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Pyrimidine-derived disulfides as potential antimicrobial agents: synthesis and evaluation in vitro

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Pyrimidine-derived disulfides as potential antimicrobial agents: synthesis and evaluation *in vitro*

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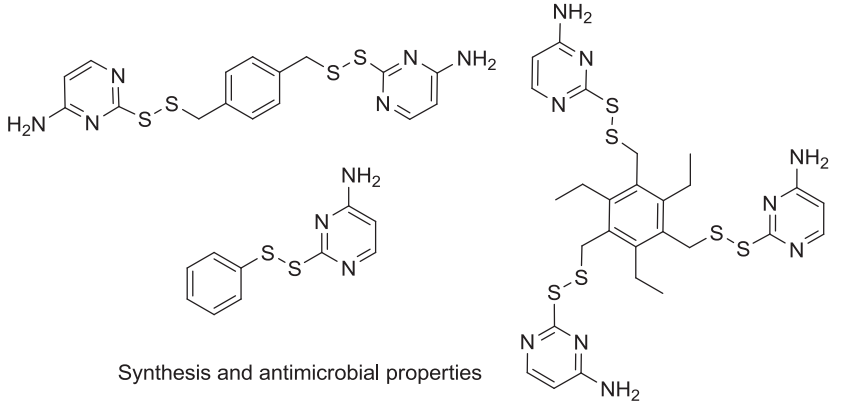
Antibiotic resistance is a worldwide problem. The synthesis and evaluation of new antimicrobial compounds, without cytotoxicity against human cells, are highly desired. In this paper, the preparation of a class of pyrimidine-derived disulfides is described. Sulfenic acids were generated from suitable precursors and used as reactive intermediates that condensed with the thiol function of thiocytosine. An introductory study on the antimicrobial activity of this disulfide family is reported. Three family components, that revealed no cytotoxicity against human erythrocytes, exhibited inhibitory activity against Gram-positive *Staphylococcus aureus*.



Keywords: antibacterial activity; cytotoxicity; disulfides; sulfenic acids; thiocytosine

1. Introduction

Several derivatives of pyrimidine have been used in the synthesis of compounds with notable antimicrobial activity. Both the antibacterial, 5-[(3,4,5-trimethoxyphenyl)methyl]-

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2,4-pyrimidinediamine, better known as trimethoprim, and the antimalarial pyrimethamine [5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine] are inhibitors of dihydrofolate reductase, causing folate deficiency in some patients.[1] A variety of cephalosporins, possessing C(3)-aminopyrimidinyl substituents, exhibited excellent antibacterial activities [2] *in vitro*. The synthesis of a family of carbapenem derivatives bearing an aminopyrimidinylthioether moiety at the C-5 position of the pyrrolidine ring was described, together with their potent antibacterial activities.[3] The bacteriostatic and fungistatic activities of pyrimidinophanes, macrocycles with pyrimidine fragments, possessing in their skeleton thiocytosine and uracil moieties, were estimated.[4] The reported data show that some of these macrocycles exhibit a remarkable selectivity toward Gram-positive bacteria, in dependence of their size and substituent nature. Finally, amino-substituted pyrimidines used as capping for gold nanoparticles induced bacterial resistance and appeared harmless to human cells.[5]

On the other hand, the existing collection of antimicrobial agents seems to be insufficient to protect the human species from infectious diseases, due to the resistance that many clinically relevant pathogens have developed against some of the well-known classes of antibacterial agents, such as the classical β-lactam antibiotic penicillins and cephalosporins. There is a real need of new compounds endowed with antimicrobial activity that show, possibly, new mechanisms of action. *Staphylococcus aureus* is perhaps the pathogen of greatest concern because of its intrinsic virulence and *its ability* to develop antibiotic resistance.[6]

In continuing our study directed to the development of the sulfenic acid chemistry for the preparation of new biologically active compounds,[7–10] we had the idea of connecting a pyrimidine residue to a planar structural skeleton by disulfide bond, as spacer, and prepare a small library of mono, di, and tri-pyrimidine-derived disulfides **2**–**8** (Figure 1) with different structural characteristics. In this paper we present the synthetic strategy, the hemolytic impact on red blood cells (RBCs), and an introductory study of the potential antibacterial and antifungal activities of compounds **2**–**8**.

