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SYNTHESIS AND *IN VITRO* ANTIMICROBIAL STUDY OF 4-THIAZOLIDINONE CONTAINING SULFANILAMIDE

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Abstract: The title compounds, 3-allyl-2-sulfanylamido-4-thiazolidinones (**2a–d**), have been synthesized after substitution of amino group of sulfanylamide with allylisothiocyanate and cyclization into 4-thiazolidinones. The synthesized compounds were tested for their antibacterial and antifungal activity (MIC) *in vitro* against microorganisms: *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, *B. subtilis*, *B. cereus* and *C. albicans* taking sulfadimidine, sulfathiazole, sulfanilamide and sulfamethizole as standard drugs. Synthesized compounds (**2a–d**) demonstrated selective activity against *B. cereus*.

Keywords: sulfanylamide, thiazolidinone, antibacterial activity, antifungal activity

The introduction of antimicrobial agents into general clinical use represents one of the landmark medical advances of modern medicine (1). Investigations of new antimicrobial substances are very useful because there are no medicinal for which bacteria will not become resistant (2). Sulfonamide drugs were the first antimicrobial drugs, which lead to the antibiotic revolution in medicine (3). Use of sulfonamides becomes rarer because of resistant bacteria and side effects [4]. Thiazoles, being an integral part of many potent biologically active molecules such as sulfathiazole (antimicrobial drug), ritonavir (antiretroviral drug), abafungin (antifungal drug) and bleomycin (antineoplastic drugs), have been explored previously. It has been noticed continuously over the years that interesting biological activities were associated with thiazole derivatives. (5). Because of similar pharmacological activity, sulfonamides and urea were used in clinical practice. According to previous literature data, scientists tried to used both pharmacophores into one molecule to receive active antibacterial agent – sulfanylamidothiourea or sulfanylamidourea. (6, 7).

EXPERIMENTAL

Materials and Metods

New compounds were synthesized at the Department of Pharmaceutical Chemistry in Lithuanian University of Health Sciences. All reactions were monitored by TLC (Kieselgel 60 F_{254}, Merck). Melting points were determined with Kofler's melting point apparatus and are uncorrected. Elemental analyses were performed with analyzator Gerhardt Vapodest 20 (nitrogen) and by Schoniger's method (sulfur). Infrared (IR) spectra were recorded on Spektrum 100 FT-IR (PerkinElmer) spectrometer. The NMR spectra were taken on a Varian Unity Inova apparatus (300 MHz for ^{1}H). Purity was checked at the Department of Analytical and Toxicological Chemistry, Lithuanian University of Health Sciences by HPLC with separation system Waters 2695 and photodiode array detector Waters 996.

Chemistry

Synthesis of allylsulfanylamides (**1a–d**) [8]

The appropriate sulfanylamide (0.1 mol) and allylisothiocyanate (0.2 mol) were dissolved in anhydrous ethanol (100 mL) (**1a–b**) or 1-butanol (**1c–d**). After heating for 3–5 h, the reaction mixture was cooled to room temperature and then the precipitate has appeared. The corresponding pure compounds (allylsulfanylamides) were obtained by filtration. For some special cases, the target compounds could be purified by recrystallization using 2-propanol (**1a**) or mixture of ethanol and DMF (1:1) (**1b**) or 1-butanol (**1c–d**).

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Table 1. **C**haracterization data of new compounds.

