Chem. Chem. Technol., 2023,
Vol. 17, No. 2, pp. 315–324

Chemistry

OBTAINING AND DETERMINING ANTIVIRAL AND ANTIBACTERIAL ACTIVITY OF S-ESTERS OF 4-R-AMINOBENZENETHIOSULFONIC ACID

Ewa Zaczynska[1], Anna Czarny[2], Olena Karpenko[3,4], Sofiya Vasylyuk[4], Nataliya Monka[4], Nataliya Stadnytska[4], Liubov Fizer[4], Olena Komarovska-Porokhnyavets[4], Maciej Jaranowski[5], Vira Lubenets[4, ✉], Michał Zimecki[1]

https://doi.org/10.23939/chcht17.02.315

Abstract. A number of S-esters of 4-R-aminobenzenethiosulfonic acids were synthesized *via* alkylation of the sodium salt of 4-acetylaminobenzenethiosulfonic acid with various alkylating agents and acylation of the corresponding esters of 4-aminobenzenethiosulfonic acid with methacryloyl chloride. For obtaining S-methyl 4-(acetylamino)benzenesulfonothioate, it was developed a synthetic technique corresponding to the basic principles of "green chemistry". The degree of compound cytotoxicity was measured by determining A-549 cell growth using colorimetric method. The antibacterial activity of the thiosulfonates was determined by the agar diffusion test and the antiviral action by their cytopathic effect at $TCID_{50}$ value.

Keywords: thiosulfonates, antibacterial activity, antiviral activity.

1. Introduction

Successes in the viral infections treatment are not progressive due to the peculiarities of the biology of viruses and viral infections compared to bacterial ones. Nowadays, antiviral drugs are not able to destruct viruses, are rarely effective in both treating viral diseases and reducing their symptoms and mortality. A successful solution to the problem is significantly complicated by microbial infections against the background of weakened immunity. Thus, patients with COVID-19 often suffer from bacterial and fungal infections, in particular mucormycosis and aspergillosis, which complicate the treatment of the virus itself.[1] Therefore, the search for new drugs with both high antiviral and antimicrobial activity is an urgent task.

In recent years, much attention in the field has been paid to the study of organosulfur compounds (Fig. 1). They are widely used in various fields, especially compounds (**2-4**) containing the -S-S- disulfide bonds.[2,3]

Compounds with the disulfide bonds are also found in some natural sources, in particular in plants of the genus *Allium*[4] and cauliflower.[2,5]

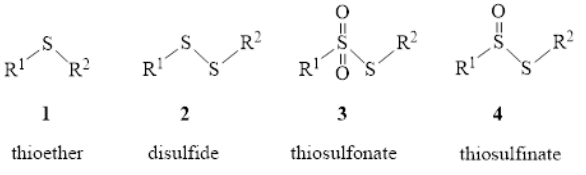


Fig. 1. General formulas of organosulfur compounds

Oxidized disulfide derivatives – thiosulfonates (**3**) and thiosulfinates (**4**) – are applied as pharmaceuticals and agrochemicals (Fig. 2).

So, thiosulfonates (**3**) contain one sulfur atom with oxidation state +II (SR_2) and another sulfur atom with oxidation state +VI ($R1SO_2$), whereas thiosulfinates (**4**) have one sulfur atom with oxidation state +II (SR_2) and another sulfur atom with oxidation state +IV (R1SO). This determines their ability to react with both nucleophiles and electrophiles. As a result, they behave like "chameleons" and are synthetically very powerful sulfenylating and sulfonylating reagents. The similarity of the reactivity, due to content in breaking the –S-S bond, allows us to make certain analogies compared to the biological proper-

[1] Department of Experimental Therapy, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Laboratory of Immunobiology, R. Weigla St. 12, Wrocław, 53-114, Poland
[2] Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Laboratory of Immunobiology, R. Weigla St. 12, Wrocław, 53-114, Poland
[3] Department of Physical Chemistry of Fossil Fuels of the Institute of Physical Organic Chemistry and Coal Chemistry named after L. M. Lytvynenko of the NAS of Ukraine, 3a, Naukova St., Lviv, 79060, Ukraine
[4] Department of Technology of Biologically Active Substances, Pharmacy & Biotechnology, Lviv National Polytechnic University, 12, S. Bandery St., Lviv, 79013, Ukraine
[5] Alpinus Chemia Sp. z o. o., 5 Garbary St., Solec Kujawski, 86-050, Poland
✉ *vlubenets@gmail.com*

ties of these compounds. Mampuys *et al.* developed methods for preparation of thiosulfonates and thiosulfinates of various structures.[3]

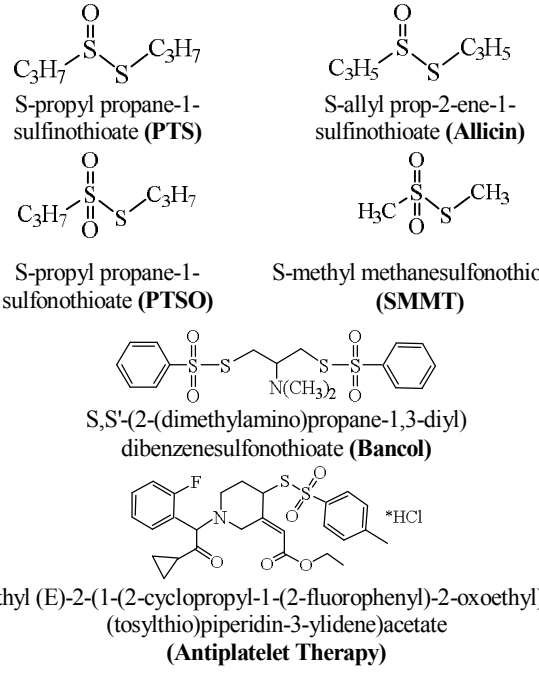


Fig. 2. Examples of disulfide derivatives in pharmacy and agrochemistry

The most promising initial substances (substrates) for the discovery of new drugs for treating various diseases are natural products, especially those that people consume. Such products are expected to cause the least harm to the body due to side effects. However, achieving the desired therapeutic effect by simple consumption of useful products is problematic as it requires the addition of large quantities to food. Based on this, it would be easier to isolate a substance that gives the desired biological effect from natural raw materials and use it for the treatment of diseases. In the search for natural substances with antimicrobial and antiviral activities, such compounds were isolated from plants of the genus *Allium*.[6]

The healing properties of *Allium* plant extracts have been known for a long time and are used in phytotherapy due to their broad spectrum of action including antibacterial, antifungal, antiviral, antitumor, antioxidant, antidiabetic, and hypotensive properties.[7-10]

The most studied thiosulfonate isolated from plants of the genus *Allium* is propylpropanethiosulfonate (PTSO), which is the carrier of the smell of freshly cut plants and has valuable physiologically active properties (antimicrobial, antioxidant, metabolic, probiotic, anti-inflammatory, and antitumor ones).

