# SYNTHESIS AND QSAR STUDIES OF NEW CONJUGATED BENZOTHIAZOLE DERIVATIVES

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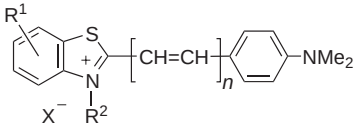
A series of new push-pull 2-(ω-phenylalkenyl)benzothiazolium compounds with methyl groups in different positions of the conjugated alkenyl bridge were synthesized by alkylation and condensation. The antimicrobial testing has been carried out and the effect of the alkenyl substituent was investigated by the QSAR Free–Wilson method.

**Keywords**: Benzothiazolium salts; Push-pull systems; Antimicrobial activity; Free–Wilson method; Benzothiazoles; Alkenes; Dienes; Substituent effects.

In the last few years a systematic study of benzothiazole derivatives with antimicrobial activity has been carried out and good structure-activity relations have been formulated[1–4]. The following structure features influencing biological activity have been suggested (Structure A).




Structure A

(*1*) the benzene ring bearing an electron-donating substituent (*secondary* amino group in *para* position is preferred),

(*2*) the conjugated bridge between the benzothiazole C-2 carbon and the benzene ring,

(*3*) the allyl, prop-2-yn-1-yl or methyl group bonded to the benzothiazole nitrogen.

Based on structure-activity information, new compounds with enhanced activity against the model microorganism *Euglena gracilis* were designed and synthesized.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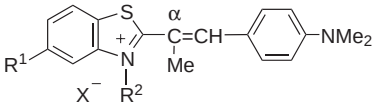
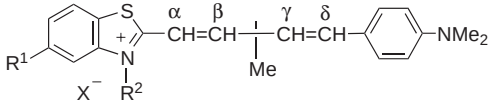
In the present paper our attention has been focused on modification of the conjugated bridge between the heterocycle and the benzene ring. The most active compounds prepared by us contain three or two double bonds ($n$ = 3 or 2) in the bridge. The electron-donor $NMe_2$ group on one and the electron-acceptor quaternary nitrogen on the opposite side of the conjugated system cause the push-pull character of the studied compounds. The assumption that the conjugation degree affects their biological activity led us to the modification of the bridge. With the aim of investigating the effect of the bridge substituents on the structure and biological activity, the new benzothiazolium salts with the methyl group in different positions of the conjugated bridge have been prepared (Structures B and C).




Structure B




Structure C

The presence of the methyl group in the bridge could decrease the conjugation and modify the biological activity. The QSAR study by the additive Free–Wilson method[5] has been carried out and quantitative contributions of structural factors to the biological activity were calculated.

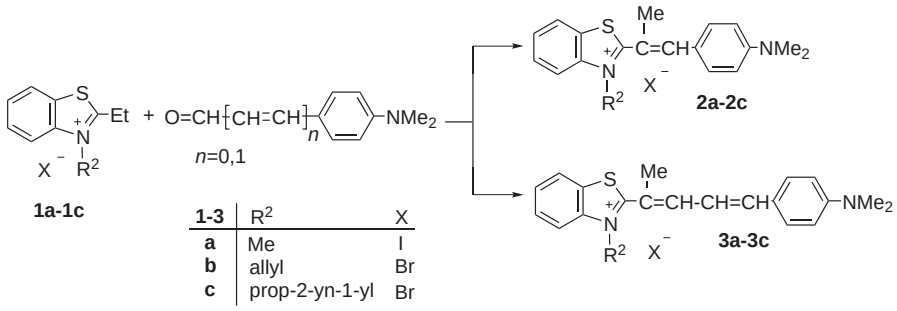
## RESULTS AND DISCUSSION

### *Chemistry*

The starting 2-methylbenzothiazole, 2,5-dimethylbenzothiazole, 3-[4-(dimethylamino)phenyl]propenal and 1-[4-(dimethylamino)phenyl]ethan-1-one are commercially available. 2,3,5-Trialkylbenzothiazolium salts **1** were prepared according to literature[6–8]. The reaction was performed either in nitromethane ($R^2$ = methyl or prop-2-yn-1-yl) or without solvent in an autoclave ($R^2$ = allyl).

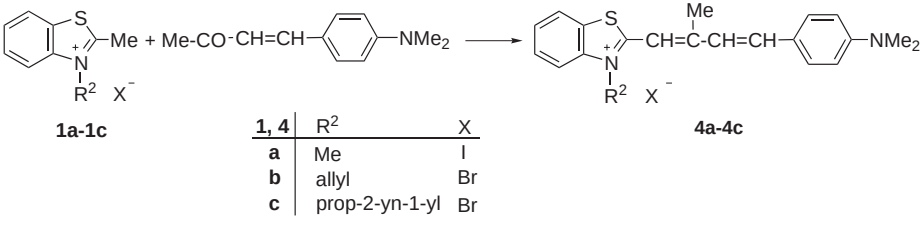
Compounds **2** and **3** with methyl in α-position to the heterocycle were synthesized by the aldol condensation of 2-ethylbenzothiazolium salts with corresponding aromatic aldehyde (Scheme 1).

β-Methyl derivatives **4a**–**4c** were prepared by the aldol condensation of 2-methylbenzothiazolium salts with 4-(dimethylamino)phenylbutene-2-one[9] (Scheme 2).




| 1-3 | $R^2$ | X |
|---|---|---|
| **a** | Me | I |
| **b** | allyl | Br |
| **c** | prop-2-yn-1-yl | Br |

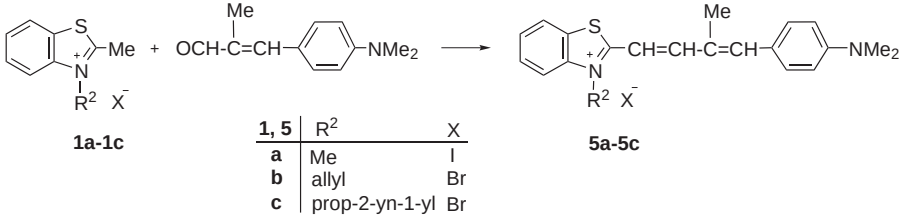
SCHEME 1




| 1, 4 | $R^2$ | X |
|---|---|---|
| **a** | Me | I |
| **b** | allyl | Br |
| **c** | prop-2-yn-1-yl | Br |

