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# Synthesis and Antibacterial Activity of Novel Fused 1,3-Thiazoles and 1,3-Thiazines Incorporating a 2,4-Dihydroxyphenyl Residue

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In an attempt to find a new class of antimicrobial agents, a series of benzothiazoles, 1,3-thiazolo[5,4-*b*]pyridines, 4*H*-3,1-benzothiazines, naphtho[2,3-*d*][1,3]thiazole-4,9-diones and other related compounds containing a 2,4-dihydroxyphenyl moiety were prepared. They were obtained *via* the reaction of aryl-modified sulfinyl[bis(2,4-dihydroxyphenylmethanethione)]s with appropriate commercial chemical reagents in the endocyclization processes. The MIC values of the compounds towards eight reference bacterial strains were assessed by the two-fold serial micro-dilution broth method. They exhibited inhibitory effects against the Gram-positive strains tested opposite to Gram-negative ones. Some compounds were more effective than the reference drug. 4-(6-Chloro-4*H*-3,1-benzothiazin-2-yl)-6-methylbenzene-1,3-diol (**5b**) due to its very good activity (MIC from 1.56 to 3.13 μg/mL) and low cytotoxicity ($IC_{50} > 50$ μg/mL) may be regarded as a promising precursor for the development of novel antibacterial agents.

**Keywords:** Antibacterial activity / Cytotoxicity / Fused thiazines / Fused thiazoles / Synthesis

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## Introduction

Fused heterocycles incorporating 1,3-thiazole and 1,3-thiazine rings have attracted a lot of attention from medicinal and agricultural chemists due to their broad-spectrum antifungal and antibacterial activities as well as action mechanism [1–4]. For example, benzothiazole derivatives are commonly known as antimycotic agents [5–9]. 6-Amino-2-pentylthiobenzothiazole (APB), is a compound characterized by *in vitro* and *in vivo* antifungal activities. An especially strong effect was observed in the combination with other commonly applied antimycotics [10, 11]. Some of these types of compounds like bentaluron, chlobenthiazone and TCMTB have been widely used in agriculture [12]. Recently a promising selective fungal *N*-myristoyltransferase inhibitor from the benzothiazole group with a strong *in vivo* antifungal effect has been described [13–15].

2-Methoxy-2,6-difluorobenzamide derivatives of thiazolo[4,5]pyridine are potent antistaphylococcal compounds with suboptimal drug-like properties [16]. They act as inhibitors of the bacterial cell division protein FtsZ. The others are described as useful antimicrobial agents effective against a variety of human and veterinary pathogens including, among others, Gram-positive and Gram-negative aerobic and anaerobic bacteria as well as mycobacteria [17]. Naphthothiazole derivatives are also known as antibacterial agents [18].

4-(4*H*-3,1-Benzothiazin-2-yl)benzene-l,3-diols obtained in our laboratory show a strong antifungal effect against various strains of moulds, yeasts and dermatophytes on the level of nystatin studied comparatively [19]. Similar properties for some benzothiazole derivatives have been found

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[20]. Extending the research in this area, we designed and obtained new fused heterocycles incorporating a 1,3-thiazole or 1,3-thiazine ring with a 2,4-dihydroxyphenyl residue. To evaluate the potential antibacterial efficacy of the compounds we determined their *in vitro* activity against the panel of reference bacterial strains. Additionally, the most active compounds were tested for their cytotoxic potential. The structure-activity relationship has been discussed.

## Results and discussion

### Chemistry

The structures of the compounds under consideration are presented in Table 1. They were obtained *via* the reaction of sulfinylbis[(2,4-dihydroxyphenyl)methanethione] (STB) or its aryl-modified analogs: sulfinylbis[(2,4-dihydroxy-3-methylphenyl)methanethione] (S3MTB), sulfinylbis[(2,4-dihydroxy-5-methylphenyl)methanethione] (S5MTB), sulfinylbis[(5-ethyl-2,4-dihydroxyphenyl)methanethione] (SETB): sulfinylbis[(5-chloro-2,4-dihydroxyphenyl)methanethione] (SClTB), sulfinylbis[(2,3,4-trihydroxyphenyl)methanethione] (S3TTB) with the appropriate commercial chemical reagents containing the amine group (Table 1, Schemes 1–6). In the case of compounds **1**, **2** and **7** cyclization proceeded by the elimination of $H_2S$, HCl or $NH_3$ (phenylamine) respectively (Schemes 1 and 2). Compounds **5** and **6** were obtained as a result of the elimination of $H_2O$ (Scheme 3). The intramolecular cyclization process proceeded according to the mechanism of nucleophilic addition to the carbonyl groups in the case of compounds **3, 4, 8** and **9** (Schemes 4, 5 and 6). The synthesis mechanism of **7** and **8** was described previously [19].

Generally, cyclization processes are connected with thiolimine isomerization and ionization capabilities of the -SH group. The reaction promotes a tendency of the compounds with the thioamide moiety toward transition into the thiolimine form compared with the analogs with an oxygen atom [21]. Purity of compounds was monitored by the reversed-phase (RP-18) HPLC chromatography with methanol/water as a mobile phase. STB and its analogues were obtained according to the method described previously *i.e.* by treatment of the corresponding dithioic acids with $SOCl_2$ [22].

The spectroscopic data of new derivatives are in agreement with the proposed structures. In the $^1$H NMR spectrum in the range of low fields as a rule two signals corresponding to the protons of the -OH groups in the resorcinol moiety are registered. OH and NH protons are sometimes invisible in the background on the base line (**3b**, **6b**, **8b**, **8c**).

The resonance signals of resorcinol moiety in the case of additional substitution in position 5 appear as characteristic two singlets in the range about 7.9 and 6.6 ppm. In the IR spectrum, there are strong bands in the region about 3400–3100 and 1635–1600 $cm^{-1}$ corresponding to the vibrations of O–H and C=N respectively. The mass spectra (EI) of the compounds gave molecular ion peaks, however, with different intensities.

### Antibacterial activity

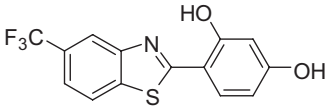
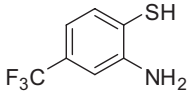
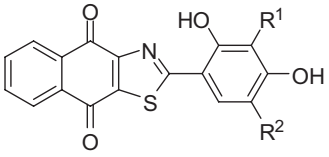
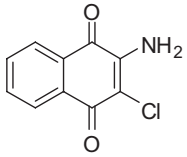
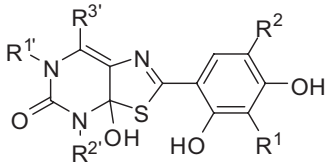
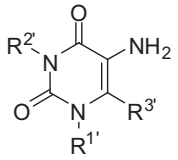
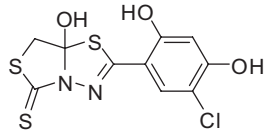
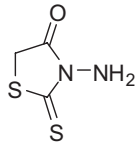
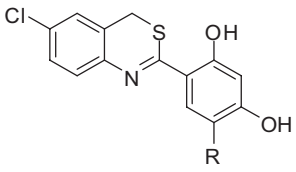
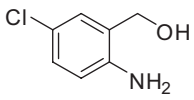
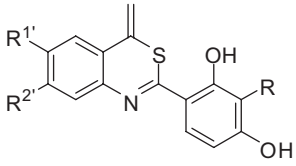
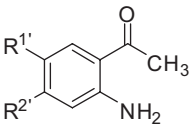
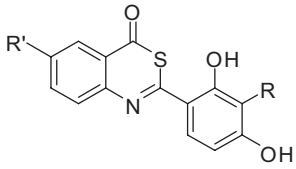
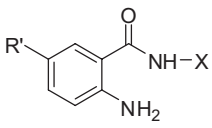
The newly synthesized (**1–4**, **6–9**) and previously described (**5**) [23] compounds (Table 1) were screened for their antibacterial properties against the panel of the reference Gram-positive and Gram-negative bacteria. First, the power of activity was assessed by the determination of the minimal inhibitory concentration (MIC) values towards both groups of strains. The obtained data are presented in Table 2. Generally, the tested compounds exert different activities against the Gram-positive bacteria (MIC ranged 0.78 – 200 μg/mL). They depend clearly on the type of fused azole (azine) ring and the presence of the additional substituents in the benzenediol moiety. In contrast, the growth of Gram-negative bacteria has not been inhibited even at the highest concentration of compounds (MIC >200 μg/mL). The selective antibacterial activity of the compounds is due to the difference in the structure of bacterial cell wall, and Gram-negative bacteria are generally more resistant to antibacterial agents than the Gram-positive ones [24].