PTSO is a volatile substance and this complicates its wide practical applications. In this regard, the search for its synthetic analogues by modifying substituents both on the side of sulfonyl and sulfide sulfur is of great interest.[11] An additional argument for the perspective of the new biologically active substances among thiosulfonates search is their low toxicity and wide spectrum of biological activity (antimicrobial, antioxidant, antithrombotic, antitumor, *etc.*).[12-21] It has been shown that some synthetic thiosulfonates have antiviral activity, in particular against the influenza virus and SARS-CoV-2 due to thiol-mediated absorption.[22]

The mechanism of the action of these compounds is not precisely established. Nevertheless, they exhibit several properties that may affect the microbial metabolism such as inhibition of acetyl-CoA synthetases,[23] decrease of glutathione levels,[24] and inhibition of RNA polymerase.[25] *Herpes simplex* viruses (HSV) cause prolonged latent infections, with terms of recurrent viral replicas, skin lesions around mouth, eyes, and genital mucosa.[26] The frequency of HSV-2 infection, a main source of genital herpes, recently increased and contributes to the risk of sexually transmitted HIV.[27] Though infections may be subclinical, HSV can elicit symptoms of various degree, mainly in immunocompromised patients, such as elderly, transplant and acquired immune deficiency syndrome subjects as well as patients experiencing severe primary illnesses and frequent symptomatic recurrences.[28] Since the prospects to find effective drugs among thiosulfonates are promising, in this work a number of S-esters of 4-R-aminobenzenethiosulfonic acid was synthesized and their antimicrobial actions against herpes simplex viruses HSV-1 and HSV-2 and several bacterial strains, were investigated.

2. Materials and Methods

2.1. Chemistry

2.1.1. Synthesis of Title Compounds

Methods of preparation and characteristics of sodium 4-acetamidobenzenesulfonothioate and A1-A3 for biological research were presented in previous works.[29,30.]

All melting points were determined in open capillary tubes and then were uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz Ultrashield Bruker in DMSO-d6; the chemical shifts were measured and tetramethylsilane was used as a standard. LC-MS (liquid chromatography–mass spectrometry) spectra were recorded on Agilent 110\DAD\HSD\VLG 119562, ionization by electrospray at the atmospheric pressure (70 eV). Elemental analysis was performed on the PerkinElmer CHN-Analyzer series 2400. IR spectra were recorded on a FTIR spectrometer (Nexus from Thermo

Nicolet, USA). The reactions and individuality of compounds were monitored by the TLC method on plates "Silufol UV 254".

2.1.2. S-Methyl 4-(Acetylamino)benzenesulfonothioate A1 ("Green Method")

2.5 g (0.01 mol) of the sodium salt of 4-acetylaminobenzenethiosulfonic acid was dissolved in 2 mL of water and 1.23 mL (0.013 mol) of dimethyl sulfate was added. The flask was placed in an ultrasonic bath, where the mixture of reagents was exposed to ultrasound irradiation at room temperature for a specified period of time (~5 h). Then the mixture was diluted with cold water. The precipitate of the target product was filtered, washed with water, dried, and recrystallized from benzene. Yield was 75 %.

2.1.3. General Procedure of Acylation of Methyl, Ethyl, and Allyl 4-Aminobenzenethiosulfoacid S-Esters by Methacryloyl Chloride

Methacryloyl chloride (0.033 mol) was added to an S-ester of 4-aminobenzenethiosulfoacid (0.025 mol) in dioxane (20 mL) containing pyridine (3 mL) at 273 K, and the mixture was stirred for 15 min. Next, the reaction mixture was poured under stirring into ice water. The residue was filtered, washed with water, dried, and recrystallized from benzene.

2.1.4. Characteristics of S-(1-Methylethyl)-4-(methacrylamido)benzenesulfonothioate M5

Yield 72.3 %, mp 385-386 K. IR (KBr, cm^{-1}): 1132, 1316 (SO_2); 1588 (Ar); 1624 (C=C); 1644 (NH); 1670 (C=O), 3232 (NH). ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm: 10.49 (s, 1H, NH), 8.09 (d, J = 9.6 Hz, 2H, ArH), 7.79 (d, J = 9.5 Hz, 2H, ArH), 5.50 (s, 1H, CH_2 methacryl.), 5.41 (s, 1H, CH_2 methacryl.), 3.13 (sp, J = 6.7 Hz, 1H, CH iPr), 2.05 (s, 3H, CH_3 methacryl), 1.32 (d, J = 6.5 Hz, 6H, $2CH_3$ iPr). ^{13}C NMR (126 MHz, DMSO-d_6) δ, ppm: 169.13 (C=O), 143.81 (C, Ar), 136.62 (C, Ar), 128.58 (2CH, Ar), 120.42 (CH_2 methacryl), 116.31 (2CH, Ar), 39.21 (CH iPr), 23.17 ($2CH_3$ iPr), 22.29 (CH_3 methacryl). Anal. caled for $C_{13}H_{17}NO_3S_2$ C 51.16; H 5.68; N 4.68; S 21.4; found: C 51.32; H 5.46; N 4.83; S 21.180. MS (EI) m/z 299.06 [M+1].

2.1.5. Characteristics of S-(2-Methylpropyl)-4-methacrylamidobenzenesulfonothioate M6

Yield 69.1 %, mp 368–369 K. IR (KBr, cm^{-1}): 1130, 1314 (SO_2); 1596 (Ar); 1622 (C=C); 1638 (NH); 1676 (C=O); 3248(NH).^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm: 10.51 (s, 1H, NH), 8.07 (d, J = 9.6 Hz, 2H, ArH), 7.69 (d, J = 9.5 Hz, 2H, ArH), 5.58 (s, 1H, CH_2 methacryl.), 5.49 (s, 1H, CH_2 methacryl.), 2.32 (d, J = 6.7 Hz, 2H, CH_2 iBu), 2.08 (s, 3H, CH_3 methacryl), 1.94 – 1.84 (m, 1H, CH iBu), 0.97 (d, J = 6.6 Hz, 6H, $2CH_3$ iBu). ^{13}C NMR (126 MHz, DMSO-d_6) δ, ppm: 168.91 (C=O), 143.61 (C, Ar), 135.11 (C, Ar), 127.02 (2CH, Ar), 118.92 (CH_2 methacryl), 117.24 (2CH, Ar), 42.13 (CH_2 iBu), 27.92 (CH iBu), 22.98 ($2CH_3$ iBu), 22.41 (CH_3 methacryl). Anal. caled for $C_{14}H_{19}NO_3S_2$ C 53.55; H 6.06; N 4.47; S 20.44, found: C 53.70; H 6.33; N 4.64; S 20.18. MS (EI) m/z 313.08 [M+1].

2.1.6. Charateristics of S-Allyl-4-methacrylamidobenzenesulfonothioate M7

Yield 76 %, mp 388 K. IR (KBr, cm^{-1}): 1138, 1316 (SO_2); 1582 (Ar); 1624 (C=C); 1630 (NH); 1670 (C=O); 3232 (NH). ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm: 10.46 (s, 1H, NH), 7.85 (s, 4H, ArH), 5.67 (dd, J = 15.5, 7.7 Hz, 1H, allyl), 5.51 (s, 1H, CH_2 methacryl.), 5.43 (s, 1H, CH_2 methacryl.), 5.19 (d, J = 16.7 Hz, 1H, allyl), 5.05 (d, J = 9.4 Hz, 1H, allyl), 3.42 (br.s, 2H, CH_2 allyl), 2.10 (s, 3H, CH_3 methacryl).^{13}C NMR (126 MHz, DMSO-d_6) δ, ppm: 169.25 (C=O), 144.30 (C, Ar), 137.66 (C, Ar), 131.39 (CH allyl), 128.21 (2CH, Ar), 119.61 (CH_2 methacryl), 118.74 (2CH, Ar), 38.27 (CH_2 allyl), 24.18 (CH_3 methacryl). Anal. caled for $C_{13}H_{15}NO_3S_2$: C 52.51; H 5.04; N 4.71; S 21.54; found: C 52.33; H 5.22; N 4.58; S 21.34. MS (EI) m/z 297.05 [M+1].