SCHEME 2

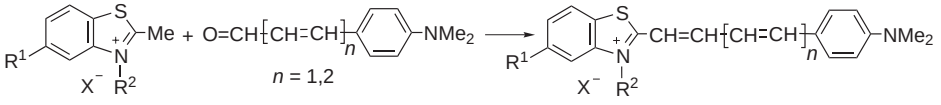
Reflux of 3-[4-(dimethylamino)phenyl]-2-methylpropenal[10] with 2-methylbenzothiazolium salts in methanol has led to γ-methyl-substituted derivatives **5a**–**5c** (Scheme 3).




| 1, 5 | $R^2$ | X |
|---|---|---|
| **a** | Me | I |
| **b** | allyl | Br |
| **c** | prop-2-yn-1-yl | Br |

SCHEME 3

The studied series was completed with the compounds without methyl substituents in the conjugated bridge, namely 3-alkyl-2-{4-[4-(dimethylamino)phenyl]buta-1,3-dien-1-yl}benzothiazolium salts **6a**–**6f**, 3-alkyl-2-{6-[4-(dimethylamino)phenyl]hexa-1,3,5-trien-1-yl}benzothiazolium salts **7a**–**7f** and 3-allyl-2-{2-[4-(dimethylamino)phenyl]ethen-1-yl}benzothiazolium bromide (**8b**). The synthesis of compounds **6** and **7** is shown in Scheme 4. The preparation of 5-[4-(dimethylamino)phenyl]penta-2,4-dienal was described previously[7,11].




**1a-1c**

| **1, 6, 7** | $R^1$ | $R^2$ | X |
|---|---|---|---|
| **a** | 5-H | Me | I |
| **b** | 5-H | allyl | Br |
| **c** | 5-H | prop-2-yn-1-yl | Br |
| **d** | 5-Me | Me | I |
| **e** | 5-Me | allyl | Br |
| **f** | 5-Me | prop-2-yn-1-yl | Br |

SCHEME 4

*Biological Activity and QSAR Study*

The compounds under study were evaluated for their toxicity against the unicellular autotrophic flagellate *Euglena gracilis*. Their biological effects, expressed as log $(1000/ED_{50})$ in $10^{-3}$ mol $l^{-1}$, as well as the structural factors used as input in QSAR calculations are given in Table I.

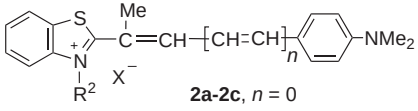
The Free–Wilson method[5] in the Fujita–Ban approximation[12] was used to calculate the toxicity contributions of substituent ($R^1$, $R^2$) as well as the structural factors (number of double bonds and position of Me group in the conjugated bridge). The results are presented in Table II.

The studied series of compounds represents benzothiazolium salts with high biological activity caused by the push-pull character of the conjugated benzothiazolium structure. Configuration arrangement on the bridge double bonds was found as *E* in all studied compounds. This fact was confirmed by the AM1 quantum-chemical calculations[13] as well as the NMR NOE experiments[14]. The activity of the reference compound is 6.218 and most of structural fragments increase it. The most important is the contribution of the conjugated bridge with three or two double bonds. The effect of 5-Me bonded to the fused benzene ring or 3-alkyl group bonded to the quaternary nitrogen is not essential and their calculated values are statistically little significant. This could mean that the overall variation in the data was not large enough to obtain a significant relations. The synthesis and the study of large series of compounds with more $R^1$ and $R^2$ substituents are in progress.

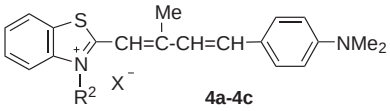
As expected, the presence of Me group in any position of the conjugated bridge lowers the activity owing to the distortion of the heterocyclic and benzene rings from planarity. This fact causes a decrease in delocalization

TABLE I

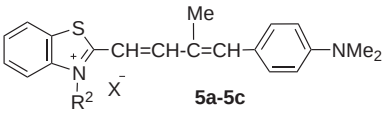
Structure and toxicity against *Euglena gracilis* (*E.g.*) of studied compounds (log ($1000/ED_{50}$) in $10^{-3}$ mol $l^{-1}$)



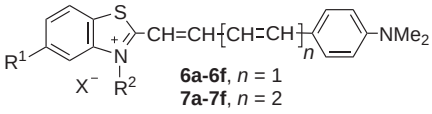

**2a-2c**, *n* = 0
**3a-3c**, *n* = 1




**4a-4c**




**5a-5c**




**6a-6f**, *n* = 1
**7a-7f**, *n* = 2

| Compound | $R^1$ | $R^2$ | X | Me | *n* | *E.g.* |
|---|---|---|---|---|---|---|
| **2a** | H | Me | I | α | 0 | 6.225 |
| **2b** | H | allyl | Br | α | 0 | 6.064 |
| **2c** | H | prop-2-yn-1-yl | Br | α | 0 | 6.017 |
| **3a** | H | Me | I | α | 1 | 6.457 |
| **3b** | H | allyl | Br | α | 1 | 6.485 |
| **3c** | H | prop-2-yn-1-yl | Br | α | 1 | 6.468 |
| **4a** | H | Me | I | β | 1 | 5.695 |
| **4b** | H | allyl | Br | β | 1 | 5.703 |
| **4c** | H | prop-2-yn-1-yl | Br | β | 1 | 5.800 |
| **5a** | H | Me | I | γ | 1 | 6.490 |
| **5b** | H | allyl | Br | γ | 1 | 6.509 |
| **5c** | H | prop-2-yn-1-yl | Br | γ | 1 | 6.503 |
| **6a**[a] | H | Me | I | – | 1 | 6.437 |
| **6b**[a] | H | allyl | Br | – | 1 | 6.445 |
| **6c**[a] | H | prop-2-yn-1-yl | Br | – | 1 | 6.821 |
| **6d** | 5-Me | Me | I | – | 1 | 6.791 |
| **6e**[a] | 5-Me | allyl | Br | – | 1 | 6.874 |
| **6f**[a] | 5-Me | prop-2-yn-1-yl | Br | – | 1 | 6.939 |
| **7a**[a] | H | Me | I | – | 2 | 6.955 |
| **7b**[a] | H | allyl | Br | – | 2 | 6.964 |
| **7c**[a] | H | prop-2-yn-1-yl | Br | – | 2 | 6.962 |
| **7d** | 5-Me | Me | I | – | 2 | 6.799 |
| **7e** | 5-Me | allyl | Br | – | 2 | 6.782 |
| **7f** | 5-Me | prop-2-yn-1-yl | Br | – | 2 | 6.757 |
| **8b**[b] | H | allyl | Br | – | 0 | 6.135 |

[a] Ref.[7]; [b] ref.[4]

but the abnormal decrease in activity observed in the case of β-Me substituent is difficult to explain.

