Synthesis and antibacterial activity of new (2,4-dioxothiazolidin-5-yl/ylidene)acetic acid derivatives with thiazolidine-2,4-dione, rhodanine and 2-thiohydantoin moieties

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A B S T R A C T

A series of new (2,4-dioxothiazolidin-5-yl/ylidene)acetic acid derivatives with thiazolidine-2,4-dione, rhodanine and 2-thiohydantoin moiety (28–65) were synthesized by the reaction of (2,4-dioxothiazolidin-5-yl/ylidene)acetic acid chlorides with 5-(hydroxybenzylidene) thiazolidine-2,4-dione, rhodanine and 2-thiohydantoin derivatives. Obtained compounds (28–65) were tested on reference strains of Gram-positive bacteria and ones of the Gram-negative bacteria. The antibacterial activity of target compounds was determined by broth microdilution method. These derivatives showed antibacterial activity generally against Gram-positive bacterial strains. Most active compounds possess MIC = 3.91 mg/L. Our results suggest that presence of electron-withdrawing substituent at phenyl ring is favorable while geometry of molecule does not play important role in antibacterial response. It was confirmed the lack of direct influence of substitution pattern at phenyl ring on antibacterial activity of closely related compounds of series 1–3. The antibacterial activity of some compounds was similar or higher than the activity of commonly used reference drugs such as oxacillin and cefuroxime.

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1. Introduction

The increasing resistance of bacteria to currently available antibiotics is an extremely serious problem in the treatment of infections. In the world, the emergence of bacteria with multiple genes for resistance has been reported, which may result in insensitivity to all available classes of antibiotics. Therefore, search of new antibacterial agents and investigation of new targets for antimicrobial drugs is an alternative to existing antimicrobial drugs (Trojanowski et al., 2014).

Thiazolidine-2,4-diones is a well-known class of biologically active compounds due to the group of antidiabetic drugs (Pioglitazone, Rosiglitazone etc.). Besides, the thiazolidine-2,4-dione is the ring with wide application as biologically active substances. It possesses a broad spectrum of biological activity, including antibacterial (Heerding et al., 2003; Ibrahim et al., 2011; Liu et al., 2011; Bozdag-Dündar et al., 2007; Aneja et al., 2011; Purohit et al., 2012; Desai et al., 2014a, 2014b; Shaikh et al., 2013; Trotsko et al., 2017), anticancer (Liu et al., 2010; Patil et al., 2010; Salamone et al., 2012), anti-inflammatory (Koppireddi et al., 2013; Barros et al., 2010), antifungal (Tuncbilek and Alatanlar, 2006; Marc et al., 2017), antioxidant (Jeong et al., 2004).

One of the directions of the search for new bioactive compounds used in medicinal chemistry is combination two biologically active heterocyclic systems into single molecule. It is known that the combination of different pharmacophore or bioactive fragments with different mechanisms of the action often showed synergistic effects (Asati et al., 2014).

Such bioactive fragment may be a thiazolidine-2,4-dione and it structural analogues: rhodanine (2-thioxothiazolidine-4-one) and 2-thiohydantoin (2-thioxoimidazolidine-4-one) due to their broad spectrum of biological activity (anticancer (Moorthy et al., 2010; Min et al., 2013; Wu et al., 2015), anti-inflammatory (Cutshall et al., 2005; Irvine et al., 2008), anticonvulsant (Gangadhara et al., 2013), antiviral (Rajamaki et al., 2009; Jiang et al., 2011), antifungal...
2.2. 4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-methoxyphenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (31)

Yield 86%, mp = 230–233 °C. CAS Registry Number: 938,895-02-4. 1H NMR δ (ppm) (DMSO-d6): 3.42–3.44 m (2H, CH–CH2); 3.82 s (3H, OCH3); 4.83 dd (1H, CH–CH2, J = 5.3, 6.8 Hz); 7.17 dd, 7.26 d, 7.38 d (3H, C6H3, J = 1.8, 8.4 Hz); 7.80 s (1H, CH=); 12.35 bs (2H, 2NH, thiazolidine). 13C NMR δ (ppm) (DMSO-d6): 36.1; 46.9; 56.5; 115.2; 122.5; 124.0; 131.6; 132.8; 140.7; 151.5; 167.8; 168.3; 168.8; 172.7; 175.8. Anal. calc. for C16H12N2O8S2 (%): C 47.61; H 2.66; N 7.38.