2.2. Biology

2.2.1. Reagents

4-Acetamidobenzenesulfonyl chloride, dimethyl sulfate, 1-bromo-2-chloroethane, and other alkyl bromides were obtained from Merck (Germany), sodium sulfide · $9H_2O$ were purchased from PE Sistema Optimum, Lviv Ukraine. RPMI-1640 medium was derived from Biowest (Nuaillé, France). Fetal calf serum (FCS) was from HyClone (Pittsburgh, PA, USA). L-glutamine, penicillin, streptomycin, and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) were delivered by Sigma-Aldrich (St. Louis, MO, USA). Cidofovir was purchased from Cayman Chemical (MI, USA) and Acyclovir was obtained from Merck (Germany). The following culture media were used: nutrient broth, nutrient agar, and 0.9 % NaCl. The media were purchased from the Institute of Immunology and Experimental Therapy (Wroclaw, Poland).

2.2.2. Cell Lines

A-549: A human lung adenocarcinoma cell line (ATCC CCL 185) was derived from the collection of cell

lines of the Institute of Immunology and Experimental Therapy (Wroclaw, Poland). The cells were cultured in RPMI-1640 medium with 100 U/mL penicillin and 100 μg/mL streptomycin, 2 mM L-glutamine and 10 % fetal calf serum.

2.2.3. Viruses

Human Herpes simplex virus type-1 (HSV-1), MacIntyre strain; ATTC VR-539™ and Human Herpes simplex virus type-2 (HSV-2), MS strain; ATTC VR-540™; *Herpesviridae*, DNA enveloped virus were a property of the Laboratory of Virology, Institute of Immunology and Experimental Therapy. A-549 cell line is routinely used as an efficient, practical, and economical cell system for the evaluation of the replication of these virus types.[31] The viruses were expanded and titrated in A-549 cells. The titer of the viruses was determined with reference to the $TCID_{50}$ (tissue culture infectious dose) value, based on the cytopathic effect (CPE) caused by this virus in about 50 % of infected cells.

2.2.4. Bacteria

The experiments were performed on strains stored in the Polish Collection of Microorganisms (PCM), (Wrocław, Poland): *Escherichia coli* PCM 2057, gram-negative *Pseudomonas aeruginosa* PCM 2058, and gram-positive *Staphylococcus aureus* PCM 2054. The following culture media were used: nutrient broth, Muller-Hinton agar, and 0.9 % NaCl. The media were manufactured at the Institute of Immunology and Experimental Therapy (Wroclaw, Poland).

2.3. Methods

2.3.1. Cell Toxicity Test

The cytotoxicity of the compounds was established by measuring the quality of growth and morphology of human lung epithelial A-549 cells (criteria of toxicity effect based on changes in cell morphology (according to EN ISO10993-5:2009. Biological evaluation. Part 5: Test for *in vitro* cytotoxicity, International Organization for Standardization, Geneva, Switzerland, 2009). The evaluation of the potential cytotoxic action of compounds was performed in a monolayer culture of epithelial lung cancer cell line A-549. The cells at a density of $5 \cdot 10^4$/well were incubated for 24 h. After the incubation, the culture supernatants were removed and appropriate doses of the compounds (200 μL/well) were added to the cell monolayers and incubated for an additional 72 h. Control cultures contained corresponding dilutions of DMSO. Cell growth, morphology, and viability were analyzed to determine the compound's cytotoxicity. The degree of cytotoxicity was defined as the highest dilution of the compounds that cause 50 % or greater destruction of cells.

2.3.2. The Colorimetric MTT Assay

Cell viability was determined by MTT [3-(4,5-dimethythiazoyl-2-yl) 2,5-diphenylte-trozolium bromide] colorimetric assay. The test is based on mitochondrial dehydrogenase cell activity reflecting cell metabolism.[32] MTT was dissolved in PBS at a concentration of 5 mg/mL. To determine cell killing, 25 μL of the solution was added per well to the culture plates, followed by incubation for 2 h in a cell incubator. Then, 100 μL of a lysing buffer (20 % SDS with 50 % DMF, pH 4.7) was added, and after an overnight incubation, the OD was measured at 550 nm with the reference wavelength of 630 nm in a Dynatech 5000 spectrophotometer.

2.3.3. Determination of Antimicrobial Activities by the Agar Diffusion Method

The following thiosulfonates concentrations were used: 50, 100 and 1000 μg/mL. The compounds were initially dissolved in DMSO (dimethyl sulfoxide) at 10 mg/mL concentration, and subsequently in distilled water. Pre-cultures of *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* in broth were incubated at 310 K. After overnight incubation the cultures were diluted 10x with a nutrient broth. The bacterial suspensions (100 μL) were plated onto Muller-Hinton agar. The plates were dried and 20 μL of the compound solutions at concentrations of 50; 100, and 1000 μg/mL were applied. Relevant dilutions of DMSO were applied to control cultures. The bacterial cultures were incubated for 24 h at 308 K. The diameters of zones were measured which reflected antimicrobial effects and the compounds' degree of activity. Conventional drugs served as reference compounds. Every experiment was performed three times.

2.3.4. Determination of the Antiviral Activity

The antiviral activity of the synthesized compounds was determined by means of A-549 cells infected with HSV-1 or HSV-2 viruses. The cells, at density of $1 \cdot 10^5$ cells/mL, were applied onto 96-flat bottom culture plate and incubated for 24 h at 37^o/5 % CO_2 atmosphere, followed by infection of the cells with HSV-1 or HSV-2 at a multiplicity of infection (MOI) = 5. After 1 h incubation the supernatants containing non absorbed viruses were removed and to the cell cultures the studied compounds and the solvent (DMSO) at respective concentrations were added. Control cultures were represented by non-infected cells, infected cells, or containing Acyclovir – a reference drug. After 48 h incubation in a cell incubator the super-

natants were harvested for determination of virus titer. The levels of HSV-1 and HSV-2 replication were determined based on the cytopathic effect (CPE) registered in 24 h monolayer culture of A-549 cells. In the first step the supernatants from the investigated cultures of infected cells and treated with the compounds, were diluted by applying a logarithmic scale (from 10^{-1} to 10^{-9}) and transferred to the A-549 cell culture. After 48 h incubation in a cell culture incubator, the cytopathic effect was analyzed using an inverted microscope. $TCID_{50}$ was defined as a highest dilution of a virus where CPE occurred in 50 % of the infected cells. The antiviral activity of the investigated compounds was determined by comparison of the logarithmic reduction (log 10) index of the virus titer with DMSO control.

3. Results and Discussion

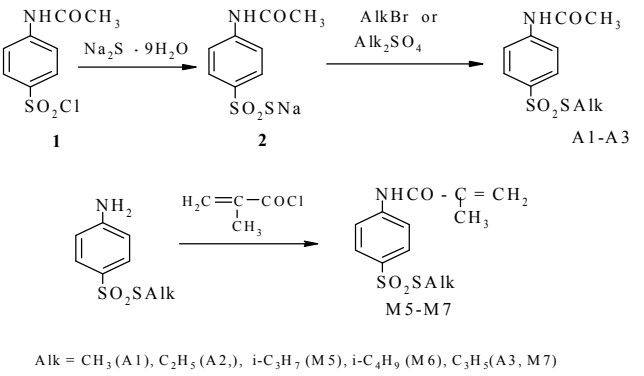
The compounds A1, A2, A3, M5, M6, and M7 were synthesized in the Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University (Fig. 1).