According to our data (Table 2), all tested naphtho[2,3-*d*][1,3]thiazole-4,9-diones (**2**) exert an inhibitory effect on the Gram-positive bacteria at promising concentrations. They are effective at the MIC 0.78–6.25 μg/mL independently of the substitution type of benzenediol residue. Significant differences in susceptibility of Gram-positive strains to the particular 4*H*-3,1-benzothiazine derivatives (**5**) are observed (MIC from 1.56 to 200 μg/mL). The most favourable is the presence of the Me or Et group in position 5 of the resorcinol moiety (**5b**, **5c**). Compound **5a** without the additional substituent exhibits the weakest potency. In the all screened groups, compounds **2a** and **2d** (naphtho[2,3-*d*][1,3]thiazole-4,9-diones) are found to be the most active (MIC = 0.78–1.56 μg/mL), however, the following compounds: **2b**, **5b**, **5c** (4*H*-3,1-benzothiazines) and **8a** (4-hydroxy-4*H*-3,1-benzothiazine) exhibit comparably high potency of bacterial growth inhibition with the MICs ranging from 0.78 to 3.13 μg/mL. As can be seen in Table 2, compounds **2a** and **2d** appear to be more effective (MIC 0.78–1.56 μg/mL) against all Gram-positive bacteria tested than ampicillin (MIC 3.13–6.25 μg/mL). Moreover, compounds **2b**, **3b**, **5b**, **5c**, and **8a** show a higher or similar activity compared to that of ampicillin depending on the strain. Good activity is also revealed by both derivatives from the 1,3-thiazolo[5,4-*d*]pyrimidin-5(4*H*)-one group (**3**).

Generally, the structure-activity analysis of the compounds under consideration reveals that antibacterial potency of bezenediols is enhanced by the naphtho[2,3-d][1,3]thiazole-4,9-dione residue. However, modification of their structure by two fused five-membered rings of 7a-hydroxy-7,7a-dihydro[1,3]thiazolo[4,3-b][1,3,4]thiadiazole-5-thione proves to be

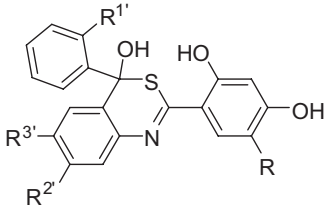
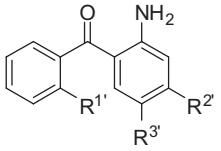
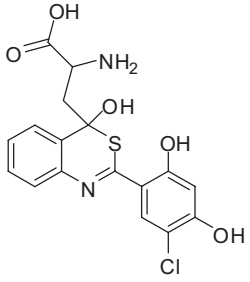
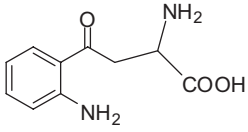
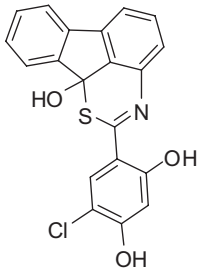
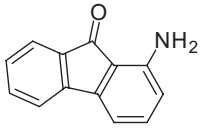
**Table 1.** The structures of compounds **1–9** and reagents applied for their syntheses

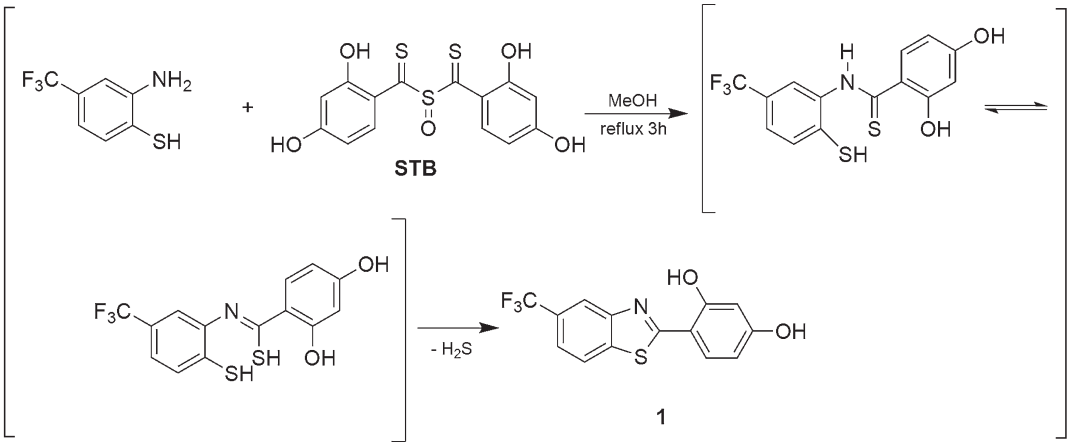
| No. | Substituents | Structure of compounds | Reagents | |
|---|---|---|---|---|
| | | | Nucleophile | Electrophile |
| **1** | |  |  | STB |
| **2a** | $R^1 = R^2 = H$ |  |  | STB |
| **2b** | $R^1 = Me$, $R^2 = H$ | | | S3MTB |
| **2c** | $R^1 = H$, $R^2 = Et$ | | | SETB |
| **2d** | $R^1 = H$, $R^2 = Cl$ | | | SClTB |
| **3a** | $R^1 = H$, $R^2 = Cl$<br>$R^{1'} = R^{2'} = Me$<br>$R^{3'} = NH_2$ |  |  | SClTB |
| **3b** | $R^1 = Me$, $R^2 = R^{1'} = R^{2'} = R^{3'} = H$ | | $R^{1'}, R^{2'} = H, Me$;<br>$R^{3'} = H, NH_2$ | S3MTB |
| **4** | |  |  | SClTB |
| **5a** | $R = H$ |  |  | STB |
| **5b** | $R = Me$ | | | S5MTB |
| **5c** | $R = Et$ | | | SETB |
| **5d** | $R = Cl$ | | | SClTB |
| **6a** | $R = R^{1'} = R^{2'} = H$ |  |  | STB |
| **6b** | $R = Me$, $R^{1'} = R^{2'} = OMe$ | | $R^{1'} = R^{2'} = H, OMe$ | S3MTB |
| **7a** | $R = OH$, $R' = H$ |  |  | S3TTB |
| **7b** | $R = H$, $R' = Cl$ | | X= H, Ph; R'= H, Cl | STB |

continued

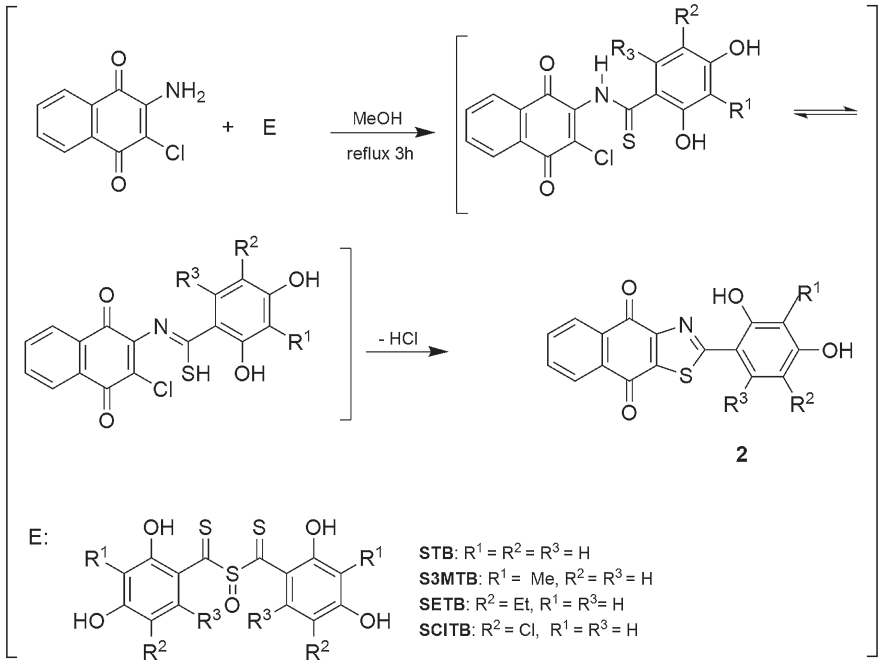
**Table 1.** (*continued*)

| No. | Substituents | Structure of compounds | Reagents | |
|---|---|---|---|---|
| | | | Nucleophile | Electrophile |
| **8a** | R = Et, $R^{1'} = R^{3'} = Cl$, $R^{2'} = H$ |  |  | SETB |
| **8b** | R = Cl, $R^{1'} = R^{3'} = H$, $R^{2'} = Me$ | | $R^{1'}$, $R^{3'}$ = H, Cl; $R^{2'}$ = H, Me | SClTB |
| **8c** | |  |  | SClTB |
| **9** | |  |  | SClTB |

