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5-Ene-rhodanine-3-carboxylic acids as potential antimicrobial and antiparasitic agents

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Aim. Design, synthesis and study of antibacterial, antifungal and trypanocidal activity of a series of novel 2-thioxo-4-thiazolidinone-3-carboxylic acids with different arylidene substituents in C5 position. **Methods.** Organic wet synthesis, analytical and spectral methods, pharmacological screening, SAR analysis. **Results.** A series of 5-(aminomethylene)-4-oxo-2-thioxothiazolidin-3-ylcarboxylic acids and their analogs **IIIa-IIIj** were synthesized in the reactions of 5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-ylcarboxylic acids **IIa,b** or ethyl 5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-ylpropanoate **IIc** with various amines and ammonium hydrogen carbonate. Five of the synthesized compounds **IIIb**, **IIIf** and **IIIh-j** were tested towards a series of Gram (+) and Gram (–) bacteria and four yeasts strains at a dose of 1mM. In general, the tested compounds are promising building scaffolds for the development of antifungal agents as all of them inhibited growth of clinical strain of *Candida albicans*. Moreover, pyridine containing 3-[5-(aminomethylene)-rhodanine-3-yl]carboxylates showed good trypanocidal activity and low cytotoxicity towards normal fibroblasts. **Conclusions.** A series of novel 3-[5-(aminomethylene)-4-oxo-2-thioxothiazolidin-3-yl]carboxylic acids derivatives were synthesized. Study of their antibacterial and antifungal action allowed identifying a hit-compound ethyl 3-[5-[(4-(fluoroanilino)methylene]-4-oxo-2-thioxothiazolidin-3-yl] propanoate **IIIf**, which is active against clinical strains of *Staphylococcus lentus* and *Candida ssp*. In general, most of the studied compounds showed good antifungal properties.

K e y w o r d s: 2-thioxo-4-thiazolidinone-3-carboxylic acids, rhodanine, synthesis, antitrypanosomal activity, antibacterial activity, antifungal activity, SAR.

Introduction

4-Thiazolidinone derivatives have been known as a source of drug-like molecules with the studied hypoglycemic, anticancer, anti-inflammatory, antituberculosis and antimicrobial effects [1–3]. The inhibition activity against protozoa such as *Trypanosoma ssp.* [4–6] or *Plasmodium falciparum* [7] had been also investigated for thiazole/thiazolidinone- and re-

lated heterocycles-based compounds. 2,4-Thiazolidinedione, rhodanine (2-thioxo-4-thiazolidinone), 2-alkyl(aryl)-substituted, and 2-R-amino(imino)-substituted 4-thiazolidinone subtypes provide the major part of antimicrobial, antidiabetic, anti-inflammatory and anticancer lead-compounds and drug candidates [1,8,9]. 5-Ene-thiazolidinones including the 4-thiazolidinone-3-carboxylic acids derivatives [10,11] are of special interest taking into account their pharmacological profiles, the feasibility of structure optimization as well as the toxicity profile [12,13]. Despite falling out of favor with medicinal chemists, 5-ene-4-thiazolidinones should not be treated as pan-assay interference compounds (PAINS) only [14] and still hold a promise for providing active drug-like molecules [15]. One more direction of the 5-ene-4-thiazolidinones study is the search for novel antibacterial agents [16], great part of which comprise rhodanine-3-carboxylic acids.

On the one hand, antibacterial drugs have been saving millions of lives since the discovery of penicillin, but on the other hand, their extensive usage had pushed the antibiotic resistance mechanisms in bacteria. Surviving of species with such mechanisms challenges therapeutic options of the infectious diseases treatment [17], which needs new classes of antibiotics based on the chemical structures different from those used today. The development of novel safe and efficacious antibacterials remains the topical task of medicinal chemistry and health care system worldwide. Reviewing the latest literature data, it should be mentioned that a class of 5-ene-2-thioxo-4-thiazolidininone-3-carboxylic acids is characterized as a source of agents with excellent antimicrobial activity including MDR strains. For example, 3-α-carboxyethyl-5-benzylidenerhodanine derivatives showed moderate to good MIC values against MRSA pathogen panel [18]; a series of 5-(2-hydroxybenzylidene)-rhodanines possessed the MIC values in 32–256 μg/mL range against *S. aureus*, *E. faecalis*, and *H. influenza* and were experimentally characterized as novel inhibitors of bacterial DNA gyrase [19]; *para-N,N*-benzylidenediphenylamine substituted rhodanine-3-alkanecarboxylic acids were active against Gram

