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ORIGINAL ARTICLE

Synthesis and antimicrobial properties of 4-acylaminobenzenethiosulfoacid S-esters

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Abstract A series of esters of 4-acetyl, 4-trifluoroacetyl- and 4-(3-chloropropionyl)aminobenzene thiosulfoacids (twenty-four compounds) were synthesized and characterized by elemental analysis, ^{1}H NMR and IR spectroscopy. The antibacterial activity of the novel candidates has been screened using the agar diffusion or serial dilution methods against representative Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus mesentericus*, *Mycobacterium* sp., *Mycobacterium luteum*), Gram-negative (*Aeromonas* sp., *Burkholderia cepacia*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*) bacteria and fungi (*Candida albicans*, *Candida tenuis*, *Candida glabrata*, *Verticillium dahliae*, *Trichophyton gypseum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium chrysogenum*). Particular potency has been discovered against all tested pathogenic bacteria and fungi by compounds **1l** and **3l** at nanomolar concentrations. Some appropriate effect of thiosulfoesters structure upon their antimicrobial activity was determined.

1. Introduction

Nowadays one of the most important issues of organic chemistry is the synthesis of new bioactive compounds and research of correlation of their structure, reactivity and biological properties. According to data of World Health Organization, bacterial and fungal infections that develop on the background of other diseases is one of the large-scale problems. For the effective treatment of this kind of diseases, target therapy and

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antiseptics and disinfectant application should be simultaneously carried out. The effectiveness of this approach is based on the research results of sensitivity of microorganisms to the effect of disinfectants of various chemical structure, and also processes of development of microbial resistance (Babu and Aravind, 2006).

Most attention is surely paid to the research of aforementioned issues on the examples of classes of compounds that have not been studied enough and still are promising in terms of practical application. A particular place in such researches is taken by various sulfur-containing organic compounds, among which thiosulfoacid S-esters are distinguished as compounds of wide range and high index of biological activity (Petrikovich et al., 1994; Maher, 2003; Sotirova et al., 2012). Quite a few of them are offered as medicines, preservatives of fruit and vegetables, effective remedies for plant protection, growth regulators, biocidal additives, insecticides and radioprotectors (Lubenets et al., 2007, 2011, 2013; Boldyrev et al., 1983; Field et al., 1964). Thiosulfoacid esters are effective sulfenilating reagents in organic synthesis, and also valuable objects for solving complex issues of molecular biology and biochemistry (Cavallini et al., 1959).

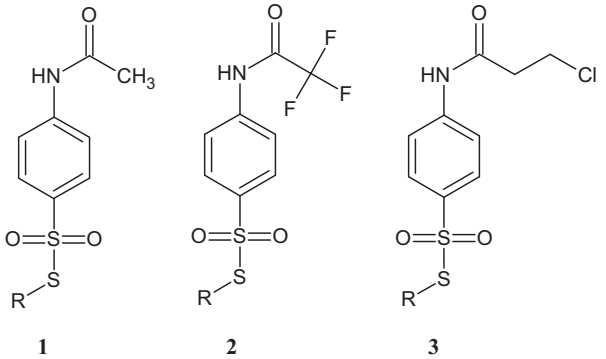
Moreover, thiosulfoesters are structural analogs of biologically active compounds of natural origin, such as volatiles of garlic (*Allium sativum* L.), onion (*Allium cepa* L.) (Block et al., 1996), and deep-sea urchin *Echinocardium cordatum* (Noboru et al., 2001) in particular, that serve as an extra benefit for their research.

Among thiosulfoacid derivatives with pronounced antimicrobial activity at relatively low toxicity (LD_{50} = 2500 mg/kg) (Lubenets et al., 2013; Boldyrev et al., 1983), alkyl S-esters of 4-aminobenzenethiosulfoacids are pointed out. Their high antimicrobial activity can probably be explained by the ability of alkyl esters of 4-aminobenzenethiosulfoacid not only to block SH- and NH_2-containing enzymes and proteins, but also to manifest antagonistic properties of p-aminobenzoic acid, similar to sulfonamides.

Acylation of amino group of sulfonamides sometimes leads to complete loss of their antimicrobial activity. However, in some cases, as in phtalazol, acylation of amino group provides stability of substance while being delivered to its destination (Mashkovskyi, 2001).

Therefore, in order to search for new promising antimicrobial substances that make an effect on both gram-positive and gram-negative bacteria and making comparative researches on determinination of "structure and biological activity" regularities of thiosulfonates, it is certainly important and interesting to synthesize and research antimicrobial properties of 4-acylaminobenzenethiosulfoacid S-esters because they are the closest structural analogs of alkyl S-esters of 4-aminobenzenethiosulfoacids.

Taking into consideration the mentioned above, S-esters of 4-acetyl, 4-trifluoroacetyl- and 4-(3-chloropropionylamino)-benzenethiosulfoacids were the objects of our researches (Scheme 1 and Table 1).



1 2 3

Scheme 1 Structures of compounds **1**, **2** and **3**.

2. Materials and methods

2.1. General experimental details

All melting points were determined in open capillary tubes and were uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer (500 MHz) in DMSO-d_6; the chemical shifts were measured relative to tetramethylsilane. IR spectra were recorded on a spectrophotometer "SPECORD M 80" in tablets with KBr. Monitoring of the reactions and individuality of compounds were performed by TLC method on plates "Silufol UV 254".

2.2. Synthesis of title compounds

2.2.1. General procedure of synthesis of alkyl esters of 4-acetylaminobenzenethiosulfoacid

The alkylating reagent (0.036 mmol) was added to the potassium salt of 4-acetylaminobenzenethiosulfoacid (0.043 mmol) in acetone (30 ml) and water (3 ml) at 20 °C, and the mixture was stirred for 2 h. The acetone was removed under vacuum. The residue was filtered, washed with water, dried and recrystallized from benzene.

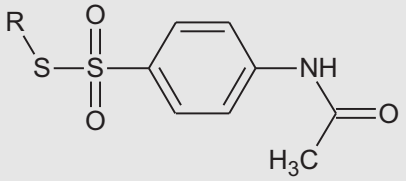
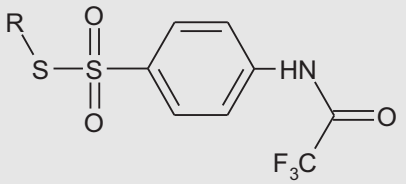
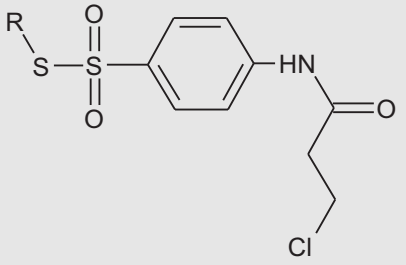
2.2.2. General procedure of synthesis of aryl and cycloalkyl esters of substituted arylthiosulfoacids

The arylsulfenchloride (0.054 mmol) was slowly added to the sodium salt of substituted arylsulfinic acid (0.07 mmol) in solvent. The reaction mixture was stirred at boiling temperature for 6 h. The insoluble residue was filtered. The solvent was removed under vacuum. Residues were combined, washed with water (250 ml) and recrystallized from ethanol.