2.2.5. 4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-ethoxyphenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (32)

Yield 61%, mp = 238–240 °C. CAS Registry Number: 938,740-47-3. 1H NMR δ (ppm) (DMSO-d6): 1.32 t (3H, OCH3J, J = 7.0 Hz); 3.42 d (2H, CH–CH2, J = 6.0 Hz); 4.05 q (2H, OCH2CH3, J = 7.0 Hz); 4.841 (1H, CH–CH2, J = 6.0 Hz); 7.16–7.36 m (3H, C6H3); 7.79 s (1H, CH=); 12.16 s, 12.57 s (2H, 2NH, thiazolidine). 13C NMR δ (ppm) (DMSO-d6): 14.8; 35.9; 46.7; 64.7; 115.9; 122.5; 123.9; 124.4; 131.6; 134.7; 149.0; 150.7; 167.7; 168.2; 170.8; 175.7. Anal. calc. for C21H14N2O8S2 (%): C 48.34; H 3.34; N 6.83. Found: c = 48.25; H: 3.33; N: 6.60.

2.2.6. 2-chloro-4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (33)

Yield 80%, mp = 234–236 °C. 1H NMR δ (ppm) (DMSO-d6): 3.51–3.54 m (2H, CH–CH2); 4.86 dd (1H, CH–CH2, J = 5.3, 6.7 Hz); 7.47 d, 7.59 dd, 7.86 d (3H, C6H3, J = 2.0, 8.5 Hz); 7.79 s (1H, CH=); 12.16 s, 12.68 s (2H, 2NH, thiazolidine). 13C NMR δ (ppm) (DMSO-d6): 36.0; 46.6; 125.4; 126.0; 127.1; 129.6; 132.8; 133.4; 147.5; 167.5; 167.9; 168.6; 172.5; 175.6. Anal. calc. for C16H14ClN2O8S2 (%): C 43.64; H: 2.20; N: 6.79. Found: C 43.66; H 2.18; N 6.77.

2.2.7. 2-bromo-4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (34)

Yield 82%, mp = 243–245 °C. 1H NMR δ (ppm) (DMSO-d6): 3.51–3.53 m (2H, CH–CH2); 4.87 dd (1H, CH–CH2, J = 5.5, 6.5 Hz); 7.46 d, 7.64 dd, 7.99 d (3H, C6H3, J = 2.1, 8.4 Hz); 7.87 s (1H, CH=); 12.34 bs (2H, 2NH). 13C NMR δ (ppm) (DMSO-d6): 36.2; 46.6; 116.8; 125.3; 126.2; 129.6; 130.1; 133.8; 148.8; 167.8; 168.1; 168.7; 172.5; 175.7. Anal. calc. for C16H14BrN2O8S2 (%): C 39.40; H 1.98; N: 6.13. Found: C 39.31; H 1.94; N 6.11.

2.2.8. 2-[(4-oxo-2-thio-1,3-thiazolidin-5-ylidene)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (35)

Yield 73%, mp = 212–214 °C. 1H NMR δ (ppm) (DMSO-d6): 3.52–3.55 m (2H, CH–CH2); 4.877 (1H, CH–CH2, J = 6.6 Hz); 7.31–7.35 m, 7.47–7.59 m (5H, C6H4–CH=); 12.14 s (1H, NH, thiazolidine). 13C NMR δ (ppm) (DMSO-d6): 36.4; 46.7; 124.0; 124.7; 127.4; 129.1; 132.5; 149.7; 169.4; 169.7; 172.5; 175.8; 196.1. Anal. calc. for C16H14N2O8S3 (%): C 45.68; H 2.56; N: 7.10. Found: C 45.61; H 2.55; N 7.02.

2.2.9. 3-[(4-oxo-2-thio-1,3-thiazolidin-5-ylidene)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (36)

Yield 74%, mp = 207–209 °C. CAS Registry Number: 938,740-53-5. 1H NMR δ (ppm) (DMSO-d6): 3.45–3.48 m (2H, CH–CH2); 4.85
13C NMR δ (ppm) (DMSO-d6): 36.5; 46.9; 56.6; 111.4; 123.4; 128.3; 132.1; 140.1; 151.2; 166.2; 168.8; 172.7; 175.8; 179.8. Anal. calc. for C17H15N3O6S2 (%): C 47.17; H 3.22; N 10.31. Found: C 46.99; H 3.17; N 10.34.

