 8452A Hewllett Packard. Column chromatography was performed using silica gel (mesh 40/100) , thin-layer chromatography was performed on silica precoated plates , Silufol UV-254 (Kavalier , Czech Republic).

2,3-Dimethylbenzothiazolium iodide 2a

A mixture of 2-methylbenzothiazole (1.0g , 6.7mmol) and freshly distilled iodomethane (3.5g , 25mmol) in nitromethane (7ml) was heated at 70 °C for 24h. The crystalline product was filtered off , washed with acetone and dried *in vacuo* to give the corresponding salt 2a (1.8g , 93%); m.p. 225-6 °C.

3-Allyl-2-methylbenzothiazolium bromide. 2b

A solution of 2-methylbenzothiazole (5g , 0.033mol) in allyl bromide (15ml , 0.174mol) and boron trifluoride etherate (3 drops) was placed into the steel autoclave and heated at 120 °C for 12h. The crude mild green crystalline product (8.3g) was filtered off , washed with acetone , dried and recrystallised from chloroform/acetone 1:1 to give white crystals 2b (7.3g , 82%); m.p. 217-18 °C.

2-Methyl-3-propargylbenzothiazolium bromide 2c

A mixture of 2-methylbenzothiazole (5.0g , 0.033mol) and propargyl bromide (15ml , 0.195mol) in nitromethane (30ml) was refluxed for 24h. The crude gray product (5.2g) was filtered off , washed with acetone , dried and recrystallised from nitromethane to give white crystals 2c (4.3g , 48%); m.p. 235-8 °C.

α,α-Bis(trimethylsilyl)-N-tert-butylacetaldimine 5

To a stirred solution of LDA (0.306mol) in anhydrous THF (150ml), was added dropwise N-tert-butylacetaldimine (15.0g , 0.151mol at –60 °C. The mixture was stirred at this temperature for 18h. Trimethylchlorosilane was then added in two portions: the first portion (20ml , 0.155mol) was added at –60 °C and the solution was stirred for 5h. at this temperature under nitrogen ; the second portion (20ml , 0.155mol) was added and the solution was stirred for 1h. under the same conditions. Slow warming to room temperature of the resulting mixture was followed by filtration through a pad of Celite and evaporation of the solvent under reduced pressure. The distilation of the remaining yellow oil gave the silylated product 5 (26.5g , 72%) b p. 90-93°C/2.7kPa.

3-(4-N,N-dimethylaminophenyl)propenal 3

To a solution of 3-(N,N-dimethylamino)propenal (10mmol) and N,N-dimethylaniline (15mmol) in chloroform (3ml) was added dropwise a solution of $POCl_3$ (10mmol) in chloroform (2ml) under nitrogen at –10 °C over a period of 30 min. The mixture was gradually allowed to warm to room temperature, then heated at 65 °C for 3h. The solvent was removed under reduced pressure before methanol (3ml) and ice (13g) are slowly added. The solution was hydrolysed by addition of aqueous sodium hydroxide (10ml , 5N) and stirred for 18h. at room temperature. The yellow suspension was extracted with chloroform (2x20ml) and the combined organic layers were filtered off , washed with water (10ml), then dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude mixture was purified by flash chromatography (silica gel , hexane:acetone-2:1) to give product 3 (44%) as an orange powder ; m.p. 137-9 °C .

5-(4-N,N-dimethylaminophenyl) penta-2,4-dienal 6

To a solution of 3-(4-N,N-dimethylaminophenyl)propenal (2.6g , 14.93mmol) and $ZnBr_2$ (3.7g , 16.42mmol) in THF (15ml), was added dropwise under nitrogen at room temperature a solution of α,α-bis(trimethylsilyl)-N-tert-butylacetaldimine 5 (4.0g , 16.42mmol) in THF (5ml). This mixture was stirred for 2h. The resulting solution was hydrolysed by addition of a solution of $ZnCl_2$ (3.0g) in a mixture of water (30ml) and ether (40ml). The red suspension was stirred for 1h. at room temperature. The precipitate was filtered through a pad of Celite. The aqueous layer was extracted with ether (3x250ml) and the combined organic extracts were washed with water (2x150ml), then dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude reaction product was purified by flash chromatography (silica gel , hexane:ethylacetate – 2:1) to give the expected product 6 (1.8g , 61%).m.p. 153-5 °C.

Preparation of compounds 4a-f and 7a-c. General Procedure.

A mixture of 3-alkyl-2-methylbenzothiazolium salt (1.3mmol) and aldehyde (1.4mmol) in methanol (3ml) was refluxed for 15h in the catalytic presence of pyridine (1drop). The mixture was allowed to cool to room temperature , the product was separated by filtration , washed with cold methanol, then acetone, dried *in vacuo* and recrystallised from ethanol to give dark crystals. Yields , physical properties and spectral data of compounds 4a-f , 7a-c are given in Tables 2,3.

Biological testing

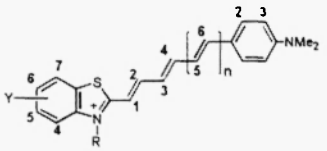
The compounds have been tested on growth of the autotrophic form of unicellular flagelate *Euglena gracilis*. The appropriate concentrations of the tested substances were prepared by dissolving in DMSO and the effect was monitored in a liquid Cramer/Myers medium. The ED_{50} toxicity values (in 10^{-3} mol.l^{-1}) were interpolated from cubic relation. The log ($1000/ED_{50}$) values are presented in Table 1.