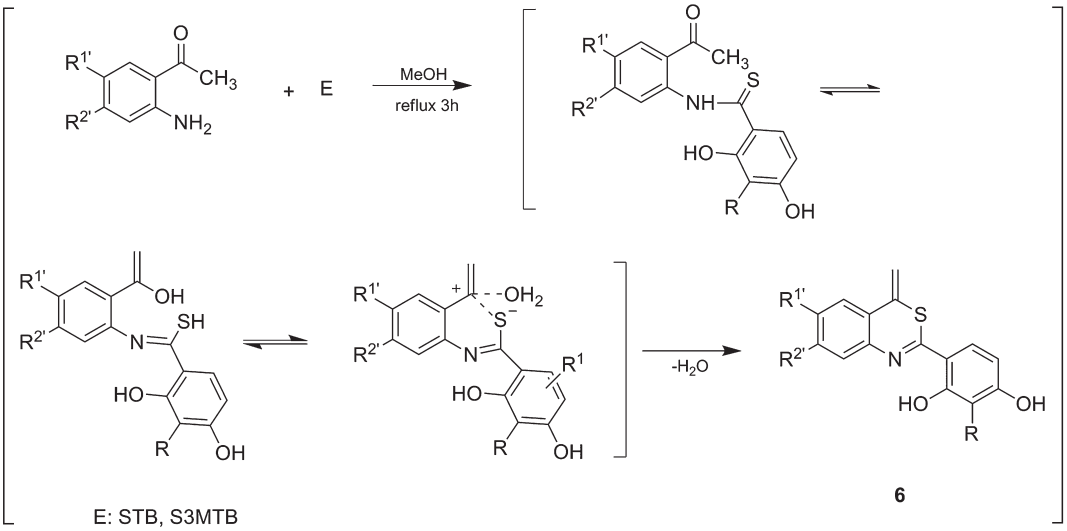



**Scheme 1.** Reaction mechanism of the formation of 4-(1,3-benzothiazol-2-yl)benzene-1,3-diol.




**Scheme 2.** Reaction mechanism of the formation of 2-(2,4-dihydroxyphenyl)naphtho[2,3-*d*][1,3]thiazole-4,9-diones.

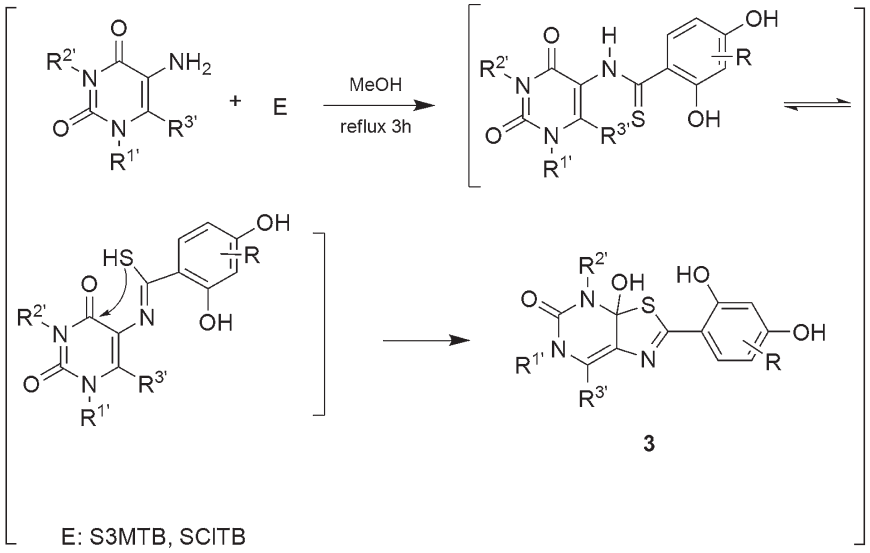



**Scheme 3.** Reaction mechanism of the formation of 4-(4-methylidene-4*H*-3,1-benzothiazin-2-yl)benzene-1,3-diols.

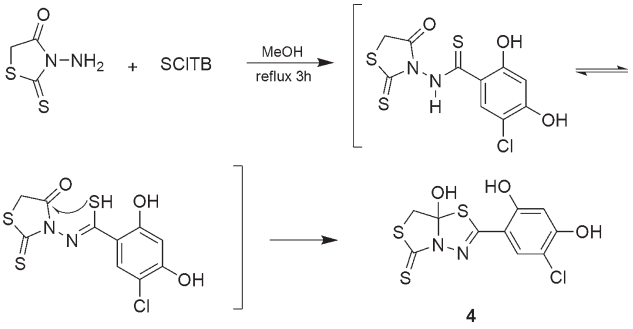
unfavourable. In the group of 4*H*-3,1-benzothiazines the strongest antibacterial potency is exhibited by the analogs with unmodified C(4) of fused ring or with the hydroxyl and phenyl substituents at this position. Analyzing an effect of the substitution of the benzenediol moiety it can be found that Et group enhances antibacterial activity of 4*H*-3,1-benzothiazines. It causes the opposite effect in the case of naphtho[2,3-d][1,3]thiazole-4,9-dione derivatives.

Moreover, the activity of the tested compounds against Gram-positive bacteria was examined in more detail by the determination of the minimal bactericidal concentration (MBC) and compared with the MIC of a particular compound. It is worth emphasizing that the low values of the MBC/MIC ratio ($\leq 4$) indicate a bactericidal agent. According to our data (Table 2) such potency against all strains is possessed only by the compounds from the 4-substituted-4*H*-3,1-benzothiazine group **8c** and **6a**. Other benzothiazines **8a** and **5d** exhibit the same effect against 2 strains (*S. epidermidis, S. aureus* and *S. epidermidis, M. luteus,* respectively). Furthermore, compound **9** (fluoreno[1,9-*de*][1,3]thiazine) exhibits bactericidal activity




**Scheme 4.** Reaction mechanism of the formation of 2-(2,4-dihydroxyphenyl)-3a-hydroxy-3a,6-dihydro[1,3]thiazolo[5,4-*d*]pyrimidin-5(6*H*)-ones.




**Scheme 5.** Reaction mechanism of the formation of 2-(5-chloro-2,4-dihydroxyphenyl)-7a-hydroxy-7,7a-dihydro[1,3]thiazolo[4,3-*b*][1,3,4]thiadiazole-5-thione.

against 3 strains (*S. epidermidis, B. subtilis, M. luteus*) (Table 2). The remaining compounds show bacteriostatic activity.

### Cytotoxicity

Cytotoxicity is one of the most important characteristics of a compound intended for biomedical applications. In our study, compounds **2a, 2b, 2d, 5b, 5c,** and **8a,** exhibiting promising activity against the Gram-positive bacteria, were included in the cytotoxicity test. The results in Table 3 reveal that the tested compounds show different levels of cytotoxicity effect. The highest cytotoxicity is observed for compounds **5c, 8a,** and **2b** ($IC_{50}$ >6.25 μg/mL, $IC_{50}$ >6.25 μg/mL, and $IC_{50}$ >12.5 μg/mL, respectively). Compound **5b** is found to possess the lowest cytotoxicity effect ($IC_{50}$ >50 μg/mL).

### Conclusion

Summing up, a series of novel fused heterocycles incorporating the 1,3-thiazole or 1,3-thiazine ring and the 2,4-dihydroxyphenyl substituent has been designed and synthesized. The compounds inhibit *in vitro* growth of the Gram-positive strains opposite to the Gram-negative ones. Very good activity against the Gram-positive bacteria and the low cytotoxicity profile of compound **5b** make them promising for further optimization in the development of novel antibacterial agents. The results of biological studies of compounds **2a, 2b, 2d, 5c,** and **8a** as the inhibitors of Gram-positive strains are certainly encouraging, but their cytotoxicity can confine the potential therapeutic applications.