The synthesis of thiosulfoesters used for the study of antiherpetic properties was carried out according Scheme I.

As a starting compound for the synthesis of esters of 4-acylamino benzenethiosulfonic acid (A1-A3), 4-(acetylamino)benzenesulfonyl chloride **1** was used. Compound **1** was converted into the corresponding sodium salt of 4-acetylamino-benzenethiosulfonic acid **2** *via* a redox reaction using sodium sulfide in an alkaline medium. To obtain the alkyl esters of 4-acetylaminobenzenethiosulfonic acid A1-A3, compound **2** was alkylated with various alkylating agents. Methyl (A1), and ethyl (A2) esters were obtained *via* alkylation of sodium 4-acetylaminobenzenethiosulfonate with dimethyl and diethyl sulfates, and allyl (A3) ester with allyl bromide.

Recently, significant attention of researchers has been focused on the obtaining of practically valuable compounds with the elimination of certain environmental restrictions and the use of new "green" methods, in particular, ultrasound-assisted reactions.[33,34,35]

It is worth noting that in the case of S-methyl 4-(acetylamino)benzenesulfonothioate (A1) we have developed a production method that corresponds to the basic principles of "green chemistry". In particular, the alkylation reaction of the sodium salt of 4-acetylaminobenzenethiosulfonic acid with dimethyl sulfate was carried out in an environmentally safe solvent (water) under the influence of ultrasound. In addition, our proposed alkylation technique, in comparison with the known technique,[30] allows us to increase the yield of the target product from 45 % to 75 % and reduce the reaction time.



Scheme 1. Synthesis of esters of 4-acetylamino- and 4-methacryloylamino benzenethiosulfonic acids

The target esters of 4-methacryloylaminobenzenethiosulfonic acid (M5-M7) were obtained *via* acylation of the corresponding esters of 4-aminobenzenethiosulfonic acid with methacryloyl chloride, analogously to the procedure of obtaining 4'-methoxyphenyl and 4'-nitrophenyl esters of 4-methacryloylaminobenzenethiosulfonic acid.[36]

3.1. Determination of Toxicity

The metabolic activity of the human epithelial cell line A-549 was evaluated using the compounds A1-A3, M5-7 (Fig. 3) at a concentration range of 200–1.57 μg/mL and applying relevant dilutions of the solvent (DMSO) in control cultures.

The results showed that the synthetic thiosulfonates were generally non-toxic up to 100 μg/mL, and the highest concentration measured was 200 μg/mL (Table 1).

A toxic effect of compounds was observed only at 200 and 100 μg/mL concentrations. The minimal concentration, toxic to approximately 50 % of the cells, was accepted as $TCCD_{50}$. The degree of cytotoxicity was determined by measuring the growth of the A-549 cells for 72 h at 310 K. The level of cell viability was assessed using the colorimetric MTT method. The conditions of the cultures incubated with the tested compounds were evaluated using image analysis. The photographs presented in Fig. 4 show cultures of A-549 epithelial cells after 72 h of incubation with the tested compounds. Fig. 4c shows the effects of nontoxic concentration (50 μg/mL) of the tested compounds on A-549 cells during the following 72 h of incubation. The cells present a normal shape and size as the cells treated with the culture medium or DMSO (Fig. 4a, b). In an experiment determining the toxicity of the compounds. a toxic effect at ≥200 μg/mL concentration was found, characterized by a change of cell morphology, the presence of granules in the cytoplasm, and subsequent cell death (Fig. 4d).

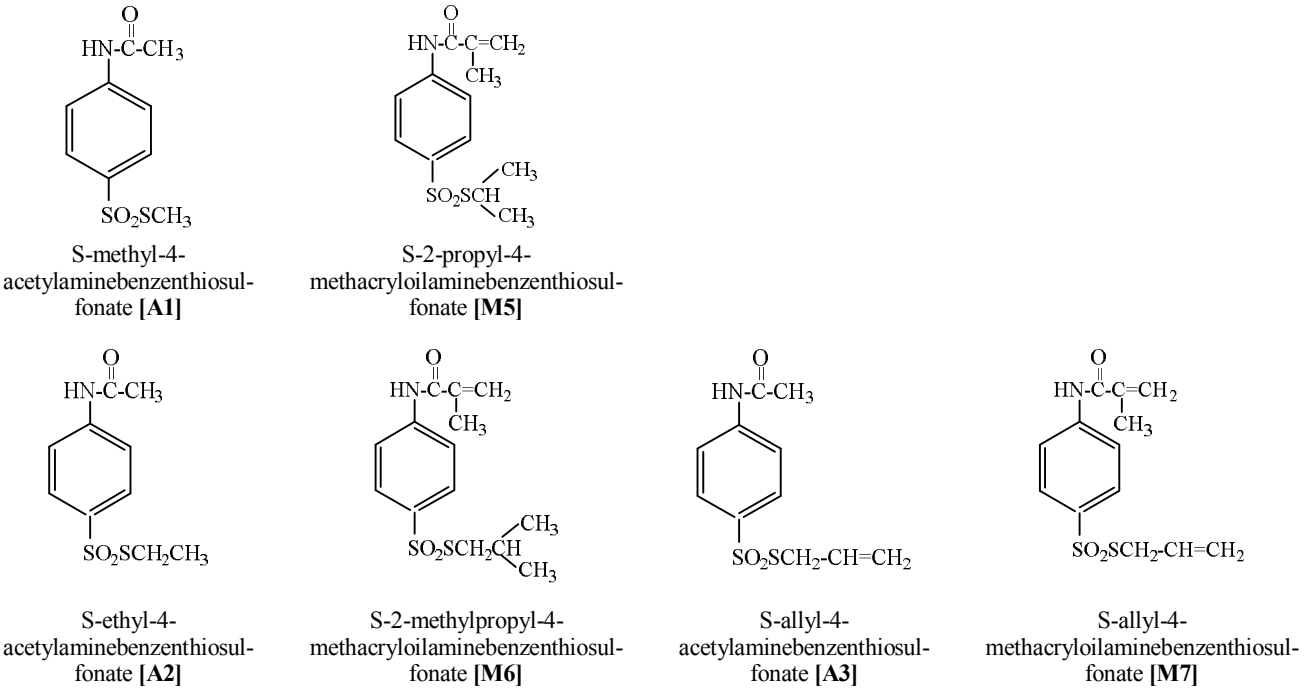
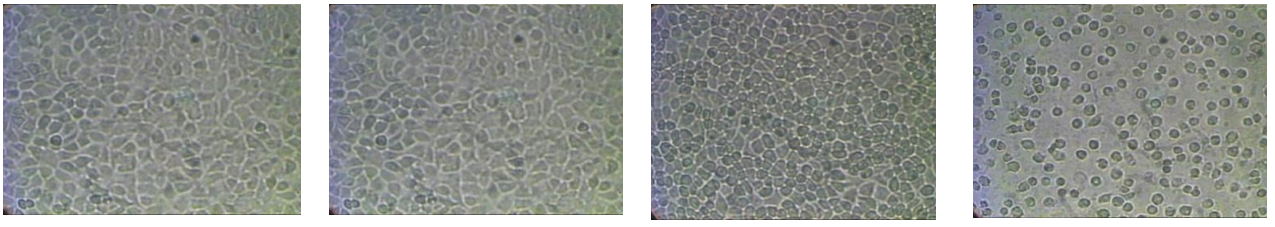