12.19. 2-ethoxy-4-[(5-oxo-2-thioxoimidazolidin-4-ylidene)methyl]phenyl methyl phenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (46)

Yield 65%, mp = 224–224 °C. 1H NMR δ (ppm) (DMSO-d6): 3.13 t (3H, OCH2CH2), 3.86 s (3H, OCH3), 4.83 dd (1H, CH–CH2–J = 8.4, 6.6 Hz); 7.19 d, 7.24 d, 7.26 d (3H, C6H3, J = 8.4 Hz); 7.67 s (1H, CH=); 12.17 s (1H, NH, rhodanine). 13C NMR δ (ppm) (DMSO-d6): 36.1; 46.6; 125.5; 127.2; 128.1; 129.4; 130.0; 132.8; 133.4; 147.7; 168.6; 170.2; 172.5; 175.7; 195.9. Anal. calc. for C17H15N3O6S2 (%): C 45.67; H 3.22; N 6.39. Found: C 46.61; H 3.18; N 6.37.

12.13. 3-chloro-4-[(4-oxo-3-thiazolidinyl)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (40)

Yield 81%, mp = 228–231 °C. 1H NMR δ (ppm) (DMSO-d6): 3.52–3.55 m (2H, CH–CH2), 4.87 dd (1H, CH–CH2–J = 5.2, 6.9 Hz); 7.49 d, 7.59 dd, 7.89 d (3H, C6H3, J = 2.1, 8.4 Hz); 7.65 s (1H, CH=); 12.17 s (1H, NH, rhodanine). 13C NMR δ (ppm) (DMSO-d6): 36.1; 46.6; 125.5; 127.2; 128.1; 129.4; 130.0; 132.8; 133.4; 147.7; 168.6; 170.2; 172.5; 175.7; 195.9. Anal. calc. for C17H15N3O6S2 (%): C 42.01; H 2.12; N 6.53. Found: C 41.83; H 2.01; N 6.55.

12.14. 2-bromo-4-[(4-oxo-3-thiazolidinyl)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (41)

Yield 79%, mp = 225–226 °C. 1H NMR δ (ppm) (DMSO-d6): 3.51 d (2H, CH–CH2–J = 5.75 Hz); 4.89 t (1H, CH–CH2–J = 5.75 Hz); 7.46 d, 7.62–7.66 m, 8.01 d (4H, CH2–CH2–J = 2.0, 8.5 Hz); 12.17 s (1H, NH, rhodanine). 13C NMR δ (ppm) (DMSO-d6): 36.3; 46.6; 117.0; 125.4; 128.0; 129.7; 130.5; 133.7; 135.9; 149.4; 168.0; 169.9; 172.5; 175.7; 195.9. Anal. calc. for C17H15BrN3O6S2 (%): C 38.06; H 1.92; N 5.92. Found: C 38.08; H 1.91; N 5.91.

12.2. 4.4-[(4-oxo-2-thioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-yl)acetate (37)

Yield 76%, mp = 223–236 °C. CAS Registry Number: 938,740-45-5. 1H NMR δ (ppm) (DMSO-d6): 3.42–3.44 m (2H, CH–CH2), 3.38 s (3H, OCH3), 4.83 dd (1H, CH–CH2–J = 5.3, 6.8 Hz); 7.18 dd, 7.27 d, 7.38 d (3H, C6H3, J = 1.8, 8.4 Hz); 7.65 s (1H, CH=); 12.13 s (1H, NH, rhodanine). 13C NMR δ (ppm) (DMSO-d6): 36.0; 46.8; 56.5; 115.5; 123.1; 124.2; 126.6; 131.2; 132.8; 140.9; 151.6; 168.7; 170.2; 172.6; 175.7; 196.3. Anal. calc. for C17H16N3O6S2 (%): C 46.58; H 2.56; N 7.10. Found: C 45.62; H 2.49; N 7.03.
bs (2H, 2NH). 13C NMR δ (ppm) (DMSO-d6): 115.9; 123.9; 124.6; 126.1; 127.3; 127.9; 128.9; 132.2; 146.4; 149.3; 164.1; 166.6; 167.5; 168.1; 169.3. Anal. calc. for C13H12N2O5S (S2 %): C 47.87; H 2.14; N 7.44. Found: C 47.92; H 2.11; N 7.34.