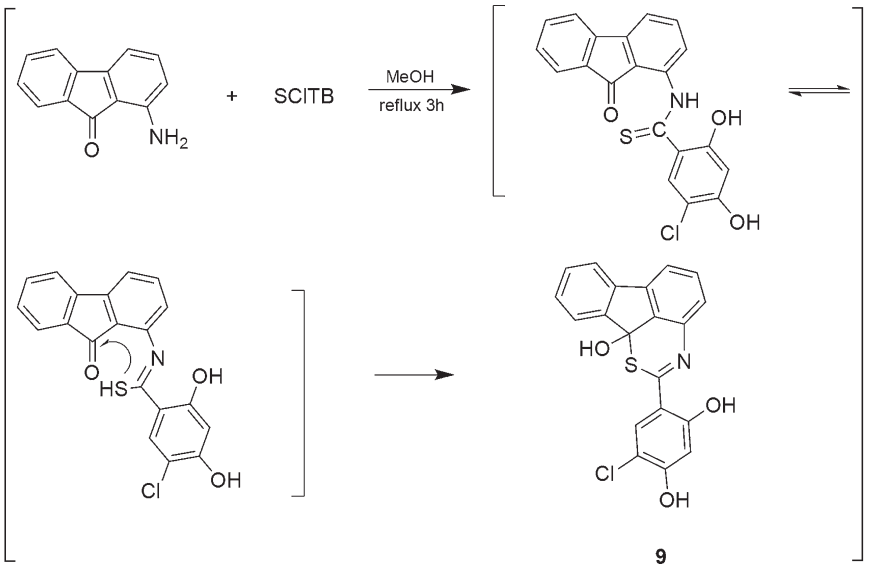
## Experimental protocol

### Analytical studies

Melting points (m.p.) were determined using a BÜCHI B-540 (Switzerland) melting point apparatus. Elemental analyses (C, H, N) were conducted using a Perkin-Elmer 2400 instrument; their results were found to be in good agreement (±0.4%) with the calculated values. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X spectrophotometer (in KBr) in the range of 600–4000 $cm^{-1}$. $^1H$ and $^{13}C$-NMR spectra were recorded in DMSO-$d_6$ by means of a Bruker DRX 500 instrument. Chemical shifts (δ, ppm) were given in relation to tetramethylsilane (TMS). The mass spectra (EI, 70 eV) were recorded with an AMD-604 instrument.

The purity of the compounds was examined by a liquid chromatograph Knauer with a dual pump, a 20 μL simple injection valve and a UV-visible detector at 280 nm. The Hypersil Gold C18 (3 μm, 100 × 3 mm) column was used as the stationary




**Scheme 6.** Reaction mechanism of the formation of 4-chloro-6-(10b-hydroxy-10b*H*-fluoreno[1,9-*de*][1,3]thiazin-2-yl)benzene-1,3-diol.

**Table 2.** The minimal inhibitory concentrations (MICs) and the minimal bactericidal concentrations (MBCs) of the tested compounds against the reference Gram-positive bacteria

| Compound | Antibacterial activity [μg/ml] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***S. epidermidis*** ATCC[a] 12228 | | ***S. aureus*** ATCC 25923 | | ***B. subtilis*** ATCC 6633 | | ***M. luteus*** ATCC 10240 | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| **1**[d] | 12.5 | >200 | 12.5 | >200 | 12.5 | 200 | 6.25 | 200 |
| **2a**[c] | 0.78 | 100 | 0.78 | 200 | 0.78 | 25 | 1.56 | 25 |
| **2b**[c] | 1.56 | 100 | 1.56 | 100 | 1.56 | 25 | 3.13 | 50 |
| **2c**[d] | 12.5 | >200 | 6.25 | 200 | 6.25 | 100 | 6.25 | 200 |
| **2d**[c] | 1.56 | 200 | 1.56 | 200 | 0.78 | 50 | 1.56 | 25 |
| **3a**[d] | 12.5 | 200 | 6.25 | 200 | 3.13 | 200 | 3.13 | 50 |
| **3b**[c] | 6.25 | 50 | 3.13 | >200 | 3.13 | 200 | 3.13 | 50 |
| **4**[e] | 25 | >200 | 12.5 | >200 | 25 | >200 | 25 | 200 |
| **5a**[e] | 100 | nd [b] | 200 | nd [b] | 200 | nd [b] | 50 | nd [b] |
| **5b**[c] | 3.13 | 50 | 1.56 | 200 | 3.13 | 25 | 1.56 | 12.5 |
| **5c**[c] | 1.56 | 12.5 | 3.13 | 25 | 1.56 | 12.5 | 1.56 | 12.5 |
| **5d**[e] | 25 | 100 | 25 | 200 | 25 | 200 | 25 | 100 |
| **6a**[d] | 12.5 | 50 | 12.5 | 25 | 6.25 | 25 | 1.56 | 12.5 |
| **6b**[d] | 12.5 | 200 | 6.25 | 200 | 6.25 | 200 | 6.25 | 50 |
| **7a**[c] | 6.25 | 200 | 6.25 | 200 | 3.13 | 200 | 6.25 | 50 |
| **7b**[d] | 6.25 | 200 | 12.5 | 200 | 12.5 | 200 | 6.25 | 25 |
| **8a**[c] | 1.56 | 6.25 | 3.13 | 6.25 | 3.13 | 50 | 0.78 | 6.25 |
| **8b**[e] | 25 | 200 | 25 | 200 | 50 | 100 | 12.5 | 100 |
| **8c**[d] | 6.25 | 25 | 6.25 | 25 | 12.5 | 25 | 6.25 | 25 |
| **9**[e] | 12.5 | 50 | 12.5 | 100 | 25 | 50 | 6.25 | 25 |
| Ampicillin | 6.25 | nd [b] | 3.13 | nd [b] | 3.13 | nd [b] | 6.25 | nd [b] |

[a] ATCC = American Type Culture Collection
[b] nd = not determined
[c] - very good activity
[d] - good activity
[e] - moderate activity

**Table 3.** Effect of the tested compounds on the cell viability in the Vero cell line

| Conc. [μg/mL] | Cell viability [%][a] | | | | | |
|---|---|---|---|---|---|---|
| | 2a | 2b | 2d | 5b | 5c | 8a |
| 100 | 12.5 ± 0.5 | 8.7 ± 1.0 | 5.7 ± 0.8 | 18.0 ± 4.0 | 8.1 ± 1.9 | 3.7 ± 1.8 |
| 50 | 21.1 ± 6.8 | 9.4 ± 0.3 | 16.7 ± 2.5 | 65.6 ± 3.6 | 8.2 ± 1.8 | 4.1 ± 2.0 |
| 25 | 54.5 ± 5.5 | 19.5 ± 4.5 | 55.7 ± 3.4 | 88.3 ± 3.7 | 8.3 ± 1.7 | 2.9 ± 1.4 |
| 12.5 | 67.3 ± 7.1 | 61.5 ± 1.5 | 85.0 ± 6.1 | 92.1 ± 0.1 | 29.7 ± 0.3 | 3.3 ± 1.6 |
| 6.25 | 86.1 ± 1.0 | 72.5 ± 2.5 | 100 ± 0.0 | 98.1 ± 1.9 | 82.1 ± 5.1 | 87.5 ± 2.5 |
| 3.13 | 98.0 ± 2.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 88.0 ± 7.7 | 100 ± 0.0 |
| 1.56 | 99.0 ± 1.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 98.6 ± 0.4 | 100 ± 0.0 |
| 0.78 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 99.5 ± 0.4 | 100 ± 0.0 |
| 0.39 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| 0 (control) | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |

[a] The results estimated as the percentage of viability of Vero cells plus compound tested to the control cell culture

phase. The mobile phase included different contents of MeOH and acetate buffer (pH 4, 20 nM) as the aqueous phase. The flow rate was 0.5 mL/min at room temperature. The retention time of an unretained solute ($t_o$) was determined by the injection of a small amount of acetone dissolved in water. Log k values for 70% of MeOH (v/v) in the mobile phase are presented.

## Synthesis of compounds

### *4-[5-(Trifluoromethyl)-1,3-benzothiazol-2-yl]-benzene-1,3-diol* ***1***

A mixture of 2-amino-4-(trifluoromethyl)benzenethiol (0.0011 mol) and STB (0.0011 mol) in MeOH (5.5 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel. The formed solid was combined with that removed after the concentration of the filtrate and the compound was recrystallized from MeOH (4 mL).

