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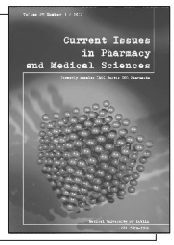


The antibacterial activity of allyl derivatives of thiosemicarbazide, N^1-thiocarbamylamidrazone, 1,2,4-triazole-5-thione, 1,3,4-(thiadiazol-2-yl) amine and derivatives of bicyclic systems: 1,2,4-triazole[3,4-b]1,3-thiazine, 1,2,4-triazole[3,2-b]1,3-thiazine, 1,3,4-thiadiazole[3,2-a]pyrimidine, thiazolo[2,3-c][1,2,4]triazole

LEOKADIA STRZEMECKA[1*], URSZULA KOSIKOWSKA[2], ANNA MALM[2]

[1] Chair and Department of Organic Chemistry, Faculty of Pharmacy with Medical Analytics Division, Medical University of Lublin, Poland
[2] Chair and Department of Pharmaceutical Microbiology, Faculty of Pharmacy with Medical Analytics Division, Medical University of Lublin, Poland

ABSTRACT

The 1-acyl(aroil)-4-(allyl)cinnamyl-thiosemicarbazides (**1-9**), N^1-allyl(cinnamyl)-thiocarbamyl-N^3-phenyl-amidrazones (**10-12**), allyl derivatives of 1,2,4-triazole-5-thione (**13-23**), 1,3,4-(thiadiazol-2-yl) amine (**24-29**), derivatives of bicyclic systems: 1,2,4-triazole[3,4-b]1,3-thiazine (**30-34**), 1,2,4-triazole[3,2-b]1,3-thiazine (**35**), 1,3,4-thiadiazole[3,2-a]pyrimidine (**36, 37**), hydrazide derivatives of substituted benzoic acid (**38**, **39**), 3,4-disubstituted-3,4,5,6-tetrahydropyrimidine-2-thione-(2-thiol) (**40**) and thiazolo[2,3-c][1,2,4]triazole (**41-43**) were screened for their *in vitro* activity against the reference strains of Gram-positive and Gram-negative bacteria. The compounds (**11**, **12**, **20**, **37**) showed moderate activity against Gram-positive bacterial species with MIC = 31.25 – 62.5 μg/mL and may be of value for searching new derivatives showing better antimicrobial activity. The structure-activity relationship has been discussed.

Keywords: allyl derivatives of thiosemicarbazide, N^1- thiocarbamyl-N^3-phenyl-amidrazones, 1,2,4-triazole-5-thione, 1,3,4-(thiadiazol-2-yl)-amine and bicyclic systems: 1,2,4-Triazole[3,4-b]1,3-thiazine, 1,2,4-triazole[3,2-b]1,3-thiazine, thiazolo[2,3-c][1,2,4]triazole. The antimicrobial activity

INTRODUCTION

The compounds bearing five-membered heterocyclic ring of 1,2,4-triazole-5-thione and 1,3,4-thiadiazole possess a wide spectrum of activity. Very often the starting material for the synthesis of these compounds were the 1,4-disubstituted thiosemicarbazide derivatives.

The 1,4-disubstituted thiosemicarbazide, 1,2,4-triazole and 1,3,4-thiadiazole were reported to possess a variety of pharmacological activities such as antibacterial [1, 4, 7, 9, 12, 14], antifungal [9, 12, 8], antiviral, antitubercular [16, 10], anticancer [5]. The literature survey revealed the anticonvulsant [12], antiinflammatory [7, 1, 14], analgesic [14] properties of these compounds. To compare the antimicrobial activity of the 1,4-disubstituted thiosemicarbazides and their cyclized derivatives – 1,2,4-triazole and 1,3,4-thiadiazole the biological investigations for these compounds were carried out simultaneously [1, 4, 7-9, 12, 14]. The literature also states that the antibacterial and antiviral activities of thiourea derivatives are due to the presence of the –NH-C(S)-NH function in the molecule and the changes in this activity depend on the nature of its substituents [13, 6].