Fig. 3. Formulas of the tested compounds of A and M group

Table 1. The determination of the cytotoxic activity of the compounds on human lung epithelial cell line A549

	Compounds							
	A1	A2	A3	M5	M6	M7	DMSO	Control
Non-toxic concentration [µg/mL]	50≥	50≥	50≥	50≥	50≥	50≥	NT	NT

NT- non-toxic effect



a) A549- not toxic effect – control [cells + culture medium]

b) A549- not toxic effect – control [cells + culture control [cells + DMSO]

c) compound A1 [50 µg/ml] – not toxic effect

d) compound A1 [200 µg/ml] - toxic effect

Fig. 4. Morphology of cell lines A-549 treated and untreated with cytotoxic doses of tested compounds

Table 2. Antibacterial activity of thiosulfonate derivatives

Compound	Concentration [µg/mL]	*Pseudomonas aeruginosa* PCM 2058	*E. coli* PCM 2057	*Staphylococcus aureus* PCM 2054
1	2	3	4	5
A 1	50	____	____	____
	100	+	+/-	+/-
	1000	+++	+/-	+++

Continuation of Table 2

1	2	3	4	5
A 2	50	-----	-----	-----
	100	+	+/-	+/-
	1000	+++	+	+++
A 3	50	____	____	-----
	100	____	____	+/-
	1000	____	____	+++
M 5	50	-----	____	-----
	100	-----	____	+
	1000	-----	------	+++
M 6	50	-----	____	-----
	100	-----	____	+
	1000	-----	------	+++
M 7	50	-----	-----	-----
	100	-----	+/-	+
	1000	+++	+	+++

Degree of antibacterial actions: – inactive, +/- weak active (opaque zone); + weak clear zone; +++ active (clear zone of growth inhibition)

3.2. Determination of Antimicrobial Activities

Antimicrobial activity of thiosulfonates A1, A2, A3 and M5, M6, M7 was determined by a method of compound diffusion into agar [37,38]. The tested thiosulfonates showed selective antibacterial activities (Table 2).

The growth of *S. aureus* was markedly inhibited by all tested thiosulfonate derivatives at 1000 μg/mL concentration. Compounds M5, and M6 showed activity at a lower concentration – 100 μg/mL, while A1, A2, and A3 derivatives were active only at a concentration of 1000 μg/mL. *Pseudomonas aeruginosa* was weakly sensitive to A1 and A2 at 100 μg/mL concentration; the growth was clearly inhibited by A1, A2, and M7 compounds at 1000 μg/mL. *E. coli* strain was resistant to A3, M5, and M6 and weakly sensitive to A1, A2, and M7 at 1000 μg/mL concentration.

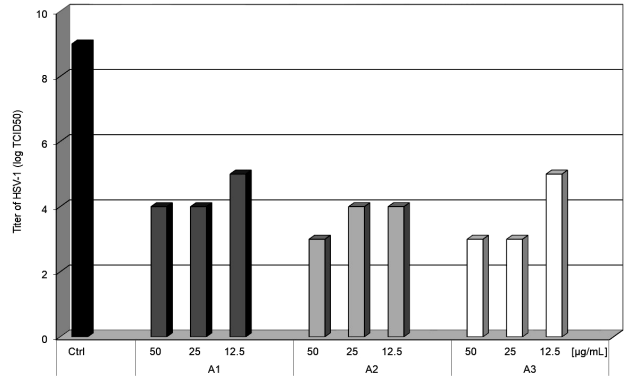
In general, the compounds were toxic with regard to both gram+ and gram– strains. Interestingly, the compounds showed stronger antibacterial effects against pathogenic bacteria than against *E. coli* predominated in the colonic microbiota, which appears to be their advantage. The effective concentrations of the compounds were comparable to the aqueous allium preparations inhibiting *Streptococci* growth.[39]

3.3. Determination of Antiviral Activity

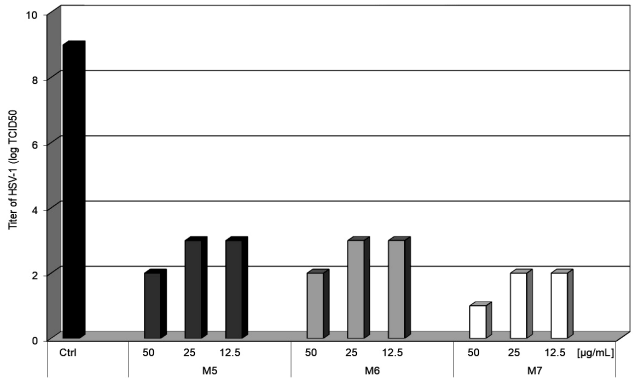
A-549 cells were infected with HSV-1 or HSV-2 and after 1 h of incubation at 310 K, the cell culture supernatants were discarded, followed by the addition of the compounds A1, A2, A3 or M5, M6, and M7 to the cultures and subsequent 48 h incubation. The results are shown as the $\log_{10}$ $TDIC_{50}$ values from quadruplicate determinations (mean ±SE) as compared with an appropriate DMSO control. The results, with a description of the experiments, are presented in Fig. 3a, b. The synthetic A1, A2, and A3 allium analogs caused a significant decline in the viral titer of HSV-1. A2 compound most effectively affected the viral replication, since already at a concentration of 50 μg/mL it reduced the virus level from 9- to 3-log and at a concentration of 25–12.5 μg/mL from

9- log to 4 -log. Next, the potential ability of M7 to inhibit viral replication was investigated in the model of A-549 cells infected with the HSV-1 virus. The results, with the description of the experiments, are presented in Fig. 5b. As shown in Fig. 5b, compounds M5, M6 and M7, as well as Acyclovir (Fig. 5c), inhibited viral titers in a dose-dependent way. The potency of Acyclovir was stronger and attained its maximal value at 22.5.0 μg/mL, when the virus titer was reduced to 8 -log (Fig. 5c).

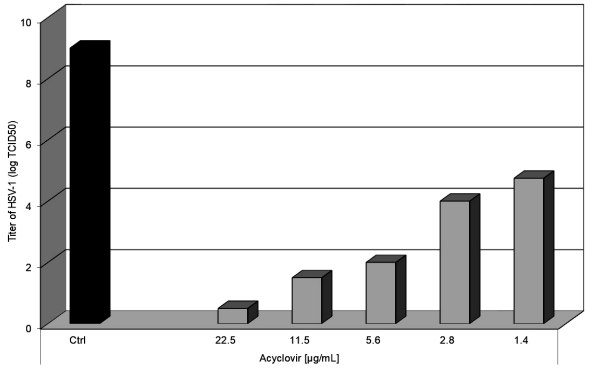
The antiviral effects of the substances were also tested in relation to the HSV-2 virus using Acyclovir as the reference drug (Fig. 6). In the group of synthetic allium analogs, compound A3 most effectively reduced the viral titer to 3 -log at concentrations of 50, 25 and 12.5 μg/mL (Fig. 6a). An inhibition of HSV-2 replication (virus titers reduced to 2 -log) was observed for M6 and M7 at 50 μg/mL. A decrease of the virus titer to 3-log at 50 and 25 μg/mL was registered for M5 and for M7 at 25 and 12.5 μg/mL, 12.5 μg/ml concentration of M5 and 25 and 12.5 μg/mL of M6 compounds reduced the level of the virus to 4 -log (Fig. 6b). The antiviral potency of Acyclovir was stronger and attained its maximal value at 22.5 μg/mL virus titers were reduced up to 5 -log (Fig. 6c).