2. Results and discussion

The sulfenic acid/thiol condensation was used to prepare the pyrimidine-derived family of disulfides shown in Figure 1. This is a well-assessed synthetic process (Scheme 1) that allowed us to obtain unsymmetrical disulfides in good yields and in three steps, starting from the suitable thiols.[11]

The syntheses of compounds **2** [11] and **8** [12,13] were previously reported. Compounds **4** and **5** have been analogously prepared from the commercially available thiophenol and 1,4-benzenedimethanethiol, respectively. Disulfides **6** and **7** have been obtained from thiols **9** and **10** (Figure 2), that can be easily prepared from the corresponding 1,3,5-tris(bromomethyl)benzenes, through the formation of the related thiouronium salts.[14] Finally, disulfide **3** has been prepared in quantitative yield by base-catalyzed deprotection of compound **2** with aqueous ammonia.

The procedure for the synthesis of 2′-[1,4-phenylenebis(methylenedithio)]bis-4-pyrimidinamine (**5**) is outlined in Scheme 2, as an example of the sulfenic acid chemistry used for the preparation of the molecules under study. The synthesis started with the preparation of the sulfenic acid precursors, such as racemic bis-sulfoxide **13**, that was obtained from bis-thiol **11** in two steps: (i) a conjugated addition in basic media of the corresponding thiolate to methyl acrylate to give **12**, obtained in high yields after column chromatography, and (ii) the subsequent controlled and quantitative oxidation of sulfide **12**–**13**, by means of *m*CPBA.[8] Thermolysis of **13** was first conducted in 1,2-dichloroethane (DCE) at 83 °C, but compound **5** was obtained in very poor yields. Side-products were isolated from the top of the chromatographic column, due

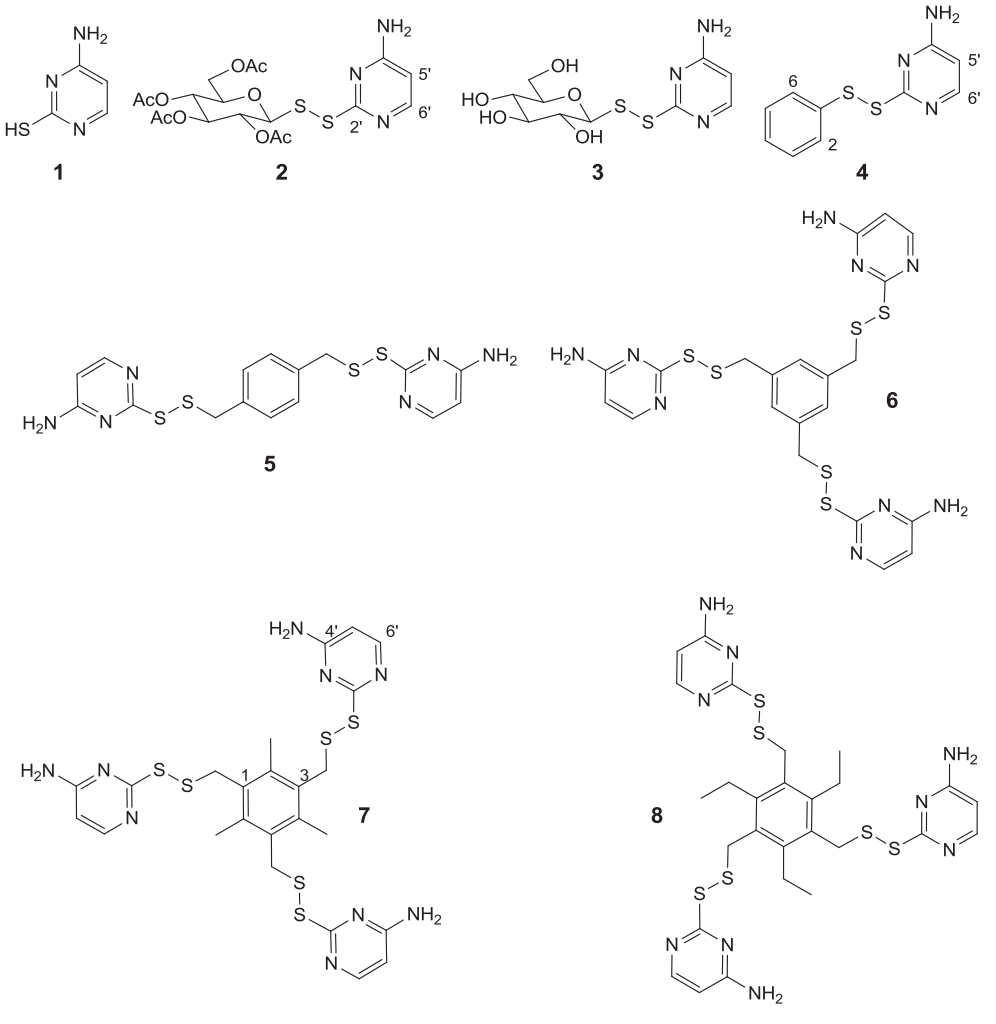
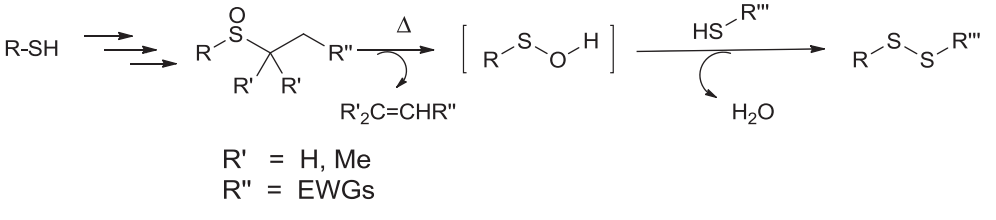