The proteins, nucleic acids, sugars and lipids - the active structures – are the main places of the actions of the drug. The electrostatic and the inductive interactions are among the other important interactions between the medicine and the active structure. Due to these interactions, the ionic and the hydrogen bonds between active structure-drug are possible to form.

In this paper the *in vitro* activity of the series of 1-acyl (aroil)-4-(allyl)cinnamyl-thiosemicarbazides, N^1-[(allyl)cinnamyl–thiocarbamyl]-N^3-phenyl-amidrazones, allyl derivatives of 1,2,4-triazole-5-thione, 1,3,4-(thiadiazol-2-yl) amine, hydrazide derivatives of substituted benzoic acid and derivatives of bicyclic systems: 1,2,4-triazole[3,4-b]1,3-thiazine, 1,2,4-triazole[3,2-b]1,3-thiazine, 1,3,4-thiadiazole[3,2-a]pyrimidine and thiazolo[2,3-c][1,2,4]triazole against some species of Gram-positive and Gram-negative bacteria has been investigated. Derivatives of bicyclic systems: 1,2,4-triazole[3,4-b]1,3-thiazine, 1,2,4-triazole[3,2-b]1,3-thiazine and thiazolo[2,3-c][1,2,4]triazole show moderate antiHIV activity [27].

Corresponding author
* Chair and Department of Organic Chemistry, Faculty of Pharmacy with Medical Analytics Division, Medical University of Lublin, 4a Chodźki Str., 20-093 Lublin, Poland
e-mail: leokadia.strzemecka@am.lublin.pl

The derivatives of 1-acyl(aroil)-4-(allyl)cinnamyl-thiosemicarbazides and N^1-[(allyl)cinnamyl–thiocarbamyl]-N^3-phenyl-amidrazones due to the presence of R-C-NH-R' or R-C=NR', R–C=O, -NH-NH-C(S)-NH, $–CH_2$-CH=CH-R groups in their structure may appear in different tautomeric and ionic forms. The structural studies of cinnamyl derivatives were described earlier [17, 18]. Due to SH, OH, R-C-NH-R', R-C=NR' group or heterocyclic pyridine ring in the molecules of the discussed compounds they may be involved in the metabolic processes of bacteria and may show the bacteriostatic or the bactericidal antibacterial activity.

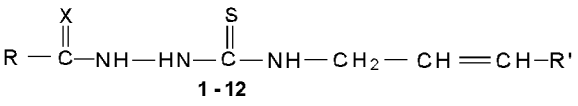
The literature survey revealed that the bioactivity of derivatives of 1,2,4-triazole depends on the substituents at 3, 4, 5 positions. The lack of the substituent at the nitrogen N4 or the presence of aryl and alkyl substituents at 3 and 4 positions, respectively [1] increases the activity. The incorporation of benzofurane [2], 1,2,4-triazole, tetrazole [12], imidazole [15] systems into the moiety containing the 1,2,4-triazole ring augments considerably the biological activity.

MATERIALS AND METHODS

Nine derivatives of 1-acyl(aroil)-4-(allyl)cinnamyl-thiosemicarbazide (**1-9**) [19, 20] and three of N^1-allyl(cinnamyl)-thiocarbamyl-N^3-phenyl-amidrazone (**10-12**) [21, 22] have been chosen for the antimicrobial investigations. They are: 1-formyl-4-cinnamyl-thiosemicarbazide (**1**) [20], 1-acyl-4-cinnamyl-thiosemicarbazide (**2**) [20], 1-phenylacyl-4-cinnamyl-thiosemicarbazide (**3**) [20],1-benzoil-4-cinnamyl-thiosemicarbazide (**4**) [20], 1-(p-bromobenzoil)-4-cinnamyl--thiosemicarbazide (**5**) [20], 1-(o-hydroxybenzoil)-4-cinnamyl-hiosemicarbazide (**6**) [20], 1-(p-nitrobenzoil)-4-cinnamyl-thiosemicarbazide (**7**) [20], 1-(p-bromobenzoil)-4-allyl-thiosemicarbazide (**8**) [19], 1-(o-hydroxybenzoil)-4-allyl--thiosemicarbazide (**9**) [19] and N^1-cinnamyl-thiocarbamyl-N^3-phenyl-benzamidrazone (**10**) [21], N^1-cinnamyl-thiocarbamyl-N^3-phenyl-(pyridin-2-yl)amidrazone (**11**) [21], N^1-allyl-thiocarbamyl-N^3-phenyl-(pyridin-2-yl)amidrazone (**12**) [22]. The chemical structures of the investigated compounds are shown on Fig. 1.