The new compounds have been tested also *in vitro* for their antimicrobial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), as well as

TABLE II
Calculated toxicity contributions of structure fragments and their standard errors *s* (reference compound: $n = 0$, $R^1$ = H, $R^2$ = allyl and X = Br, bridge without Me). Statistics: number of compounds $n = 25$, multiple correlation coefficient $R = 0.958$, standard deviation of regression $s = 0.140$ and $F$-value = 22.39

| Structure fragment | Toxicity contribution | *s* |
|---|---|---|
| Reference compound | 6.218 | 0.099 |
| Number of double bonds | | |
| $n = 0$ | 0.000 | |
| $n = 1$ | 0.423 | 0.093 |
| $n = 2$ | 0.589 | 0.113 |
| $R^1$ | | |
| 5-H | 0.000 | |
| 5-Me | 0.073 | 0.079 |
| $R^2$ and X | | |
| allyl, Br | 0.000 | |
| Me, I | 0.013 | 0.069 |
| prop-2-yn-1-yl, Br | 0.065 | 0.069 |
| Me in bridge | | |
| without Me | 0.000 | |
| α-Me | –0.170 | 0.090 |
| β-Me | –0.935 | 0.104 |
| γ-Me | –0.167 | 0.104 |

the yeast (*Candida albicans*) and the mould (*Microsporum gypseum*). Minimum inhibitory concentrations (MIC in μg/ml) are given in Table III.

In accordance with our previous findings[7,8], the compounds are inactive against Gram-negative but active against Gram-positive microorganisms. Compounds **3a**, **3b**, **4b**, **6d** and **7d–7f** show interesting fungicidal and fungistatic activity against *Microsporum gypseum*.

TABLE III
Antimicrobial activities (MIC in μg/ml) of compounds **2–5**, **6d** and **7d–7f**

| Compound | MIC, μg/ml | | | | | |
|---|---|---|---|---|---|---|
| | *S.a.* | *B.s.* | *E.c.* | *P.a.* | *C.a.* | *M.g.* |
| **2a** | 2 | 50 | 250 | >250 | 50 | 10/2[a] |
| **2b** | 0.4 | 10 | 250 | >250 | 10 | 10/2 |
| **2c** | 2 | 50 | 250 | >250 | 50 | 10/2 |
| **3a** | 2 | 10 | 250 | >250 | 50 | 2/0.4 |
| **3b** | 0.4 | 10 | 50 | >250 | 50 | 2/0.4 |
| **3c** | 0.4 | 10 | 50 | >250 | 50 | 10/2 |
| **4a** | 2 | 10 | 250 | >250 | 250 | 50/10 |
| **4b** | 2 | 10 | 250 | >250 | 50 | 2/0.4 |
| **4c** | 2 | 10 | 250 | >250 | 50 | 10/2 |
| **5a** | 2 | 10 | 250 | >250 | 250 | 10/2 |
| **5b** | 2 | 2 | 50 | >250 | 50 | 10/2 |
| **5c** | 2 | 2 | 250 | >250 | 50 | 10/2 |
| **6d** | 2 | 10 | 250 | >250 | 10 | 2/0.4 |
| **7d** | 2 | 10 | 250 | >250 | 10 | 2/0.4 |
| **7e** | 2 | 10 | 250 | >250 | 10 | 2/0.4 |
| **7f** | 2 | 10 | 250 | >250 | 10 | 2/0.4 |
| Ampicilin | 0.08 | 4 | 10 | >250 | | |
| Nystatine | | | | | 10 | 2/0.4 |

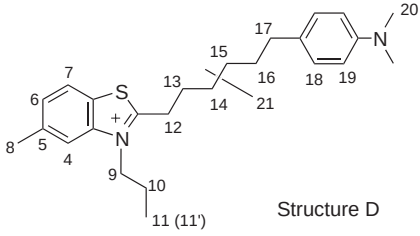
[a] Fungicidal/fungistatic activity. (*S.a.*, *Staphylococcus aureus* CCM3953; *B.s.*, *Bacillus subtilis* 18/64; *E.c.*, *Escherichia coli* CCM3988; *P.e.*, *Pseudomonas aeruginosa* CCM8221; *C.a.*, *Candida albicans* Pn-10; *M.g.*, *Microsporum gypseum*)

## EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. $^1$H NMR spectra (δ, ppm; *J*, Hz) were measured on a Varian Gemini 300 (300 MHz) instrument in DMSO-$d_6$ using TMS as an internal standard. UV-VIS spectral data ($\lambda_{max}$, nm) were recorded on an HP 8452A spectrophotometer. Column chromatography was performed on silica precoated plates, Silufol UV-254 (Kavalier, Czech Republic). Elemental analysis was determined on an Erba Science 1106 instrument.

### Preparation of Target Compounds. General Procedure

A mixture of substituted benzothiazolium salt **1** (1.3 mmol) and corresponding carbonyl compound (1.4 mmol) in methanol (3 ml) was refluxed for 15 h in the catalytic presence of pyridine (15 mg). The catalyst is not suitable for the cases: $n$ = 0, Me in α-position (**2a**–**2c**) and $n$ = 1, Me in β-position (**4a**–**4c**). The mixture was allowed to cool to room temperature, the product was separated by filtration (compound **4b** was separated by column chromatography of the reaction mixture on silica gel (chloroform–methanol 5:1)), washed sequentially with cold methanol and acetone, dried *in vacuo* and crystallized from ethanol to give dark crystals. Yields of the prepared salts **2**–**7** were determined after crystallization of the products in ethanol. The proton numbering in the NMR interpretation is indicated by the numbering of the corresponding carbon of the general skeleton shown in Structure D.