2.2.22. 3-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-ylidene)acetate (54)
Yield 83%, mp = 278–279 °C. 1H NMR δ (ppm) (DMSO-d6): 7.12 s (1H, =CH–COO); 7.56 d, 7.65 dd, 8.01 d (3H, C6H3, J = 2.0, 8.5 Hz); 7.81 s (1H, CH=); 12.67 bs (2H, 2NH). 13C NMR δ (ppm) (DMSO-d6): 115.5; 116.7; 125.3; 126.1; 129.8; 130.3; 134.0; 135.4; 146.9; 148.7; 163.4; 166.3; 167.5; 168.0; 169.0. Anal. calc. for C13H12N2O5S (S2 %): C 43.86; H 1.72; N 6.82. Found: C 43.77; H 1.68; N 6.79.

2.2.23. 4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl (2,4-dioxo-1,3-thiazolidin-5-ylidene)acetate (55)
Yield 86%, mp = 240–242 °C. 1H NMR δ (ppm) (DMSO-d6): 7.19 s (1H, =CH–COO); 7.44–7.67 m (5H, C6H4, CH3); 13.54 s (2H, 2NH). 13C NMR δ (ppm) (DMSO-d6): 116.1; 124.0; 124.1; 126.0; 128.1; 129.1; 129.4; 132.5; 146.2; 149.5; 164.1; 166.4; 169.2; 169.6; 196.0. Anal. calc. for C16H12N2O4S (S3 %): C 45.91; H 2.05; N 7.14. Found: C 45.83; H 1.97; N 7.11.
Klebsiella pneumoniae
Pseudomonas aeruginosa
Bacillus cereus
ATCC 6633, ATCC 6538, S. calc. for C17H13N3O6S2 (%): C 48.68; H 3.12; N 10.02. Found: C 48.73; H 3.09; N 9.97.

Staphylococcus aureus bacteria (Culture Collection (ATCC) were used in the study: Gram-positive bacteria (Deghenghi and Daneault, 1960). The acids were prepared from acetic acid chlorides (3). 1H NMR (ppm) (DMSO-\(\text{d}_{6}\)): 1.25 t (3H, OCH2CH3, J = 6.9 Hz); 4.11 q (2H, OCH2CH3, J = 6.9 Hz); 6.50 s (1H, CH=);introduction of thiazoledione, rhodanine and 2-oxo-

Microbiology tests

The following reference strains of bacteria from American Type Culture Collection (ATCC) were used in the study: Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 12228, Bacillus subtilis ATCC 6633, Bacillus cereus ATCC 10876, Micrococcus luteus ATCC 10240) and Gram-negative bacteria (Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 13883, Proteus mirabilis ATCC 12453, Pseudomonas aeruginosa ATCC 9027). Microbial suspensions were prepared in sterile 0.85% NaCl with an optical density of 0.5 McFarland standard – 150 × 10^6 CFU/mL (CFU - colony forming units).

All stock solutions of detected compounds were dissolved in dimethyl sulfoxide (DMSO) at final concentration with no inhibitory effect on the growth of bacteria (negative control). The medium with DMSO at the final concentration and without the tested compounds served as negative control. A ciprofloxacin and oxacillin were used as reference antimicrobials.

The tested compounds (28–65) antibacterial activity was assayed in two steps. Firstly, it was screened using the agar dilution method on the basis of the microbial growth inhibition. Preliminary antibacterial effect of all compounds was screened on the Petri plates with the Mueller-Hinton agar medium with the tested compounds at concentrations 1000 mg/L. Then the antibacterial activity of the selected compounds with inhibitory effect with 1000 mg/L concentration was determined by broth microdilution technique using 96-well microplates with series of twofold dilution of the tested compounds, as well as the ciprofloxacin and oxacillin in the range of final concentrations from 0.007 to 1000 mg/L, according to described earlier (Trotsko et al., 2012).