The following allyl derivatives of 1,2,4-triazole-5-thoine (**13-23**) and 1,3,4-thiadiazole (**24-29**) have been selected for the investigations (Figs 2 and 3). They are: 4-cinnamyl-1,2,4-triazole-5-thione (**13**) [20], 3-methyl-4-cinnamyl-1,2,4-triazole -5-thione (**14**) [23], 3-phenylmethyl-4-cinnamyl-1,2,4-triazole-5-thione (**15**) [23], 3-phenyl-4-cinnamyl-1,2,4-triazole-5-thione (**16**) [21], 3-p-bromo-phenyl-4-cinnamyl-1,2,4-triazole-5-thione (**17**) [24], 3-(pyridin-2-yl)-4-cinnamyl--1,2,4-triazole-5-thione (**18**) [23], 3-phenylmethyl-4-allyl-1,2,4-triazole-5-thione (**19**) [23], 3-phenyl-4-allyl-1,2,4-triazole-5-thione (**20**) [23], 3-p-bromo-phenyl-4-allyl-1,2,4-triazole-5-thione (**21**) [19], 3-o-hydroxy-phenyl-4-allyl-1,2,4-triazole-5-thione (**22**) [19], 3-(pyridin-2-yl)-4-allyl-1,2,4-triazole-5-thione (**23**) [23] (Fig. 2).

The selected derivatives of 1,3,4-thiadiazole for the investigations are as follows: 2N-cinnamyl-(5-phenylmethyl-[1,3,4]-thiadiazol-2-yl) amine (**24**) [23], 2N-cinnamyl-(5-phenyl-[1,3,4]-thiadiazol-2-yl) amine (**25**) [20, 21], 2N-cinnamyl-[5-(pyridin-2-yl)-[1,3,4]-thiadiazol-2-yl] amine (**26**) [21], 2N-allyl-(5-phenylmethyl-[1,3,4]-thiadiazol-2-yl) amine (**27**) [23], 2N-allyl-(5-phenyl-[1,3,4]-thiadiazol-2-yl) amine (**28**) [23], 2N-allyl-[5-(pyridin-2-yl)-[1,3,4]-thiadiazol-2-yl] amine (**29**) [22] (Fig. 3).



1 - 12

1. R = H
2. R = CH_3-
3. R = C_6H_5-CH_2-
4. R = C_6H_5-
5. R = p-Br-C_6H_4-
6. R = o-HO-C_6H_4-
7. R = p-NO_2-C_6H_4-

X = O, R' = C_6H_5 (**1 - 7**)

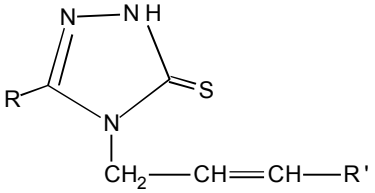
8. R = p-Br-C_6H_4-
9. R = o-HO-C_6H_4-

X = O, R' = H (**8,9**)

10. R = C_6H_5-
11, 12. R = pyridin-2-yl-

X = N-C_6H_5-, R' = C_6H_5- (**10,11**)
X = N-C_6H_5-, R' = H (**12**)

Fig. 1. The chemical structures of the studied 1-acyl(aroil)-4-(allyl)cinnamyl-thiosemicarbazides and N^1-allyl(cinnamyl)-thiocarbamyl-N^3-phenyl-amidrazones