a

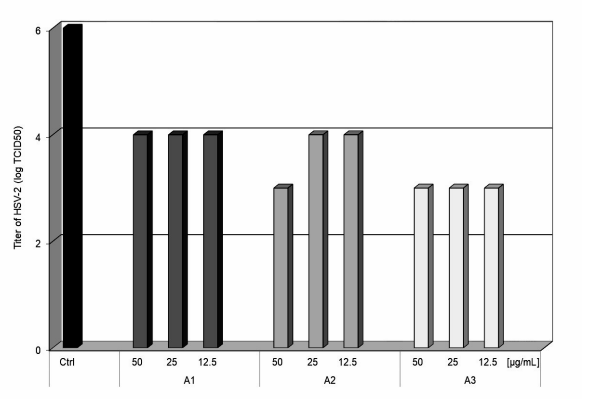


b

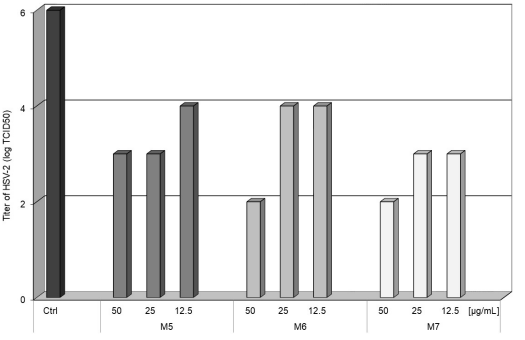


c

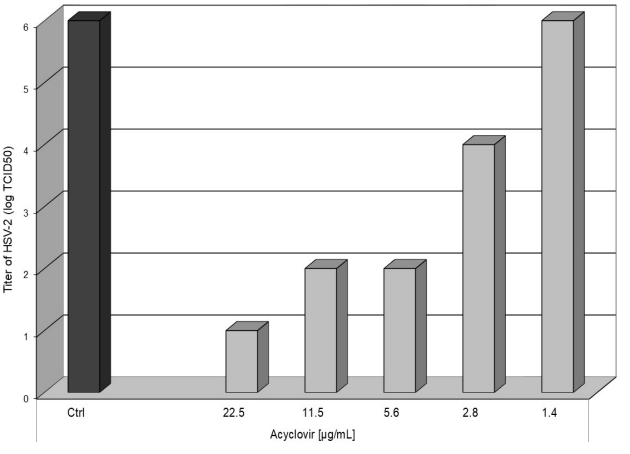
Fig. 5. The effect of the compound on *in vitro* herpesvirus (HSV-1) replication in A-549 cell. A-549 cells were infected with HSV-1 of MOI=5 and after 1 h of virus absorption at 37^{0}C, the virus inoculums were removed and the infected cells were incubated for 48 h with (25; 12.5; 6.2; 3.1 µg/mL) or DMSO as the solvent control (a, b). Acyclovir (22.5;11.5; 5.6; 2.8 and 1.4 µg/mL) was used as a reference drug (c). The viral titer was expressed with reference to the $TCID_{50}$ value, which is based on the cytopathic effect caused by this virus in approximately 50 % of infected cells. The results were obtained from quadruplicate determinations (mean ±SE), and presented as the $TDIC_{50}$ values in relation to the appropriate DMSO control



a



b



c

Fig. 6. The effect of synthetic analogs of garlic (A1-A3 and M5-M7) on *in vitro* HSV-2 replication in A-549 cell. A-549 cells were infected with HSV-2 of MOI=5 and after 1h of virus absorption at 37^{0}C, the virus inoculums were removed and the infected cells were incubated for 48 h with compounds (50, 25, and 12.5 µg/mL) or DMSO as the solvent control (Fig. 6A, B). Acyclovir (22.5; 11.5; 5.6; 2.8, and 1.4 µg/mL) was used as a reference drug (Fig. 6C) The viral titer was expressed with reference to the $TCID_{50}$ value, which is based on the cytopathic effect caused by this virus in approximately 50 % of infected cells. The results were obtained from quadruplicate determinations (mean ±SE), and presented as the $TDIC_{50}$ values in relation to the appropriate DMSO control

In this investigation we demonstrated the antiviral properties of S-esters of 4-R-aminobenzenthiosulfonic acid in the model of lung epithelial A549 cells infected with HSV-1 or HSV-2 viruses, using appropriate reference drugs. The antiviral properties of the compounds were comparable to classical drugs, which directly interfere with assembly of viral genetic material. The 4-$\log_{10}$ reduction of the virus titer by the compounds, demonstrated in this work, meets the European standards for efficient antiviral drugs.

Only isolated reports relate to in vitro effects of garlic-derived compounds on viral replication [5, 40]. However, prior investigations employed, besides various types of garlic extracts, less stable thiosulfinates [7]. In this work we applied for the first time thiosulfonates with a predicted higher resistance to external environment and storage.

4. Conclusions

A series of S-esters of 4-R-aminobenzenthiosulfonic acid were synthesized and evaluated for their antiviral and antibacterial activities. In the case of obtaining of S-methyl 4-(acetylamino)benzenesulfonothioate, it was developed a production method that corresponds to the basic principles of "green chemistry". This made it possible to increase the yield of the target product from 45 % to 75 % and shorten the reaction time. The structures of new thiosulfoesters were characterized by spectroscopic methods (^{1}H NMR, ^{13}C NMR, and IR) and elemental analysis. In the presented work, it was shown that all the synthetic compounds have the ability to inhibit viral replication *in vitro* of HSV-1 and HSV-2 strains, M7 compound being more effective. The lack of toxicity *in vitro* at a high concentration, with reference to A-549 cells, predisposes the compound for further preclinical studies. Of note, the chemical structure of thiosulfonates renders them more resistant to changes in the external environment in comparison with thiosulfinates. The synthesized esters showed selective action in relation to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Taking into account the relatively low antibacterial efficacy of the compounds at low doses, one can envisage using them as disinfectants at high concentrations. Alternatively, they may be applied topically to infected skin or in cases of vaginal dysbiosis.

Acknowledgments

The project was supported by Alpinus Chemia LTD and by a statuary grant from the Hirszfeld Institute of Immunology and Experimental Therapy # 501-4.

References

[1] Kubin, C.J.; McConville, T.H.; Dietz, D.; Zucker, J.; May, M.; Nelson, B.; Istorico, E.; Bartram, L.; Small-Saundres, J.; Sobieszczyk, M.E.; *et al*. Characterization of Bacterial and Fungal Infections in Hospitalized Patients with Coronavirus Disease 2019 and Factors Associated with Health Care-Associated Infections. *Open Forum Infect. Dis*. **2021**, *8*, ofab201. https://doi.org/10.1093/ofid/ofab201

[2] Reddy, R.J.; Waheed, Md.; Kumar J.J. A Straightforward and Convenient Synthesis of Functionalized Allyl Thiosulfonates and Allyl Disulfanes. *RSC Adv*. **2018**, *8*, 40446-40453. https://doi.org/10.1039/c8ra06938g

[3] Mampuys, P.; McElroy, C.R.; Clark, J.; Orru, R.V.A.; Maes, B.U.W. Thiosulfonates as Emerging Reactants: Synthesis and Applications. *Adv. Synth. Catal*. **2020**, *362*, 3-64. https://doi.org/10.1002/adsc.201900864

[4] Batiha, G.E.S.; Beshbishy, A.M.; Wasef, L.G.; Elewa Y.H.A., Al-Sagan, A.A.; Abd El-Hack, M.E.; Taha, A.E.; Abd-Elhakim, Y.M.; Devkota, H.P. Chemical Constituents and Pharmacological Activities of Garlic (*Allium sativum* L.). *Nutrients* **2020**, *12*, 872. https://doi.org/10.3390/nu12030872

[5] Gabriele, E.; Ricci, C.; Meneghetti, F.; Ferri, N.; Asai, A.; Sparatore, A. Methanethiosulfonate Derivatives as Ligands of the STAT3-SH2 Domain. *J Enzyme Inhib Med Chem*. **2017**, *32*, 337-344. http://dx.doi.org/10.1080/14756366.2016.1252757

[6] Alseeni, M.N., Allheani, E. M., Qusti, S.Y., Qusti, N. Y., Alotaibi, S.A., Alotaibi, H.A.. Antimicrobial Activities of Some Plant Extracts against Some. *Int. j. pharm. biol. sci*. **2019**, *14*, 01-10.