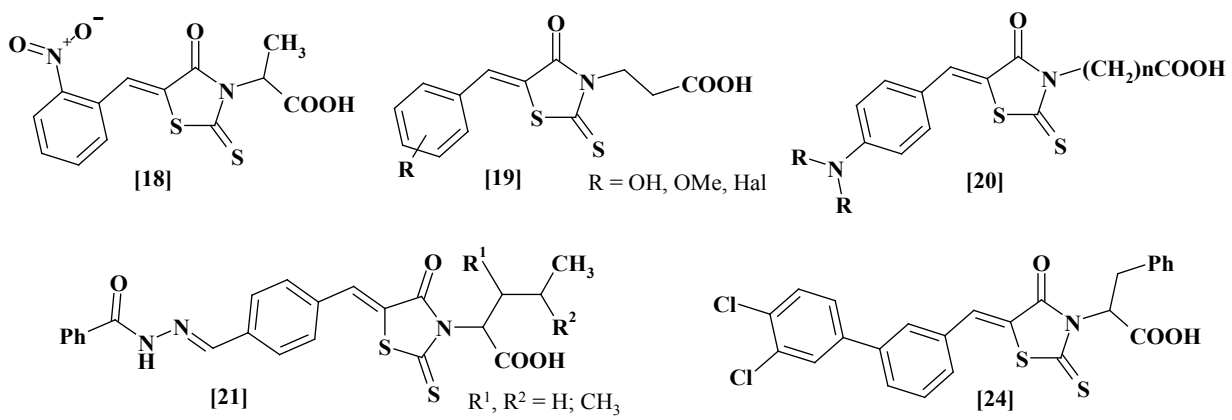


Fig. 1. Examples of rhodanine-3-carboxylic acids with the antimicrobial activity.

(+) pathogens with MIC = 1.95 μg/mL [20]. Rhodanine-3-carboxylic acids with an arylhydrazone fragment displayed the excellent activity against MDR methicillin-resistant and quinolone-resistant *S. aureus* with MIC of 2–4 μg mL^{-1} [21].

With a broad exposure to antibiotics and immunosuppression, the incidence of opportunistic fungal pathogens such as *Candida albicans* has increased [22,23]. The compounds revealing antifungal activity are also presented among 2-thioxo-4-thiazolidinones. A derivative of 2-(rhodanine-3-yl)-3-phenylpropanoic acid showed the micromolar ranges of MIC towards the isolates of Gram (+) and Gram (–) bacteria including the vancomycin resistant strains as well as *Candida albicans* and was not toxic to mouse murine macrophages and human keratinocytes [24]. Taking into account the literature data on antibacterial, antifungal and antiparasitic properties of thiazolidinone derivatives, the feasibility of their synthesis and further chemical optimization, the aim of presented research was the design of potentially active antimicrobials on the base of rhodanine-3-carboxylic acids.

Materials and Methods

Chemistry

All chemicals were of the analytical grade and commercially available. All reagents and solvents were used without further purification and drying. NMR spectra were determined with Varian Mercury 400 (400 MHz) spectrometer, in DMSO-d_6 using tetramethylsilane as an internal standard. Elemental analyses (C, H, N) were performed at the Perkin-Elmer 2400 CHN analyzer and the results were within ± 0.4 % of the theoretical values. Mass spectra were obtained using electrospray ionization (ESI) techniques on an Agilent 1100 Series LCMS. The purity of the compounds was checked by thin-layer chromatography performed with Merck Silica Gel 60 F254 aluminum sheets.

Synthesis of ethyl 3-(4-oxo-2-thioxothiazolidin-3-yl)propanoate (Ic). The 3-(4-oxo-2-thioxothiazolidin-3-yl)propanoic acid (0.1 mole) was refluxed in the ethanol medium (200 mL) with adding catalytic amounts of concentrated sulfuric acid for 5 hours. After cooling the reaction mixture to the room temperature, it was neutralized with potassium carbonate to pH 7.0 and the formed precipitate (potassium sulfate) was filtered off. The obtained filtrate was evaporated under vacuo to yield the product as viscous yellow liquid that was used in further reactions without additional purification.

General procedure of 5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-carboxylic acids (IIa, IIb) and ethyl 3-[5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-yl]propanoate (IIc) synthesis

A mixture of 4-oxo-2-thioxothiazolidine-3-acetic acid (0.1 mole), ethyl orthoformate (0.12 mole) and 125 ml of acetic anhydride was refluxed for 1.5 hour. After cooling, the reaction mixture was concentrated and small amount of chloroform was added to cause the precipitate formation. The precipitate was filtered off and recrystallized from ethyl acetate to give 5-(ethoxymethylene)-4-oxo-2-thioxothiazolidine-3-acetic acid [25].

In the case of **IIb** and **IIc** synthesis, a mixture of 4-oxo-2-thioxothiazolidine-3-propanoic acid or its ethyl ester (0.1 mol), ethyl ortho-

formate (0.12 mol) and 40-50 ml of acetic anhydride was refluxed for 1.5 hour. The reaction mixture was poured into water and the product was extracted with ethylacetate. The ethylacetate layer was evaporated under vacuo and the obtained solid product was recrystallized at first from the mixture of acetic acid/water with the second recrystallization from the mixture of toluene/hexane for **IIb** (*method* A) or from ethanol only for **IIc**. Purification of the mixture of products after the first crystallization (acetic acid/water) by column chromatography was performed in the system of solvents: aceton/hexane/NH_4OH = 50:50:1.

2-[5-(Ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid (IIa). Yield 61 %, mp 188-189 °C, lit. 190-192 °C [25]. ^{1}H NMR (DMSO-d_6) *d*: 1.25 (t, 3H, CH_3), 4.18 (q, 2H, CH), 4.41 (s, 2H, CH_2COO), 7.58 (s, 1H, -CH=). Calcd. for $C_8H_9NO_4S_2$: C, 38.86; H, 3.67; N, 5.66; Found: C, 39.35; H, 3.74; N, 5.61.

3-[5-(Ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-yl]propanoic acid (IIb). Yield 85 %, mp 113-115 °C. ^{1}H NMR (DMSO-d_6) *d*: 1.28 (t, 3H, CH_2CH_3), 1.81 (t, 1H, CH_2), 2.28 (t, 1H, CH_2), 3.96 (t, 2H, CH_2), 4.36 (q, 2H, CH_2CH_3), 7.99 (s, 2H, CH=), 12.01 (s, 1H, COOH). LCMS (ESI+): *m/z* 262.0 (96.6 %, $[M+H]^+$).