Yield: 66%; m.p.: 235–236°C; HPLC: log $k$ = 0.551; IR (KBr): 3411 (OH), 3032 (Ar-H), 2922 (CH), 2837 (CH), 1619 (C=N), 1582 (C=C), 1543 (C=C), 1500 (C=C), 1483, 1459, 1398, 1334, 1263, 1258, 1215, 1177, 1136, 1080, 1057, 1005, 987, 962, 863, 815, 774, 737, 713; $^1$H-NMR δ: 11.48 (s, 1H, OH), 10.33 (s, 1H, OH), 8.31 (d, $J$ = 8.3 Hz, 1H, Ar-H), 8.28 (t, $J$ = 0.8 Hz, 1H, Ar-H), 8.07 (d, $J$ = 8.7 Hz, 1H, Ar-H), 7.68 (dd, $J$ = 8.4 and 1.2 Hz, 1H, Ar-H), 6.55 (d, $J$ = 2.3 Hz, 1H, Ar-H), 6.49 (dd, $J$ = 8.8 and 2.3 Hz, 1H, Ar-H); $^{13}$C-NMR δ: 167.6, 162.2, 158.4, 151.0, 138.1, 130.1, 127.2, 125.5, 123.35, 120.1, 117.9, 110.2, 108.6, 102.7; EI-MS $m/z$ (%): 311 ($M^+$, 100), 292 (3), 282 (3), 254 (31), 222 (4), 157 (3), 142 (5). Anal. Calcd ($C_{14}H_8F_3NO_2S$): C, 54.02; H, 2.59; N, 4.50. Found: C, 54.23; H, 2.61; N, 4.48.

### *2-(2,4-Dihydroxyphenyl)naphtho[2,3-d][1,3]-thiazole-4,9-dione* ***2a***

A mixture of 2-amino-3-chloronaphthalene-1,4-dione (0.0024 mol) and STB (0.0048 mol) in MeOH (12 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel and the filtrate was left at room temperature (24 h). The formed solid was inserted into $CHCl_3$ (25 mL) and refluxed for 2 h. The hot mixture was filtered and the product was recrystallized from MeOH/$H_2O$ (2:1, 15 mL).

Yield: 69%; m.p.: 172–173°C; HPLC: log $k$ = 0.529; IR (KBr): 3460 (OH), 3318 (OH), 2922 (CH), 2843 (CH), 1682 (C=O), 1617 (C=N), 1578 (C=C), 1510 (C=C), 1478, 1459, 1417, 1385, 1322, 1304, 1275, 1221, 1174, 1125, 1058, 1017, 1005, 978, 918, 852, 818, 785, 760, 721; $^1$H-NMR δ: 11.13 (s, 1H, OH), 10.19 (s, 1H, OH), 8.35 (d, $J$ = 8.4 Hz, 1H, Ar-H), 8.27 (d, $J$ = 8.0 Hz, 1H, Ar-H), 7.99 (d, $J$ = 8.8 Hz, 1H, Ar-H), 7.75 (t, $J$ = 7.5 Hz, 1H, Ar-H), 7.3 (t, $J$ = 7.2 Hz, 1H, Ar-H), 6.57 (dd, $J$ = 8.4 and 2.4 Hz, 1H, Ar-H), 6.51 (d, $J$ = 2.1 Hz, 1H, Ar-H); $^{13}$C-NMR δ: 181.0, 179.2, 175.1, 147.0, 134.8 (2C), 132.6 (2C), 132.3, 129.8, 126.1 (2C), 125.8 (2C), 117.9, 108.8, 102.4; EI-MS $m/z$ (%): 323 ($M^+$, 100), 292 (28), 264 (3), 235 (6), 184 (36), 153 (8), 136 (10), 129 (9), 124 (11), 101 (6), 69 (4), 36 (10), 85 (23), 80 (7), 69 (17), 63 (7), 51 (10), 44 (48), 40 (39), 36 (22). Anal. Calcd ($C_{17}H_9NO_4S$): C. 63.15; H, 2.81; N, 4.33. Found: C, 63.28; H, 2.79; N, 4.31.

### *2-(2,4-Dihydroxy-3-methylphenyl)naphtho[2,3-d][1,3]-thiazole-4,9-dione* ***2b***

A mixture of 2-amino-3-chloronaphthalene-1,4-dione (0.0024 mol) and S3MTB (0.0048 mol) in MeOH (12 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel and the filtrate was concentrated. The formed solid was recrystallized from MeOH (5 mL).

Yield: 71%; m.p.: 197–200°C; HPLC: log $k$ = 0.515; IR (KBr): 3413 (OH), 3305 (OH), 3120 (OH), 2920 (CH), 2843 (CH), 1685 (C=O), 1638 (C=N), 1610 (C=N), 1578 (C=C), 1560 (C=C), 1480, 1458, 1386, 1334, 1306, 1276, 1228, 1177, 1103, 1040, 1006, 963, 852, 820, 783, 719; $^1$H-NMR δ: 12.30 (s, 1H, OH), 9.53 (s, 1H, OH), 8.10–8.08 (m, 2H, Ar-H), 7.53 (d, $J$ = 8.8, 1H, Ar-H), 7.49–7.46 (m, 1H, Ar-H), 7.40–7.36 (m, 1H, Ar-H), 6.53 (d, $J$ = 8.7, 1H, Ar-H), 2.01 (s, 3H, $CH_3$); EI-MS $m/z$ (%): 337 ($M^+$, 65), 323 (45), 315 (100), 307 (44), 298 (46), 286 (18), 270 (19), 258 (12), 240 (7), 209 (17), 182 (15), 172 (42), 167 (9), 160 (22), 151 (37), 131 (13), 124 (11), 105 (54), 89 (22), 76 (32), 51 (12), 44 (14), 36 (82), 44 (48), 40 (39), 36 (22). Anal. Calcd ($C_{18}H_{11}NO_4S$): C, 64.09; H, 3.29; N, 4.15. Found: C, 64.31; H, 3.27; N 4.13.

### *2-(5-Ethyl-2,4-dihydroxyphenyl)naphtho[2,3-d][1,3]-thiazole-4,9-dione* ***2c***

A mixture of 2-amino-3-chloronaphthalene-l,4-dione (0.0024 mol) and SETB (0.0048 mol) in MeOH (12 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel. The filtrate was concentrated, $CHCl_3$ (20 mL) was added to

the mixture and refluxed for 0.5 h. The formed solid was recrystallized from MeOH (5 mL).

Yield: 59%; m.p.: 163–165°C; HPLC: log $k = 0.520$; IR (KBr): 3447 (OH), 2923 (CH), 2942 (CH), 1687 (C=O), 1638 (C=N), 1591 (C=C), 1513 (C=C), 1460, 1438, 1387, 1331, 1291, 1286, 1217, 1158, 1136, 1107, 1018, 936, 857, 755, 718; $^1$H-NMR δ: 11.45 (s, 1H, OH), 10.38 (s, 1H, OH), 8.12–8.10 (m, 1H, Ar-H), 7.99–7.97 (m, 1H, Ar-H), 7.91–7.88 (m, 1H, Ar-H), 7.50–7.48 (m, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 6.61 (s, 1H, Ar-H), 2.45 (m, $J = 7.0$ Hz, 2H, $CH_2CH_3$), 1.17 (t, $J = 7.2$ Hz, 3H, $CH_2CH_3$); EI-MS *m/z* (%): 351 ($M^+$, 43), 336 ($M^+$–$CH_3$, 92), 322 (19), 266 (9), 173 (19), 155 (10), 146 (8), 138 (42), 123 (100), 105 (19), 89 (13), 77 (15), 69 (11), 64 (34), 48 (11), 40 (14), 38 (17), 36 (51). Anal. Calcd ($C_{19}H_{13}NO_4S$): C, 64.95; H, 3.73; N, 3.98. Found: C, 65.18; H, 3.71; N, 3.96.

### *2-(5-Chloro-2,4-dihydroxyphenyl)naphtho[2,3-d][1,3]-thiazole-4,9-dione* ***2d***

A mixture of 2-amino-3-chloronaphthalene-1,4-dione (0.0024 mol) and SClTB (0.0048 mol) in MeOH (12 mL) was treated to reflux for 3 h, left at room temperature (24 h) and filtrated. $CHCl_3$ (40 mL) was added to the precipitate and treated to reflux for 1 h. The reaction mixture was filtered off and the formed solid was recrystallized from MeOH (15 mL).

Yield: 63%; m.p.: 181–183°C; HPLC: log $k = 0.347$; IR (KBr): 3419 (OH), 3257 (OH), 2921 (CH), 2844 (CH), 1654 (C=O), 1622 (C=N), 1587 (C=C), 1514 (C=C), 1480, 1393, 1373, 1298, 1262, 1093, 951, 883, 864, 842, 742, 718; $^1$H-NMR δ: 11.93 (s, 1H, OH), 11.15 (s, 1H, OH), 8.21 (s, 1H, Ar-H), 8.19–8.17 (m, 1H, Ar-H), 8.12–8.10 (m, 1H, Ar-H), 7.92–7.88 (m, 2H, Ar-H), 6.78 (s, 1H, Ar-H); EI-MS *m/z* (%): 357 ($M^+$, 100), 323 (6), 294 (5), 266 (13), 188 (27), 160 (11), 137 (16), 104 (28), 89 (7), 76 (20), 55 (5), 44 (17), 40 (12), 36 (6), 69 (17), 63 (7), 51 (10), 44 (48), 40 (39), 36 (22). Anal. Calcd ($C_{17}H_8ClNO_4S$): C, 57.07; H, 2.25; N, 3.92. Found: C, 57.21; H, 2.26; N, 3.94.