*2.2.2.1. S-Methyl 4-(acetylamino)benzenesulfonothioate (**1a**).* Yield 45%, mp: 61 °C; IR (KBr, cm^{-1}): 1140, 1324 (SO_2), 1568, 1576, 1592 (Ar—H), 1644 (NH), 1680 (C=O), 3236 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.92 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 7.48 (d, J = 7.80 Hz, 2H, Ar—H), 7.84 (d, J = 7.80 Hz, 2H, Ar—H), 10.22 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 15.77, 23.87, 119.09, 124.13, 136.97, 144.16, 169.76. Anal. calcd for $C_9H_{11}NO_3S_2$: C, 44.07; H, 4.52; N, 5.71; S, 26.14; found: C, 44.14; H, 4.63; N, 5.64; S, 26.03. MS (EI) m/z 246.33 [M + 1].

*2.2.2.2. S-Ethyl 4-(acetylamino)benzenesulfonothioate (**1b**).* Yield 68%, mp: 89 °C; IR (KBr, cm^{-1}): 1144, 1328 (SO_2), 1570, 1582, 1604 (Ar—H), 1636 (NH), 1688 (C=O), 3248 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.26 (t, J = 7.4 Hz, 3H, CH_3), 1.86 (s, 3H, CH_3), 3.06 (q, J = 7.3 Hz, 2H, CH_2), 7.52 (d, J = 7.80 Hz, 2H, Ar—H),

Table 1 Structures of compounds **1a-o**, **2a,b** and **3a, d-f, l-n**.

Compounds		R
1a		CH_3
1b		C_2H_5
1c		C_3H_5
1d		C_3H_7
1e		*i*-C_3H_7
1f		C_4H_9
1g		*i*-C_4H_9
1h		CH_2COOCH_3
1i		CH_2-CH(OH)-CH_2Cl
1j		cycl-C_5H_9
1k		cycl-C_6H_{11}
1l		C_6H_5
1m		C_6H_4Cl-4
1n		$C_6H_4NO_2$-4
1o		$C_6H_4NO_2$-2
2a		CH_3
2b		C_2H_5
3a		CH_3
3d		C_3H_7
3e		i-C_3H_7
3f		C_4H_9
3l		C_6H_5
3m		C_6H_4Cl-4
3n		$C_6H_4NO_2$-4

7.82 (d, J = 7.80 Hz, 2H, Ar—H), 10.20 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 16.09, 24.46, 26.37, 119.11, 119.11, 123.99, 123.99, 138.56, 142.34, 171.17. Anal. calcd for $C_{10}H_{13}NO_3S_2$: C, 46.31; H, 5.05; N, 5.40; S, 24.73; found: C, 46.44; H, 5.17; N, 5.35; S, 24.56. MS (EI) m/z 260.31 [M + 1].

*2.2.2.3. S-Prop-2-en-1-yl 4-(acetylamino)benzenesulfonothioate (**1c**)*. Yield 67%, mp: 59 °C. IR (KBr, cm^{-1}): 1136, 1320 (SO_2), 1564, 1572, 1592 (Ar—H), 1624 (NH), 1684 (C═O), 3246 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.88 (s, 3H, CH_3), 3.88 (d, J = 6.8 Hz, 2H, —CH_2S—), 5.14–5.26 (dd, J = 7.0 Hz, 2H, CH_2), 5.88 (m, 1H, CH), 7.46 (d, J = 7.8 Hz, 2H, Ar—H), 7.82 (d, J = 7.8 Hz, 2H, Ar—H), 10.22 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.56, 31.83, 119.00, 119.00, 120.10, 123.95, 123.95, 134.05, 137.33, 143.56, 170.23. Anal. calcd for $C_{11}H_{13}NO_3S_2$: C, 48.69; H, 4.83; N, 5.16; S, 23.63; found: C, 48.81; H, 4.99; N, 5.06; S, 23.49. MS (EI) m/z 272.34 [M + 1].

*2.2.2.4. S-Propyl 4-(acetylamino)benzenesulfonothioate (**1d**)*. Yield 45%, mp: 48 °C. IR (KBr, cm^{-1}): 1120, 1312 (SO_2), 1586, 1592, 1608 (Ar—H), 1640 (NH), 1690 (C═O), 3250 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.34 (t, J = 6.8 Hz, 3H, CH_3), 1.89 (m, 2H, CH_2), 1.90 (s, 3H, CH_3), 3.72 (t, J = 6.3 Hz, 2H, S-CH_2), 7.42 (d, J = 7.9 Hz, 2H, Ar—H), 7.88 (d, J = 7.9 Hz, 2H, Ar—H), 10.18 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 13.20, 23.34, 23.43, 31.92, 118.75, 118.75, 124.04, 124.04, 137.48, 141.89, 170.25. Anal. calcd for $C_{11}H_{15}NO_3S_2$: C, 48.33; H, 5.53; N, 5.12; S, 23.46; found: C, 48.51; H, 5.69; N, 5.04; S, 23.58. MS (EI) m/z 274.36 [M + 1].

*2.2.2.5. S-(1-Methylethyl)4-(acetylamino)benzenesulfonothioate (**1e**)*. Yield 47%, mp: 112 °C. IR (KBr, cm^{-1}): 1132, 1324 (SO_2), 1576, 1588, 1602 (Ar—H), 1632 (NH), 1688 (C═O), 3238 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.90 (d, J = 6.8 Hz, 3H, $CH(CH_3)_2$), 1.03 (d, J = 6.9 Hz, 3H, $CH(CH_3)_2$), 1.88 (s, 3H, CH_3), 2.40 (m, 1H, $CH(CH_3)_2$), 7.40 (d, J = 7.92 Hz, 2H, Ar—H), 7.82 (d, J = 8.0 Hz, 2H, Ar—H), 10.22 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.24, 24.63, 25.15, 36.47, 119.13, 119.13, 123.85, 123.85, 140.13, 144.39, 169.29. Anal. calcd for $C_{11}H_{15}NO_3S_2$: C, 48.33; H, 5.53; N, 5.12; S, 23.46; found: C, 48.49; H, 5.64; N, 5.01; S, 23.54. MS (EI) m/z 274.33 [M + 1].

