Evaluation of the antimicrobial activity of *N*-acylated 4-chloro-2-mercaptobenzenesulfonamide derivatives

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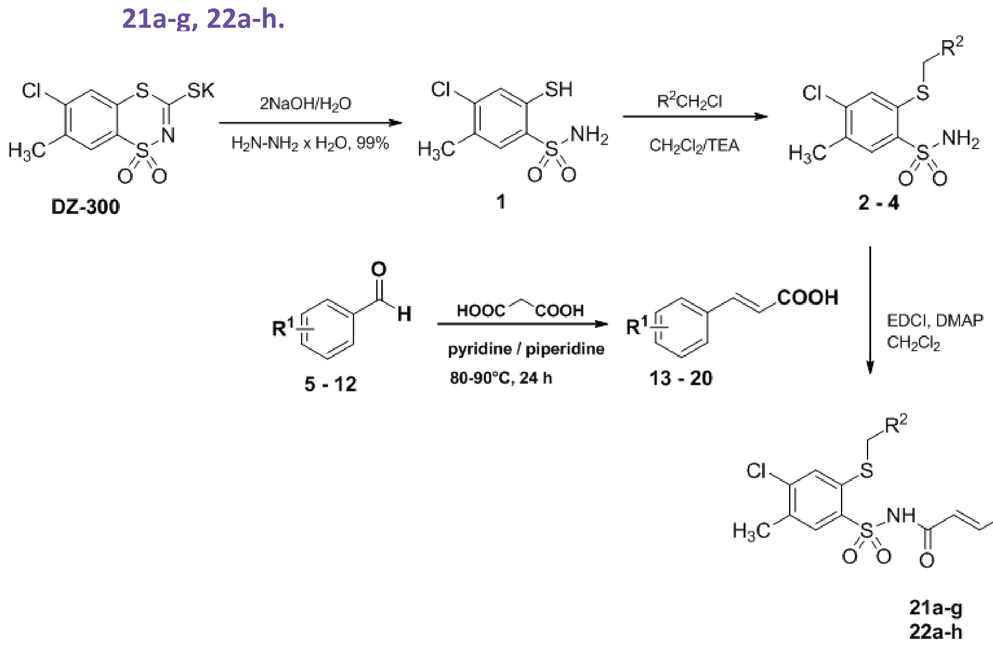
Introduction

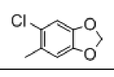
Aryl/heteroarylsulfonamides are an important group of compounds with different directions of biological activity. The number of literature reports on the antibacterial activity of sulfonamides is steadily increasing, bringing a lot of interesting data on the diverse structures and mechanisms of their pharmacological action [1]. The presented research joins the stream of the search for new hybrid molecules being created as a result of the combination of various pharmacophores with interesting biological profiles [2, 3]. Particular attention was paid to their antibacterial activity.

Synthesis

The new compounds were designed and obtained based on the structure of the pharmacophore group of 4-chlorobenzenesulfonamide functionalized in the 2-position on sulfur atom and the structure of chalcone (**Scheme 1**). Taking into account the previous results of own research and available literature data, the synthesis of *N*-(2-arylmethylthio-4-chloro-5-methylphenylsulfonyl)cinnamamide derivatives (**21a-g**, **22a-h**) were synthesized. The efficiency of the carried out reactions were in the range of 39-85%. The substrates used for the syntheses were 4-chloro-2-arylmethylthio-5-methylbenzenesulfonamide derivatives (**2**-**4**). In turn, the derivatives of cinnamic acid (**13**-**20**) were obtained by reacting the appropriate benzaldehyde derivatives (**5**-**12**) with malonic acid. The structures of the final compounds were confirmed using the spectroscopic methods: IR, ^{1}H NMR and elemental analysis C, H, N.

Scheme 1. Synthesis of *N*-(2-arylmethylthio-4-chloro-5-methylphenylsulfonyl)cinnamamide 21a-g, 22a-h.