Calcd. for $C_9H_{19}NO_4S_2$: C, 41.37; H, 4.24; N, 5.36; Found: C, 41.25; H, 4.24; N, 5.34.

Ethyl 3-[5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-yl]propanoate (IIc). Yield 87 %, mp 111-113°C. ^{1}H NMR (DMSO-d_6) *d*: 1.15-1.17 (m, 3H, CH_2CH_3), 1.28 (t, 3H, CH_3), 2.64-2.66 (m, 2H, CH_2), 4.03 (q, 2H, CH_2CH_3), 4.19 (t, 2H, CH_2), 4.30 (q, 2H, CH_2), 7.98 (s, 2H, CH=). Calcd. for $C_{11}H_{15}NO_4S_2$: C, 45.66; H, 5.22; N, 4.84; Found: C, 41.25; H, 5.19; N, 4.89.

General procedure of 2-[5-[aminomethylene]-4-oxo-2-thioxothiazolidin-3-yl]carboxylic acids (IIIa-IIId) and their esters (IIIf-IIIj) synthesis

To 0.01 mol of 5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-carboxylic acid (**IIa** or **IIb**) or ethyl 3-[5-(ethoxymethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]propanoate (**IIc**) the equimolar quantity of appropriate amine (4-(ethoxycarbonyl)aniline, 4-chloroaniline, 4-fluoroaniline or 4-(6-methyl-1,3-benzothiazol-2-yl)aniline), 3/4-aminopyridines or 4-pyridinecarboxylic acid hydrazide) was added and refluxed in the ethanol medium for 1.5-2 hours. After cooling, the formed precipitate was filtered off and recrystallized from the mixture of acetic acid/water (1:1) (**IIIa**) or acetic acid (**IIIb, IIIc, IIIg**) or the mixture of DMF/ethanol (1:3) (**IIId, IIIh-IIIj**), or ethanol (**IIIf**).

2-[5-[(4-Ethoxycarbonylanilino)methylene]-4-oxo-2-thioxothiazolidin-3-yl]acetic acid (IIIa). Yield 55 %, mp 170-172°C. ^{1}H NMR (DMSO-d_6) *d*: 1.28 (t, 3H, CH_2CH_3, J = 6.9 Hz), 4.25 (q, 2H, CH_2CH_3), 4.63 (s, 2H, CH_2), 7.39 (d, 2H, J = 8.2 Hz, arom.), 7.91 (d, 2H, J = 8.2 Hz, arom.), 8.36 (d, 1H, CH=), 10.57 (д, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 191.9, 168.1, 166.7, 165.6, 144.4, 135.1, 131.4, 131.3, 125.0, 116.6, 113.0, 96.7, 60.9, 45.2, 14.6. LCMS (ESI+): *m/z* 367.0 (98.2 %, $[M+H]^+$). Calcd. for $C_{15}H_{14}N_2O_5S_2$: C, 49.17; H, 3.85; N, 7.65; Found: C, 49.87; H, 3,92; N, 7.50.

3-[5-[(4-Ethoxycarbonylphenylamino) methylene]-4-oxo-2-thioxothiazolidin-3-yl] propanoic acid (IIIb). Yield 57 %, mp 192-193°C. ^{1}H NMR (DMSO-d_6) *d*: 1.30 (brs, 3H, CH_2CH_3), 2.67 (t, 2H, CH_2), 4.05-4.18 (m, 2H,

CH_2), 4.26 (q, 2H, CH_2CH_3), 7.48 (m, 2H, arom.), 7.92 (m, 2H, arom.), 8.15 (s, 1H, CH=). ^{13}C NMR (100 MHz, DMSO-d_6): 191.2, 170.8, 165.7, 162.6, 141.9, 136.4, 131.4 (2H, arom.), 124.7, 117.0 (2H, arom.), 98.1, 61.0, 50.6, 31.6, 14.7. Calcd. for $C_{16}H_{16}N_2O_5S_2$: C, 50.51; H, 4.24; N, 7.36; Found: C, 51,00; H, 4.19; N, 7.39.

3-[5-[(4-Chlorophenylamino)methylene]-4-oxo-2-thioxothiazolidin-3-yl]propanoic acid (IIIc). Yield 84 %, mp 208-210°C. ^{1}H NMR (DMSO-d_6) *d*: 2.48-2.54 (m, 2H, CH_2), 4.02-4.13 (m, 2H, CH_2), 7.28-7.31 (m, 4H, arom.), 7.98 (s, 1H, CH=). ^{13}C NMR (100 MHz, DMSO-d_6): 191.4, 172.3, 166.9, 139.6, 135.9, 135.9, 129.9, 128.2, 118.9, 118.9, 97.6, 95.3, 31.5. LCMS (ESI+): *m/z* 343.0/345.0 (96.6 %, $[M+H]^+$). Calcd. for $C_{13}H_{11}ClN_2O_3S_2$: C, 45.55; H, 3.23; N, 8.17; Found: C, 45,83; H, 3.28; N, 8.09.