Figure 1. Thiocytosine (**1**) and derived disulfides **2**–**8**.



Scheme 1. The general procedure for the synthesis of disulfides **2**–**8**.

to the nucleophilic attack of the sulfur atom of thiocytosine (**1**) to electron-poor carbon atoms of DCE. In order to avoid this side-reaction, several other solvents were tested, such as acetonitrile, THF or 1,4-dioxane (bp 101 °C) and the last one resulted in the most efficient in term of yield and work-up of the thermolysis crude. Thermolysis of bis-sulfoxide **13**, in refluxing 1,4-dioxane, allowed the stepwise generation of the transient sulfenic functions in **14** and **15**, not isolated,[15] that in turn condensed with an excess of commercially available thiocytosine (**1**), giving rise to

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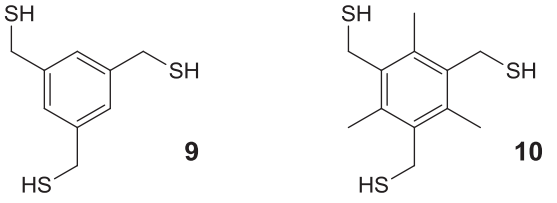
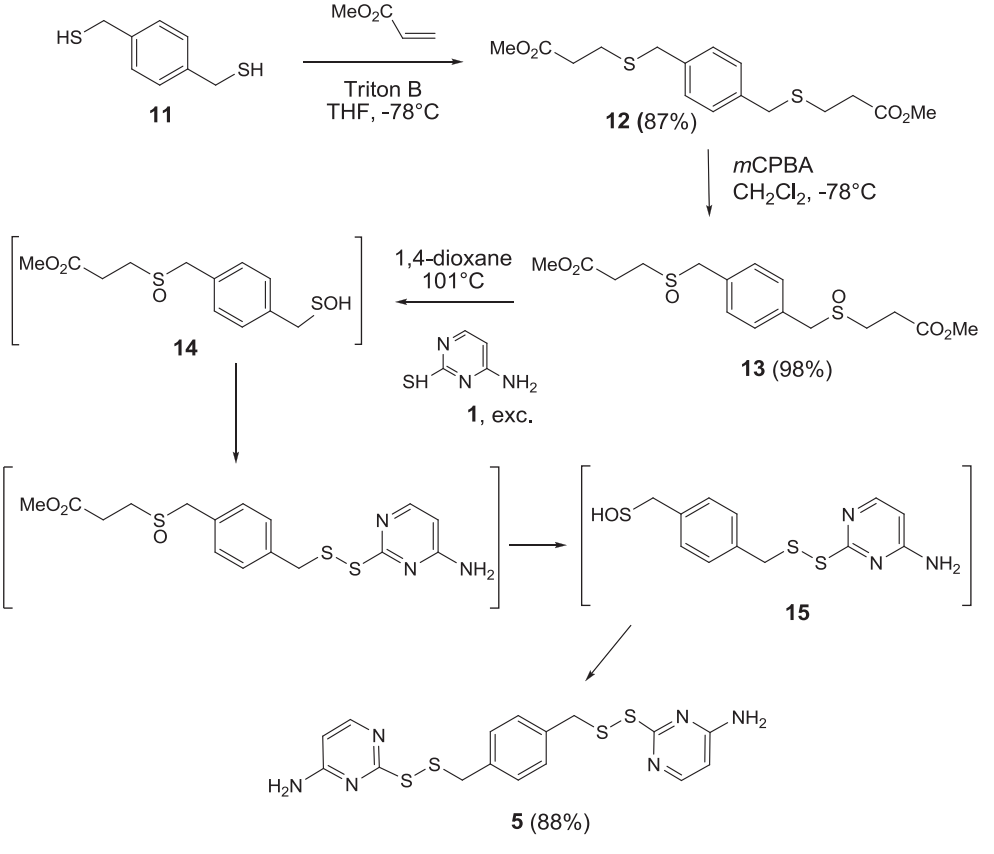


Figure 2. Starting products for the synthesis of 2,2′,2″-[1,3,5-benzenetriyltris(methylenedithio)]tris-4-pyrimidinamines **6** and **7**.