[7] Sharma, N.; Behl, T.; Singh, S.; Bansal, A.; Kumari Singh, S.; Zahoor, I. Expatiating the Therapeutic Profile of Garlic (*Allium sativum*): a Bench to Bedside Approach. *Biointerface Res. Appl. Chem*. **2021**, *11*, 14225-14239. https://doi.org/10.33263/BRIAC116.1422514239

[8] Sorlozano-Puerto, A.; Albertuz-Crespo, M.; Lopez-Machado, I.; Gil-Martinez, L.; Ariza-Romero, J. J.; Maroto-Tello, A.; Baños-Arjona, A.; Gutierrez-Fernandez, J. Antibacterial and Antifungal Activity of Propyl-Propane-Thiosulfinate and Propyl-Propane-Thiosulfonate, Two Organosulfur Compounds from *Allium* cepa: In Vitro Antimicrobial Effect via the Gas Phase. *Pharmaceuticals* **2021**, *14*, 21. https://doi.org/10.3390/ph14010021

[9] Gruhlke, M.C.H.; Nicco, C.; Batteux, F.; Slusarenko, A.J. The Effects of Allicin, a Reactive Sulfur Species from Garlic, on a Selection of Mammalian Cell Lines. *Antioxidants (Basel)* **2016**, *6*, 1. https://doi.org/10.3390/antiox6010001

[10] Llana-Ruiz-Cabello, M.; Gutiérrez-Praena, D.; Puerto, M.; Pichardo, S.; Javier Moreno, F.; Baños, A.; Nuñez, C.; Guillamón, E.; Cameán, A. M. Acute Toxicological Studies of the Main Organosulfur Compound Derived from *Allium* sp. Intended to be Used in Active Food Packaging. *Food Chem. Toxicol*. **2015**, *82*, 1-11. https://doi.org/10.1016/j.fct.2015.04.027

[11] Zilbeyaz, K.; Oztekin, A.; Kutluana, E.G. Design and Synthesis of Garlic-Related Unsymmetrical Thiosulfonates as Potential Alzheimer's Disease Therapeutics: *In Vitro* and *In Silico* Study. *Bioorg. Med. Chem*. **2021**, *40*, 116194. https://doi.org/10.1016/j.bmc.2021.116194

[12] Oriabinska, L.B.; Starovoitova, S.O.; Vasylyuk, S.V.; Novikov, V.P.; Lubenets, V.I. Ethylthiosulfanilate Effect on Candida Tropicalis. *Ukr. Biochem. J*. **2017**, *89*, 70-76. https://doi.org/10.15407/ubj89.05.070

[13] Martyrosian, I.A.; Pakholiuk, O.V.; Semak, B.D.; Komarovska-Porokhnyavets, O.; Lubenets, V.I.; Pambuk, S.A. New Technologies of Effective Protection of Textiles Against Microbiological Damage. *Nanosistemi, Nanomateriali, Nanotehnologii* **2019**, *17*, 621-636. https://doi.org/10.15407/nnn

[14] Lubenets, V.; Stadnytska, N.; Baranovych, D.; Vasylyuk, S.; Karpenko, O.; Havryliak, V.; Novikov, V. Thiosulfonates. The Prospective Substances against Fungal Infections. In *Fungal Infection*; Silva de Loreto, É.; Moraes Tondolo, J.S., Eds.; 2019. http://dx.doi.org/10.5772/intechopen.84436

[15] Liubas, N.; Iskra, R.; Stadnytska, N.; Monka, N.; Havryliak, V.; Lubenets, V. Antioxidant Activity of Thiosulfonate Compounds in Experiments *In Vitro* and *In Vivo*. *Biointerface Res. Appl. Chem* **2022**, *12*, 3106-3116. https://doi.org/10.33263/BRIAC123.31063116