### *7-Amino-2-(5-chloro-2,4-dihydroxyphenyl)-3a-hydroxy-4,6-dimethyl-3a,6-dihydro[1,3]thiazolo[5,4-d]-pyrimidin-5(4H)-one* ***3a***

A mixture of 5,6-diamino-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (0.0015 mol) and SClTB (0.0015 mol) in MeOH (7.5 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel. The formed solid was combined with that removed after the concentration of the filtrate and the compound was recrystallized from MeOH (4 mL).

Yield: 63%; m.p. 328–330°C; HPLC: log $k = -1.111$; IR (KBr): 3539 (NH), 3297 (OH), 3190 (OH), 2962 (CH), 2837 (CH), 1690 (C=O), 1638 (C=N), 1611 (C=N), 1564 (C=C), 1516 (C=C), 1468, 1437, 1413, 1383, 1361, 1300, 1246, 1228, 1203, 1135, 1077, 1043, 980, 941, 915, 864, 770, 759; $^1$H-NMR δ: 11.96 (s, 1H, OH), 10.90 (s, 1H, OH), 10.54 (s, 1H, OH), 8.06 (s, 1H, Ar-H), 6.78 (br. s, 2H, $NH_2$), 6.57 (s, 1H, Ar-H), 3.37 (s, 3H, $CH_3$), 3.34 (s, 3H, $CH_3$); EI-MS *m/z* (%): 356 ($M^+$, 5), 339 (100), 322 (31), 306 (3), 282 (61), 265 (15), 255 (5), 248 (12), 224 (9), 219 (6), 208 (6), 187 (84), 170 (34), 153 (25), 131 (9), 126 (5), 85 (23), 80 (7), 69 (17), 63 (7), 51 (10), 44 (48), 40 (39), 36 (22). Anal. Calcd ($C_{13}H_{13}ClN_4O_4S$): C, 43.76; H, 3.67; N, 15.70. Found: C, 43.69; H, 3.69; N, 15.64.

### *2-(2,4-Dihydroxy-3-methylphenyl)-3a-hydroxy-3a,6-dihydro[1,3]thiazolo[5,4-d]pyrimidin-5(4H)-one* ***3b***

A mixture of 5-aminouracil (0.002 mol) and S3MTB (0.002 mol) in methanol (10 mL) was refluxed for 3 h. The hot reaction mixture was filtered *via* a Büchner funnel. The filtrate was concentrated, the formed solid was filtered off and recrystallized from methanol (4 mL).

Yield: 69%; m.p.: 244–246°C; HPLC: log $k = -0.731$; IR (KBr): 3436, 3310 (OH), 2957 (CH), 1691 (C=O), 1618 (C=N), 1552 (C=C), 1500, 1482, 1427, 1367, 1388, 1303, 1270, 1214, 1182, 1119, 1079, 1067, 1003, 963, 892, 818, 780, 762, 704; $^1$H-NMR δ: 11.51 (s, 1H, OH), 11.33 (s, 1H, OH), 10.97 (s, 1H, OH), 7.74 (d, $J = 8.6$ Hz, 1H, Ar-H), 6.55 (d, $J = 8.6$ Hz, 1H, Ar-H), 6.48 (s, 1H, CH), 2.05 (s, 3H, $CH_3$); $^{13}$C-NMR δ: 193.7, 167.5, 160.8, 160.0, 157.0, 150.2, 136.5, 127.5, 115.3, 114.2, 111.4, 107.0; EI-MS *m/z* (%): 293 ($M^+$, 49), 275 (39), 260 (38), 232 (14), 217 (18), 181 (11), 167 (78), 151 (100), 144 (11), 122 (8), 85 (8), 77 (6), 65 (5), 39 (4). Anal. Calcd ($C_{12}H_{11}N_3O_4S$): C, 49.14; H, 3.79; N, 14.33. Found: C, 49.22; H, 3.77; N, 14.29.

### *2-(5-Chloro-2,4-dihydroxyphenyl)-7a-hydroxy-7,7a-dihydro[1,3]thiazolo[4,3-b][1,3,4]thiadiazole-5-thione* ***4***

A mixture of 3-amino-2-thioxothiazolidin-4-one (0.0017 mol) and SClTB (0.0017 mol) in MeOH (8.5 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel and the filtrate was left at room temperature (24 h). The formed solid was combined with that removed after the concentration of the filtrate and the compound was recrystallized from MeOH (4 mL).

Yield: 78%; m.p.: 235–237°C; HPLC: log $k = -0.508$; IR (KBr): 3421 (OH), 2923 (CH), 2836 (CH), 1604 (C=N), 1508 (C=C), 1438, 1417, 1396, 1298, 1273, 1220, 1194, 1182, 1089, 1004, 888, 876, 809, 775, 732; $^1$H-NMR δ: 11.98 (s, 1H, OH), 11.39 (s, 1H, OH), 10.87 (s, 1H, OH), 8.03 (s, 1H, Ar-H), 6.75 (s, 1H, Ar-H), 3.68 (s, 2H, $CH_2$); $^{13}$C-NMR δ: 168.5, 162.7, 162.0, 156.3, 154.5, 127.3, 111.8, 109.1, 103.5, 52.5; EI-MS *m/z* (%): 334 ($M^+$, 42), 333 (15), 332 (100), 300 (98), 272 (33), 218 (7), 196 (14), 170 (10), 169 (19), 109 (6), 69 (5), 45 (5), 36 (2). Anal. Calcd ($C_{10}H_7ClN_2O_3S_3$): C, 35.87; H, 2.11; N, 8.36. Found: C, 36.00; H, 2.10; N, 8.33.

Compounds: *4-(6-chloro-4H-3,1-benzothiazin-2-yl)benzene-1,3-diol* **5a**, *4-(6-chloro-4H-3,1-benzothiazin-2-yl)-6-methylbenzene-1,3-diol* **5b**, *4-(6-chloro-4H-3,1-benzothiazin-2-yl)-6-ethylbenzene-1,3-diol* **5c**, *4-chloro-6-(6-chloro-4H-3,1-benzothiazin-2-yl)benzene-1,3-diol* **5d** were described previously [23].

### *4-(4-Methylidene-4H-3,1-benzothiazin-2-yl)-benzene-1,3-diol* ***6a***

A mixture of o-amino(acetophenone) (0.00185 mol) and STB (0.0014 mol) in MeOH (15 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel. The filtrate was concentrated, the formed solid was filtered off and recrystallized from MeOH (5 mL).

Yield: 71%; m.p.: 206–208°C; HPLC: log $k = 1.235$; IR (KBr): 3067 (OH), 1611 (C=N), 1542 (C=C), 1509 (C=C), 1452, 1376, 1326, 1292, 1261, 1196 (C-OH), 1132, 1035, 981, 943, 843, 805, 760; $^1$H-NMR δ: 14.38 (s, 1H, OH), 10.42 (s, 1H, OH), 7.65 (d, $J = 8.6$ Hz, 1H, Ar-H), 7.54–7.39 (m, 4H, Ar-H), 6.74 (s, 1H, CH), 6.34 (d, $J = 2.3$ Hz, 1H, Ar-H), 6.28 (m, 2H, Ar-H, CH); $^{13}$C-NMR δ: 162.8, 159.8, 139.6, 129.2, 128.1, 127.0, 126.7, 124.6, 120.2, 111.2, 110.2, 108.1, 107.9, 102.9, 47.7; EI-MS *m/z* (%): 269 ($M^+$, 100), 236 (34), 225 (11), 212 (16), 180 (11), 167 (8), 154 (3), 135 (3), 108 (4), 89 (11), 76 (5), 63 (5), 44 (8). Anal. Calcd ($C_{15}H_{11}NO_2S$) C, 66.89; H, 4.12; N, 5.20. Found: C, 66.72; H, 4.13; N, 5.18.

### *4-(6,7-Dimethoxy-4-methylidene-4H-3,1-benzothiazin-2-yl)-2-methylbenzene-1,3-diol* ***6b***

A mixture of 2′-amino-4′,5′-dimethoxyacetophenone (0.0013 mol) and S3MTB (0.0013 mol) in MeOH (12 mL) was treated to reflux

for 3 h. The hot mixture was filtered *via* a Büchner funnel. The filtrate was concentrated and the formed solid was recrystallized from MeOH/$H_2O$ (3:1, 4 mL).