*2.2.2.6. S-Butyl 4-(acetylamino)benzenesulfonothioate (**1f**)*. Yield 42%, mp: 75 °C. IR (KBr, cm^{-1}): 1128, 1332 (SO_2), 1584, 1588, 1592 (Ar—H), 1628 (NH), 1686 (C═O), 3256 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.89 (t, J = 7.3 Hz, 3H, $(CH_2)_3C\underline{H}_3$), 1.30 (m, 2H, $(CH_2)_2C\underline{H}_2CH_3$),

1.82 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 1.94 (s, 3H, CH_3), 2.72 (m, 2H, $C\underline{H}_2(CH_2)_2CH_3$), 7.40 (d, J = 7.6 Hz, 2H, Ar—H), 7.82 (d, J = 7.8 Hz, 2H, Ar—H), 10.20 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 13.24, 20.60, 24.21, 30.72, 32.41, 119.05, 119.05, 124.02, 124.02, 137.49, 142.47, 168.54. Anal. calcd for $C_{12}H_{17}NO_3S_2$: C, 50.15; H, 5.96; N, 4.87; S, 22.31; found: C, 50.30; H, 6.11; N, 4.69; S, 22.45. MS (EI) m/z 288.41 [M + 1].

*2.2.2.7. S-(2-Methylpropyl) 4-(acetylamino)benzenesulfonothioate (**1g**).* Yield 32%, mp: 80 °C. IR (KBr, cm^{-1}): 1128, 1312 (SO_2), 1582, 1596, 1604 (Ar—H), 1644 (NH), 1692 (C═O), 3248 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.14 (d, J = 5.3 Hz, 6H, —$CH_2CH(CH_3)_2$), 1.86 (s, 3H, CH_3), 2.56–2.69 (m, 1H, —$CH_2CH(CH_3)_2$), 3.27 (m, 2H, —$CH_2CH(CH_3)_2$), 7.44 (d, J = 7.8 Hz, 2H, Ar—H), 7.88 (d, J = 7.8 Hz, 2H, Ar—H), 10.18 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 20.27, 21.07, 24.51, 29.97, 43.17, 118.87, 118.87, 123.83, 123.83, 137.37, 142.96, 168.97. Anal. calcd for $C_{12}H_{17}NO_3S_2$: C, 50.15; H, 5.96; N, 4.87; S, 22.31; found: C, 50.33; H, 6.12; N, 4.71; S, 22.39. MS (EI) m/z 288.35 [M + 1].

*2.2.2.8. Methyl ({[4-(acetylamino)phenyl]sulfonyl}sulfanyl) acetate (**1h**).* Yield 47%, mp: 105 °C. IR (KBr, cm^{-1}): 1136, 1292 (SO_2), 1556, 1592, 1608 (Ar—H), 1636 (NH), 1688, 1698 (C═O), 3252 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.90 (s, 3H, CH_3), 2.88 (d, J = 6.8 Hz, 2H, CH_2), 3.61 (s, 3H, CH_3), 7.40 (d, J = 7.8 Hz, 2H, Ar—H), 7.82 (d, J = 7.8 Hz, 2H, Ar—H), 10.24 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 22.59, 33.29, 52.18, 119.29, 119.29, 124.23, 124.23, 137.06, 143.91, 167.57, 169.57. Anal. calcd for $C_{11}H_{13}NO_5S_2$: C, 43.55; H, 4.32; N, 4.62; S, 21.14; found: C, 43.72; H, 4.41; N, 4.55; S, 21.24. MS (EI) m/z 304.33 [M + 1].

*2.2.2.9. S-(3-Chloro-2-hydroxypropyl) 4-(acetylamino)benzenesulfonothioate (**1i**).* Methods of obtaining the compound and its characteristics are presented in the previous work (Baranovych et al., 2001).

*2.2.2.10. S-Cyclopentyl 4-(acetylamino)benzenesulfonothioate (**1j**).* Yield 58%, mp: 88 °C. IR (KBr, cm^{-1}): 1126, 1304 (SO_2), 1572, 1604, 1612 (Ar—H), 1640 (NH), 1696 (C═O), 3248 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.81 (dd, J = 13.7, 12.0 Hz, 2H, H-3,4 cyclopenthyl); 1.89 (s, 3H, CH_3), 2.02 (m, 2H, H-3,4 cyclopenthyl), 2.11–2.23 (m, 2H, H-2,5 cyclopenthyl), 2.32 (dd, J = 17.0, 9.4 Hz, 2H, H-2,5 cyclopenthyl), 4.13 (dt, J = 13.7, 6.7 Hz, 1H, H-1 cyclopenthyl), 7.38 (d, J = 7.9 Hz, 2H Ar—H), 7.80 (d, J = 7.8 Hz, 2H, Ar—H), 10.18 (br. s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.65, 23.83, 24.09, 37.12, 38.02, 47.02, 118.41, 118.41, 124.51, 124.51, 137.73, 143.12, 171.59. Anal. calcd for $C_{13}H_{17}NO_3S_2$: C, 52.15; H, 5.72; N, 4.68; S, 21.42; found: C, 52.48; H, 5.78; N, 4.57; S, 21.25. MS (EI) m/z 300.39 [M + 1].

*2.2.2.11. S-Cyclohexyl 4-(acetylamino)benzenesulfonothioate (**1k**).* Yield 41%, mp: 137 °C. IR (KBr, cm^{-1}): 1128, 1320 (SO_2), 1564, 1584, 1602 (Ar—H), 1624 (NH), 1672 (C═O), 3236 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.55–1.84 (m, 6H, H-4 cyclohexyl), 1.91 (s, 3H, CH_3), 2.04 (d, 2H, H-2, 6 cyclohexyl), 2.22 (d, J = 12.7 Hz, 2H, H-2, 6 cyclohexyl), 3.77 (t, 1H, H-1 cyclohexyl), 7.34 (d, J = 7.8 Hz, 2H, Ar—H), 7.84 (d, J = 7.8 Hz, 2H, Ar—H), 10.20 (br. s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 22.88, 24.23, 24.54, 24.54, 32.81, 32.81, 47.25, 118.71, 118.71, 123.93, 123.93, 138.03, 144.17, 171.11. Anal. calcd for $C_{14}H_{19}NO_3S_2$: C, 53.65; H, 6.11; N, 4.47; S, 20.46; found: C, 53.94; H, 6.20; N, 4.39; S, 20.78. MS (EI) m/z 314.46 [M + 1].

*2.2.2.12. S-Phenyl 4-(acetylamino)benzenesulfonothioate (**1l**).* Yield 57%, mp: 156 °C. IR (KBr, cm^{-1}): 1144, 1328 (SO_2), 1576, 1588, 1592, 1608 (Ar—H), 1646 (NH), 1684 (C═O), 3244 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.92 (s, 3H, CH_3), 7.28–7.92 (m, 9H, Ar—H), 10.20 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.81, 118.57, 118.57, 124.54, 124.54, 127.67, 128.53, 129.26, 129.26, 129.38, 129.38, 135.09, 144.01, 172.31. Anal. calcd for $C_{14}H_{13}NO_3S_2$: C, 54.70; H, 4.26; N, 4.56; S, 20.86; found: C, 54.82; H, 4.34; N, 4.45; S, 20.94. MS (EI) m/z 308.4 [M + 1].