13 - 23

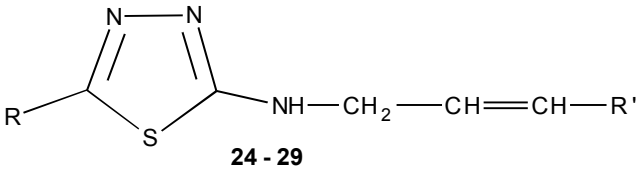
13. R = H -
14. R = CH_3 -
15. R = C_6H_5-CH_2 -
16. R = C_6H_5-
17. R = p-Br-C_6H_4-
18. R = pyridin-2-yl

13 - 18 R' = C_6H_5-

19. R = C_6H_5-CH_2 -
20. R = C_6H_5-
21. R = p-Br-C_6H_4-
22. R = o-HO-C_6H_4-
23. R = pyridin-2-yl

19 - 23 R' = H

Fig. 2. The chemical structure of the studied 3-substituted-4-allyl(cinnamyl)-1,2,4-triazole-5-thiones



24 - 29

24. R = C_6H_5-CH_2 -
25. R = C_6H_5-
26. R = pyridin-2-yl

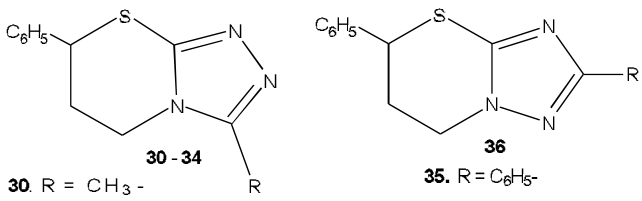
24 - 26 R' = C_6H_5-

27. R = C_6H_5-CH_2 -
28. R = C_6H_5-
29. R = pyridin-2-yl

27 - 29 R' = H

Fig. 3. The chemical structure of the studied 2N-allyl(cinnamyl)-(5- substituted-[1,3,4]-thiadiazol-2-yl) amine

The derivatives of the bicyclic systems:5H-3,7-disubstituted-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**30-34**), 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (**35**), 4,5,6,7-tetrahydro-2,5-disubstituted-1,3,4-thiadiazole [3,2-a]pyrimidine (**36, 37**) are shown on Fig. 4.

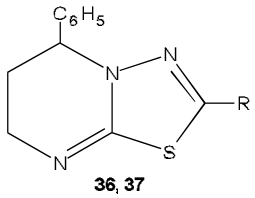


30 - 34

30. R = CH_3 -
31. R = C_6H_5-CH_2 -
32. R = C_6H_5-
33. R = p-Br-C_6H_4-
34. R = pyridin-2-yl

36

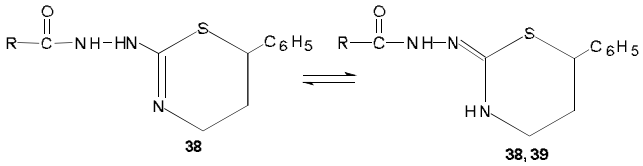
35. R = C_6H_5-



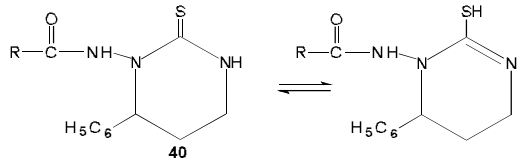
36, 37

36. R = pyridin-2-yl
37. R = C_6H_5-

Fig. 4. The chemical structures of the studied 5H-3-substituted -7-phenyl-6,7-dihydro-1,2,4-triazole[3,4-b] 1,3-thiazines, 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine and 4,5,6,7-tetrahydro-2,5-disubstituted-1,3,4-thiadiazole[3,2-a]pyrimidines