The activity was expressed as the minimal inhibitory concentration (MIC) of the compound that inhibits the visible growth of the bacteria. MIC was assayed spectrophotometrically by optical density determination (OD_{500}) using a broth microdilution technique. The MBC (minimal bactericidal concentration), defined as the lowest concentration of each compound that resulted in >99.9% reduction in CFU of the initial inoculum, was also determined. MBC was determined by plating out the contents of wells (5 µL) that showed no visible growth of bacteria, onto Mueller-Hinton agar plates and incubating at 35°C for 18 h. The compounds were classified as bacteriostatic when the MBC/MIC ratio was greater than or equal to 8 and bactericidal when the MBC/MIC ratio is less than or equal to 4 (Jones, 2006).

2.4. Computational part

Conformational search was performed using the RM1 semiempirical parametrization as implemented in HyperChem 8.0.3 (2007) and default convergence criteria.

3. Results and discussion

3.1. Chemistry

In the present research as a starting material were (2,4-dioxo-thiazolidin-5-yl)acetic acid (1) and (2,4-dioxothiazolidin-5-ylidene)acetic acid (2). The (2,4-dioxothiazolidin-5-yl)acetic acid was synthesized by the reaction of cyclocondensation of thiourea with maleic anhydride in presence of concentrated hydrochloric acid (Lesyk et al., 2001). (2-Dioxothiazolidin-5-ylidene)acetic acid (2) was prepared by the reaction of compound (1) with bromine in acetic acid medium (Deghenghi and Daneault, 1960). The acids (1, 2) by the reaction with thionyl chloride in anhydrous 1,4-dioxane medium were transformed into acid chlorides (3, 4). Scheme 1 illustrates reactions’ pathway.

Next step of synthesis was obtaining of 5-benzylidene derivatives of thiazolidine-2,4-dione, rhodanine and 2-thiohydantoin. These compounds (8–27) were synthesized by Knoevenagel condensation of thiazolidine-2,4-dione, rhodanine and 2-thiohydantoin with corresponding hydroxymethylhydrazides. The reactions are shown in Scheme 2.

Final step of synthesis was a connection 5-benzylidene derivatives (8–27) with acetic acids (3, 4). Target compounds (28–65) were prepared from acetic acid chlorides (3, 4) and series of hydroxymethylidene derivatives of thiazolidine-2,4-dione, rhodanine and 2-thiohydantoin with corresponding hydroxymethylhydrazides. The synthesis of these compounds (28–65) was achieved through synthetic route outlined in Scheme 3.

The structure of target compounds (28–65) was confirmed by elemental analysis, \(^\text{1}H\) NMR and \(^{13}C\) NMR spectra.

The fragment CH2CH of compounds (28–47) that are derivatives of 2-(2,4-dioxothiazolidin-5-yl)acetic acid appeared on \(^1H\) NMR spectra as two multiplets in 3.41–3.61 and 4.83–4.97 ppm ranges. For the compounds 32, 39, 41, 42, 46 and 47 signals of protons of 28
the fragment CH₂CH were visible as doublet in 3.43–3.52 ppm and triplet in 4.84–4.91 ppm ranges.

The proton CH⁻ benzylidene group appeared in the 7.55–7.87 ppm range as singlet. For the 2-thiohydantoin derivatives (42–47 and 62–65) proton of CH⁻ group was visible at δ ~ 6.34–6.56 ppm.

Protons signals of CH⁻A COO group of compounds 48–65 were visible as a singlet at 7.06–7.21 ppm range.

Protons of NH group of heterocyclic rings appeared in the spectra as singlet or broad singlet in the range 12.06–13.63 ppm. For the compounds (35–41) that are rhodanine derivatives, proton signal of NH group was observed at 13.78–13.92 ppm range.

The presence of all carbon atoms for compounds (28–65) is confirmed by ¹³C NMR spectra. For the compounds 28–47, that are derivatives of 2-(2,4-dioxothiazolidin-5-yl)acetic acid, carbon signal of all C=O groups appeared in the 167.4–176.0 ppm region. Signals of all C=O groups for the 2-(2,4-dioxothiazolidin-5-ylidene) acetic acid derivatives 48–65 were visible at δ ~ 163.3–169.3 ppm range. Signals of C=S groups of rhodanine ring (35–41 and 55–61) were observed at 195.7–196.3 ppm range but 2-thiohydantoin derivatives (42–47 and 62–65) signals of C=S groups appeared in the 179.8–180.1 ppm region.