Structure D

*2-Ethyl-3-methylbenzothiazolium iodide* (**1a**): Yield 89%, m.p. 174–175 °C. For $C_{10}H_{12}INS$ (305.2) calculated: 39.36% C, 3.96% H, 4.59% N, 10.51% S; found: 39.30% C, 4.01% H, 4.52% N, 10.42% S. $^1$H NMR: 8.45 d, 1 H, *J*(6,7) = 8.3 (H-7); 8.31 d, 1 H, *J*(4,5) = 8.1 (H-4); 7.92 dd, 1 H, *J*(5,6) = 7.8, *J*(4,5) = 8.1 (H-5); 7.82 dd, 1 H, *J*(5,6) = 7.8, *J*(6,7) = 8.3 (H-6); 4.22 s, 3 H (H-9); 3.49 q, 2 H, *J*(12,13) = 7.5 (H-12); 1.48 t, 3 H, *J*(12,13) = 7.5 (H-13).

*3-Allyl-2-ethylbenzothiazolium bromide* (**1b**): Yield 71%, m.p. 163–165 °C. For $C_{12}H_{14}BrNS$ (284.2) calculated: 50.71% C, 4.96% H, 28.11% Br, 4.93% N, 11.28% S; found: 50.66% C, 5.10% H, 28.01% Br, 4.91% N, 11.20% S. $^1$H NMR: 8.50 d, 1 H, *J*(6,7) = 8.1 (H-7); 8.32 d, 1 H, *J*(4,5) = 8.2 (H-4); 7.90 dd, 1 H, *J*(5,6) = 7.8, *J*(4,5) = 8.2 (H-5); 7.83 dd, 1 H, *J*(5,6) = 7.8, *J*(6,7) = 8.1 (H-6); 6.08 bm, 1 H (H-10); 5.46 d, 2 H, *J*(9,10) = 4.5 (H-9); 5.39 d, 1 H, *J*(10,11′) = 10.2 (H-11′); 5.33 d, 1 H, *J*(10,11) = 16.5 (H-11); 3.53 q, 2 H, *J*(12,13) = 7.4 (H-12); 1.49 t, 3 H, *J*(12,13) = 7.4 (H-13).

*2-Ethyl-3-(prop-2-yn-1-y)lbenzothiazolium bromide* (**1c**): Yield 39%, m.p. 167–168 °C. For $C_{12}H_{12}BrNS$ (282.2) calculated: 51.07% C, 4.29% H, 28.31% Br, 4.96% N, 11.36% S; found: 50.64% C, 4.27% H, 28.14% Br, 4.99% N, 11.32% S. $^1$H NMR: 8.52 d, 1 H, *J*(6,7) = 7.7 (H-7); 8.38 d, 1 H, *J*(4,5) = 8.4 (H-4); 7.97 dd, 1 H, *J*(5,6) = 7.3, *J*(4,5) = 8.4 (H-5); 7.85 dd, 1 H,

*J*(5,6) = 7.3, *J*(6,7) = 7.7 (H-6); 5.80 d, 2 H, *J*(9,10) = 2.5 (H-9); 3.85 t, 1 H, *J*(9,10) = 2.5 (H-10); 3.62 q, 2 H, *J*(12,13) = 7.4 (H-12); 1.51 t, 3 H, *J*(12,13) = 7.4 (H-13).

*2,3,5-Trimethylbenzothiazolium iodide* (**1d**): Yield 94%, m.p. 267–270 °C. For $C_{10}H_{12}INS$ (305.2) calculated: 39.36% C, 3.96% H, 4.59% N, 10.51% S; found: 39.02% C, 3.90% H, 4.58% N, 10.50% S. $^1$H NMR: 8.30 d, 1 H, *J*(6,7) = 8.1 (H-7); 8.14 s, 1 H (H-4); 7.64 d, 1 H, *J*(6,7) = 8.1 (H-6); 4.17 s, 3 H (H-9); 3.15 s, 3 H (H-12); 2.58 s, 3 H (H-8).

*3-Allyl-2,5-dimethylbenzothiazolium bromide* (**1e**): Yield 86%, m.p. 220–222 °C. For $C_{12}H_{14}BrNS$ (284.2) calculated: 50.71% C, 4.96% H, 28.11% Br, 4.93% N, 11.28% S; found: 50.56% C, 5.07% H, 27.65% Br, 4.87% N, 11.07% S. $^1$H NMR: 8.33 d, 1 H, *J*(6,7) = 8.4 (H-7); 8.12 s, 1 H (H-4); 7.64 d, 1 H, *J*(6,7) = 8.4 (H-6); 6.07 bm, 1 H (H-10); 5.40 d, 2 H, *J*(9,10) = 4.8 (H-9); 5.36 d, 1 H, *J*(10,11′) = 10.5 (H-11′); 5.29 d, 1 H, *J*(10,11) = 17.4 (H-11); 3.18 s, 3 H (H-12); 2.53 s, 3 H (H-8).

*2,5-Dimethyl-3-(prop-2-yn-1-yl)benzothiazolium bromide* (**1f**): Yield 51%, m.p. 231–232 °C. For $C_{12}H_{12}BrNS$ (282.2) calculated: 51.07% C, 4.29% H, 28.31% Br, 4.96% N, 11.36% S; found: 50.77% C, 4.31% H, 28.20% Br, 4.90% N, 11.21% S. $^1$H NMR: 8.35 d, 1 H, *J*(6,7) = 8.5 (H-7); 8.22 s, 1 H (H-4); 7.68 d, 1 H, *J*(6,7) = 8.5 (H-6); 5.72 d, 2 H, *J*(9,10) = 2.4 (H-9); 3.85 t, 1 H, *J*(9,10) = 2.4 (H-10); 3.25 s, 3 H (H-12); 2.59 s, 3 H (H-8).