Scheme 2. The synthetic pathway to 2′-[1,4-phenylenebis(methylenedithio)]bis-4-pyrimidinamine (**5**).

product **5** in 88% yield. All the thiocytosine-derived disulfides **2**, **4**–**8** were obtained under the same reaction conditions and in good yields, separated from the excess of thiocytosine (**1**) by simply washing the crude with acetone and, finally, purified by column chromatography.

The *in vitro* antibacterial and antifungal activity of the family of pyrimidine derived disulfides **2**–**8** was investigated against representative Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), Gram-positive (*Bacillus cereus* ATCC 11778, *S. aureus* ATCC 29213 including three our clinical isolates MRSA: CI26428, CI25967, CI26348) and yeast (*Candida albicans* ATCC 10231, *Cryptococcus neoformans* ATCC MYA-565) strains. Thiocytosine glucosides **2** and **3** are part of a project in which we intended to prepare small glucosides to be tested as antimicrobial and/or anticancer therapeutics.[16] However, they are inactive against all the bacteria and fungi tested in this study, together with thiocytosine (**1**). In compounds **4**–**8**

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Table 1. Percentage of hemolysis by compounds **4**, **5**, and **8** (50 µg/mL).

	Osmolarity (mOsm/Kg)				
	300	200	150	100	0
Control	0	0.3	34	100	100
Compound **4**	0	0.1	22	98	100
Compound **5**	0	0.2	36	99	100
Compound **8**	0	0.2	31	98	100

(Figure 1) pyrimidine residues are connected, by disulfide bonds, to phenyl or benzylic skeletons. The monosubstituted benzene derivative **4** and the elongated benzenedimethanedisulfide **5** were synthesized taking into account the previously obtained results with similarly shaped biologically active molecules.[7,8] *In vitro* susceptibility data showed that *E. coli*, *P. aeruginosa*, and *B. cereus* strains grew well in all tubes containing these compounds and, therefore, were considered to be resistant. Only *S. aureus* isolates showed inhibition of the growth. A total inhibition of the four examined *S. aureus* strains was observed at a concentration of 50 µg/mL using compound **5**, whereas minimal inhibitory concentration (MIC) values obtained for compound **4** were lower (25 µg/mL). Molecules possessing a C_3 symmetry, such as the multi-armed benzene derivatives **6**–**8** (Figure 1), have been tested as potential antibacterials on the basis of literature data.[17] Noteworthy, whereas molecules **6** and **7** were inactive against all the bacteria studied, disulfide **8**, a hexafunctionalized 1,3,5$(R^1)_3$–2,4,6-$(R^2)_3$-benzene (R^1 = ethyl) that adopts the most thermodynamically stable tripodal conformation,[12] with an alternate geometrical orientation of the ring substituents above and below the benzene plane, exhibited inhibitory activity against Gram-positive *S. aureus*. MIC values were comparable with those of compound **5**. In addition, no antifungal effect was observed for the two pathogenic fungi included in this study indicating that compounds **5**, **8** and, in particular, **4** can be opportunely modified to enhance a species-specific inhibitory activity of these pyrimidine derivatives against the important human pathogen *S. aureus*. Finally, the potential toxicity of the biologically active compounds **4**, **5**, and **8** against human erythrocytes was evaluated (Table 1). The trypan blue assay showed a viability of 92–100% in all experiments (data not shown). Hemolytic activity was determined by hemoglobin release in the plasma after exposure to the molecules. No cytotoxic activity was observed. There were no statistical differences in the viability at different concentrations of the molecules.

3. Conclusions

In conclusion, we have described the synthesis of a small family of pyrimidine derivatives, disulfides **2**–**8**, with chemical moieties in common but distinct structural and spatial characteristics. Three of these compounds, disulfides **4**, **5**, and **8**, that reveal no cytotoxicity activity against human erythrocytes, are promising candidates for the development of new antibacterial agents.

4. Experimental

Solvents were purified according to standard procedures. All reactions were monitored by thin layer chromatography (TLC) on commercially available precoated plates (Aldrich silica gel 60 F254) and the products were visualized with vanillin [1 g dissolved in MeOH (60 mL) and conc. H_2SO_4 (0.6 mL)]. Silica gel used for column chromatography was Aldrich 60. ^1H and ^{13}C NMR

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spectra were recorded with a Varian 500 spectrometer at 500 and 125 MHz, respectively; *J* are given in Hz; the attributions are supported by Heteronuclear Single Quantum Coherence and correlation spectroscopy experiments. Proton and carbon nuclei identified by apex' pertain to thiocytosine residues (for numbering see Figure 1).