Compound code	Molecular formula	Molecular weight	Yield %	Melting point (°C)	Elemental analysis calcd./found (%)	
					N, %	S, %
1a	$C_{16}H_{19}N_3O_2S_2$	377.49	85	182–185	11.19 11.5	17.08 17.5
1b	$C_{13}H_{14}N_4O_2S_3$	354.47	91	192–195	15.81 16.0	27.14 27.4
1c	$C_{10}H_{13}N_3O_2S_2$	271.36	78	192–193	15.48 15.5	23.63 24.3
1d	$C_{13}H_{15}N_3O_2S_3$	369.49	79	179–181	18.95 18.7	26.03 25.7
2a	$C_{19}H_{23}N_3O_3S_2$	433.55	57	72–78	14.79 15.2	16.15 16.6
2b	$C_{16}H_{18}N_4O_3S_3$	410.54	52	63–65	13.65 13.3	23.43 22.8
2c	$C_{13}H_{17}N_3O_3S_2$	327.43	52	68–70	12.83 12.7	19.59 19.3
2d	$C_{16}H_{19}N_3O_3S_3$	425.55	40	75–77	16.46 16.6	22.60 22.8

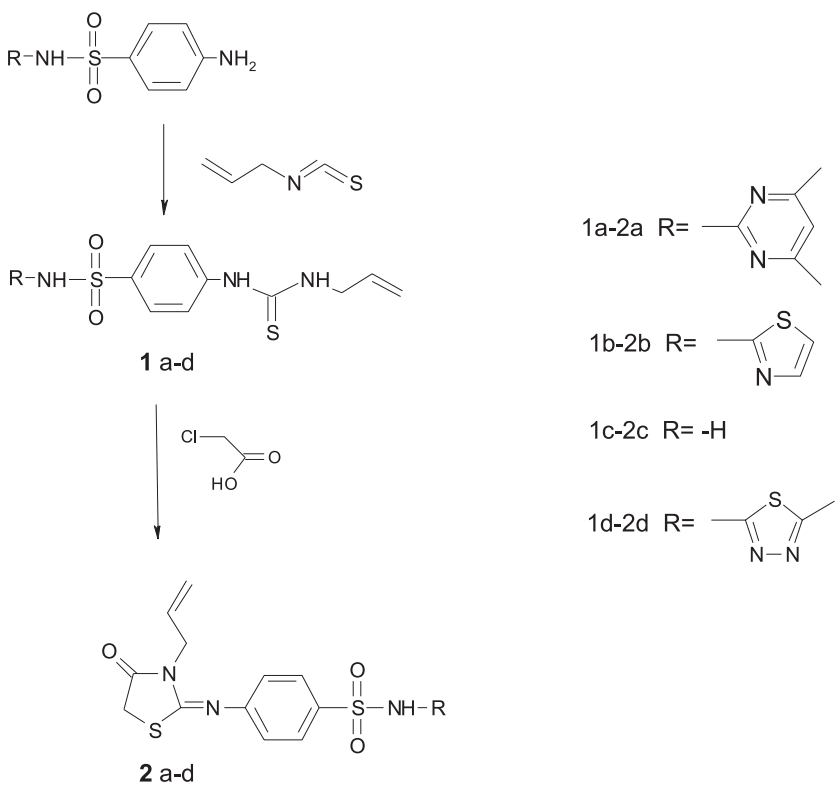

Figure 1. Synthesis of 4-thiazolidinones containing sulfanylamides

Table 2. Spectral data of new compounds.