3-[(5Z)-5-[[4-(6-methyl-1,3-benzothiazol-2-yl)anilino]methylene]-4-oxo-2-thioxo-thiazolidin-3-yl]propanoic acid (IIId). Yield 85 %, mp 229-230°C. ^{1}H NMR (DMSO-d_6) *d*: 2.44 (s, 3H, CH3), 2.58 (t, 2H, CH2), 4.18 (t, 2H, CH2), 7.33 (d, 1H, J = 7.3 Hz, arom.), 7.45 (d, 2H, J = 7.1 Hz, arom.), 7.84-7.90 (m, 2H, arom.), 8.01 (d, 2H, J = 7.1 Hz, arom.), 8.13 (s, 1H, =CH), 10.52 (br.s, 1H, NH), 12.52 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): 191.6, 172.3, 167.0, 165.9, 152.3, 142.9, 135.6, 134.8, 134.9, 129.0, 128.6, 128.5, 122.7, 122.2, 117.4 (2H, arom.), 101.2, 96.5, 49.7, 31.5, 21.6. LCMS (ESI+): LCMS (ESI+): *m/z* 456.0 (98.1 %, $[M+H]^+$). Calcd. for $C_{21}H_{17}N_3O_3S_3$: C, 55.36; H, 3.76; N, 9.22; Found: C, 55.69; H, 3.81; N, 9.15.

Ethyl 3-[5-[(4-(fluorophenylamino) methylene]-4-oxo-2-thioxothiazolidin-3-yl] propanoate (IIIf). Yield 50 %, mp 164-165°C. ^{1}H NMR (DMSO-d_6) *d*: 1.16-1.18 (m, 3H, CH_2CH_3), 2.63-2.66 (m, 2H, CH_2), 4.04 (q, 2H, CH_2CH_3), 4.21 (t, 2H, CH_2, *J* = 7.2 Hz), 7.19-7.22 (m, 2H, arom.), 7.33-7.37 (m, 2H, arom.), 8.06 (brs, 2H, CH=). ^{13}C NMR (100 MHz, DMSO-d_6): 192.0, 168.1, 166.7, 165.7, 144.5, 135.3, 131.4 (2H, arom.), 125.1, 116.7 (2H, arom.), 96.7, 61.0, 45.3, 14.7. LCMS (ESI+): *m/z* 355.0 (99.6 %, $[M+H]^+$). Calcd. for $C_{15}H_{15}FN_2O_3S_2$: C, 50.83; H, 4.27; N, 7.90; Found: C, 51.05; H, 4.35; N, 7.94.

Ethyl 3-{5-[(Z)-1-(4-ethyloxycarbonyl-phemylamino)methylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}propanoate (IIIg). Yield 45 %, mp 160-162°C. ^{1}H NMR (DMSO-d_6) *d*: 1.16 (brs, 3H, CH_2CH_3), 1.30 (brs, 3H, CH_2CH_3), 2.64 (t, 2H, CH_2), 4.05 (t, 2H, CH_2), 4.18 (q, 2H, CH_2CH_3), 4.28 (q, 2H, CH_2CH_3), 7.38 (m, 2H, arom.), 7.91 (m, 2H, arom.), 8.16 (s, 1H, CH=). ^{13}C NMR (100 MHz, DMSO-d_6): 191.7, 171.5, 165.7, 163.6, 141.9, 136.1 131.4 (2H, arom.), 129.0, 124.7, 116.9 (2H, arom.), 61.1, 60.8, 50.3, 32.1, 14.7, 14.1. LCMS (ESI+): *m/z* 409.0 (98.6 %, $[M+H]^+$). Calcd. for $C_{18}H_{20}N_2O_5S_2$: C, 52.92; H, 4.93; N, 6.86; Found: C, 52.63; H, 4.86; N, 6.89.

Ethyl 3-[4-oxo-5-[(3-pyridylamino) methylene]-2-thioxothiazolidin-3-yl]propanoate (IIIh). Yield 69 %, mp 193-195°C, lit. 192-194 °C [26]. ^{1}H NMR (DMSO-d_6) *d*: 2.55 (t, 3H, *J* =6.8 Hz, CH_3), 3.01 (q, 2H, *J* =6.8 Hz, OCH_2), 3.75 (t, 2H, CH_2, *J* =6.9 Hz), 5.50 (q, 2H, CH_2, *J* =6.9 Hz), 7.34 (brs,1H, pyrid.), 7.66 (d, 1H, *J*= 7.9 Hz, pyrid.), 7.73 (s, 1H, pyrid.), 7.80 (d, 1H, *J* = 7.3 Hz, pyrid.), 7.83 (brs, 1H, CH=), 10.42 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 193.7, 172.1, 167.2, 145.8, 144.7, 139.6, 133.1, 129.3, 123.7, 114.5, 60.7, 49.8, 32.2, 14.1. Calcd. for $C_{14}H_{15}N_3O_3S_2$:

C, 49.83; H, 4.48; N, 12.45; Found: C, 50.02; H, 4.42; N, 12.35.

Ethyl 3-[(4-oxo-5-[(4-pyridylamino) methylene]-2-thioxothiazolidin-3-yl]propanoate (IIIi). Yield 45 %, mp 182-184°C lit. 183-185°C [26]. ^{1}H NMR (DMSO-d_6) *d*: 1.16 (t, 3H, CH_3, *J* = 7.0 Hz), 2.60 (q, 2H, OCH_2, *J* = 7.0 Hz), 4.00 (t, 2H, CH_2, *J* = 7.3 Hz), 4.19 (t, 2H, CH_2, *J* = 7.3 Hz), 6.71 (d, 2H, *J* = 6.9 Hz, pyrid.), 7.57 (s, 1H, CH=), 8.07 (d, 2H, *J* = 6.9 Hz, pyrid.), 10.41 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 192.6, 171.6, 166.5, 149.8 (2H, arom.), 140.1, 132.8, 129.1, 118.1 (2H, arom.), 60.9, 49.5, 32.3, 14.1. Calcd. for $C_{14}H_{15}N_3O_3S_2$: C, 49.83; H, 4.48; N, 12.45; Found: C, 49.80; H, 4.46; N, 12.38.