1-(4-Amino-2-pyrimidinyldithio)-1-deoxy-β-D-glucopyranose (3)

To a solution of disulfide **2** [11] (60 mg, 0.12 mmol) in MeOH/THF (16 mL, 5:5) aqueous ammonia (30%, 5 mL) was added under stirring, and stirring was maintained for 16 h, at r.t. The reaction was monitored by TLC (EtOAc/MeOH 9.5:0.5) and ^{1}H NMR. The solvent was evaporated and the crude was washed with MeOH (3 × 5 mL), to remove acetamide. Yield 92%. TLC: R_f 0.20 (EtOAc/MeOH 9.5:0.5). White solid. M.p. 198–202 °C. ^{1}H NMR (DMSO-d_6): δ 7.96 (d, 1H, $J_{5',6'}$ 5.9, H-6′), 7.36 (br s, 2H, NH_2), 6.29 (d, 1H, H-5′), 6.03 (d, 1H, J_{vic} 3.4, 2-OH), 5.04 (d, 1H, J_{vic} 4.9, 4-OH), 4.99 (d, 1H, J_{vic} 5.4, 3-OH), 4.56 (t, 1H, J_{vic} 5.6, 6-OH), 4.41 (d, 1H, $J_{1,2}$ 8.8, H-1), 3.66 and 3.40 (split AB system, 2H, H_2-6), 3.29–3.12 (m, 2H, H-3,5), 3.12 (m, 1H, H-2), 3.02 (m, 1H, H-4). ^{13}C NMR (DMSO-d_6): δ 168.9 (C-2′), 163.2 (C-4′), 154.7 (C-6′), 102.9 (C-5′), 86.9 (C-1), 81.7 and 76.3 (C-3,5), 70.8 (C-2), 69.8 (C-4), 61.0 (C-6). Anal. Calcd for $C_{10}H_{15}N_3O_5S_2$ (321.37): C, 37.37; H, 4.70; N, 13.08. Found: C, 37.24; H, 4.71; N, 13.12.

2-(Phenyldithio)-4-pyrimidinamine (4)

3-(phenylsulfinyl)propanenitrile [18] (200 mg, 1.12 mmol) was dissolved in 1,4-dioxane (6 mL) and thiocytosine (**1**) (426 mg, 3.35 mmol) was added, forming a suspension that was heated up to the reflux (101 °C) and maintained for 2 h at this temperature. During this time, the reaction was monitored via TLC (DCM/EtOAc 8:2). The excess of thiocytosine (**1**) was filtered. The solid residue was washed with acetone (3 × 5 mL) and the solution was recovered. The solvent was removed and the crude was purified by column chromatography (DCM/EtOAc 9.5:0.5). Yield 80%. TLC: R_f 0.65 (DCM/EtOAc 8:2). White solid. M.p. 133–135 °C. ^{1}H NMR (acetone-d_6): δ 7.99 (d, 1H, $J_{5',6'}$ 5.4, H-6′), 7.58 (d, 2H, J_{ortho} 7.3, H-2,6), 7.33 (t, 2H, J_{ortho} 7.3, H-3,5), 7.26 (t, 1H, H-4), 6.51 (br s, 2H, NH_2), 6.37 (d, 1H, H-5′). ^{13}C NMR (acetone-d_6): δ 170.0 (C-2′), 165.6 (C-4′), 157.4 (C-6′), 139.0 (C-1), 130.4, 130.0, and 128.8 (C-2–6), 104.0 (C-5′). Anal. Calcd for $C_{10}H_9N_3S_2$ (235.33): C, 51.04; H, 3.85; N, 17.86. Found: C, 50.91; H, 3.84; N, 17.90.

2,2′-[1,4-Phenylenebis(methylenedithio)]bis-4-pyrimidinamine (5)

A solution of dimethyl 3,3′-[1,4-phenylenebis(methylenesulfinyl)]bispropanoate [8] (**12**) (150 mg, 0.40 mmol) and thiocytosine (**1**) (212.3 mg, 1.67 mmol) in 3 mL of 1,4-dioxane was maintained under stirring, at reflux temperature (101 °C) for 3 h and 40 min. The reaction was monitored via TLC (DCM/EtOAc 7.5:2.5) and ^{1}H NMR. The excess of thiocytosine (**1**) was filtered. The solid residue was washed with acetone (3 × 5 mL) and the solution was recovered. The solvent was removed and the crude was purified by column chromatography (acetone/hexane 4:6). Yield 88%. TLC: R_f 0.30 (acetone/hexane 8:2). White solid. M.p. 85–90 °C. ^{1}H NMR (acetone-d_6): δ 8.01 (d, 2H, $J_{5',6'}$ 5.9, 2 × H-6′), 7.32 (s, 4H, benzeneH), 6.46 (br s, 4H, 2 × NH_2), 6.36 (d, 2H, 2 × H-5′), 4.12 (s, 4H, 2 × CH_2). ^{13}C NMR (acetone-d_6): δ 171.1 (2 × C-2′), 165.4 (2 × C-4′), 157.3 (2 × C-6′), 137.7 (C-1,4), 131.1 (C-2,3,5,6), 103.7 (2 × C-5′), 43.5 (2 × CH_2). Anal. Calcd for $C_{16}H_{16}N_6S_4$ (420.60): C, 45.69; H, 3.83; N, 19.98. Found: C, 45.56; H, 3.83; N, 19.95.