*2.2.2.13. S-(4-Chlorophenyl) 4-(acetylamino)benzenesulfonothioate (**1m**).* Yield 52%, mp: 202 °C. IR (KBr, cm^{-1}): 1136, 1336 (SO_2), 1586, 1592, 1600, 1608 (Ar—H), 1634 (NH), 1676 (C═O), 3268 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.98 (s, 3H, CH_3), 7.32–7.98 (m, 8H, Ar—H), 10.22 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 24.01, 117.95, 117.95, 124.54, 124.54, 127.27, 128.65, 128.65, 129.61, 129.61, 130.67, 135.09, 144.01, 170.27. Anal. calcd for $C_{14}H_{12}ClNO_3S_2$: C, 49.19; H, 3.54; N, 4.10; S, 18.76; Cl, 10.37; found: C, 49.32; H, 3.69; N, 4.02; S, 18.88; Cl, 10.29. MS (EI) m/z 342.85 [M + 1].

*2.2.2.14. S-(4-Nitrophenyl) 4-(acetylamino)benzenesulfonothioate (**1n**).* Yield 53%, mp: 220 °C. IR (KBr, cm^{-1}): 1128, 1320 (SO_2), 1566, 1578, 1588, 1600 (Ar—H), 1632 (NH), 1688 (C═O), 3268 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.92 (s, 3H, CH_3), 7.30–7.92 (m, 8H, Ar—H), 10.20 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.38, 118.57, 118.57, 123.46, 123.46, 124.54, 124.54, 129.88, 129.88, 133.65, 135.09, 144.01, 145.66, 171.23. Anal. calcd for $C_{14}H_{12}N_2O_5S_2$: C, 47.72; H, 3.43; N, 7.95; S, 18.20; found: C, 47.84; H, 3.51; N, 7.88; S, 18.29. MS (EI) m/z 353.35 [M + 1].

*2.2.2.15. S-(2-Nitrophenyl) 4-(acetylamino)benzenesulfonothioate (**1o**).* Yield 48%, mp: 145 °C. IR (KBr, cm^{-1}): 1126, 1318 (SO_2), 1586, 1590, 1596, 1600, 1608 (Ar—H), 1636 (NH), 1674 (C═O), 3256 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.88 (s, 3H, CH_3), 7.22–7.88 (m, 8H, Ar—H), 10.18 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 24.43, 118.34, 118.34, 123.46, 123.75, 124.28, 124.28, 128.54, 129.88, 134.57, 135.26, 141.67, 144.01, 170.92. Anal. calcd for $C_{14}H_{12}N_2O_5S_2$: C, 47.72; H, 3.43; N, 7.95; S, 18.20; found: C, 47.86; H, 3.56; N, 7.90; S, 18.26. MS (EI) m/z 353.37 [M + 1].

2.2.3. General procedure of deacylation

S-ester of 4-acylaminobenzenethiosulfoacid (0.0039 mmol) was added to 40% sulfuric acid (4 ml to 0.0195 mmol) and heated for 3 h. Hot reaction mixture was filtered, and then filtrate was cooled. The excess of acid was neutralized with sodium bicarbonate to pH 6–7. The residue of 4-aminobenzenethiosulfoacid S-ester was filtered and washed with water.

2.2.4. General procedure of acylation of methyl and ethyl 4-aminobenzenethiosulfoacid S-esters by trifluoroacetic anhydride

Trifluoroacetic anhydride (0.0025 mmol) was added to the S-ester of 4-aminobenzenethiosulfoacid (0.0025 mmol) in benzene (10 ml) at 20 °C, and the mixture was stirred for 3.5 h. The benzene was removed under vacuum. The residue was filtered, washed with water and dried.

*2.2.4.1. S-Methyl 4-[(trifluoroacetyl)amino]benzenesulfonothioate (**2a**)*. Yield 95%, mp: 99 °C. IR (KBr, cm^{-1}): 1144, 1304 (SO_2), 1576, 1592, 1600 (Ar—H), 1644 (NH), 1744 (C═O), 3296 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.55 (s, 3H, CH_3), 7.52 (d, J = 8 Hz, 2H, Ar—H), 7.99, (d, J = 8 Hz, 2H, Ar—H), 11.74 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ, 114.48, 119.81, 119.81, 125.09, 125.09, 136.97, 143.35, 157.83. Anal. calcd for $C_9H_8F_3NO_3S_2$: C, 36.12; H, 2.69; N, 4.68; S, 21.43; found: C, 36.28; H, 2.78; N, 4.59; S, 21.85. MS (EI) m/z 300.3 [M + 1].

*2.2.4.2. S-Ethyl 4-[(trifluoroacetyl)amino]benzenesulfonothioate (**2b**)*. Yield 92%, mp: 109 °C. IR (KBr, cm^{-1}): 1152, 1312 (SO_2), 1592, 1600, 1608 (Ar—H), 1648 (NH), 1740 (C═O), 3288 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.21 (t, J = 7.4 Hz, 3H, CH_3), 3.05 (q, J = 7.3 Hz, 2H, CH_2), 7.56 (d, J = 8.0 Hz, 2H, Ar—H), 8.01 (d, J = 8.0 Hz, 2H, Ar—H), 11.74 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 16.35, 27.43, 114.48, 119.83, 119.83, 124.95, 124.95, 137.11, 143.35, 157.83. Anal. calcd for $C_{10}H_{10}F_3NO_3S_2$: C, 38.34; H, 3.22; N, 4.47; S, 20.47; found: C, 38.51; H, 3.34; N, 4.29; S, 20.68. MS (EI) m/z 314.35 [M + 1].

2.2.5. General procedure of synthesis of thiosulfate esters of 4-(3chloropropionylamino) –benzenethiosulfoacid

Pyridine was added to the solution of appropriate ester of thiosulfanyl acid (0.01 mmol) in absolute dioxane, and while stirring, chloroanhydride of β-chloropropionic acid (0.01 mmol) was slowly added into the mixture to keep the temperature of reaction mixture at 40 °C for 30 min. While the reaction mixture being stirred, it was put into a mixture of water with ice. The residue was filtered and washed with cool water. Obtained thiosulfoesters were recrystallized from the appropriate solvent.