Ethyl 3-[(4-oxo-5-[[2-(pyridine-4-carbonyl)hydrazino]methylene]-2-thioxothiazolidin-3-yl]propanoate (IIIj). Yield 73 %, mp 235-237°C [26]. ^{1}H NMR (DMSO-d_6) *d*: 1.15 (t, 3H, CH_3, *J* = 7.1 Hz), 2.60 (t, 2H, CH_2, *J* = 7.1 Hz), 4.02 (q, 2H, CH_2, *J* = 7.1 Hz), 4.16 (t, 2H, CH_2, *J* = 7.1 Hz), 7.85 (m, 3H, CH=, pyrid.), 8.84 (brs, 2H, pyrid.), 10.27 (brs, 1H, NH), 11.40 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 192.3, 172.2, 170.7, 167.1, 164.8, 150.8 (2H, arom.), 139.6, 131.2, 121.8 (2H, arom.), 89.8, 60.7, 31.5, 14.4. Calcd. for $C_{15}H_{16}N_4O_4S_2$: C, 47.36; H, 4.24; N, 14.73; Found: C, 47.56; H, 4.26; N, 14.77.

Method of the ethyl 3-[5-(aminomethylene)-4-oxo-2-thioxothiazolidin-3-yl]propanoate (IIIe) synthesis

The mixture of equimolar amounts of ethyl 3-[5-(ethoxymethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]propanoate **IIc** and ammonium hydrogen carbonate (0.01 mol) was refluxed in the ethanol medium (15 mL) for 2 hours. After cooling the reaction mixture, the formed precipitate was filtered off and recrystallized from acetic acid.

Ethyl 3-[5-(aminomethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]propanoate (IIIe). Yield 65 %, mp 113-115°C. ^{1}H NMR (DMSO-d_6) *d*: 1.15 (t, 3H, CH_3, *J* = 7.1 Hz), 2.67 (t, 2H, CH_2, *J* = 7.1 Hz), 4.08 (q, 2H, CH_2), 4.17 (m, 2H, CH_2), 8.14 (s, 1H, CH=). ^{13}C NMR (100 MHz, DMSO-d_6): 191.9, 172.3, 165.2, 132.4, 129.1, 60.9, 50.3, 32.6, 14.1. Calcd. for $C_9H_{12}N_2O_3S_2$: C, 41.52; H, 4.65; N, 10.76; Found: C, 41.74; H, 4.72; N, 10.85.

Pharmacology

Antibacterial and antifungal activity screening

The synthesized compounds were screened for their *in vitro* antibacterial and antifungal activities using the agar diffusion method and the broth microdilution method (Resazurin Reduction-Based Assay) [27]. A total of 12 microorganisms were tested. They consisted of 4 Gram (–) bacteria: *Pseudomonas aeruginosa* ATCC 27853 (F-51) and *Escherichia coli* ATCC 25922 used as reference strains and clinical strains of *Pseudomonas aeruginosa* and *Escherichia coli*; 4 Gram (+) bacteria: clinical strains of *Staphylococcus lentus*, *Staphylococcus lugnuniensis*, *Staphylococcus simulans* and the reference strain – *Staphylococcus aureus* ATCC 25923 (F-49); four yeasts: *Candida albicans (ATCC 885-653)* used as reference strain, clinical strains of *Candida albicans*, *Candida dubliniensis* and *Candida membranifaciens*. The Clinical and rarely found clinical strains were multidrug resistant [28] and isolated from patients with health-care-associated infections.

The tested compounds (at the concentration of 1mM) were inoculated into a 5.5±0.5 mm diameter well (50 μL per well) containing suspension of the culture of microorganisms (McFarland 2.0) on the agar plate (meat peptone agar or Saburo agar for fungi). DMSO was used as a control. A compound with the diameter of growth inhibition more than 10 mm was considered as a hit-compound [27].

Antitrypanosomal activity screening

Bloodstream forms of *Trypanosoma brucei brucei* strain 90-13 and *Trypanosoma brucei gambiense* Feo strain were cultured in HMI9 medium supplemented with 10 % FCS at 37 °C in an atmosphere of 5 % CO_2 [29]. In all experiments, log-phase cell cultures were harvested by centrifugation at 3000×g and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells [30]. Drug stock solutions were prepared in DMSO. *Trypanosoma brucei* bloodstream forms (10^5 cells/mL) were cultured in 96-well plates either in the absence or in the presence of different concentrations of inhibitors in a final volume of 200 mL. After the 72 h incubation, resazurin solution was added in each well at the final concentration of 45 mM and fluorescence was measured at 530 nm and 590 nm absorbance after further 4 h incubation. The percentage of inhibition of the parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that in the absence of drug. DMSO was used as a control. The concentration inhibiting 50 % of parasite growth (IC_{50}) was given as the mean +/- the standard deviation of three independent experiments.