Yield 68%; m.p.: 195–197°C; HPLC: log $k$ = 1.216; IR (KBr, $cm^{-1}$): 3435 (OH), 2922 (CH), 1614 (C=N), 1502 (C=C), 1480, 1463, 1338, 1314, 1272, 1218, 1089, 1064, 1058, 1026, 942, 877, 822, 780; $^1$H-NMR δ: 10.25 (br.s, 1H, OH), 7.80 (d, $J$ = 8.8 Hz, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 6.65 (s, 1H, CH), 6.55 (d, $J$ = 8.7 Hz, 1H, Ar-H), 6.40 (s, 1H, CH), 3.91–3.80 (m, 6H, $OCH_3$), 1.18 (s, 3H, $CH_3$); EI-MS *m/z* (%): 343 ($M^+$, 43), 321 (17), 297 (17), 277 (31), 249 (22), 222 (36), 162 (43), 151 (100), 114 (15), 77 (13), 57 (61), 40 (60). Anal. Calcd ($C_{18}H_{17}NO_4S$) C, 62.96; H, 4.99; N, 4.08. Found: C, 63.28; H, 5.01; N, 4.06.

### *2-(2,3,4-Trihydroxyphenyl)-4H-3,1-benzothiazin-4-one* **7a**

A mixture of 2-amino-*N*-phenylbenzamide (0.0012 mol) and S3TTB (0.0017 mol) in MeOH (6 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel. The obtained product was combined with that removed after the concentration of the filtrate and the compound was recrystallized from MeOH (4 mL).

Yield: 74%; m.p.: 246–248°C; HPLC: log $k$ = 0.061; IR (KBr): 3559 (OH), 3068 (Ar-H), 2856 (CH), 1705 (C=O), 1645 (C=N), 1598 (C=C),1543 (C=C), 1504 (C=C), 1474, 1442, 1363, 1322, 1282, 1261, 1215, 1181, 1116, 1091, 995, 931, 916, 894, 865, 812, 792, 765, 747, 720, 709; $^1$H-NMR δ: 13.35 (s, 1H, OH), 10.07 (s, 1H, OH), 8.67 (s, 1H, OH), 8.12–8.11 (m, 1H, Ar-H), 7.95–7.93 (m, 1H, Ar-H), 7.81 (d, $J$ = 8.7 Hz, 1H, Ar-H), 7.62–7.61 (m, 1H, Ar-H), 7.16–7.15 (m, 1H, Ar-H), 6.51 (d, $J$ = 8.6 Hz, 1H, Ar-H); EI-MS *m/z* (%): 287 ($M^+$, 100), 259 (3), 227 (59), 202 (12), 140 (4), 153 (3), 143 (5), 136 (2), 115 (3), 104 (2), 77 (18), 65 (3), 51 (4), 39 (4). Anal. Calcd ($C_{14}H_9NO_4S$): C, 58.53; H, 3.16; N, 4.88. Found: C, 58.60; H, 3.14; N, 4.86.

### *6-Chloro-2-(2,4-dihydroxyphenyl)-4H-3,1-benzothiazin-4-one* **7b**

A mixture of 2-amino-5-chlorobenzamide (0.0015 mol) and STB (0.0015 mol) in MeOH (7.5 mL) was treated to reflux for 3 h. The hot mixture was filtered *via* a Büchner funnel. The obtained product was combined with that removed after the concentration of the filtrate and the compound was recrystallized from MeOH (4 mL).

Yield: 78%; m.p.: 249–250°C; HPLC: log $k$ = 0.347; IR (KBr): 3398 (OH), 3082 (Ar-H), 2843 (CH), 1703 (C=O), 1631 (C=N), 1598 (C=C), 1562 (C=C), 1538 (C=C), 1516 (C=C), 1470, 1448, 1403, 1382, 1326, 1314, 1247, 1194, 1135, 1069, 980, 937, 870, 835, 796, 745, 704; $^1$H-NMR δ: 12.93 (s, 1H, OH), 10.59 (s, 1H, OH), 8.06 (d, $J$ = 2.5 Hz, 1H, Ar-H), 7.99–7.97 (m, 1H, Ar-H), 7.93 (m, 1H, Ar-H), 7.67 (d, $J$ = 8.8 Hz, 1H, Ar-H), 6.47 (dd, $J$ = 8.8 and 2.4 Hz, 1H, Ar-H), 6.38 (d, $J$ = 2.3 Hz, 1H, Ar-H); $^{13}$C-NMR δ: 178.4, 177.5, 167.4, 157.3, 156.5, 152.3, 139.7, 134.3, 134.0, 132.9, 128.5, 126.0, 111.8, 103.4; EI-MS *m/z* (%): 305 ($M^+$, 100), 277 (4), 245 (60), 217 (13), 182 (7), 154 (11), 108 (16), 75 (6), 69 (5), 63 (5), 52 (4), 39 (5). Anal. Calcd ($C_{14}H_8ClNO_3S$): C, 55.00; H, 2.64; N, 4.58. Found: C, 54.86; H, 2.65; N, 4.60.

### *4-[6-Chloro-4-(2-chlorophenyl)-4-hydroxy-4H-3,1-benzothiazin-2-yl]-6-ethylbenzene-1,3-diol* **8a**

A mixture of 2-amino-2′,5-dichlorobenzphenone (0.0013 mol) and SETB (0.0013 mol) in MeOH (9.5 mL) was refluxed for 3 h. The hot reaction mixture was filtered *via* a Büchner funnel. The filtrate was concentrated, the formed solid was filtered off and recrystallized from MeOH (4 mL).

Yield: 62%; m.p.: 188–190°C; HPLC: log $k$ = 1.141; IR (KBr): 3438 (OH), 3197 (OH), 2969 (CH), 1610 (C=N), 1582 (C=C), 1546 (C=C), 1521 (C=C), 1493, 1466, 1402, 1377, 1338, 1267, 1219, 1198, 1153, 1088, 1057, 989, 951, 880, 828, 758, 732; $^1$H-NMR δ: 13.66 (s, 1H, OH), 11.50 (s, 1H, OH), 10.47 (s, 1H, OH), 8.14 (d, $J$ = 8.7 Hz, 1H, Ar-H), 7.56–7.54 (m, 3H, Ar-H), 7.52–7.50 (m, 2H, Ar-H), 7.46 (s, 1H, Ar-H), 6.81–6.79 (m, 1H, Ar-H), 6.42 (s, 1H, Ar-H), 2.46–2.44 (m, $J$ = 7.5 Hz, 2H, $CH_2CH_3$), 1.09 (t, $J$ = 7.5 Hz, 3H, $CH_2CH_3$); EI-MS *m/z* (%): 445 (6), 427 (53), 414 (22), 394 (4), 306 (7), 296 (6), 261 (7), 246 (3), 230 (4), 212 (6), 195 (5), 186 (4), 164 (6), 139 (43), 111 (10), 91 (3), 69 (7), 45 (44), 36 (100). Anal. Calcd ($C_{22}H_{17}Cl_2NO_3S$): C, 59.20; H, 3.84; N, 3.14; found: C, 59.28; H, 3.82; N, 3.12.

### *4-Chloro-6-(4-hydroxy-7-methyl-4-phenyl-4H-3,1-benzothiazin-2-yl)benzene-1,3-diol* **8b**

A mixture of 2-amino-4-methylbenzphenone (0.0012 mol) and SClTB (0.002 mol) in MeOH (6 mL) was refluxed for 3 h. The hot reaction mixture was filtered *via* a Büchner funnel. The filtrate was concentrated, NaOH solution (5%) was added (5 mL) and the mixture was filtrated. HCl solution (5%) was added to the filtrate to afford the product. The compound was recrystallized from MeOH (4 mL).

Yield: 64%; m.p.: 154–156°C; HPLC: log $k$ = 0.488; IR (KBr): 3432 (OH), 2922 (CH), 2843 (CH), 1617 (C=N), 1534 (C=C), 1490, 1441, 1339, 1298, 1257, 1207, 1159, 1078, 1043, 964, 923, 882, 845, 778, 742; $^1$H-NMR δ: 14.35 (s, 1H, OH), 11.72 (s, 1H, OH), 11.35 (s, 1H, OH), 7.94 (s, 1H, Ar-H), 7.69–7.67 (m, 1H, Ar-H), 7.57–7.52 (m, 3H, Ar-H), 7.43–7.40 (m, 2H, Ar-H), 6.99–6.96 (m, 1H, Ar-H), 6.76 (s, 1H, Ar-H), 6.57–6.53 (m, 1H, Ar-H), 1.75 (s, 3H, $CH_3$); $^{13}$C-NMR δ: 160.8, 160.5, 159.2, 158.1, 140.2, 139.4, 135.9, 129.9, 129.2, 128.6, 128.2, 127.3, 126.6, 119.7, 115.0, 112.1, 111.5, 111.3, 104.4, 58.3, 19.5 ($CH_3$); EI-MS *m/z* (%): 397 ($M^+$, 5), 381 (100), 348 (6), 313 (5), 304 (9), 218 (53), 186 (46), 142 (35), 130 (15), 114 (9), 107 (5), 87 (6), 79 (11), 77 (7), 53 (11), 51 (20), 44 (15), 39 (9). Anal. Calcd ($C_{21}H_{16}ClNO_3S$): C, 63.39; H, 4.05; N, 3.52. Found: C, 63.13; H, 4.03; N, 3.54.