Compound code	IR wave number (cm^{-1})	^1H NMR (δ, ppm)
1a	1425 (CH_2), 1141 (C=S)	2.58 (6H, s, -CH_3), 3.91 (2H, d, -CH_2-), 5.06 (1H, dd, CH_2=), 5.13 (1H, dd, CH_2=), 5.87 (1H, m, =CH-), 6.56 (1H, s, =CH-), 7.40–7.65 (4H, m, ArH)
1b	1422 (CH_2), 1143 (C=S)	3.90 (2H, d, -CH_2-), 5.06 (1H, dd, CH_2=), 5.10 (1H, dd, CH_2=), 5.87 (1H, m, =CH-), 7.12 (1H, d, -CH=), 7.36 (1H, d, =CH-), 7.95–8.11 (4H, m, ArH)
1c	1428 (CH_2), 1140 (C=S)	3.90 (2H, d, -CH_2-), 5.06 (1H, dd, CH_2=), 5.10 (1H, dd, CH_2=), 5.87 (1H, m, =CH-), 7.93–8,10 (4H, m, ArH)
1d	1421 (CH_2), 1145 (C=S)	2.17 (3H, s, -CH_3), 3.90 (2H, d, -CH_2-), 5.06 (1H, dd, CH_2=), 5.10 (1H, dd, CH_2=), 5.87 (1H, m, =CH-), 7.95–8.13 (4H, m, ArH)
2a	1716 (C=O), 1542 (C=N), 1420 (CH_2)	2.58 (6H, s, -CH_3), 4.26 (2H, d, -CH_2-), 4.80 (2H, d, -CH_2-), 5.00 (2H, dd, CH_2=), 6.18 (1H, m, =CH-), 6.56 (1H, s, =CH-), 7.27–7.61 (4H, m, ArH)
2b	1715 (C=O), 1544 (C=N), 1425 (CH_2)	4.24 (2H, d, -CH_2-), 4.80 (2H, d, -CH_2-), 5.00 (2H, dd, CH_2=), 6.18 (1H, m, =CH-), 7.66 (1H, d, =CH-), 7.24–7,61 (4H, m, ArH), 8.25 (1H, d, =CH-)
2c	1710 (C=O), 1543 (C=N), 1422 (CH_2)	4.22 (1H, d, -CH_2-), 4.31 (1H, d, -CH_2-), 4.79 (2H, d, -CH_2-), 5.00 (2H, dd, CH_2=), 6.18 (1H, m, =CH-), 7.23–7.60 (4H, m, ArH)
2d	1714 (C=O), 1544 (C=N), 1430 (CH_2)	2.17 (3H, s, -CH_3), 4.25 (2H, d, -CH_2-), 4.80 (2H, d, -CH_2-), 5.00 (2H, dd, CH_2=), 6.18 (1H, m, =CH-), 7.21–7,61 (4H, m, ArH)

Table 3. Minimal inhibitory concentrations of synthesized compounds.

Compound	MIC, μg/mL							
	S. aureus	*E. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*B. subtilis*	*P. mirabilis*	*C. albicans*
1a	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
1b	150	800	150	800	> 1000	150	> 1000	400
1c	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
1d	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
2a	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
2b	> 1000	> 1000	> 1000	> 1000	> 1000	900	> 1000	> 1000
2c	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
2d	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
SA-1	500	500	800	> 1000	> 1000	100	500	500
SA-2	> 1000	1000	100	> 1000	300	100	100	500
SA-3	> 1000	> 1000	> 1000	> 1000	800	300	>1000	800
SA-4	800	300	300	> 1000	800	100	300	800

SA-1 – sulfadimidine, SA-2 – sulfathiazole, SA-3 – sulfanilamide, SA-4 – sulfamethizole

Synthesis of 3-allyl-2-sulfanylamido-1,3-thiazolidin-4-one (2a–d) [9]

The appropriate allylsulfanylamide (0.1 mol), chloracetic acid (0.1 mol) and ammonia acetate (0.1 mol) were dissolved in glacial acetic acid (25 mL). After heating for 10–15 min, the white precipitate has appeared. After heating from 2–3 h, hot reaction mixture was filtrated, filtrate was cooled until room temperature and then the precipitate has appeared. The corresponding pure compounds (3-allyl-2-sulfanylamido-1,3-thiazolidin-4-ones) were obtained by filtration. For some special cases, the target compounds could be purified by recrystallization using glacial acetic acid.

Determination of antimicrobial activity

Antimicrobial activity experiments were carried out at the Department of Microbiology, Lithuanian University of Health Sciences.

Antimicrobial susceptibility tests

Antimicrobial activity of new compounds was tested *in vitro* in Mueller-Hinton Agar (Mueller-Hinton II Agar, BBL, Cockeysville, USA) in standard bacteria cultures: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 33499, *Proteus mirabilis* ATCC 12459, *Bacillus subtilis* ATCC 6633 and standard fungal culture: *Candida albicans* ATCC 60193. These bacterial and fungal strains were selected for research because of different structure and functions. Also they are used as standard microorganisms for determination of antimicrobial activity.