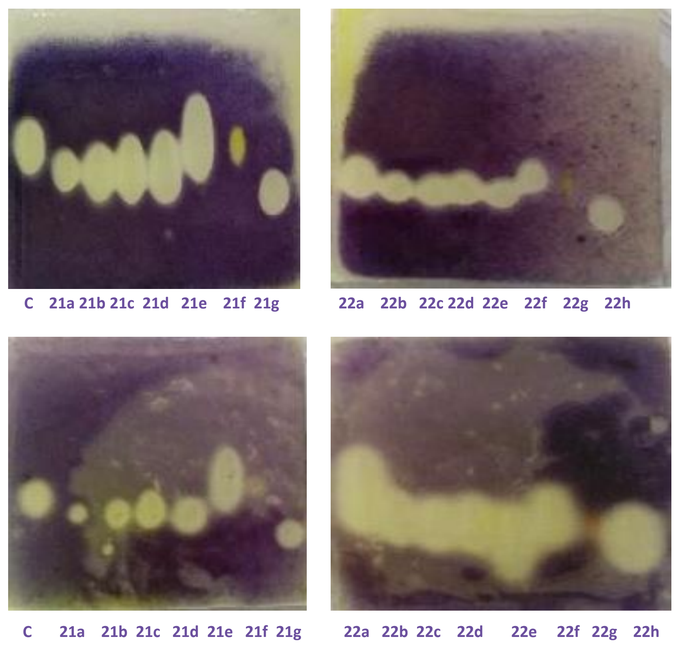


zw.	21a	21b	21c	21d	21e	21f	21g	22a	22b	22c	22d	22e	22f	22g	22h
R^1	4-CH_3	4-Br	4-NO_2	4-F	4-Cl	3-F, 4-OCH_3	4-$N(CH_3)_2$	4-CH_3	4-Br	4-NO_2	4-F	4-Cl	3-F, 4-OCH_3	4-$N(CH_3)_2$	H
R^2															

Biological activity

Preliminary microbiological analysis performed by *TLC-bioautography* showed the activity of **21a-g** and **22a-h** derivatives against Gram-positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus* (**Figure 1**). Then, the antibacterial activity of 15 obtained compounds was confirmed in *in vitro* tests against Gram-positive bacteria: *S. aureus*, *S. epidermidis*, *Enterococcus hirae*, *Enterococcus faecalis* and *B. subtilis* and *Corynebacterium diphtheriae*. Co-trimoxazole was used as the reference compound. The tested compounds showed activity against standard strains of *Staphylococcus* bacteria (MIC from 0.2 to 16 µg/ml), *Enterococcus* (MIC from 1 to 128 µg/ml), *B. subtilis* (MIC from 64 to 128 µg/ml) and *C. diphtheriae* (MIC from 64 to 128 µg/ml). Activity against Gram-negative bacteria was not observed.

Figure 1. Preliminary study of microbial activity by *TLC-bioautography* of compounds 21a-g and 22a-h.