Results and Discussion

Chemistry

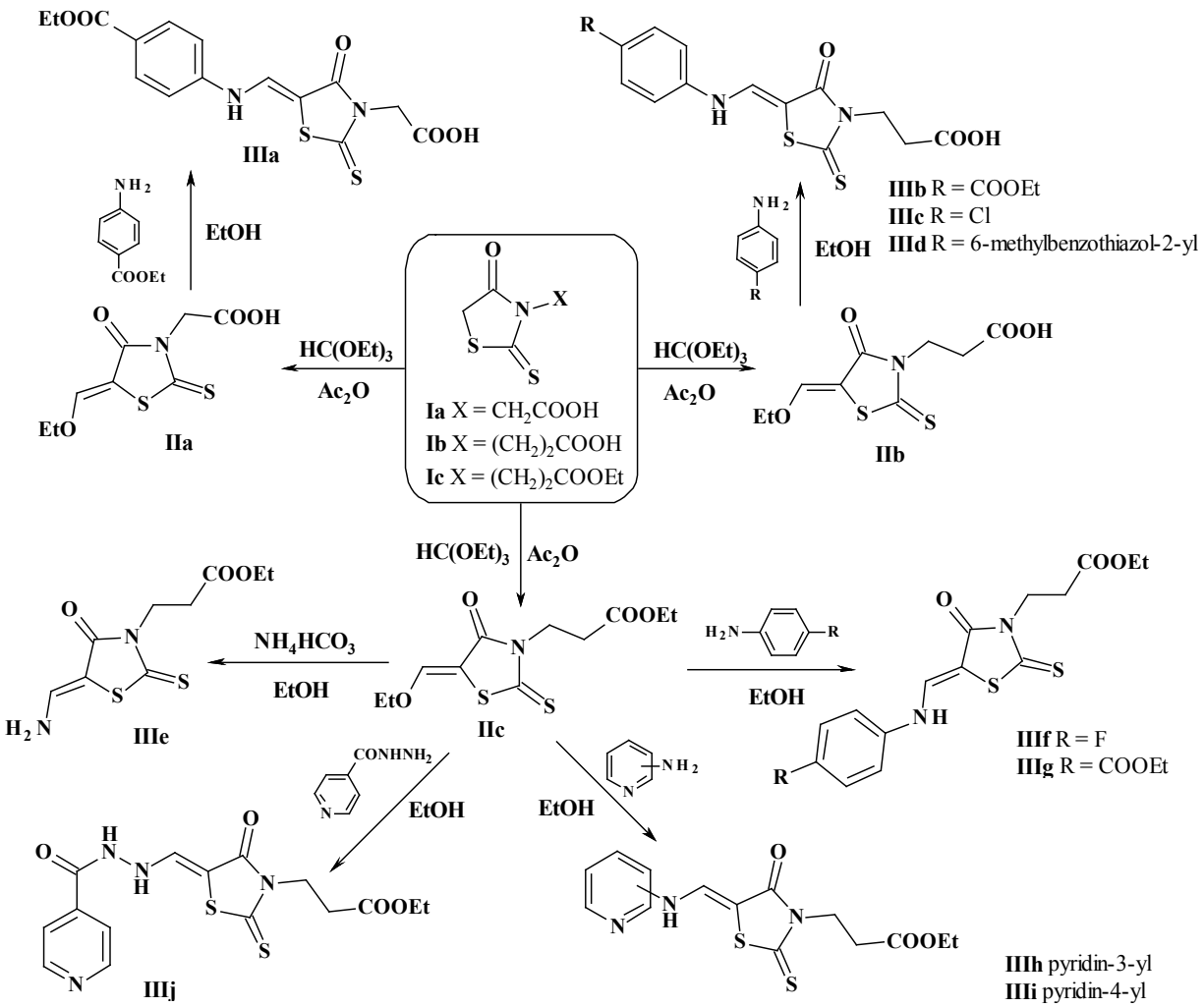
The synthesis of 3-carboxyalkylrhodanines (Scheme 1) was conducted according to the modified procedure proposed by Körner [31] from glycine or β-alanine and CS_2 in an alkaline medium. The next step involved [2+3]-cyclocondensation of formed dithiocarbamates (*S,N*-binucleophiles) with chloroacetic acid (equivalent to dielectrophilic synton $[C_2]^{2+}$) and yielded the corresponding 2-thioxo-4-thiazolidone-3-alkancarboxylic acids. Synthesis of 5-ethoxymethylidene derivatives **II** was performed in the reaction of rhodanine-3-carboxylic acids **Ia-b** and ethyl ester **Ic** with triethyl orthoformate in the acetic anhydride medium. There was observed the formation of the mixture of ethyl 3-[5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-yl]propanoate and an acid itself unlike in the same reaction of rhodanine-3-phenylpropionic acid, when only ethyl ester of 2-(5-ethoxymethylidene-2-(4-oxo-2-thioxo-thiazolidine-3-yl)-3-phenyl-propionic acid was formed [32]. Therefore, besides the method of column chromatography, to obtain pure compound **IIc**, an ethyl 3-(4-oxo-2-thioxothiazolidin-3-yl)propanoate **Ic** (synthesized in the reaction of esterification) was used as a starting agent in the reaction with triethyl orthoformate.