The detailed results of ¹H NMR and ¹³C NMR spectra are presented in the experimental part.

3.2. Antimicrobial activity

Using the agar broth dilution method, it was shown that most tested compounds inhibited the growth of one to several reference species of bacteria at 1000 mg/L concentration. Only 32, 46, 48–50 and 55 compounds had no activity against all the tested bacteria.

Next, the compounds with potential inhibitory effect against bacteria was determined using broth dilution method. The variable antibacterial in vitro activity against the growth of the tested reference species of Gram-positive bacteria was shown as both concentration and species dependent (Table 1). The tested derivatives had mainly bacteriostatic effect (MBC/MIC > 4) towards the sensitive bacteria.

Among Gram-negative species only P. mirabilis ATCC 12453 had moderate sensitivity on 35, 44, 45 compounds with MIC = 125 mg/L, and on 62 and 63 compounds with MIC = 250 mg/L. None of the tested compound had inhibitory effect against the growth of Gram-negative E. coli, K. pneumoniae and P. aeruginosa reference species.
In the absence of detailed information about the molecular target, in order to identify the privileged scaffold for antibacterial activity among various thiazolidine-2,4-dione-based derivatives, we selected five compounds representing series of thiazolidine-2,4-dione-phenyl-thiazolidine-2,4-dione hybrids (series 1). As indicated from results collected in Table 1, the compound 30 was inactive against all tested Gram-positive bacteria. Adding electron-donating methoxy group to phenyl ring provided compound 31 with potent activity against *S. epidermidis* (MIC 15.63 mg/L) and mild to weak inhibitory activity against remaining Gram-positive bacteria (MICs range from 62.5 to 250 mg/L). Increasing the carbon chain from methyl to ethyl (compound 32) completely reduced the activity. In turn, the replacement of methoxy group in 31 with electron-withdrawing chloro group as in 33 improved the activity by twofold against *S. aureus* and fourfold against *B. subtilis*. Important to note, 33 inhibited the growth of *B. cereus* at MIC of 3.91 mg/L thereby indicating more effective action than those standard drugs oxacillin and cefuroxime. Finally, incorporation of bromo group within phenyl ring furnished compound 34 with the best activity among all compounds series 1. Indeed, as seen from results collected in Table 1, 34 exhibited antibacterial potency at MIC of 3.91 and 7.81 mg/L against *S. aureus* and *S. epidermidis* reference strains. Moreover, it had activity equipotent to that of oxacillin and cefuroxime against *B. cereus*. These results collectively suggest that the presence of electron-withdrawing substituent at phenyl ring is favorable while geometry of molecule does not play important role in antibacterial response. Indeed, as illustrated in Fig. 1 for representative model compounds, the superposition of the most stable conformers of active and inactive revealed only minor deviations. Taking into consideration the results highlighted above, the next set of structures included adding double bond between thiazolidine-2,4-dione and ester core. Hence, compounds 50–54 (series 2) were synthesized. Within series 2 the best antibacterial response was noted both for compounds with electron-donating ethoxy group 52 and for ones with electron-withdrawing bromo group 54 with MICs in the range from 7.81 to 125 mg/L against all Gram-positive bacterial strains tested, following by 53 with MICs at 31.25 mg/L against *S. aureus* and *S. epidermidis* and 51 with...
Table 1
The antibacterial activity of (2,4-dioxo-1,3-thiazolidin-5-yl)acid acetate derivatives (28–65).