[16] Kotyk, B.I.; Iskra, R.Ya.; Slivinska, O.M.; Liubas, N.M.; Pylypetes, A.Z.; Lubenets, V.I.; Pryimych, V.I. Effects of Ethylthiosulfanylate and Chromium (VI) on the State of Pro/Antioxidant System in Rat Liver. *Ukr. Biochem. J.* **2020**, *92*, 78-86. https://doi.org/10.15407/ubj92.05.078
[17] Halenova, T.I.; Nikolaeva, I.V.; Nakonechna, A.V.; Bolibrukh, K.B.; Monka, N.Y.; Lubenets, V.I.; Savchuk, O.M.; Novikov, V.P.; Ostapchenko, L.I. The Search of Compounds with Antiaggregation Activity Among S-esters of Thiosulfonic Acids. *Ukr. Biochem. J.* **2015**, *87*, 83-92. https://doi.org/10.15407/ubj87.05.083
[18] Dmitryjuk, M.; Szczotko, M.; Kubiak, K.; Trojanowicz, R.; Parashchyn, Z.; Khomitska, H.; Lubenets, V. S-Methyl-(2-Methoxycarbonylamino-Benzimidazole-5) Thiosulfonate as a Potential Antiparasitic Agent-Its Action on the Development of Ascaris suum Eggs In Vitro. *Pharmaceuticals (Basel)* **2020**, *13*, 332. https://doi.org/10.3390/ph13110332
[19] Lubenets, V.I.; Vasylyuk, S.V.; Novikov, V.P. Synthesis of S-(3-chloroquinoxalin-2-YL) Esters of Aliphatic and Aromatic Thiosulfonic Acids. *Chem. Heterocycl. Compd.* **2005**, *41*, 1547-1548. https://doi.org/10.1007/s10593-006-0039-9
[20] Vasylyuk, S.; Komarovska-Porokhnyavets, O.; Novikov, V.; Lubenets, V. Modification of Alkyl Esters of 4-Aminobenzenethiosulfonic Acid by S-triazine Fragment and Investigation of their Growth-Regulative Activity. *Chem. Chem. Technol.* **2018**, *12*, 24-28. https://doi.org/10.23939/chcht12.01.024
[21] Kuz'menko, L.; Avdeenko, A.; Konovalova, S.; Vasylyuk, S.; Fedorova, O.; Monka, N.; Krychkovska, A.; Lubenets, V. Synthesis and Study of Pesticidal Activity of Some N-Arylthio-1,4-Benzoquinone Imines. *Biointerface Res. Appl. Chem.* **2019**, *9*, 4232-4238. https://doi.org/10.33263/BRIAC95.232238
[22] Cheng, Y.; Pham, A.-T.; Kato, T.; Lim, B.; Moreau, D.; Lopez-Andarias, J.; Zong, L.; Sakai, N.; Matile, S. Inhibitors of Thiol-Mediated Uptake. *Chem. Sci*. **2021**, *12*, 626-631. https://doi.org/10.1039/D0SC05447J
[23] Focke, M.; Feld, A.; Lichtenthaler, K. Allicin, a Naturally Occurring Antibiotic from Garlic, Specifically Inhibits Acetyl-CoA Synthesis. *FEBS Lett*. **1990**, *261*, 106-108. https://doi.org/10.1016/0014-5793(90)80647-2
[24] Roth, P.J.; Theato, P. Thiol–Thiosulfonate Chemistry in Polymer Science: Simple Fictionalization of Polymers via Disulfide linkages. In *Thiol-X Chemistries in Polymer and Materials Science*; Lowe, A., Bowman, C., Eds.; Abingdon: Nashville, TN, USA, 2013; pp 76-94.
[25] Borlinghaus, J.; Albrecht, F.; Gruhlke, M.C.H.; Nwachukwu, I.D; Slusarenko, A.J. Allicin: Chemistry and Biological Properties. *Molecules* **2014**, *19*, 12591-12618. https://doi.org/10.3390/molecules190812591
[26] Whitley, R.J.; Roizman, B. Herpes Simplex Virus Infections. *Lancet* **2001**, *357*, 1513-1518. https://doi.org/10.1016/S0140-6736(00)04638-9
[27] Looker, K.J.; Welton, N.J.; Sabin, K. M.; Dalal, S.; Vickerman, P.; Prof, Turner, K.M.E.; Boily, M.-C.; Gottlieb, S. L. Global and Regional Estimates of the Contribution of Herpes Simplex Virus Type 2 Infection to HIV Incidence: A Population Attributable Fraction Analysis Using Published Epidemiological Data. *Lancet Infect. Dis*. **2019**, *20*, 240-249. https://doi.org/10.1016/S1473-3099(19)30470-0
[28] Reid, G.E.; Lynch, J.P.; Weigt, S.; Sayah, D.; Belperio, J.A.; Grim, S. A.; Clark, N.M. Herpesvirus Respiratory Infections in Immunocompromised Patients: Epidemiology, Management, and Outcomes. *Semin Respir Crit Care Med*. **2016**, *37*, 603-630. https://doi.org/10.1055 %2Fs-0036-1584793
[29] Lubenets, V.; Karpenko, O.; Ponomarenko, M.; Zahoriy, G.; Krychkovska, A, Novikov V. Development of New Antimicrobial Compositions of Thiosulfonate Structure. *Chem. Chem. Technol*. **2013**, *7*, 119-124. https://doi.org/10.23939/chcht07.02.119
[30] Lubenets, V.; Vasylyuk, S.; Monka, N.; Bolibrukh, K.; Komarovska-Porokhnyavets, O.; Baranovych, D.; Musyanovych, R.; Zaczynska, E,; Czarny, A.; Nawrot, U. *et al*. Synthesis and Antimicrobial Properties of 4-Acylaminobenzenethiosulfoacid S-Esters. *Saudi. Pharm. J.* **2017**, *25*, 266-274. https://doi.org/10.1016/j.jsps.2016.06.007
[31] Smith, C.D.; Craft, D.W.; Shiromoto, R.S.; Yan, P.O. Alternative Cell Line for Virus Isolation. *J. Clin. Microbiol*. **1986**, *24*, 265-268. https://doi.org/10.1128/jcm.24.2.265-268.1986
[32] Hansen, M.B.; Nielsen, S.E.; Berg, K. Re-examination and Further Development of a Precise and Rapid dye Method for Measuring Cell Growth/cell Kill. *J. Immunol. Methods* **1989**, *119*, 203-210. https://doi.org/10.1016/0022-1759(89)90397-9
[33] Heydari, S.; Habibi, D.; Faraji, A. A Green and Efficient Solvent- and Catalyst-Free Ultrasonic Dibenzylation Procedure. *Chem. Chem. Technol*. **2022**, *16*, 126-132. https://doi.org/10.23939/chcht16.01.126
[34] Kusuma, H.; Putri, D.; Dewi, I.; Mahfud, M. Solvent-free Microwave Extraction as the Useful Tool for Extraction of Edible Essential Oils. *Chem. Chem. Technol*. **2016**, *10*, 213-218. https://doi.org/10.23939/chcht10.02.213
[35] Starchevskyy, V.; Bernatska, N.; Typilo, I.; Oliynyk, L.; Strogan, O. Establishment of the Regularities of the Concentrations Change of Microorganisms and Water-Soluble Compounds in Polluted Water After Ultrasound Treatment. *Chem. Chem. Technol*. **2021**, *15*, 408–413. https://doi.org/10.23939/chcht15.03.408
[36] Lubenets, V.; Yarish, M.E.; Vid, L.V. ChemInfrom Abstract: Synthesis of Acylation Sulfanilic Esters. *Zh. Org. Khim*. **1987**, *23*, 157-161. https://doi:10.1002/chin.198723133
[37] *Manual of Clinical Microbiology*, 6th edition; Murray, P.R.; Baron, E.J.; Pfaller, M.A., Eds.; ASM Press: Washington, 1995.
[38] Pfaller, M.A. National Committee for Clinical Laboratory Standard, Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard – second edition M-38. Conidium Forming Filamentous Fungi: Proposed Standard. NCCLS. 1998, 22, M27-A2.
[39] Cutler, R.R.; Odent, M.; Hajj, H.; Maharjan, S.; Bennett, N.; Josling, P.; Ball, V.; Hatton, P.; Dall'antonia, M. *In vitro* Activity of an Aqueous Allicin Extract and a Novel Allicin Topical Gel Formulation against Lancefield Group B Streptococci. *J. Antimicrob. Chemother*. **2009**, *63*, 151-154. https://doi.org/10.1093/jac/dkn457
[40] Weber, N.D.; Andersen, D.O.; North, J.A.; Murray, B.K.; Lawson, L.D.; Hughes, B.G. *In vitro* Virucidal Effects of Allium Sativum (Garlic) Extract and Compounds. *Planta Med*. **1992**, *58*, 417-423. https://doi.org/10.1055/s-2006-961504

Received: August 23, 2022 / Revised: January 11, 2023 / Accepted: February 11, 2023

ОДЕРЖАННЯ ТА ВИЗНАЧЕННЯ ПРОТИВІРУСНОЇ Й АНТИБАКТЕРІАЛЬНОЇ АКТИВНОСТІ S-ЕСТЕРІВ 4-R-АМІНОБЕНЗЕНТІОСУЛЬФОКИСЛОТИ

Анотація. *Алкілуванням натрієвої солі 4-ацетиламінобензентіосульфокислоти різними алкілувальними агентами та ацилюванням відповідних естерів 4-амінобензентіосульфокислоти метакрилоїлхлоридом синтезовано ряд S-естерів 4-R-амінобензентіосульфокислот. Для одержання S-метил 4-(ацетиламіно)бензенсульфонотіоату розроблено методику синтезу, яка відповідає основним принципам "зеленої хімії". Ступінь цитотоксичності сполук оцінювали через визначення росту клітин А-549 колориметричним методом. Антибактеріальну активність тіосульфонатів визначали в агаровому дифузійному тесті, а противірусну дію – за цитопатичним ефектом за значенням TCID50.*

Ключові слова: *тіосульфонати, антибактеріальна активність, противірусна активність.*