*2-{2-[4-(Dimethylamino)phenyl]-1-methylvinyl}-3-methylbenzothiazolium iodide* (**2a**): Yield 48%, m.p. 138–140 °C. For $C_{19}H_{21}IN_2S$ (436.4) calculated: 52.30% C, 4.85% H, 6.42% N, 7.35% S; found: 51.71% C, 4.84% H, 6.46% N, 7.28% S. $^1$H NMR: 8.42 d, 1 H, *J*(6,7) = 7.7 (H-7); 8.26 d, 1 H, *J*(4,5) = 8.4 (H-4); 7.92 dd, 1 H, *J*(5,6) = 7.2, *J*(4,5) = 8.4 (H-5); 7.81 dd, 1 H, *J*(5,6) = 7.2, *J*(6,7) = 7.7 (H-6); 7.59 d, 2 H, *J*(18,19) = 9.0 (H-18); 7.35 s, 1 H (H-13); 6.84 d, 2 H, *J*(18,19) = 9.0 (H-19); 4.25 s, 3 H (H-9); 3.04 s, 6 H (H-20); 2.54 s, 3 H (H-21). UV: 448 ($H_2O$), 482 ($CHCl_3$). TLC: $R_F$ 0.30 (chloroform–methanol 5:1).

*3-Allyl-2-{2-[4-(dimethylamino)phenyl]-1-methylvinyl}benzothiazolium bromide* (**2b**): Yield 61%, m.p. 122–125 °C. For $C_{21}H_{23}BrN_2S$ (415.4) calculated: 60.72% C, 5.58% H, 19.24% Br, 6.74% N, 7.72% S; found: 60.12% C, 5.62% H, 18.79% Br, 6.71% N, 7.61% S. $^1$H NMR: 8.45 d, 1 H, *J*(6,7) = 7.9 (H-7); 8.12 d, 1 H, *J*(4,5) = 8.4 (H-4); 7.88 dd, 1 H, *J*(5,6) = 7.3, *J*(4,5) = 8.4 (H-5); 7.80 dd, 1 H, *J*(5,6) = 7.3, *J*(6,7) = 7.9 (H-6); 7.54 d, 2 H, *J*(18,19) = 9.0 (H-18); 7.31 s, 1 H (H-13); 6.83 d, 2 H, *J*(18,19) = 9.0 (H-19); 6.23 bm, 1 H (H-10); 5.44 d, 1 H, *J*(10,11′) = 10.8 (H-11′); 5.38 d, 2 H, *J*(9,10) = 4.4 (H-9); 5.31 d, 1 H, *J*(10,11) = 17.2 (H-11); 3.03 s, 6 H (H-20); 2.54 s, 3 H (H-21). UV: 440 ($H_2O$), 486 ($CHCl_3$). TLC: $R_F$ 0.30 (chloroform–methanol 5:1).

*2-{2-[4-(Dimethylamino)phenyl]-1-methylvinyl}-3-(prop-2-yn-1-yl)benzothiazolium bromide* (**2c**): M.p. 126–129 °C. For $C_{21}H_{21}BrN_2S$ (413.4) calculated: 61.02% C, 5.12% H, 19.33% Br, 6.78% N, 7.76% S; found: 60.58% C, 5.14% H, 19.18% Br, 6.70% N, 7.69% S. $^1$H NMR: 8.46 d, 1 H, *J*(6,7) = 7.9 (H-7); 8.26 d, 1 H, *J*(4,5) = 8.4 (H-4); 7.92 dd, 1 H, *J*(5,6) = 7.2, *J*(4,5) = 8.4 (H-5); 7.81 dd, 1 H, *J*(5,6) = 7.2, *J*(6,7) = 7.7 (H-6); 7.59 d, 2 H, *J*(18,19) = 9.0 (H-18); 7.35 s, 1 H (H-13); 6.84 d, 2 H, *J*(18,19) = 9.0 (H-19); 5.61 d, 2 H, *J*(9,11) = 2.1 (H-9); 3.94 t, 1 H, *J*(9,11) = 2.1 (H-11); 3.06 s, 6 H (H-20); 2.61 s, 3 H (H-21). UV: 452 ($H_2O$), 486 ($CHCl_3$). TLC: $R_F$ 0.30 (chloroform–methanol 5:1).

*2-{4-[4-(Dimethylamino)phenyl]-1-methylbuta-1,3-dien-1-yl}-3-methylbenzothiazolium iodide* (**3a**): Yield 89%, m.p. 217–218 °C. For $C_{21}H_{23}IN_2S$ (462.4) calculated: 54.55% C, 5.01% H, 6.06% N, 6.93% S; found: 54.15% C, 5.13% H, 5.99% N, 6.88% S. $^1$H NMR: 8.40 d, 1 H, *J*(6,7) = 7.5 (H-7); 8.25 d, 1 H, *J*(4,5) = 8.4 (H-4); 7.90 dd, 1 H, *J*(5,6) = 7.3, *J*(4,5) = 8.4 (H-5); 7.79 dd, 1 H, *J*(5,6) = 7.3, *J*(6,7) = 7.5 (H-6); 7.59 d, 2 H, *J*(18,19) = 9.0 (H-18); 7,32 d, 1 H, *J*(13,14) = 10.4 (H-13); 7.23 d, 1 H, *J*(14,15) = 14.8 (H-15); 7.20 dd, 1 H, *J*(13,14) = 10.4,