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2,2′,2″-[1,3,5-Benzenetriyltris(methylenedithio)]tris-4-pyrimidinamine (6)

To a solution of trimethyl 3,3′,3″-[1,3,5-benzenetriyltris(methylenesulfinyl)]trispropanoate [8] (65 mg, 0.12 mmol) in 1,4-dioxane (3 mL) thiocytosine (**1**) was added (146 mg, 1.15 mmol). The suspension was refluxed (101 °C) under stirring for 4 h and monitored by TLC (acetone/hexane 3:7). The excess of thiocytosine (**1**) was filtered. The solid residue was washed with acetone (3 × 5 mL) and the solution was recovered. The solvent was removed and the crude was purified by column chromatography (acetone/hexane 4:6).Yield 60%. TLC: R_f 0.32 (acetone/hexane 6:4). White solid. M.p. 95–100 °C. ^{1}H NMR (acetone-d_6): δ 8.01 (d, 3H, $J_{5',6'}$ 5.9, 3 × H-6′), 7.31 (s, 3H, benzeneH), 6.47 (br s, 6H, 3 × NH_2), 6.34 (d, 3H, 3 × H-5′), 4.11 (s, 6H, 3 × CH_2). ^{13}C NMR (acetone-d_6): δ 171.0 (3 × C-2′), 165.5 (3 × C-4′), 157.3 (3 × C-6′), 139.0 (C-1,3,5), 131.1 (C-2,4,6), 103.7 (3 × C-5′), 44.0 (3 × CH_2). Anal. Calcd for $C_{21}H_{21}N_9S_6$ (591.84): C, 42.62; H, 3.58; N, 21.30. Found: C, 42.51; H, 3.57; N, 21.36.

2,2′,2″-[(2,4,6-Trimethyl-1,3,5-benzenetriyl)tris(methylenedithio)]tris-4-pyrimidinamine (7)

To a solution of trimethyl 3,3′,3″-[(2,4,6-trimethyl-1,3,5-benzenetriyl)tris(methylenesulfinyl)] trispropanoate [8] (100 mg, 0.18 mmol) in 1,4-dioxane (3.6 mL) thiocytosine (**1**) was added (203 mg, 1.60 mmol). The suspension was refluxed (101 °C) under stirring for 3 h and monitored by TLC (EtOAc/MeOH 9.5:0.5). The excess of thiocytosine (**1**) was filtered. The solid residue was washed with acetone (3 × 5 mL) and the solution was recovered. The solvent was removed and the crude was purified by column chromatography (acetone/hexane 4:6). Yield 63%. TLC: R_f 0.42 (acetone/hexane 6:4). White solid. M.p. 208–212 °C. ^{1}H NMR (acetone-d_6): δ 8.08 (d, 3H, $J_{5',6'}$ 5.9, 3 × H-6′), 6.41 (br s, 6H, 3 × NH_2), 6.39 (d, 3H, 3 × H-5′), 4.30 (s, 6H, 3 × CH_2), 2.74 (s, 9H, 3 × CH_3). ^{13}C NMR (acetone-d_6): δ 171.3 (3 × C-2′), 165.5 (3 × C-4′), 157.4 (3 × C-6′), 139.5 (C-1,3,5), 132.7 (C-2,4,6), 103.7 (3 × C-5′), 40.8 (3 × CH_2), 17.3 (3 × CH_3). Anal. Calcd for $C_{24}H_{27}N_9S_6$ (633.92): C, 45.47; H, 4.29; N, 19.89. Found: C, 45.39; H, 4.30; N, 19.91.