| Compound | Gram-positive bacteria | Gram-negative bacteria |
|----------|------------------------|------------------------|
|          | S. aureus 6538         | S. aureus 25,923       | S. epidermidis 12,228 | B. subtilis 6633 | B. cereus 10,876 | M. luteus 10,240 | P. aeruginosa 1,2453 |
|          | MIC mg/L               | MBC µM                | MIC mg/L               | MBC µM                | MIC mg/L               | MBC µM                | MIC mg/L               | MBC µM                |
| 28       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 29       | >2642.8                | >2642.8                | >2642.8                | >2642.8                | >2642.8                | >2642.8                | >2642.8                | >2642.8                |
| 30       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 31       | 125                    | 250                   | 250                    | 250                    | 250                    | 250                    | 250                    | 250                    |
| 32       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 33       | 125                    | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 34       | 3.91                   | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 35       | >2186.9                | >2186.9                | >2186.9                | >2186.9                | >2186.9                | >2186.9                | >2186.9                | >2186.9                |
| 36       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 37       | 62.5                   | 125                   | 125                    | 125                    | 125                    | 125                    | 125                    | 125                    |
| 38       | 147.2                  | >294.5                | >294.5                 | >294.5                 | >294.5                 | >294.5                 | >294.5                 | >294.5                 |
| 39       | >125                   | >62.5                 | >62.5                  | >62.5                  | >62.5                  | >62.5                  | >62.5                  | >62.5                  |
| 40       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 41       | 3.91                   | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 42       | >2112.6                | 31.0                   | 1056.3                 | 31.0                   | 1056.3                 | 31.0                   | 1056.3                 | 31.0                   |
| 43       | >2649.7                | 2649.7                 | >1000 nd               | >2649.7                | >1000 nd               | >2649.7                | >1000 nd               | >2649.7                |
| 44       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 45       | >2454.5                | >2454.5                | >2454.5                | >2454.5                | >2454.5                | >2454.5                | >2454.5                | >2454.5                |
| 46       | 7.81                   | 31.25                  | 100                    | 31.25                  | 100                    | 31.25                  | 100                    | 31.25                  |
| 47       | >17.1                  | >2649.7               | >1324.9                | >2649.7                | >1656.6                | >2649.7                | >1324.9                | >2649.7                |
| 48       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 49       | >2649.7                | >2649.7                | >2649.7                | >2649.7                | >2649.7                | >2649.7                | >2649.7                | >2649.7                |
| 50       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 51       | >2443.2                | 43.3                   | >2434.7                | 76.1                   | >2434.7                | 304.3                 | >2434.7                | 304.3                 |
| 52       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 53       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 54       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 55       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 56       | 31.25                  | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 57       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 58       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 59       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 60       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 61       | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 62       | 3.91                   | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               | >1000 nd               |
| 63       | >2212.7                | 2121.7                 | >2121.7                | 265.2                  | >2121.7                | 326.2                 | >2121.7                | 326.2                 |
| 64       | >2384.2                | 2384.2                 | >2384.2                | 74.5                   | >2384.2                | 1192.5                | >2384.2                | 1192.5                |
| 65       | >2201.3                | >2201.3                | >2201.3                | >2201.3                | >2201.3                | >2201.3                | >2201.3                | >2201.3                |

(continued on next page)
Abbreviations: Sa - Staphylococcus aureus; Se - Staphylococcus epidermidis; Bs - Bacillus subtilis; Bc - Bacillus cereus; Ml - Micrococcus luteus; Pm - Proteus mirabilis

**Table 1 (continued)**

| Compound | Gram-positive bacteria | Gram-negative bacteria |
|----------|------------------------|------------------------|
|          | Sa ATCC 6538 | Sa ATCC 25,923 | Se ATCC 12,228 | Bs ATCC 6633 | Bc ATCC 10,876 | Ml ATCC 10,240 | Pm ATCC 12,453 |
|          | MIC (µg/L) | MBC (µg/L) | MIC (µg/L) | MBC (µg/L) | MIC (µg/L) | MBC (µg/L) | MIC (µg/L) | MBC (µg/L) |
| Oxacillin | 0.06 | 0.06 | nd | nd | 0.12 | 0.12 | 0.06 | 0.12 | 62.5 | 62.5 | 0.98 | 0.98 | 0.24 | 0.49 |
| Ciprofloxacin | 0.49 | 0.49 | nd | nd | 0.49 | 0.49 | 0.015 | 0.12 | 0.12 | 0.12 | 0.98 | 1.95 | 0.015 | 0.24 |
| Cefuroxime | 1.5 | 1.5 | 0.49 | nd | 0.49 | 0.49 | 0.05 | 0.4 | 0.4 | 0.4 | 3.0 | 5.9 | 0.05 | 0.7 |

**Fig. 1.** Superposition diagram of compounds active (34) and inactive (30) (left) and active (54) and inactive (50) (right). Remaining compounds within series 1, 4 and 5 share geometry of molecule very similar to that of compounds 34 and 30 while all compounds series 2 and 3 share geometry of molecule very similar to that of compounds 54 and 50.