*J*(14,15) = 14.8 (H-14); 6.75 d, 2 H, *J*(18,19) = 9.0 (H-19); 4.25 s, 3 H (H-9); 3.01 s, 6 H (H-20); 2.46 s, 3 H (H-21). UV: 460 ($H_2O$), 540 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*3-Allyl-2-{4-[4-(dimethylamino)phenyl]-1-methylbuta-1,3-dien-1-yl}benzothiazolium bromide* (**3b**): Yield 88%, m.p. 197–202 °C. For $C_{23}H_{25}BrN_2S$ (441.4) calculated: 62.58% C, 5.71% H, 18.10% Br, 6.35% N, 7.26% S; found: 62.51% C, 5.69% H, 17.91% Br, 6.37% N, 7.13% S. $^1$H NMR: 8.42 d, 1 H, *J*(6,7) = 7.9 (H-7); 8.08 d, 1 H, *J*(4,5) = 8.4 (H-4); 7.86 dd, 1 H, *J*(5,6) = 7.3, *J*(4,5) = 8.4 (H-5); 7.78 dd, 1 H, *J*(5,6) = 7.3, *J*(6,7) = 7.9 (H-6); 7.60 d, 2 H, *J*(18,19) = 9.0 (H-18); 7.26 d, 1 H, *J*(13,14) = 10.3 (H-13); 7.22 d, 1 H, *J*(14,15) = 14.3 (H-15); 7.12 dd, 1 H, *J*(13,14) = 10.3, *J*(14,15) = 14.3 (H-14); 6.75 d, 2 H, *J*(18,19) = 9.0 (H-19); 6.27 bm, 1 H (H-10); 5.45 d, 1 H, *J*(10,11′) = 10.8 (H-11′); 5.36 d, 2 H, *J*(9,10) = 4.4 (H-9); 5.25 d, 1 H, *J*(10,11) = 17.4 (H-11); 3.01 s, 6 H (H-20); 2.46 s, 3 H (H-21). UV: 476 ($H_2O$), 538 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*2-{4-[4-(Dimethylamino)phenyl]-1-methylbuta-1,3-dien-1-yl}-3-(prop-2-yn-1-yl)benzothiazolium bromide* (**3c**): Yield 83%, m.p. 187–189 °C. For $C_{23}H_{23}BrN_2S$ (439.4) calculated: 62.87% C, 5.28% H, 18.18% Br, 6.38% N, 7.30% S; found: 62.91% C, 5.22% H, 17.88% Br, 6.29% N, 7.29% S. $^1$H NMR: 8.42 d, 1 H, *J*(6,7) = 7.8 (H-7); 8.24 d, 1 H, *J*(4,5) = 8.4 (H-4); 7.93 dd, 1 H, *J*(5,6) = 7.2, *J*(4,5) = 8.4 (H-5); 7.83 dd, 1 H, *J*(5,6) = 7.2, *J*(6,7) = 7.8 (H-6); 7.62 d, 2 H, *J*(18,19) = 8.9 (H-18); 7,42 d, 1 H, *J*(13,14) = 10.5 (H-13); 7.25 d, 1 H, *J*(14,15) = 14.7 (H-15); 7.15 dd, 1 H, *J*(13,14) = 10.5, *J*(14,15) = 14.7 (H-14); 6.76 d, 2 H, *J*(18,19) = 8.9 (H-19); 5.58 d, 2 H, *J*(9,11) = 1.9 (H-9); 3.96 t, 1 H, *J*(9,11) = 1.9 (H-11); 3.02 s, 6 H (H-20); 2.49 s, 3 H (H-21). UV: 490 ($H_2O$), 540 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*2-{4-[4-(Dimethylamino)phenyl]-2-methylbuta-1,3-dien-1-yl}-3-methylbenzothiazolium iodide* (**4a**): Yield 25%, m.p. 178–179 °C. For $C_{21}H_{23}IN_2S$ (462.4) calculated: 54.55% C, 5.01% H, 6.06% N, 6.93% S; found: 54.36% C, 5.09% H, 5.98% N, 6.87% S. $^1$H NMR: 8.37 d, 1 H, *J*(6,7) = 7.7 (H-7); 8.20 d, 1 H, *J*(4,5) = 8.1 (H-4); 7.86 dd, 1 H, *J*(5,6) = 7.6, *J*(4,5) = 8.1 (H-5); 7.73 dd, 1 H, *J*(5,6) = 7.6, *J*(6,7) = 7.7 (H-6); 7.59 d, 2 H, *J*(18,19) = 8.5 (H-18); 7.50 d, 1 H, *J*(14,15) = 15.9 (H-15); 7.48 s, 1 H (H-12); 7.36 d, 1 H, *J*(14,15) = 15.9 (H-14); 6.80 d, 2 H, *J*(18,19) = 8.5 (H-19); 4.22 s, 3 H (H-9); 3.03 s, 6 H (H-20); 2.55 s, 3 H (H-21). UV: 506 ($H_2O$), 604 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*3-Allyl-2-{4-[4-(dimethylamino)phenyl]-2-methylbuta-1,3-dien-1-yl}benzothiazolium bromide* (**4b**): Yield 6%, m.p. 172–174 °C. For $C_{23}H_{25}BrN_2S$ (441.4) calculated: 62.58% C, 5.71% H, 18.10% Br, 6.35% N, 7.26% S; found: 62.74% C, 5.66% H, 18.12% Br, 6.34% N, 7.20% S. $^1$H NMR: 8.38 d, 1 H, *J*(6,7) = 7.7 (H-7); 8.18 d, 1 H, *J*(4,5) = 8.2 (H-4); 7.84 dd, 1 H, *J*(5,6) = 7.4, *J*(4,5) = 8.2 (H-5); 7.71 dd, 1 H, *J*(5,6) = 7.4, *J*(6,7) = 7.7 (H-6); 7.57 d, 2 H, *J*(18,19) = 8.6 (H-18); 7.53 d, 1 H, *J*(14,15) = 15.8 (H-15); 7.47 s, 1 H (H-12); 7.36 d, 1 H, *J*(14,15) = 15.8 (H-14); 6.80 d, 2 H, *J*(18,19) = 8.6 (H-19); 6.02 bm, 1 H (H-10); 5.50 d, 2 H, *J*(9,10) = 4.0 (H-9); 5.31 d, 1 H, *J*(10,11′) = 11.2 (H-11′); 5.21 d, 1 H, *J*(10,11) = 16.8 (H-11); 3.04 s, 6 H (H-20); 2.49 s, 3 H (H-21). UV: 540 ($H_2O$), 604 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*2-{4-[4-(Dimethylamino)phenyl]-2-methylbuta-1,3-dien-1-yl}-3-(prop-2-yn-1-yl)benzothiazolium bromide* (**4c**): Yield 32%, m.p. 186–188 °C. For $C_{23}H_{23}BrN_2S$ (439.4) calculated: 62.87% C, 5.28% H, 18.18% Br, 6.38% N, 7.30% S; found: 61.96% C, 5.31% H, 18.01% Br, 6.34% N, 7.22% S. $^1$H NMR: 8.39 d, 1 H, *J*(6,7) = 7.7 (H-7); 8.22 d, 1 H, *J*(4,5) = 8.5 (H-4); 7.88 dd, 1 H, *J*(5,6) = 7.5, *J*(4,5) = 8.5 (H-5); 7.74 dd, 1 H, *J*(5,6) = 7.5, *J*(6,7) = 7.7 (H-6); 7.63 d, 2 H, *J*(18,19) = 9.1 (H-18); 7.61 d, 1 H, *J*(14,15) = 15.7 (H-15); 7.51 s, 1 H (H-12); 7.38 d, 1 H, *J*(14,15) = 15.7 (H-14); 6.81 d, 2 H, *J*(18,19) = 9.1 (H-19); 5.72 d, 2 H, *J*(9,11) = 2.2 (H-9);