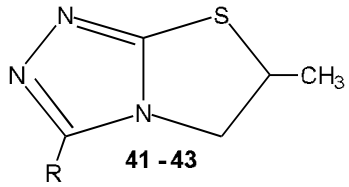
38 **38, 39**



40

38. R = o-HO-C_6H_4-
39. R = p-NO_2-C_6H_4-
40. R = o-HO-C_6H_4-

Fig. 5. The chemical structures of the studied hydrazide derivatives of substituted benzoic acid and 3-(o-hydroxybenzoilamino)-4-phenyl-3,4,5,6-tetrahydropyrimidine-2-thione-(2-thiol)



41 - 43

41. R = C_6H_5-CH_2 -
42. R = C_6H_5-
43. R = o-HO-C_6H_4-

Fig. 6. The chemical structures of the studied 3-substituted-6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4]triazole

5H-3-Methyl-7-phenyl-6,7-dihydro-1,2,4-triazole[3,4-b] 1,3-thiazine (**30**) [20], 5H-3-phenylmethyl-7-phenyl-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**31**) [20], 5H-3, 7-diphenyl-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**32**) [20], 5H-3-(4-bromo-phenyl)-7-phenyl-6,7-dihydro-1,2,4-triazole [3,4-b]1,3-thiazine (**33**) [20], 5H-3-(pyridin-2-yl)-7-phenyl--6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**34**) [21], 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (**35**) [24, 27] and 4,5,6,7-tetrahydro-2-(pyridin-2-yl)-5--phenyl-1,3,4-thiadiazole[3,2-a]pyrimidine (**36**) [21], 4,5,6,7-tetrahydro-2,5-diphenyl-1,3,4-thiadiazole[3,2-a]pyrimidine (**37**) [21] have been chosen for the investigations.

The hydrazide derivatives of the substituted benzoic acid: o-hydroxybenzoic acid [5,6-dihydro-6-phenyl-4H-1,3-thiazin-2-yl] hydrazide, o-hydroxybenzoic acid [5,6-dihydro-6-phenyl-4H-1,3-thiazin-2-ylidene] hydrazide (**38**) [20], p-nitrobenzoic acid [5,6-dihydro-6-phenyl-4H-1,3-thiazin-2-ylidene] hydrazide (**39**) [20] and 3-(o-hydroxybenzoilamino)-4--phenyl-3,4,5,6-tetrahydropyrimidine-2-thione-(2-thiol) (**40**) [20] are shown on Fig. 5.

The 3-phenylmethyl-6-methyl-5,6-dihydrothiazolo[2,3-c] [1,2,4]triazole (**41**) [19], 3-phenyl-6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4]triazole (**42**) [19], 3-(o-hydroxy-phenyl-)6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4]triazole (**43**) [19] and 7H-2,5-diphenyl 5,6-dihydro-1,2,4-triazole[3,2-b] 1,3-thiazine (**35**) [24] origin from the 4-(allyl)cinnamyl-3-substituted [1,2,4] triazole-5-thiones derivatives [19, 24]. They are presented on Figs 6 and 4.

Antibacterial activity tests were carried out against a panel of 8 reference strains of aerobic bacterial species including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240) or Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 9027). All these strains, routinely used for evaluation of antimicrobials, came from American Type Culture Collection (ATCC). The standardized microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of 0.5 McFarland standard (150 x 10^6 CFU [Colony Forming Units/mL]).

All stock solutions of the tested compounds were dissolved in dimethyl sulfoxide (DMSO). It was found that DMSO at the final concentration had no influence on growth of the tested microorganisms.

Preliminary *in vitro* antimicrobial potency of the tested compounds was screened using the agar dilution method on the basis of the bacterial growth inhibition on the Mueller-Hinton agar to which the tested compounds at concentration of 1000 μg/mL were added. The plates were poured on the day of testing; 10 μL of the standarized bacterial suspension was put onto the prepared solid media. The plates were incubated at 35°C for 18 h.