*2.2.5.1. S-Methyl 4-[(3-chloropropanoyl)amino]benzenesulfonothioate (**3a**)*. Yield 74%, mp: 122 °C. IR (KBr, cm^{-1}): 1128, 1328 (SO_2), 1572, 1584, 1600 (Ar—H), 1636 (NH), 1668 (C═O), 3244 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.12 (s, 3H, CH_3), 2.8 (t, J = 6.0 Hz, 2H, CH_2), 3.7 (t, J = 6.0 Hz, 2 HCH_2), 7.54 (d, J = 7.80 Hz, 2H, CH_3), 7.92 (d, J = 7.80 Hz, 2H, Ar—H), 10.32 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 17.33, 28.11, 38.86, 40.34, 119.55, 119.55, 123.89, 123.89, 139.31, 144.55, 170.65. Anal. calcd for $C_{10}H_{12}ClNO_3S_2$: C, 40.88; H, 4.12; N, 4.77; S, 21.83; Cl, 12.07; found: C, 40.99; H, 4.26; N, 4.67; S, 21.97; Cl, 11.92. MS (EI) m/z 308.83 [M + 1].

*2.2.5.2. S-Propyl 4-[(3-chloropropanoyl)amino]benzenesulfonothioate (**3d**)*. Yield 67%, mp: 155 °C. IR (KBr, cm^{-1}): 1140, 1328 (SO_2), 1584, 1592, 1600, 1608 (Ar—H), 1624 (NH), 1686 (C═O), 3274 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.4 (t, J = 6.8 Hz, 3H, CH_3), 1.85 M (m, 2H, CH_2), 2.76 (t, J = 5.8 Hz, 2H, CH_2), 3.62 (t, J = 6.3 Hz, 2H, S-CH_2), 3.96 (t, J = 5.8 Hz, 2H, CH_2), 7.52 (d, J = 7.8 Hz, 2H, Ar—H), 7.90 (d, J = 7.9 Hz, 2H, Ar—H), 10.28 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 14.25, 24.11, 31.82, 38.86, 40.34, 119.19, 119.19, 123.94, 123.94, 136.76, 144.55, 170.65. Anal. calcd for $C_{12}H_{16}ClNO_3S_2$: C, 44.78; H, 5.01; N, 4.35; S, 19.93; Cl, 11.02; found: C, 44.85; H, 5.09; N, 4.37; S, 20.01; Cl, 11.12. MS (EI) m/z 322.81 [M + 1].

*2.2.5.3. S-(1-Methylethyl) 4-[(3-chloropropanoyl)amino]benzenesulfonothioate (**3e**)*. Yield 48%, mp: 163 °C. IR (KBr, cm^{-1}): 1138, 1314 (SO_2), 1552, 1596, 1608 (Ar—H), 1632 (NH), 1692 (C═O), 3272 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.00 (d, J = 7.0 Hz, 3H, $CH(CH_3)_2$), 1.18 (d, J = 7.0 Hz, 3H, $CH(CH_3)_2$), 2.68 (t, J = 6.0 Hz, 2H, CH_2), 3.14 (m, 1H, $CH(CH_3)_2$), 3.96 (t, J = 6.0 Hz, 2H, CH_2), 7.46 (d, J = 7.8 Hz, 2H, Ar—H), 8.80 (d, J = 7.8 Hz, 2H, Ar—H), 10.32 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 25.01, 25.01, 36.12, 38.86, 40.34, 119.57, 119.57, 123.75, 123.75, 141.25, 144.55, 170.65. Anal. calcd for $C_{12}H_{16}ClNO_3S_2$: C, 44.78; H, 5.01; N, 4.35; S, 19.93; Cl, 11.02; found: C, 44.88; H, 5.15; N, 4.28; S, 20.09; Cl, 11.14. MS (EI) m/z 322.82 [M + 1].

*2.2.5.4. S-Butyl 4-[(3-chloropropanoyl)amino]benzenesulfonothioate (**3f**)*. Yield 36%, mp: 175 °C. IR (KBr, cm^{-1}): 1136, 1340 (SO_2), 1584, 1592, 1600 (Ar—H), 1648 (NH), 1676 (C═O), 3256 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.83 (t, J = 7.1 Hz, 3H, $(CH_2)_3C\underline{H}_3$), 1.34 (m, 2H, $(CH_2)_2C\underline{H}_2CH_3$), 1.80 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 2.7 (m, 2H, $C\underline{H}_2(CH_2)_2CH_3$), 2.96 (t, J = 5.8 Hz, 2 H, CH_2), 3.96 (t, J = 5.8 Hz, 2H, CH_2), 7.48 (d, J = 7.8 Hz, 2H, Ar—H), 7.98 (d, J = 7.8 Hz, 2H, Ar—H), 10.30 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 13.67, 21.23, 31.12, 32.56, 38.86, 40.34, 119.49, 119.49, 123.92, 123.92, 137.10, 144.55, 170.65. Anal. calcd for $C_{13}H_{18}ClNO_3S_2$: C, 46.49; H, 5.40; N, 4.17; S, 19.09; Cl, 10.56; found: C, 46.61; H, 5.54; N, 4.09; S, 19.26; Cl, 10.69. MS (EI) m/z 336.85 [M + 1].

*2.2.5.5. S-Phenyl 4-[(3-chloropropanoyl)amino]benzenesulfonothioate (**3l**)*. Yield 56%, mp: 76 °C. IR (KBr, cm^{-1}): 1144, 1306 (SO_2), 1544, 15828, 1596, 1608 (Ar—H), 1644 (NH), 1686 (C═O), 3250 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.7 (t, J = 6.0 Hz, 2H, CH_2), 3.71 (t, J = 6.0 Hz, 2H, CH_2), 7.18–8.12 (m, 9H, Ar—H), 10.32 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 38.86, 40.34, 119.01, 119.01, 124.44, 124.44, 127.67, 128.53, 129.26, 129.26, 129.38, 129.38, 135.09, 145.05, 167.56. Anal. calcd for $C_{15}H_{14}ClNO_3S_2$: C, 50.63; H, 3.97; N, 3.94; S, 18.09; Cl, 9.96; found: C, 50.80; H, 4.11; N, 3.78; S, 18.06; Cl, 10.24. MS (EI) m/z 356.83 [M + 1].

*2.2.5.6. S-(4-Chlorophenyl) 4-[(3-chloropropanoyl)amino]benzenesulfonothioate (**3m**)*. Yield 51%, mp: 146 °C. IR (KBr, cm^{-1}): 1142, 1318 (SO_2), 1568, 1592, 1600, 1608 (Ar—H), 1628 (NH), 1688 (C═O), 3246 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.64 (t, J = 6.0 Hz, 2H, CH_2), 3.71 (t, J = 6.0 Hz, 2H, CH_2), 7.32–8.0 (m, 8H, Ar—H), 10.28 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 38.86, 40.34, 119.01, 119.01, 124.44, 124.44, 127.27, 128.65, 128.65, 129.61, 129.61, 130.67, 135.09, 145.05, 167.56. Anal. calcd for $C_{15}H_{13}Cl_2NO_3S_2$: C, 46.16; H, 3.36; N, 3.59; S, 16.43; Cl, 18.17; found: C, 46.38;

H, 3.57; N, 3.55; S, 16.56; Cl, 18.04. MS (EI) *m/z* 391.32 [M + 1].