Preparation of standard microorganism cultures

Standard bacteria and fungal cultures were cultivated 20–24 h on Mueller-Hinton Agar at 35–37^{o}C. Bacterial and fungal suspensions were prepared from cultivated cultures in physiological solution according to turbinity standard 0.5 McFarland.

Preparation of test compounds solutions

Test compounds were dissolved in dimethyl sulfoxide (20 mg/mL) and then diluted to obtain final concentration ranging from 1 to 1000 μg/mL. Diluted solutions were mixed with 10 mL of Mueller-Hinton Agar. Petri plates were incubated for 20–24 h at 35–37^{o}C. The minimal concentration of antimicrobial (antifungal) compound that prevents any growth of tested bacteria (fungi) was indicated as minimal inhibitory concentration (MIC). Sulfanylamides (sulfadimidine, sulfathiazole, sulfanilamide and sulfamethizole) were used as standard drugs.

RESULTS

All new compounds were synthesized successfully. It was determined that purity of new compounds is not less than 95% (for the purest compounds was 98.5%.). The structures of new compounds were confirmed by elemental analysis (we have determined quantity of nitrogen (N) and sulfur (S) in each compound) and spectral analysis (IR and ^{1}H NMR). All characterization data (molecular formula and molecular weight, yield, melting point, quantity of nitrogen (N) and sulfur (S)) are shown in Table 1, spectral data are shown in Table 2.

Synthesis of intermediate compounds (**1a–d**) was performed in alcohol (ethanol or butanol), because of ready solubility of allylisothiocyanate and sulfanylamide. Compounds were purified by recrystallization from 2-propanol or mixture of ethanol and DMF (1:1, v/v) or 1-butanol (**1c–d**). Yield of this reaction was 78–91%. Yield depended on reaction time. By this experiment we determined that the best conditions is heating for 3–5 h. It was found out that yield of the product, obtained after heating for 1–2 h, was less than 30–50%.

Synthesis of final compounds (**2a–d**) was performed in glacial acetic acid with the presense of ammonia acetate. Compounds were purified by recrystallization from acetic acid. Yield of this reaction was 40–57%. In this experiment we have found that yield of the product depended on quantity of solvent and catalyst. It was found out that using sodium acetate instead of ammonium acetate, yield of reaction was less than 50%. The influence of solvent was very important because of solubility of final product in it. So our goal was to use as small quantity of glacial acetic acid as possible (it depended on solubility of initial reagents too).

The results of antimicrobial activity showed that new compounds can be characterized as antimicrobial and antifungal agents. No one of the tested compounds showed activity against *Pseudomonas aeruginosa* (MIC > 1 mg/mL) and *Proteus mirabilis* (MIC > 1 mg/mL). One of new compounds (**1c**) hadn't shown antimicrobial activity. Data of antibacterial and antifungal activity are shown in Table 3.

DISCUSSION AND CONCLUSIONS

In our experiements we tried to integrate some pharmacophores into one molecule and to investigate their antimicrobial activity. By modification of molecule of known medicinal products – sulfanylamides, we expected to synthesized new more active antimicrobial agents. We used substituted aromatic amino group, which is responsible for antimicrobial activity, with allyl group (compounds **1a–d**) (4). Our experiments showed that such substitution eliminated antimicrobial properties, but with the exception for one compound (**1b**), which showed better activity than initial sulfanylamide (sulfathiazole) against *S. aureus, E. coli, B. subtilis* and *C. albicans*. New 4-thiazolidinones containing sulfanylamide were inactive against tested bacteria and fungi.

We concluded that substitution of amino group in some cases did not eliminate antimicrobial properties as it was noted in previous experiments.

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