MIC = 31.25 mg/L against *B. cereus*. Again, no antibacterial response was detected for compound without substitution at phenyl ring (50) suggesting that such core is not tolerated. The second important result of these studies is that geometry of molecule is not detrimental for activity; in spite of the fact that compounds of series 2 differ in the geometry of the molecule from the compounds of series 1, their activity is not uniquely favorable.

Subsequently, series 3, structurally very similar to that of series 2 was synthesized. The compounds were obtained by replacement thiazolidine-2,4-dione with rhodanine ring. Again, the observed trend in antibacterial activity cannot be not easily explain. In contrast to series 2, within this chemical series the best antibacterial response (MIC = 7.81 mg/L) was detected for compound with electron-withdrawing chloride substitution (60). However, the potent antibacterial effect for 60, even comparable to oxacillin and cefuroxime, was observed only against *S. aureus* and *B. cereus*; remaining Gram-positive bacterial strains were able to grow at high concentrations (MICs from 31.25 to 62.50 mg/L) or even were almost insensitive to 60 (MICs = 250 mg/L). In turn, compound 58 with electron-donating methoxy substitution showed fourfold better activity than oxacillin and twofold better than cefuroxime against *B. cereus*. The same level of its activity with MIC at 15.63 mg/L was also observed against *M. luteus*. In contrast to series 1, increasing the carbon chain from methyl to ethyl gave compound 59 with comparable activity to 58; MICs in the range from 15.63 to 62.5 mg/L. Surprisingly, for the first time antibacterial response was also observed for derivative with phenyl core, compound 57. Important to note, although activity of 57 was not impressive, MICs in the range from 31.25 to 125 mg/L, it was still comparable to these obtained for 61 with bromo substitution. This is very important result, because it further confirms the lack of direct influence of substitution pattern at phenyl ring on antibacterial activity of closely related compounds of series 1–3. Unfortunately, the results for series 4 exclude the direct relationship between geometry and antibacterial activity as well. Indeed, although two compounds of series 4 were inactive (37 and 40) and two other had only marginal activity (38 and 39; MICs at 62.5 mg/L or higher), this series still contains bromo derivative 41 ranked among the most potent antibacterial agents tested so far. It is important to note that also within series 1, that is structurally closely related to series 4, the best antibacterial activity was found also for bromo derivative 34.

Next, the rhodanine ring of series 4 was replacement with 2-thiohydantoin ring and compounds series 5 were obtained. Similar to the SAR with series 1 and 4, compound with bromo substitution 47 was effective against most of the Gram-positive strains tested with MICs in the range from 3.91 to 31.25 mg/L. Remaining compounds with this chemical series were inactive even at high concentration. A point worth highlighting is that adding double bond to compound 47 significantly reduced antibacterial activity of the compound 65 (MICs = 500–1000 mg/L), which is consistent with the results highlighted above.

Finally, in order to clearly exclude phenyl core from future research, two series of regiosomers, series 6 with meta substitution and series 7 with ortho substitution were obtained and subsequently tested. As we expected, with the exception of 56 with MICs in the range from 7.81 to 15.63 mg/L against most of the tested Gram-positive bacteria and 43 with marginal activity, no antibacterial response was observed.

To the same conclusion are provided by the analysis of structure-activity relationships of studied compounds in terms of their micromolar concentrations.

### 4. Conclusions

A series of new thiazolidine-2,4-dione-phenyl-azoles hybrids were synthesized. These derivatives were assayed for antibacterial activity. Most tested compounds were shown antibacterial activity against Gram-positive bacteria. As a result of antibacterial evaluation our research showed that the presence of electron-
withdrawing substituent at phenyl ring for thiazolidine-2,4-dione-phenyl-azoles hybrids is favorable while geometry of molecule does not play important role in antibacterial response. The second important result of these studies is that geometry of molecule is not detrimental for activity. Further confirmed the lack of direct influence of substitution pattern at phenyl ring on antibacterial activity of closely related compounds of series 1–3. Unfortunately, the results for series 4 exclude the direct relationship between geometry and antibacterial activity as well. The antibacterial activity of some compounds was similar or higher than the activity of

Conflic of interest

None.

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