The 5-ethoxymethylenerhodanines can easily react with the *N*-nucleophilic reagents as primary or secondary amines. Thus, the target compounds **IIIa-IIId, IIIf-IIIg** were synthesized in the reactions of 5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-carboxylic acids **IIa,b** and ethyl 3-[5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-yl]propanoate **IIc** with

appropriate amines, such as 4-(ethoxycarbonyl)aniline, halogen-substituted anilines and 4-(6-methyl-1,3-benzothiazol-2-yl)aniline. The reaction of **IIc** with ammonium hydrogen carbonate in the ethanol medium yielded ethyl 3-[5-(aminomethylene)-4-oxo-2-thioxothiazolidin-3-yl]propanoate **(IIIe)**. To analyze the structure-activity relationship in this series of compounds the pyridine fragment was introduced in the aminomethylene group via the reaction of ethyl 3-[5-(aminomethylene)-4-oxo-2-thioxothiazolidin-3-yl]propanoate **IIc** with aminopyridines and 4-pyridinecarboxylic acid hydrazide.

Antibacterial and antifungal activity

Five of the synthesized compounds **III** were tested towards a series of Gram (+) and Gram



Scheme 1. Synthesis of 5-substituted derivatives of 2-(4-oxo-2-thioxothiazolidin-3-yl)-3-acetic and propanoic acid.

(–) bacteria and four yeasts strains (Table 1) at the concentration of 1mM (50 mcL per well). The synthesized compounds showed different mean zones of inhibition in the range of 0–15.0 mm against tested microorganisms. 5-Aminomethylenerhodanine-3-propanoic acid derivatives did not inhibited the growth of Gram (–) bacteria. Only two of the tested compounds **IIIb** and **IIIf** were active against clinical strains of Gram (+) *Staphylococcus simulans* and *Staphylococcus lentus* respectively. *Candida ssp*. turned out to be the most sensitive to the action of all studied compounds exhibiting the highest inhibition rates under the action of ethyl 3-[5-[(4-(fluoroanilino) methylene]-4-oxo-2-thioxothiazolidin-3-yl] propanoate **IIIf**. Substitution of the aryl moiety with pyridine fragment led to the total loss of antibacterial effects, although compounds **IIIh-IIIj** showed antifungal activity towards reference and clinical *Candida* strains. In general, the tested compounds are promising building scaffolds for the development of antifungal agents as all of them inhibited the growth of clinical strain of *Candida albicans*.

An interesting additional research of the pyridine containing compounds **IIIh-IIIj** was performed against *Trypanosoma ssp*. (Table 2) that corresponds to the concept of polypharmacological approach [33]. Noteworthy, such small molecules as the studied rhodanine analogs hold a privileged position for polypharmacology and may be successfully utilized in the fragment-based drug discovery too [34,35].

Table 1. **Antibacterial and antifungal activity of synthesized compounds**

Comp.	Diameter of zone inhibition, mm											
	Gram (–) bacteria				Gram (+) bacteria				Fungi			
	reference strains		clinical strains		reference strain	clinical strains		rarely found clinical strains	reference strain	clinical strains		rarely found clinical strain
	Pseudomonas aeruginosa (ATCC 27853, F-51)	*Escherichia coli* (ATCC 25922)	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus* (ATCC 25923, F-49)	*Staphylococcus lentus*	*Staphylococcus lugnuniensis*	*Staphylococcus simulans*	*Candida. albicans* (ATCC 885-653)	*Candida albicans*	*Candida dubliniensis*	*Candida membranifaciens*
IIIb	9	0	NT	0	0	0	0	10	8	12	0	10
IIIf	0	0	NT	0	0	10	0	0	7	15	11	10
IIIh	0	0	0	0	0	0	0	0	0	10	0	0
IIIi	0	0	0	0	0	0	0	0	12	10	0	0
IIIj	0	0	NT	0	0	0	0	0	0	10	0	10
DMSO	0	0	0	0	0	0	0	0	10	7	10	10

Table 2. **Antitrypanosomal activity of ethyl 3-[4-oxo-5-aminomethylene]-2-thioxothiazolidin-3-yl] propanoates IIIh-IIIj**

	Trypanosoma brucei brucei IC_{50}, μM	*Trypanosoma brucei gambiense* IC_{50}, μM	*Cytotoxicity on fibroblast* CC_{50}, μM	SI/Tbb	SI/Tbg
IIIh	19.19±6.33	5.03±1.85	182.41±12.36	9.5	36.2
IIIi	>148	21.04±0.42	155.00±29.33	<1	7.4
IIIj	>131	71.89±19.52	>262	1,0	>3.6
Pentamidine,nM	1.36±0.46	1.46±0.65	7900.00±282.84	5793.9	5396.9
Nifurtimox	2.39±0.61	4.64±0.73	65.09±2,95	27.2	14.0

The best *in vitro* inhibitory activity of *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense* was observed for ethyl 3-[4-oxo-5-[(3-pyridylamino)methylene]-2-thioxothiazolidin-3-yl]propanoate **IIIh** with the micromolar IC_{50} values comparable to the reference drugs Pentamidine and Nifurtimox. The selectivity indexes calculated as a ratio of cytotoxic concentration against normal fibroblasts CC_{50} to the antitrypanosomal IC_{50} values, indicate very good perspective for the study of rhodanine-3-carboxylic acids as potential antitrypanosomals with low toxicity parameters.

The established dual antitrypanosomal and antifungal actions are consistent with the recently developed new effective oral monotherapy of HAT with antifungal drug Fexinidazole [5].