The *in vitro* antibacterial activity of the potentially active tested compounds were determined on the basis of MIC (minimal inhibitory concentration), usually defined as the

lowest concentration of the compound at which there was no visible growth of the tested microorganisms. Determination of the MIC value was achieved by broth microdilution method with a series of two-fold dilutions of the tested substances in the range of final concentrations from 3.91 to 1000 μg/mL. In this technique 96-well microplates were used; 198 μL of Mueller-Hinton broth without or with the tested compound was inoculated with 2 μL of the standardized microbial suspension (total volume per each well – 200 μL). After incubation (at 35°C for 18 h), spectrophotometric measurements of optical density (OD_{600}) of the bacterial cultures with or without the tested compounds were performed in order to determine MIC. The blank control wells with two-fold dilution of each tested compound added to Mueller-Hinton broth without bacteria were incubated under the same conditions. Cefuroxime, belonging to 2nd generation of cephalosporins, was used as control antimicrobial agent at final concentrations from 0.063 to 500 μg/mL.

The MBC (minimal bactericidal concentration), defined as the lowest concentration of the compound that resulted in > 99.9% reduction in CFU of the initial inoculum, was also assessed. MBC was determined using broth microdilution technique by plating out onto Mueller Hinton agar plates the contents of wells (20 μL) with the tested compounds that showed no visible growth of bacteria after previous incubation at 35°C for 18 h. Then, the plates were incubated at 35°C for 18 h. Both MIC and MBC values were defined in μg/mL.

RESULTS AND DISCUSSION

The 1-acyl(aroil)-4-(allyl)cinnamyl-thiosemicarbazides and N^1-allyl(cinnamyl)-thiocarbamyl-N^3-phenyl-amidrazones due to the presence of R–C=O, R-C-NH-R' or R-C=NR', -NH-NH-C(S)-NH and $–CH_2$-CH=CH-R groups may show antimicrobial activity. The synthesis of the linear derivatives were described earlier [19-22]. They were used for the investigation of the cyclization with hydrochloric acid to afford various heterocyclic systems [20-22]. The cyclization reaction of the mentioned linear compounds provide the following heterocyclic systems: 4-cinnamyl-3-substituted [1,2,4] triazole-5-thione (**16**) [21], 2N-cinnamyl-(5-substituted-[1,3,4]-thiadiazol-2-yl) amines (**25**, **26**) [20, 21], (**29**) [22], derivatives of bicyclic systems: 5H-3,7-disubstituted-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**30-34**) [20, 21], 4,5,6,7-tetrahydro-2,5-disubstituted-[1,3,4]-thiadiazole[3,2-a] pyrimidine (**36, 37**) [21], hydrazide derivatives of substituted benzoic acid: o-hydroxybenzoic acid [5,6-dihydro-6-phenyl-4H-1,3-thiazin-2-yl] hydrazide, o-hydroxybenzoic acid [5,6-dihydro-6-phenyl-4H-1,3-thiazin-2-ylidene] hydrazide (**38**), p-nitrobenzoic acid [5,6-dihydro-6-phenyl-4H-1,3-thiazin-2-ylidene] hydrazide (**39**) [20] and 3-(o-hydroxybenzoilamino)-4-phenyl-3,4,5,6-tetrahydropyrimidine-2-thione-(2-thiol) (**40**) [20]. The biological activity of these compounds has not been the subject of the studies.

The *in vitro* antimicrobial activity of the compounds (**1**-**43**) was screened using agar dilution method against the reference strains of Gram-negative and Gram-positive bacterial species with 1000 μg/mL concentration of the agent in the medium. Some of these compounds (**1**, **9**, **11**, **12**, **19**, **20**, **22**, **23**, **36**, **37**) possessed activity only against the tested Gram-positive bacteria – *Staphylococcus* species, *Bacillus subtilis* ATCC 6633 or *Micrococcus luteus* ATCC 10240; no activity against Gram-negative bacteria was found.