*2.2.5.7. S-(4-Nitrophenyl) 4-[(3-chloropropanoyl)amino]benzenesulfonothioate (**3n**).* Yield 42%, mp: 100 °C. IR (KBr, cm^{-1}): 1128, 1346 (SO_2), 1560, 1578, 1592, 1604 (Ar—H), 1642 (NH), 1688 (C═O), 3262 (NH). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.68 (t, J = 8.3 Hz, 2H, CH_2), 3.82 (t, J = 6.0 Hz, 2H, CH_2), 7.30–8.18 (m, 8H, Ar—H), 10.32 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 38.86, 40.34, 119.01, 119.01, 123.46, 123.46, 124.44, 124.44, 129.88, 129.88, 133.65, 135.09, 145.05, 145.66, 167.56. Anal. calcd for $C_{15}H_{13}ClN_2O_5S_2$: C, 44.94; H, 3.27; N, 6.99; S, 15.99; Cl, 8.84; found: C, 45.08; H, 3.46; N, 7.26; S, 16.20; Cl, 8.64. MS (EI) *m/z* 401.87 [M + 1].

The melting points of obtained esters of *4-[(3-chloropropanoyl)amino]benzenesulfonothioates* **3a,d, l-n** correspond to the melting points of similar compounds which were obtained in another way previously described (Lubenets et al., 1987).

2.3. Biology

The synthesized compounds were tested for their in vitro antimicrobial activity against bacteria *Aeromonas* sp. 9615, *Burkholderia cepacia*, *Alcaligenes faecalis*, *Staphylococcus aureus* 209-P, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus mesentericus*, *Mycobacterium* sp., *Mycobacterium luteum* B-917, *Escherichia coli* C-600, *Proteus vulgaris* and fungi *Candida albicans*, *Candida tenuis* VCM Y-70, *Verticillium dahliae*, *Trichophyton gypseum*, *Aspergillus niger* VCM F-1119, *Penicillium chrysogenum* VCM F-245 by agar diffusion method (method A) and by serial dilution method (method B).

Antifungal activity of thiosulfoesters against *C. albicans* ATCC 90028, *Candida glabrata* ATCC 90030 and *Aspergillus fumigatus* IHEM 13934 was tested by method C.

2.3.1. Method A. Determination of antimicrobial and antifungal activities by agar diffusion method

Antimicrobial and antifungal activities have been studied by diffusion in agar on solid nutrient medium (beef-extract agar for bacteria, wort agar for fungi). Petri plates containing 20 ml of nutrient medium were used for all the microorganisms that were tested. The inoculums (the microbial loading – 10^9 cells (spores)/1 ml) were spread on the surface of the solidified media and Whatman No. 1 filter paper disks (6 mm in diameter) impregnated with the test compound (0.1% and 0.5%) were placed on the plates. The duration of bacteria incubation was 24 h at 35 °C and of fungi incubation 48–72 h at 28–30 °C. The antimicrobial effect and degree of activity of the tested compounds were evaluated by measuring the zone diameters and the results were compared with well-known drugs (Table 2). Every experiment was repeated three times.

2.3.2. Method B. Determination of minimal inhibitory (MIC), minimal bactericidal (MBC) and minimal fungicidal (MFC) concentrations using serial dilution method

The tested compounds were added to the nutrient medium (beef-extract broth for bacteria and wort for fungi) as solutions in dimethyl sulfoxide (DMSO) in ensuring needed concentration (0.9–500.0 μg/ml). Bacteria and fungi inoculum was inoculated into nutrient medium. The microbial loading was 10^6 cells (spores)/1 ml (for making bacteria suspension, 10 units turbidility standard of DNDISK named by L.A. Tarasevych was used; calculation of fungus cells (spores) was carried out in the chamber of Goryayev). The duration of bacteria incubation was 24 h at 35 °C and of fungi incubation – 48–72 h at 28–30 °C. The results were estimated by the microorganism growth measured by degree of microbial turbidity in nutrient medium. Minimal inhibitory concentration (MIC) of any compound is defined as the lowest concentration which completely inhibits visible growth (turbidity on liquid nutrient medium).

Table 2 Parameters of result evaluation by the method of compound diffusion in agar.

No	Zone diameter of microorganism growth inhibition, mm	Degree of microorganism sensitivity
1.	11–15	Low-sensitive
2.	16–25	Sensitive
3.	>25	Highly sensitive

Determination of MBC and MFC: Nutrient medium solutions being visually transparent had been sowed on the sterile agar medium (beef-extract agar for bacteria, wort agar for fungi). The duration of bacteria incubation was 24 h at 35 °C and of fungi incubation – 48–72 h at 28–30 °C. In the absence of microorganism colony growth on the incubated Petri plate minimal bactericidal (MBC) and minimal fungicidal (MFC) concentrations of the investigated compounds were identified. The test was repeated three times.

2.3.3. Method C. Determination of minimal inhibitory concentrations (MIC) using serial dilution method

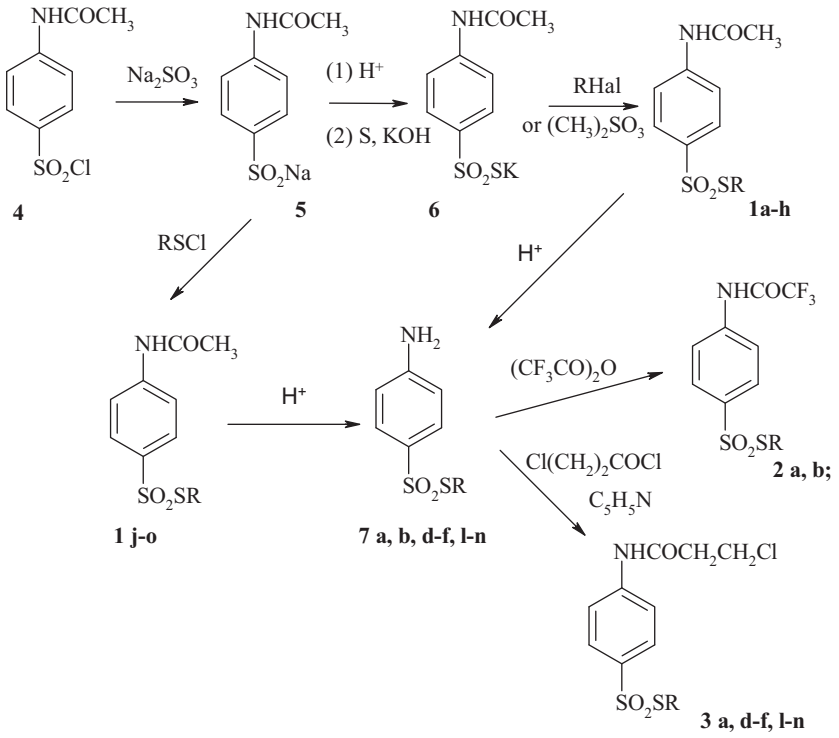
The assay was performed in the liquid medium RPMI1640 (2% glucose, L-Glu, pH 7.0, buffered by MOPS). Stock solutions at a concentration of 5 mg/ml. of tested samples were prepared in dimethyl sulfoxide. The serial, twofold dilutions of compounds were prepared in RPMI1640 and each concentration was dispensed into three wells (100 μl each) of the sterile flat-bottomed, 96-well plates (Nunc). The 24 h – old fungal cultures on Sabouraud's dextrose agar were used to prepare the cell suspensions of the density 0.5MF in sterile physiological saline. The suspensions were subsequently diluted in RPMI1640 to obtain 1–2000 CFU/ml, and used for inoculation of the plates (100 μl for one well). The final concentrations of compounds ranged from 100 to 6.25 μg/ml. Drug-free medium with and without fungi was used as a controls. The plates were incubated at 35 °C for 48 h and evaluated visually. MIC was defined as the lowest drug concentration that causes 80% growth inhibition.