In vitro antimicrobial activities were measured by the plate diffusion method using Mueller-Hinton and Sabouraud agar, or by the standard dilution method in Saboraud medium (10). MIC values in mg/ml are given in Table 1.

Table 2. Physico-chemical data for compounds 4a-f, 7a-c

	Formula	M.w.	Yield [%]	m.p.[°C]	λ_{max} [nm]	ε_{max} [$mol^{-1}m^2$]
4a	$C_{20}H_{21}N_2SI$	448.6	68	226-9	560	7759
4b	$C_{22}H_{23}N_2SBr$	427.4	82	217-9	574	9828
4c	$C_{22}H_{21}N_2SBr$	425.4	92	213-4	592	8054
4d	$C_{23}H_{25}N_2SBr$	441.4	65	214-7	574	8064
4e	$C_{23}H_{23}N_2SBr$	439.4	96	206-8	584	6020
4f	$C_{23}H_{25}N_2OSBr$	457.4	82	221-6	566	8102
7a	$C_{22}H_{23}N_2SI$	474.6	77	223-6	580	4290
7b	$C_{24}H_{25}N_2SBr$	453.4	56	207-8	592	2700
7c	$C_{24}H_{23}N_2SBr$	451.4	74	213-7	612	4838

Table 3 ^{1}H NMR spectral data of studied compounds (300MHz, DMSO-d_6, TMS, δ ppm; s, d or dd, [J]= Hz).
N =0 in compounds 4, n =1 in compounds 7



	heterocycle				conjugated bridge						phenyl			Y	R
	H4	H5	H6	H7	H1	H2	H3	H4	H5	H6	H2	H3	NMe_2		
4a	8.14 8.2	7.81 8.2 7.2	7.71 7.9 7.2	8.33 7.9	7.33 14.6	7.99 14.6 10.7	7.20 15.1 10.7	7.46 15.1			7.55 8.9	6.79 8.9	3.04		4.16 (s, 3H, CH_3-N^+)
4b	8.12 8.4	7.80 8.4 6.9	7.71 7.8 6.9	8.36 7.8	7.29 14.7	8.05 14.7 11.1	7.21 15.1 11.2	7.50 15.2			7.57 8.8	6.78 8.8	3.06		6.09 (m, 1H, -CH=), 5.38 (d, J^3=3.8, 2H, -CH_2-N^+), 5.34 (d, J^3=10.5, 1H, =CH_2cis), 5.24 (d, J^3 =17.4, 1H,=CH_2trans)
4c	8.19 8.3	7.85 8.3 7.4	7.73 8.2 7.4	8.36 8.2	7.38 14.3	8.10 14.3 11.6	7.24 14.8 11.6	7.56 14.8			7.61 8.8	6.82 8.8	3.07		5.68 (d, J^4=1.9, 2H, -CH_2-N^+), 3.79 (t, J^4=1.9, 1H, ≡CH)
4d	8.04		7.55 8.4	8.20 8.4	7.33 14.3	8.05 14.3 11.2	7.21 15.1 11.2	7.51 15.1			7.60 8.9	6.80 8.9	3.06	2.54	6.07 (m, 1H, -CH=), 5.36 (d, J^3=4.2, 2H, -CH_2-N^+), 5.34 (d, J^3=10.5, 1H, =CH_2cis), 5.23(d, J^3=17.4, 1H, =CH_2trans)
4e	7.95		7.53 8.2	8.21 8.2	7.24 14.3	8.00 14.3 10.9	7.19 14.9 10.9	7.46 14.9			7.54 8.8	6.70 8.8	3.05	2.52	5.62 (d, J^4=1.9, 2H, -CH_2-N^+) , 3.76 (t, J^4=1.9, 1H, ≡CH)
4f	8.02 9.2	7.38 9.2 2.5		7.96 2.5	7.23 14.5	7.94 14.5 10.9	7.16 14.9 10.9	7.42 14.9			7.53 9.0	6.70 9.0	3.04	3.90	6.05 (m, 1H, -CH=), 5.34 (d, J^3=5.1, 2H, -CH_2-N^+), 5.33 (d, J^3=10.6, 1H, =CH_2cis), 5.21(d, J^3=17.4, 1H, =CH_2trans)
7a	8.14 8.2	7.82 8.2 7.5	7.72 7.7 7.5	8.34 7.7	7.37 14.6	7.94 14.6 11.3	6.76 14.2 11.3	7.33 14.2 10.6	7.05 15.9 10.6	7.00 15.9	7.50 8.8	6.70 8.8	3.00		4.18 (s, 3H, CH_3-N^+)
7b	8.12 8.3	7.80 8.3 7.2	7.71 7.4 7.2	8.37 7.4	7.36 14.6	7.99 14.6 11.3	6.79 13.9 11.2	7.36 13.8 10.6	7.06 15.8 10.6	7.02 15.8	7.51 8.8	6.74 8.8	3.01		6.06 (m, 1H, -CH=), 5.41 (d, J^3=4.1, 2H, -CH_2-N^+), 5.33 (d, J^3=10.4, 1H, =CH_2cis), 5.24(d, J^3=17.3, 1H, =CH_2trans)
7c	8.19 8.5	7.85 8.5 7.2	7.73 7.4 7.2	8.36 7.4	7.43 14.6	8.04 14.6 11.3	6.78 14.0 11.3	7.38 14.0 10.5	7.10 15.9 10.6	7.04 15.9	7.53 9.0	6.75 9.0	3.02		5.69 (d, J^4=2.1, 2H, -CH_2-N^+), 3.76 (t, J^4=2.1, 1H, ≡CH)

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