### *2-Amino-3-[2-(5-chloro-2,4-dihydroxyphenyl)-4-hydroxy-4H-3,1-benzothiazin-4-yl]propanoic acid* **8c**

A mixture of 2-amino-4-(2-aminophenyl)-4-oxobutanoic acid (0.0012 mol) and SClTB (0.0012 mol) in MeOH (6 mL) was refluxed for 3 h. The reaction mixture was left at room temperature (24 h) and the formed solid was filtered off. The obtained product was combined with that removed after the filtrate concentration. The compound was recrystallized from MeOH/$H_2O$ (1:1) (4 mL).

Yield: 66%; m.p.: 189–191°C; HPLC: log $k$ = −0.568; IR (KBr): 3423 (NH), 3240 (OH), 2920 (CH), 1700 (C=O), 1615 (C=N), 1539 (C=C), 1506 (C=C), 1490, 1428, 1342, 1297, 1256, 1198, 1162, 1084, 1047, 1023, 992, 847, 762; $^1$H-NMR δ: 7.72 (d, $J$ = 8.1 Hz, 1H, Ar-H), 7.31–7.24 (m, 3H, Ar-H), 7.21 (br.s, 2H, $NH_2$), 6.79 (d, $J$ = 8.4 Hz, 1H, Ar-H), 6.56 (t, $J$ = 7.2 Hz, 1H, Ar-H), 4.46 (m, 1H, CH), 3.59 (d, $J$ = 6.2 Hz, 2H, $CH_2$); $^{13}$C-NMR δ: 170.8, 160.5, 158.1, 155.6, 151.4, 148.5, 134.9, 132.0, 117.1, 114.7, 111.6, 110.9, 104.4, 104.2, 104.0, 58.3, 52.2; EI-MS *m/z* (%): 394 ($M^+$, 5), 381 (100), 348 (6), 313 (5), 304 (9), 218 (53), 186 (46), 142 (35), 130 (15), 114 (9), 107 (5), 87 (6), 79 (11), 77 (7), 53 (11), 51 (20), 44 (15), 39 (9). Anal. Calcd ($C_{17}H_{15}ClN_2O_5S$): C, 51.71; H, 3.83; N, 7.10. Found: C, 51.98; H, 3.81; N, 7.13.

### *4-Chloro-6-(10b-hydroxy-10bH-fluoreno[1,9-de][1,3]-thiazin-2-yl)benzene-1,3-diol* **9**

A mixture of 1-amino-9*H*-fluoren-9-one (0.0013 mol) and SClTB (0.0022 mol) in MeOH (10.5 mL) was refluxed for 3 h. The hot reaction mixture was filtered *via* a Büchner funnel. The filtrate was concentrated, NaOH solution (5%) was added (5 mL) and the mixture was filtrated. HCl solution (5%) was added to the filtrate to afford the product. The compound was recrystallized from MeOH (4.5 mL).

Yield: 74%; m.p.: 126–128°C; HPLC: $\log k = 0.851$; IR (KBr): 3376 (OH), 3055 (Ar-H), 2924 (CH), 2853 (CH), 1648 (C=N), 1611 (C=N), 1576 (C=C), 1506 (C=C), 1490, 1452, 1420, 1343, 1298, 1257, 1193, 1162, 1045, 961, 928, 896, 848, 797, 755; $^1$H-NMR δ: 13.70 (s, 1H, OH), 11.94 (s, 1H, OH), 10.57 (s, 1H, OH), 7.91 (s, 1H, Ar-H), 7.69–7.64 (m, 2H, Ar-H), 7.55–7.49 (m, 1H, Ar-H), 7.35–7.30 (m, 1H, Ar-H), 7.26–7.21 (m, 1H, Ar-H), 6.90 (d, $J = 7.0$ Hz, 1H, Ar-H), 6.64 (d, $J = 7.3$ Hz, 1H, Ar-H), 6.63 (s, 1H, Ar-H); $^{13}$C-NMR δ: 193.3, 168.2, 160.6, 159.3, 148.3, 143.4, 142.7, 136.2, 134.5, 133.5, 130.6, 128.7, 122.5, 120.7, 117.8, 115.0, 112.6, 111.6, 108.77, 103.8; EI-MS *m/z* (%): 381 ($M^+$, 43), 365 ($M^+$−OH, 100), 330 (3), 211 (14), 168 (18), 152 (20), 140 (16), 139 (35), 83 (7), 51 (5), 44 (9), 36 (7). Anal. Calcd ($C_{20}H_{12}ClNO_3S$): C, 62.91; H, 3.17; N, 3.67. Found: C, 63.04; H, 3.15; N, 3.65.

### Antibacterial assay *in vitro*

MICs of all synthesized compounds for eight reference strains, including the Gram-positive bacteria (*Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, and *Micrococcus luteus* ATCC 10240) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, and *Proteus mirabilis* ATCC 12453) were assessed by the micro-dilution broth method according to Skalicka *et al* [25]. Briefly, the stock solutions of the synthesized compounds were made in DMSO (Sigma). Then the series of two-fold dilutions of these stock solutions, ranging from 0.39 to 200 μg/mL, were prepared in the Mueller-Hinton broth (Biocorp, Poland) in 96-well microtiter plates (NUNC). The wells were inoculated with the bacterial suspension (the final inoculum size of $10^6$ colony forming units – CFU/mL) and incubated at 35°C for 24 h. MIC was defined as the lowest concentration of the compound that completely suppressed visible growth. Ampicillin was used as the reference compound. MBC of the tested compounds (except **5a**) towards Gram-positive bacteria was determined by subculturing 100 μL from each well that showed complete bacterial growth inhibition on Mueller-Hinton agar (Biocorp, Poland) plates. After the incubation (35°C for 24 h), the MBC value was defined as the lowest concentration of the compound at which there was no bacterial growth. The experiment was carried out in triplicates.

### Cytotoxicity assay

Cytotoxicity of the tested compounds was estimated with the use of the MTT method described by Takenouchi and Munekata [26]. The MTT method is a quantitative colorimetric toxicity test, based on the transformation of yellow, soluble tetrazolium salts (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to purple-blue insoluble formazane. This process occurs naturally in mitochondria of living cells. The Vero cell culture from the American Type Culture Collection (ECACC-84113001) was used in the experiment. The media in the culture (Minimum Essential Medium Eagle, Sigma) were supplemented with 10% foetal bovine serum (FBS, Sigma), 100 U/mL of penicillin and 0.1 mg/mL of streptomycin (Polfa-Tarchomin, Poland). The cell culture was incubated at 37°C in the 5% $CO_2$ atmosphere. All the investigated compounds were dissolved in DMSO (Sigma) to obtain the concentration of 10 mg/mL. 100 μL of the Vero cell culture prepared were plated into 96-well plastic plates (NUNC) at the density $2 \times 10^4$ cells per well. After incubation at 37°C for 24 h the media were removed and the cells were treated with a solution of the examined compounds diluted in the media with 2% of serum. The following final concentrations were applied: 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 μg/mL. At the same time the cytotoxicity of solvents in compounds was examined. The control cell culture was supplemented with media including 2% of serum only. The culture cells were incubated for 48 h at 37°C in the 5% $CO_2$ atmosphere. After 48-h incubation with the compounds, cell cultures were supplemented with 10 μL of 5 mg/mL MTT solution per well, and further incubated for 4 h at 37°C. Afterwards 100 μL of water solution, including 50% dimethylformamide and 20% SDS per well, were added and after overnight incubation the absorbance was measured by the 96-well plastic plate reader (Organon Teknika) at the wavelengths of 540 and 620 nm. The obtained results were presented as percentage of cell viability in comparison to the control. The investigation was carried out in triplicates. Cytotoxicity was assessed using standard procedure ISO 10993-5 [27].

*The authors have declared no conflict of interest.*

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