3.74 t, 1 H, *J*(9,11) = 2.2 (H-11); 3.05 s, 6 H (H-20); 2.59 s, 3 H (H-21). UV: 522 ($H_2O$), 610 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*2-{4-[4-(Dimethylamino)phenyl]-3-methylbuta-1,3-dien-1-yl}-3-methylbenzothiazolium iodide* (**5a**): Yield 61%, m.p. 254–255 °C. For $C_{21}H_{23}IN_2S$ (462.4) calculated: 54.55% C, 5.01% H, 6.06% N, 6.93% S; found: 54.48% C, 5.04% H, 5.96% N, 6.96% S. $^1$H NMR: 8.34 d, 1 H, *J*(6,7) = 7.9 (H-7); 8.15 d, 1 H, *J*(4,5) = 8.3 (H-4); 7.96 d, 1 H, *J*(12,13) = 15.2 (H-13); 7.81 dd, 1 H, *J*(5,6) = 7.2, *J*(4,5) = 8.3 (H-5); 7.72 dd, 1 H, *J*(5,6) = 7.2, *J*(6,7) = 7.9 (H-6); 7.54 d, 2 H, *J*(18,19) = 9.1 (H-18); 7.38 s, 1 H (H-15); 7.16 d, 1 H, *J*(12,13) = 15.2 (H-12); 6.81 d, 2 H, *J*(18,19) = 9.1 (H-19); 4.24 s, 3 H (H-9); 3.02 s, 6 H (H-20); 2.30 s, 3 H (H-21). UV: 504 ($H_2O$), 586 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*3-Allyl-2-{4-[4-(dimethylamino)phenyl]-3-methylbuta-1,3-dien-1-yl}benzothiazolium bromide* (**5b**): Yield 50%, m.p. 211–212 °C. For $C_{23}H_{25}BrN_2S$ (441.4) calculated: 62.58% C, 5.71% H, 18.10% Br, 6.35% N, 7.26% S; found: 62.71% C, 5.65% H, 17.90% Br, 6.29% N, 7.13% S. $^1$H NMR: 8.34 d, 1 H, *J*(6,7) = 7.7 (H-7); 8.10 d, 1 H, *J*(4,5) = 8.2 (H-4); 7.99 d, 1 H, *J*(12,13) = 14.8 (H-13); 7.78 dd, 1 H, *J*(5,6) = 7.1, *J*(4,5) = 8.2 (H-5); 7.69 dd, 1 H, *J*(5,6) = 7.1, *J*(6,7) = 7.7 (H-6); 7.52 d, 2 H, *J*(18,19) = 8.8 (H-18); 7.40 s, 1 H (H-15); 7.08 d, 1 H, *J*(12,13) = 14.8 (H-12); 6.80 d, 2 H, *J*(18,19) = 8.8 (H-19); 6.04 bm, 1 H (H-10); 5.52 d, 2 H, *J*(9,10) = 4.2 (H-9); 5.32 d, 1 H, *J*(10,11′) = 11.0 (H-11′); 5.22 d, 1 H, *J*(10,11) = 16.5 (H-11); 3.04 s, 6 H (H-20); 2.27 s, 3 H (H-21). UV: 514 ($H_2O$), 584 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*2-{4-[4-(Dimethylamino)phenyl]-3-methylbuta-1,3-dien-1-yl}-3-(prop-2-yn-1-yl)benzothiazolium bromide* (**5c**): Yield 73%, m.p. 227–229 °C. For $C_{23}H_{23}BrN_2S$ (439.4) calculated: 62.87% C, 5.28% H, 18.18% Br, 6.38% N, 7.30% S; found: 62.35% C, 5.27% H, 18.26% Br, 6.31% N, 7.22% S. $^1$H NMR: 8.36 d, 1 H, *J*(6,7) = 8.0 (H-7); 8.19 d, 1 H, *J*(4,5) = 8.3 (H-4); 8.05 d, 1 H, *J*(12,13) = 14.8 (H-13); 7.84 dd, 1 H, *J*(5,6) = 7.5, *J*(4,5) = 8.3 (H-5); 7.73 dd, 1 H, *J*(5,6) = 7.5, *J*(6,7) = 8.0 (H-6); 7.57 d, 2 H, *J*(18,19) = 9.1 (H-18); 7.46 s, 1 H (H-15); 7.23 d, 1 H, *J*(12,13) = 14.8 (H-12); 6.83 d, 2 H, *J*(18,19) = 9.1 (H-19); 5.81 d, 2 H, *J*(9,11) = 2.0 (H-9); 3.74 t, 1 H, *J*(9,11) = 2.0 (H-11); 3.06 s, 6 H (H-20); 2.32 s, 3 H (H-21). UV: 522 ($H_2O$), 596 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*2-{4-[4-(Dimethylamino)phenyl]buta-1,3-dien-1-yl}-3,5-dimethylbenzothiazolium iodide* (**6d**): Yield 72%, m.p. 211–215 °C. For $C_{21}H_{23}N_2SI$ (462.4) calculated: 54.55% C, 5.01% H, 6.06% N, 6.93% S; found: 54.12% C, 5.10% H, 6.00% N, 6.81% S. $^1$H NMR: 8.18 d, 1 H, *J*(6,7) = 8.2 (H-7); 7.98 s, 1 H (H-4); 7.95 dd, 1 H, *J*(13,14) = 11.1, *J*(12,13) = 14.5 (H-13); 7.57 d, 1 H, *J*(6,7) = 8.2 (H-6); 7.54 d, 2 H, *J*(18,19) = 8.9 (H-18); 7.39 d, 1 H, *J*(12.13) = 14.5 (H-12); 7.31 d, 1 H, *J*(14,15) = 15.0 (H-15); 6.74 dd, 1 H, *J*(13,14) = 11.1, *J*(14,15) = 15.0 (H-14); 6.78 d, 2 H, *J*(18,19) = 8.9 (H-19); 4.13 s, 3 H (H-9); 3.04 s, 6 H (H-20); 2.51 s, 3 H (H-8). UV: 521 ($H_2O$), 607 ($CHCl_3$). TLC: $R_F$ 0.20 (chloroform–methanol 5:1).