Conclusions

A series of novel 3-[5-(aminomethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]carboxylic acids and their esters were designed and synthesized starting from 5-ethoxymethylene-rhodanine-3-carboxylic acids. Study on the antibacterial action of 5 compounds revealed a significant antifungal activity against the clinical strain of *Candida albicans* with the most active ethyl 3-[(5Z)-5-[(4-(fluoroanilino) methylene]-4-oxo-2-thioxo-thiazolidin-3-yl] propanoate **IIIf**. The pyridine-rhodanine-3-carboxylic hybrids showed good ratios of *Trypanosoma ssp*. inhibition and low cytotoxicity towards human fibroblasts. Such dual action of the rhodanine-3-carboxylic acids derivatives is an important issue that may be used in the polypharmacological approach as well as the fragment-based drug discovery.

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5-Ен-роданін-3-карбонові кислоти як потенційні протимікробні та протипаразитарні агенти

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Мета. Розробка, синтез та дослідження антибактеріальної, протигрибкової та трипаноцидної активності ряду нових 2-тіоксо-4-тіазолідинон-3-карбонових кислот з різними ариліденовими замісниками у положенні С5. **Методи.** Органічний синтез, аналітичні та спектральні методи, фармакологічний скринінг, SAR аналіз. **Результати.** Ряд 5-(амінометилен)-4-оксо-2-тіоксотіазолідин-3-ілкарбонових кислот та їх аналогів **IIIa-IIIj** синтезовано у реакціях 5-(етоксиметилен)-4-оксо-2-тиоксотіазолідин-3-ілкарбонових кислот **IIa,b** або етил 5- (етоксиметилен)-4-оксо-2-тіоксотіазолідин-3-ілпропаноату **IIc** із різноманітними амінами та гідрокарбонатом амонію. Для п'яти синтезованих сполук **IIIb**, **IIIf** та **IIIh-j** досліджувалася інгібуюча активність щодо ряду Грам (+) та Грам (–) бактерій та чотирьох штамів дріжджів у дозі 1 мМ. Загалом, досліджувані сполуки є перспективними будівельними блоками для розробки протигрибкових засобів, оскільки всі вони пригнічували ріст клінічного штаму *Candida albicans*. Крім того, для піридинвмісних 3-[5-(амінометилен)-роданін-3-іл]карбоксилати характеризувалися доброю трипаноцидною активністю та низькою цитотоксичністю щодо нормальних фібробластів. **Висновки.** Синтезовано ряд нових похідних 3-[5-(амінометилен)-

4-оксо-2-тіоксотіазолідин-3-іл]карбонових кислот. Вивчення їх антибактеріальної та протигрибкової дії дозволило виявити сполуку-хіт етиловий естер 3-[5-[(4-(флюороаніліно)метилен]-4-оксо-2-тіоксотіазолідин-3-іл]пропанової кислоти **IIIf**, що інгібував ріст клінічних штамів *Staphylococcus lentus* та *Candida ssp.* Загалом, більшість досліджуваних сполук виявляли добрі протигрибкові властивості.

Ключові слова: 2-тіоксо-4-тіазолідинон-3-карбонові кислоти, роданін, синтез, антитрипаносомна активність, антибактеріальна активність, протигрибкова активність, SAR.

5-Ен-роданин-3-карбоновые кислоты как потенциальные противомикробные и противопаразитарные агенты

А. П. Крищишин-Дилевич

Цель. Разработка, синтез и исследование антибактериальной, противогрибковой и трипаноцидной активности ряда новых 2-тиоксо-4-тиазолидинон-3-карбоновых кислот с различными арилиденовимы заместителями в положении С5. **Методы.** Органический синтез, аналитические и спектральные методы, фармакологический скрининг, SAR анализ. **Результаты.** Ряд 5-(аминометилен)-4-оксо-2-тиоксотиазолидин-3-илкарбонових кислот и их аналогов **IIIa-IIIj** синтезированы в реакциях 5- (етоксиметилен)-4-оксо-2-тиоксотиазолидин-3-илкарбонових кислот **IIa,b** или этил 5-(етоксиметилен)-4-оксо-2-тиоксотиазолидин-3-илпропаноата **IIc** с различными аминами и гидрокарбонатом аммония. Для пяти синтезированных соединений **IIIb, IIIf и IIIh-j** исследовалась ингибирующая активность в отношении ряда Грам (+) и Грам (–) бактерий и четырех штаммов дрожжей в дозе 1 мМ. Исследуемые соединения являются перспективными строительными блоками для разработки противогрибковых средств, поскольку все они подавляли рост клинического штамма *Candida albicans*. Кроме того, пиридинсодержащие 3-[5-(аминометилен)-роданин-3-ил]карбоксилаты характеризовались хорошей трипаноцидной активностью и низкой цитотоксичностью к нормальным фибробластам. **Выводы.** Синтезирован ряд новых производных 3-[5-(аминометилен)-4-оксо-2-тиоксотиазолидин-3-ил]карбоновых кислот. Изучение их антибактериальной и противогрибковой активности позволило выявить соединение-хит этиловый эфир 3-[5-[(4-(флюороанилино)метилен]-4-оксо-2-тиоксотиазолидин-3-ил]пропановой кислоты **IIIf**, который ингибировал рост клинических штаммов *Staphylococcus lentus* и *Candida ssp*. В целом, большинство исследуемых соединений проявляли хорошие противогрибковые свойства.

Ключевые слова: 2 тиоксо-4-тиазолидинон-3-карбоновые кислоты, роданин, синтез, антитрипаносомная активность, антибактериальная активность, противогрибковая активность, SAR.

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