According to our data obtained by broth microdilution method (Table 1), the compounds (**1**, **9**, **11**, **12**, **19**, **20**, **22**, **23**, **36**, **37**) had differential activity against Gram-positive bacteria with MIC values from 31.25 to >1000 μg/mL; MBC values ranged from 62.5 – >1000 μg/mL. Based on the obtained values of MBC/MIC ratio, the tested compounds had bactericidal (MBC/MIC ≤ 4) or bacteriostatic (MBC/MIC > 4) effect [3, 28]; when MIC was 500 μg/mL and MBC > 1000 μg/mL this effect was not possible to assess. It was shown that the highest activity against Gram-positive bacteria was assigned to compound (**12**) with MIC = 31.25 – 62.5 μg/mL and MBC = 62.5 – 500 μg/mL. This compound was bactericidal against *Staphylococcus epidermidis* ATCC 12228 (MIC = MBC = 62.5 μg/mL; MBC/MIC = 1), *B. subtilis* ATCC 6633 (MIC = MBC = 62.5 μg/mL; MBC/MIC = 1) or *M. luteus* ATCC 10240 (MIC = 31.25 μg/mL, MBC = 62.5 μg/mL, MBC/MIC = 2) and bacteriostatic against *Staphylococcus aureus* ATCC 25923 (MIC = 62.5 μg/mL, MBC = 500 μg/mL, MBC/MIC = 8). The slightly lower inhibitory effect on Gram-positive species used was assigned to compound (**11**) showing bacteriostatic effect (MIC = 31.25 – 62.5 μg/mL, MBC > 1000 μg/mL; MBC/MIC > 16 – 32). Compound (**9**) inhibited the growth of Gram-positive bacteria with MIC = 125 – 500 μg/mL; it had bactericidal effect but only against *B. subtilis* ATCC 6633 (MBC/MIC = 2). The compound (**1**) was active only against *B. subtilis* ATCC 6633 showing bactericidal effect (MIC = MBC = 125 μg/mL, MBC/MIC = 1).

Table 1. The influence of compounds (**1**, **9**, **11**, **12**, **19**, **20**, **22**, **23**, **36**, **37**) on the growth of Gram-positive bacteria on the basis of **MIC** (μg/mL) or **MBC** (μg/mL) values determined by broth microdilution method

	Sa25923		Se12228		Bs6633		MI 10240	
Comp.	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	>1000	nd	>1000	nd	125	125	>1000	nd
9	125	>1000	500	>1000	500	1000	500	>1000
11	62.5	>1000	62.5	>1000	31.25	>1000	62.5	>1000
12	62.5	500	62.5	62.5	62.5	62.5	31.25	62.5
19	500	>1000	250	>1000	250	1000	1000	1000
20	500	>1000	500	1000	62.5	250	250	1000
22	500	>1000	1000	>1000	1000	>1000	1000	>1000
23	500	>1000	500	>1000	250	1000	500	>1000
36	250	1000	250	250	250	250	125	125
37	125	>1000	125	>1000	62.5	62.5	62.5	125

Abbreviations: nd – not determined, Sa25923 – *Staphylococcus aureus* ATCC 25923, Se12228 - *Staphylococcus epidermidis* ATCC 12228, Bs6633 – *Bacillus subtilis* ATCC 6633, Ml10240 – *Micrococcus luteus* ATCC 10240

In series of 3-substituted-4-allyl-1,2,4-triazole-5-thione derivatives, the compounds (**19**, **20**, **22**, **23**) showed activity against Gram-positive bacteria with MIC values from 62.5 μg/mL to 1000 μg/mL and MBC ranged from 250 μg/mL to ≥1000 μg/mL. The compound (**20**) showed moderate activ-

ity against Gram-positive bacteria with MIC = 62.5 – 500 μg/mL and MBC = 250 μg/mL to ≥ 1000 μg/mL and had bactericidal effect against *S. epidermidis* ATCC 12228 MIC = 500 μg/mL, MBC = 1000 μg/mL (MBC/MIC = 2), *B. subtilis* ATCC 6633 MIC = 62.5 μg/mL, MBC = 250 μg/mL (MBC/MIC = 4) and *M. luteus* ATCC 10240 MIC = 250 μg/mL, MBC = 1000 μg/mL (MBC/MIC = 4). The lower inhibitory effect against Gram-positive species used was exhibited by compounds (**19**) and (**23**). They inhibited the growth of the investigated species with MIC = 250 – 1000 μg/mL, MBC ≥ 1000 μg/mL. The derivative (**19**) was bactericidal against *B. subtilis* ATCC 6633 (MIC = 250 μg/mL, MBC = 1000 μg/mL, MBC/MIC = 4) and *M. luteus* ATCC 10240 (MIC = MBC = 1000 μg/mL, MBC/MIC = 1). The compound (**23**) had bactericidal effect against *B. subtilis* ATCC 6633 with MIC = 250 μg/mL, MBC = 1000 g/mL and MBC/MIC = 4.