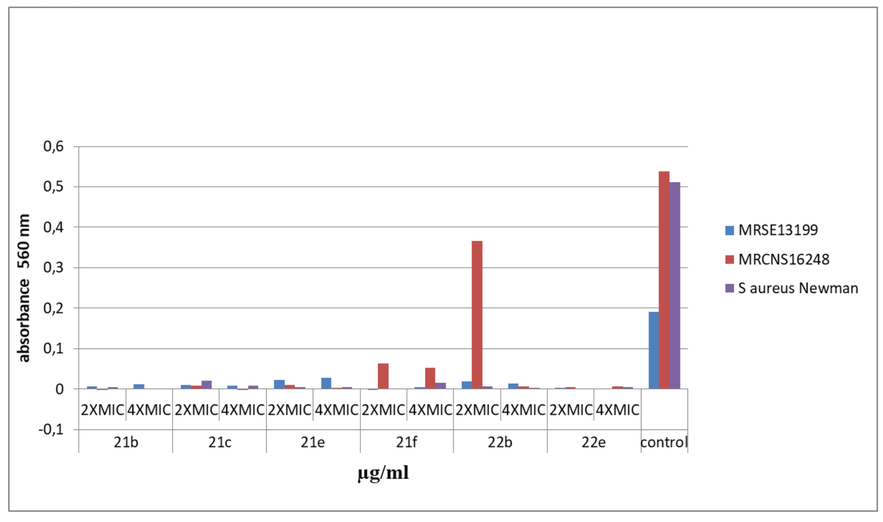


Bacillus subtilis

Staphylococcus aureus

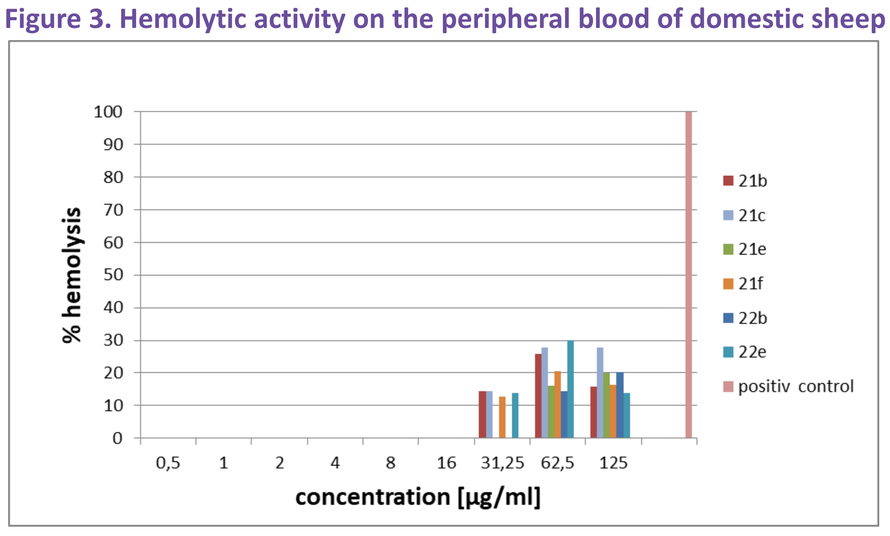
Based on the obtained results, tests were carried out against clinical strains: MRSA, MRCNS and *Enterococcus sp*. The influence of selected derivatives (**21b-c**, **21e-f**, **22b**, **22e**) on the formation of bacterial biofilm was also assessed. Compounds were tested at 2XMIC and 4XMIC concentrations. Inhibition of biofilm formation by MRCNS strains by 70-90%, compared to the control, at the concentrations of 2xMIC and 4xMIC was observed for most of the compounds selected for the study (the exception is **22b** at the concentration of 2xMIC) (**Figure 2**). The tested compounds show bacteriostatic properties and affect *quorum sensing* in biofilm formation, especially the compound **22e**.

Figure 2. Inhibition of biofilm formation of selected coagulase negative *Staphylococcus* strains.



Most of the tested compounds caused hemolysis in the blood at a concentration of 31.25 – 125 µg/ml (**Figure 3**). The hemolytic activity was demonstrated at the level of 12-30% relative to the positive control (1% triton-x 100). The obtained results require pharmacokinetic, bioavailability and mechanisms of action studies.

Figure 3. Hemolytic activity on the peripheral blood of domestic sheep (*Ovis aries*).



Literature: 1. Carta F., Scazzafava A., Supuran C.T., *Expert Opin. Ther. Pat.* (2012) 22, 747 – 758; 2. Sławiński J., Żołnowska B., Pirska D., Kędzia A., Kwapisz E., *J. Enz. Inhib. Med. Chem.* (2013) 28, 41-51.; 3. Żołnowska B., Sławiński J., Garbacz K., Jarosiewicz M., Kawiak A., *Int. J. Mol. Sci.* (2020) 21 (210) 1 - 13.