Antimicrobial activity test

Antimicrobial activity of compounds **2**–**8** was assessed against representative Gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853), Gram-positive (*B. cereus* ATCC 11778, *S. aureus* ATCC 29213 including three of our clinical isolates MRSA: CI26428, CI25967, CI26348) and yeast (*C. albicans* ATCC 10231, *C. neoformans* ATCC MYA-565) strains. Bacterial MIC of all compounds was determined *in vitro* using the broth dilution method in Mueller Hinton medium as described in Clinical and Laboratory Standard Institute (CLSI) document M07-A9.[19] Briefly, test compounds were dissolved in dimethyl sulfoxide and serial double dilutions with Mueller Hinton broth were performed to obtain concentrations varying from 50 to 0.39 µg in a final volume of 1 mL. Each tested strain was previously grown in tryptic soy agar for 24 h at 35 °C and bacterial suspensions were prepared to the turbidity of 0.5 McFarland (1.5 × 10^8 CFU/mL) with sterile saline solution. Suspensions were subsequently diluted to provide a final inoculum density of 5 × 10^5 CFU/mL in the tube. All inoculated tubes, including a control without any inhibitory substance, were incubated at 35 ± 2 °C for 24 h. For yeast strains antifungal activity was tested according to National Committee on Clinical Laboratory Standards (NCCLS) reference document M27-A2.[20] The serial doubling dilutions of all compounds were prepared in the RPMI-1640 medium supplemented with 2% of glucose and buffered to pH 7.0 with 0.165 M 4-morpholinepropanesulfonic acid in concentrations ranging from 50 to 0.39 µg/mL. Compound-free and yeast-free controls were also included. Cultures (24 h-old) of

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yeast strains in Sabouraud dextrose broth were used to prepare a 0.5 McFarland standard turbidity (approximately 5×10^6 CFU/mL) and 5 µl was used to inoculate each tube containing 1 mL of RPMI-1640 medium and a specific compound. The tubes were incubated aerobically at 30 °C for 48–72 h and MICs were determined. Bacterial and fungal MICs were calculated by two independent observers as the lowest compound concentration at which microbial growth was inhibited. All experiments were performed in triplicate.

Cytotoxicity Assay. Fresh human blood (Hb) was collected from healthy adult donors. After extraction, the whole blood was taken into anticoagulant ethylenediaminetetraacetic acid, were incubated in a saline solution [composition in mM: 125 NaCl, 5 KCl, 1 $MgSO_4$, 32 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5 D-(+)-glucose, 1 $CaCl_2$; pH 7.4 and π 300 mOsm/kg] [21] Erythrocytes were resuspended in saline solution with compounds **4**, **5**, and **8** at concentrations of 50 µg/mL.

Trypan blue assay

Cell viability by the trypan blue exclusion method was performed. The percentage of unstained cells represented the percentage of viable cells in the suspension. This assay was performed in different cell types and cytotoxicity based on alterations in plasma membrane permeability was measured.

Cell viab. (%) = number of viable cells (unstained cells) × 100/total number of cells (stained and unstained).

Hemolysis test

Hb samples were incubated with compounds and, after incubation at 37 °C overnight, the samples were centrifuged and the supernatants were harvested. As a measure of hemolysis, Hb concentration of the supernatants was determined photometrically ($\lambda = 540$ nm). The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% hemolysis. In the treatment of blood with each of compounds **4**, **5**, and **8**, hemolysis did not occur at 300 and 200 mOsm/kg, while it became clearly visible at 100 mOsm/kg to accomplish itself at 0 mOsm/kg, as in the control conditions (Table 1). The maintenance of integrity of RBC membrane up to 150 mOsm/kg and the hemolysis occurrence at identical concentrations of the control conditions suggest that none of the compounds under study showed toxicity effects in human erythrocytes at the concentration tested.