3. Results and discussion

Twenty-four compounds that belong to the three small rows (**1a-o**, **2a,b** and **3a, d-f, l-n**, Scheme 1 and Table 1) were synthesized and their antimicrobial activity was determined as described below.

3.1. Chemistry

As a starting product for the synthesis of 4-acylaminobenzene thiosulfoacid S-esters, it was selected 4-acetylaminobenzenesul



Scheme 2 General synthesis of acylaminobenzenethiosulfoacid esters **1a-o**; **2a,b** and **3a, d-f, l-n**.

Table 3 Antibacterial and antifungal activities of investigated compounds by the diffusion method (method A).

Compound	Concentration, %	Diameter of inhibition zones of growth of microorganism, mm						
		Fungi			Bacteria			
		A	B	C	D	E	F	G
1a	0.5	8	10	10	14	15	16	16
	0.1	0	0	0	8	10	10	12
1b	0.5	16	17	14	12	13	13	18
	0.1	7	8	0	9	9	9	12
2a	0.5	16	19	18	13	15	15	24
	0.1	7	8	7	9	9	7	10
2b	0.5	12	13	12	8	11	14	21
	0.1	8	10	8	0	8	8	10
Ethylthiosul fanylate	0.5	27	16	20	29	19	17	25
	0.1	13	0	12	18	17	16	17

A: *Candida tenuis* VCMY-70; B: *Aspergillus niger* VCMF-1119; C: *Penicillium chrysogenum* VCMF-245; D: *Escherichia coli* C-600; E: *Bacillus mesentericus*; F: *Staphylococcus aureus*; G: *Mycobacterium luteum* B-917.

Table 4 Values of minimal inhibitory concentrations (MIC, μM) determined by the serial dilution method (methods B and C).

Compound	MIC						
	Fungi			Bacteria			
	A	B	C	D	E	F	G
1a	0.2	–[a]	0.1	0.41	4.08	–[a]	–[a]
1b	0.1	–[a]	0.05	3.86	3.86	–[a]	–[a]
1c	0.37	–[a]	0.05	3.69	3.69	–[a]	–[a]
1i	–[a]	–[a]	–[a]	–[a]	–[a]	0.31	0.31
2a	0.17	0.33	0.08	–[a]	–[a]	–[a]	–[a]
2b	0.16	0.16	0.08	–[a]	–[a]	–[a]	–[a]

A: *Candida albicans* ATCC 90028; B: *Candida glabrata* ATCC 90030; C: *Aspergillus fumigatus* IHEM 13934.
D: *Burkholderia cepacia*; E: *Alcaligenes faecalis*; F: *Aeromonas* sp. 9615; G: *Escherichia coli* C-600.
[a] Not tested.

fochloride **4**, and by further reduction using sodium sulfite in alkaline medium, it was converted to the appropriate sodium salt of 4-acetylaminobenzenesulfinic acid **5**.

For obtaining alkyl esters of 4-acetylaminobenzenethiosul foacid **1f-h**, compound **5**, by acidification with hydrochloric acid, was previously converted to 4-acetylaminoben zenesulfinic acid, from which by further boiling with sulfur in alkaline medium, potassium salt of 4-acetylaminobenzene thiosulfoacid **6** was obtained.

By alkylation of salt **6** in acetone-water medium using alkyl halides, and on obtaining methyl ester – using dimethyl sulfate, target alkyl thiosulfoesters **1f-h** were synthesized.

For obtaining cycloalkyl and aryl esters of 4-acetylamino benzenethiosulfoacids **1j-o**, it was used a method that involves interaction of sulfen halogenides with sulfinic acids and their salts (Koval, 1995; Markley and Dunbar, 1972; Wandel, 1974).

It was applied anhydrous sulfinate **5** for preventing hydrolysis of sulfenyl chlorides. The reaction was carried out in dry tetrachloromethane at the ratio of reactants sulfenylchloride:-

Table 5 Values of minimal bactericidic and fungicidic concentrations (MBC and MFC, μM) determined by the serial dilution method (method B).

Compound	MFC			MBC					
	A	B	C	D	E	F	G	H	I
1a	0.41	0.16	0.8	0.41	0.41	0.41	0.41	0.41	0.41
1b	0.15	0.15	0.04	0.15	1.54	0.08	0.08	–[a]	–[a]
1f	0.07	0.03	0.03	0.03	1.39	0.03	0.03	0.7	1.39
1j	0.01	–[a]	–[a]	0.03	–[a]	–[a]	0.03	–[a]	–[a]
1k	0.01	–[a]	–[a]	0.06	–[a]	–[a]	0.32	–[a]	–[a]
1l	0.03	0.01	0.01	0.03	–[a]	0.07	0.01	–[a]	–[a]
1m	0.03	0.01	0.06	0.01	–[a]	0.29	0.01	–[a]	–[a]
1n	–[a]	–[a]	–[a]	1.14	1.14	–[a]	–[a]	–[a]	0.28
1o	–[a]	1.14	0.11	–[a]	1.14	–[a]	1.14	–[a]	0.28
3a	0.14	0.14	–[a]	1.36	1.36	1.36	1.36	–[a]	–[a]
3d	1.24	0.62	0.62	0.31	1.24	0.62	0.62	–[a]	–[a]
3e	0.62	–[a]	–[a]	0.62	1.24	1.24	0.62	1.24	–[a]
3f	1.19	–[a]	–[a]	0.12	–[a]	0.06	0.29	–[a]	–[a]
3l	0.11	0.03	0.03	0.06	–[a]	0.06	0.11	–[a]	–[a]
3m	0.51	0.51	0.26	0.02	–[a]	–[a]	0.51	–[a]	–[a]
3n	0.49	0.25	0.49	0.25	–[a]	–[a]	0.49	–[a]	–[a]

A: *Candida albicans*; B: *Verticillium dahliae*; C: *Trichophyton gypseum*; D: *Staphylococcus aureus*; E: *Pseudomonas aeruginosa*; F: *Bacillus subtilis*; G: *Mycobacterium* sp.; H: *Escherichia coli*; I: *Proteus vulgaris*.