*2-{6-[4-(Dimethylamino)phenyl]hexa-1,3,5-trien-1-yl}-3,5-dimethylbenzothiazolium iodide* (**7d**): Yield 70%, m.p. 194–196 °C. For $C_{23}H_{25}IN_2S$ (488.4) calculated: 56.56% C, 5.16% H, 5.74% N, 6.56% S; found: 56.20% C, 5.22% H, 5.68% N, 6.50% S. $^1$H NMR: 8.19 d, 1 H, *J*(6,7) = 8.1 (H-7); 7.99 s, 1 H (H-4); 7.90 dd, 1 H, *J*(13,14) = 11.2, *J*(12,13) = 14.4 (H-13); 7.56 d, 1 H, *J*(6,7) = 8.1 (H-6); 7.49 d, 2 H, *J*(18,19) = 8.8 (H-18); 7.34 d, 1 H, *J*(12.13) = 14.4 (H-12); 7.30 dd, 1 H, *J*(15,16) = 10.7, *J*(14,15) = 14.0 (H-15); 7.04 dd, 1 H, *J*(15,16) = 10.7, *J*(16,17) = 15.8 (H-16); 6.99 d, 1 H, *J*(16,17) = 15.8 (H-17); 6.74 dd, 1 H, *J*(13,14) = 11.2, *J*(14,15) = 14.0 (H-14); 6.73 d, 2 H, *J*(18,19) = 8.8 (H-19); 4.14 s, 3 H (H-9); 3.00 s, 6 H (H-20); 2.52 s, 3 H (H-8). UV: 516 ($H_2O$), 624 ($CHCl_3$). TLC: $R_F$ 0.15 (chloroform–methanol 5:1).

*3-Allyl-2-{6-[4-(dimethylamino)phenyl]hexa-1,3,5-trien-1-yl}-5-methylbenzothiazolium bromide* (**7e**): Yield 52%, m.p. 204–206 °C. For $C_{25}H_{27}BrN_2S$ (467.5) calculated: 64.23% C, 5.82% H, 17.09% Br, 5.99% N, 6.86% S; found: 64.39% C, 5.84% H, 17.20% Br, 5.93% N, 6.80% S. $^1$H NMR: 8.22 d, 1 H, *J*(6,7) = 8.3 (H-7); 7.96 s, 1 H (H-4); 7.96 dd, 1 H, *J*(13,14) = 11.1, *J*(12,13) = 14.7 (H-13); 7.55 d, 1 H, *J*(6,7) = 8.3 (H-6); 7.50 d, 2 H, *J*(18,19) = 8.7 (H-18); 7.32 dd, 1 H, *J*(15,16) = 10.6, *J*(14,15) = 14.1 (H-15); 7.30 d, 1 H, *J*(12.13) = 14.7 (H-12); 7.05 dd, 1 H, *J*(15,16) = 10.6, *J*(16,17) = 15.9 (H-16); 7.00 d, 1 H, *J*(16,17) = 15.9 (H-17); 6.74 dd, 1 H, *J*(13,14) = 11.1, *J*(14,15) = 14.1 (H-14); 6.74 d, 2 H, *J*(18,19) = 8.7 (H-19); 6.05 bm, 1 H (H-10); 5.37 d, 2 H, *J*(9,10) = 4.2 (H-9); 5.33 d, 1 H, *J*(10,11′) = 10.3 (H-11′); 5.21 d, 1 H, *J*(10,11) = 16.9 (H-11); 3.00 s, 6 H (H-20); 2.53 s, 3 H (H-8). UV: 532 ($H_2O$), 622 ($CHCl_3$). TLC: $R_F$ 0.15 (chloroform–methanol 5:1).

*2-{6-[4-(Dimethylamino)phenyl]hexa-1,3,5-trien-1-yl}-5-methyl-3-(prop-2-yn-1-yl)benzothiazolium bromide* (**7f**): Yield 77%, m.p. 186–188 °C. For $C_{25}H_{25}BrN_2S$ (465.5) calculated: 64.51% C, 5.41% H, 17.17% Br, 6.02% N, 6.89% S; found: 64.38% C, 5.47% H, 17.15% Br, 5.91% N, 6.77% S. $^1$H NMR: 8.22 d, 1 H, *J*(6,7) = 8.3 (H-7); 8.05 s, 1 H (H-4); 8.01 dd, 1 H, *J*(13,14) = 11.3, *J*(12,13) = 14.3 (H-13); 7.58 d, 1 H, *J*(6,7) = 8.3 (H-6); 7.52 d, 2 H, *J*(18,19) = 8.9 (H-18); 7.40 d, 1 H, *J*(12.13) = 14.3 (H-12); 7.37 dd, 1 H, *J*(15,16) = 10.5, *J*(14,15) = 13.9 (H-15); 7.09 dd, 1 H, *J*(15,16) = 10.5, *J*(16,17) = 15.8 (H-16); 7.03 d, 1 H, *J*(16,17) = 15.8 (H-17); 6.77 dd, 1 H, *J*(13,14) = 11.3, *J*(14,15) = 13.9 (H-14); 6.75 d, 2 H, *J*(18,19) = 8.9 (H-19); 5.65 d, 2 H, *J*(9,11) = 2.0 (H-9); 3.75 t, 1 H, *J*(9,11) = 2.0 (H-11); 3.01 s, 6 H (H-20); 2.55 s, 3 H (H-8). UV: 542 ($H_2O$), 632 ($CHCl_3$). TLC: $R_F$ 0.15 (chloroform–methanol 5:1).

## Biological Studies

The toxicity against *Euglena gracilis* was monitored in a liquid Cramer–Myers medium containing appropriate concentration (2.0–300 μg/ml) of the tested substances. Fresh solution of substances were prepared by dissolving in DMSO. The inoculum *Euglena gracilis* was taken from the exponential growth phase and cultivation was performed 96 h under permanent illumination at 26 ± 2 °C. The $ED_{50}$ toxicity values (in $10^{-3}$ mol $l^{-3}$) were interpolated from nonlinear cubic functions.

Antimicrobial activities were tested by the standard plate diffusion method using Mueller–Hinton and Sabouraud agar, or by the standard dilution method in Sabouraud medium[15]. MIC values were determined.

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