Statistic analysis

The experiments were performed in triplicates and statistical analyses performed using Student's *t*-test, with a confidence level of 95% ($p < .05$) considered statistically significant.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Then RL. History and future of antimicrobial diaminopyrimidines. J. Chemother. 1993;5:361–368.
[2] Lee C-S, Ryu E-J, Oh SH, Paek K-S, Kim MY, Youn H. Synthesis and antibacterial activities of novel C(3)-aminopyrimidinyl substituted cephalosporins including against respiratory tract pathogens. Bioorg Med Chem Lett. 2000;10:2123–2127. doi:10.1016/S0960-894X(00)00425-X
[3] Lee KS, Lim JH, Kang YK, Yoo KH, Kim DC, Shin KJ, Kim DJ. Synthesis and antibacterial activities of new 1β-methylcarbapenems having aminopyrimidinylthioether moiety. Eur J Med Chem. 2006;41:1347–1351. doi:10.1016/j.ejmech.2006.08.003
[4] Semenov VE, Mikhailov AS, Voloshina AD, Kulik NV, Nikitashina AD, Zobov VV, Kharlamov SV, Latypov SK, Reznik VS. Antimicrobial activity of pyrimidinophanes with thiocytosine and uracil moieties. Eur J Med Chem. 2011;46:4715–4724. doi:10.1016/j.ejmech.2011.05.034
[5] Zhao Y, Tian Y, Cui Y, Liu W, Ma W, Jiang X. Small molecule-capped gold nanoparticles as potent antibacterial agents that target gram-negative bacteria. J Am Chem Soc. 2010;132:12349–12356. doi:10.1021/ja1028843
[6] Palavecino EL. Clinical, epidemiologic, and laboratory aspects of methicillin-resistant *Staphylococcus aureus* infections. Methods Mol Biol. 2014;1085:1–24. doi:10.1007/978-1-62703-664-1_1
[7] Aversa MC, Barattucci A, Bonaccorsi P, Marino-Merlo F, Mastino A, Sciortino MT. Synthesis and biological testing of thioalkane- and thioarene-spaced bis-β-D-glucopyranosides. Bioorg Med Chem. 2009;17:1456–1463.
[8] Bonaccorsi P, Marino-Merlo F, Barattucci A, Battaglia G, Papaianni E, Papalia T, Aversa MC, Mastino A. Synthesis and biological evaluation of a new class of glycoconjugated disulfides that exhibit potential anticancer properties. Bioorg Med Chem. 2012;20:3186–3195.
[9] Barattucci A, Deni E, Bonaccorsi P, Ceraolo MG, Papalia T, Santoro A, Sciortino MT, Puntoriero F. Oligo(phenylene ethynylene) Glucosides: modulation of cellular uptake capacity preserving light ON. J Org Chem. 2014;79:5113–5120.
[10] Papalia T, Siracusano G, Colao I, Barattucci A, Aversa MC, Serroni S, Zappalà G, Campagna S, Sciortino MT, Puntoriero F, Bonaccorsi P. Cell internalization of BODIPY-based fluorescent dyes bearing carbohydrate residues. Dyes Pigments. 2014;110:67–71.
[11] Aversa MC, Barattucci A, Bonaccorsi P. Efficient synthesis of unsymmetrical disulfides through sulfenic acids. Eur J Org Chem. 2009;2009(36):6355–6359. doi:10.1002/ejoc.200900986
[12] Aversa MC, Barattucci A, Bonaccorsi P. From transient sulfenic acids to disulfide-functionalized tripodal structures. Synlett. 2011;2011(2):254–258. doi:10.1055/s-0030-1259306
[13] Bonaccorsi P, Aversa MC, Barattucci A, Papalia T, Torre A, Trischitta F, Faggio C. Sulfenic acid - derived glycoconjugated disulfides and sulfoxides: a biological evaluation on human red blood cells. J Sulfur Chem. 2013;34:684–691.
[14] Vacca A, Nativi C, Cacciarini M, Pergoli R, Roelens S. A new tripodal receptor for molecular recognition of monosaccharides. A paradigm for assessing glycoside binding affinities and selectivities by ^{1}H NMR spectroscopy. J Am Chem Soc. 2004;126:16456–16465.
[15] Barattucci A, Di Gioia ML, Leggio A, Minuti L, Papalia T, Siciliano C, Temperini A, Bonaccorsi P. Stereoselective synthesis of Dithia[3.3]cyclophane S,S′-dioxides with planar and central chirality. Eur J Org Chem. 2014;2099–2104. doi:10.1002/ejoc.201301636
[16] Calvaresi EC, Hergenrother PJ. Glucose conjugation for the specific targeting and treatment of cancer. Chem Sci. 2013;4:2319–2333. doi:10.1039/c3sc22205e
[17] Hayes W, Osborn HMI, Osborne SD, Rastall RA, Romagnoli B. One-pot synthesis of multivalent arrays of mannose mono- and disaccharides. Tetrahedron. 2003;59:7983–7996.
[18] Adams H, Anderson JC, Bell R, Jones DN, Peel MR, Tomkinson NCO. The synthesis and Diels-Alder reactions of (*E*)- and (*Z*)-1-methoxy-3-(phenylsulfinyl)-1,3-butadienes. J Chem Soc Perkin Trans. 1998;1(23):3967–3974.
[19] Clinical and Laboratory Standards Institute. M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 9th ed. Wayne, PA: CLSI; 2012.
[20] NCCLS - National Committee for Clinical Laboratory Standards. *Reference Method For Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved standard*. 2nd ed. Wayne, PA: NCCLS document M27-A2; 2002.
[21] Mineo P, Faggio C, Micali N, Scamporrino E, Villari V. A star polymer based on a polyethylene glycol with a porphyrinic core as a photosensitizing agent for application in photodynamic therapy: tests in vitro on human erythrocytes. R Soc Chem Adv. 2014;4:19389–19395.

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