[a] Not tested.

sulfinate – 1:1.3 and the reaction mixture was heated for 5–6 h at the boiling point of the solvent.

In order to obtain esters of 4-trifluoroacetyl- and 4-(3-chloropropionylamino)-benzenethiosulfoacids, corresponding 4-acetylaminobenzenethiosulfoacid esters by acidic deacylation in 40% solution of sulfuric acid, while heating (Lubenets et al., 2006) were converted to the 4-aminobenzenethiosulfoacid esters **7a,b, d-f, l-n** (see Scheme 2 and Table 3).

Thiosulfoesters **2a,b** were obtained by acylation of alkyl esters of 4-aminobenzenethiosulfoacid **2a,b** using trifluoroacetic anhydride in benzene at equimolar ratio of reactants and the boiling temperature of the reaction mixture with nearly quantitative yield (90–95%).

With a bit more moderate yields of 36–74%, there were obtained S-esters of 4-(3-chloropropionylamino)-benzenethiosulfoacid **3a, d-f, l-n** by acylation of the appropriate esters of 4-aminobenzenethiosulfoacid using chloroanhydride of β-chloropropionic acid in dioxane in the presence of pyridine.

3.2. Biological results

Antimicrobial activity of thiosulfoesters **1a,b** and **2a,b** was studied by a method of compound diffusion into agar (Murray et al., 1995; National Committee for Clinical Laboratory Standard, 1998).

The research results are presented in Table 2. Ethyl S-ester of 4-aminobenzenethiosulfoacid (ethyl thiosulfanylate) was used as the object of comparison, and it was offered to be applied as an effective biocidal agent (Boldyrev et al., 1983; Shved et al., 2006).

Analysis of the results indicates that thiosulfoesters in which the acyl residue contains a fluorine atom, show higher fungicidal activity than other tested compounds. In particular, while an effect of 4 trifluoroacetylaminobenzenethiosulfoacid methyl S-ester **2a** was on fungi growth, an inhibitory effect is observed at concentration of 0.1%, and there is no growth inhibition of test cultures while the effect of appropriate 4-acetylaminobenzenethiosulfoacid S-ester **1a** was in this concentration.

As for the bactericidal activity of the tested compounds, it was found out that fluorine-containing thiosulfoesters exhibit selective effect on certain strains of bacteria unlike the similar 4-acetylaminobenzenethiosulfoacid S-esters. In particular, thiosulfonates have the least inhibitory effect on growth of gram-negative bacterial strain *Escherichia coli*, and the most bactericidal effect – on the strain of gram-positive bacteria *M. luteum*.

It should be noted that the tested S-esters of thiosulfoacids in comparison with ethyl thiosulfanylate are of weaker fungicidal and bactericidal properties. Thus, acylation of amino thiosulfoesters reduces their antimicrobial activity.

With dilution methods (methods B and C) for synthesized thiosulfoesters **1f-c,i** and **2a,b** there were determined minimal inhibitory concentrations against bacterial cultures *B. cepacia*, *A. faecalis*, *Aeromonas* sp. 9615, *E. coli* C-600, and cultures of fungi *C. albicans* ATCC 90028, *C. glabrata* ATCC 90030, *A. fumigatus* IHEM 13.934 (Table 4).

In general, MICs were in the micromolar range while the most active candidates showed MICs in nanomolar range (50 and 80 nM by compounds **1b,c** and **2a,b** against *A. fumigatus*, respectively) which reflect the availability of the novel candidates.

Evaluation of the antibacterial activity of the compounds **1a-c,i** and **2a,b** shows that in relation to the tested cultures of fungi, all esters are of high fungicidal activity. The highest activity was observed for ethyl ester of 4-acetylaminobenzene thiosulfoacid **1b**, for which the MIC against the *C. albicans* was 0.1 μM, and as for *A. fumigatus* – 0.05 μM, while the MIC concerning mentioned fungi of appropriate thiosulfoester of 4-trifluoroacetylaminobenzenethiosulfoacid **2a** was 0.17 and 0.08 μM correspondingly.

All tested bacteria test-cultures turned out to be insensitive to the effect of thiosulfoesters of 4-acetylaminobenzenethiosul foacid **1f-c**, and it is proved by low figures of MIC.

For the number of thiosulfoesters **1a,b, j-o** and **3a, d-f, n** by serial dilution (method B), there were determined minimal bactericidal and minimal fungicidal concentrations against the bacteria *S. aureus*, *P. aeruginosa*, *B. subtilis*, *Mycobacterium* sp., *E. coli*, *P. vulgaris* and fungi *C. albicans*, *V. dahliae*, *T. gypseum* (Table 5).

The obtained results allow to characterizing tested thiosulfoesters as promising antimicrobial substances. At the same time, cycloalkyl and aryl esters of 4-acylaminobenzenethiosul foacids happened to be more active in comparison with alkyl esters, and among them phenyl esters **1l** and **3l** should be singled out for which MBC were within 0.01–0.07 μM for **1l** and 0.06–0.11 μM for **3l**, and MFC were within 0.01–0.03 μM and 0.03–0.11 μM correspondingly.

Analyzing the impact of acyl fragment on antimicrobial activity of thiosulfoesters, it can be concluded that the compounds with acetyl fragment in their structure **1** show higher antimicrobial activity than thiosulfoesters with 3-chloropropionyl fragment **3**.

4. Conclusions

A series of thiosulfonate derivatives (**1, 2, 3**) were synthesized and evaluated for their antibacterial and antifungal activities. The structures of new products were characterized by spectroscopic methods (^{1}H NMR, ^{13}C NMR, IR) and elemental analysis. In the presented work it is shown that some S-esters of 4-acylaminobenzenethiosulfoacids may be proposed for use as an effective antimicrobial substances. All the synthesized compounds showed significant antimicrobial properties against tested microorganisms. Particularly, cycloalkyl and aryl esters of 4-acylaminobenzenethiosulfoacids **1j-l**, **3l** exhibited promising activity (at nanomolar concentrations) against all tested pathogenic bacteria and fungi. However most synthesized esters showed selective action against certain microorganisms (MFC or MBC were nanomolar range against some specific bacteria or fungi).

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