The derivatives of 4,5,6,7-tetrahydro-2-substituted-5-phenyl-1,3,4-thiadiazole[3,2-a]pyrimidine, compounds (**36**, **37**) inhibited the growth of the species used with MIC = 62.5 – 250 μg/mL, MBC = 62.5 – ≥1000 μg/mL. The compound (**36**) inhibited the growth of Gram-positive bacteria with MIC = 125 – 250 μg/mL, MBC = 125 – 1000 μg/mL. It had bactericidal effect against all tested Gram-positive bacteria: *S. aureus* ATCC 25923 (MIC = 250 μg/mL, MBC = 1000 μg/mL, MBC/MIC = 4), *S. epidermidis* ATCC 12228 and *B. subtilis* ATCC 6633 (MIC = 250 μg/mL, MBC = 250 μg/mL, MBC/MIC = 1), *M. luteus* ATCC 10240 (MIC = 125 μg/mL, MBC = 125 μg/mL, MBC/MIC = 1). The compound (**37**) had the activity against Gram-positive bacteria with MIC = 62.5 – 125 g/mL, MBC = 62.5 – >1000 μg/mL; it had bactericidal effect only against *B. subtillis* ATCC 6633 with MIC = MBC = 62.5 μg/mL (MBC/MIC = 1) and *M. luteus* ATCC 10240 with MIC = 62.5 μg/mL, MBC = 125 μg/mL (MBC/MIC = 2).

In our experiments MICs of available antibiotic such as cefuroxime, that has been extensively used to treat of bacterial infections, was also estimated; it was 0.49–0.98 μg/mL for the tested Gram-positive bacteria.

On the basis of our results, the compounds (**11**, **12**, **20**, **37**) exhibited moderate antibacterial activity against Gram-positive bacteria, including both pathogenic *S. aureus* (compounds **11**, **12**) or opportunistic *S. epidermidis* (compounds **11**, **12**), *B. subtilis* (compounds **11**, **12**, **20**, **37**) and *M. luteus* (compounds **11**, **12**, **37**) species, and may be of value for searching new derivatives showing better antimicrobial activity.

From the point of a structure-activity relationship, the results obtained revealed that the antibacterial property is connected with the differences in the internal structure of the investigated derivatives. The 1-acyl(aroil)-4-(allyl)cinnamyl-thiosemicarbazides and N^1-allyl(cinnamyl)-thiocarbamyl--N^3-phenyl-amidrazones exsist in various tautomeric modifications [17, 18]. The proton tautomeric equilibrium plays an important role in the enzyme catalysis [11]. The ^{1}H NMR investigations of the linear compounds support the C=S and C-SH forms. Different tautomeric structures of the linear compounds due to the inductive interactions may take part in the hydrogen bonds.

In the series of thiosemicarbaside (**1-9**) compounds (**1**) and (**9**) show activity against Gram–positive bacteria. The substituents at the nitrogen atom N1, the aldehyde group for (**1**) and ketone group for compounds (**2-9**) are the cause of the difference of polarization of the carbonyl group. As a result of the polarization the carbon and oxygen atoms acquire a certain positive and negative charge, respectively and the strong ionic bonds may appear due to the electrostatic interactions. The presence of the electron-donating group in the aroyl radical HO-C_6H_4- increases considerably the electron density of the nitrogen at position 2 [17, 20]. On the other hand the allyl radical at N4, compound (**9**) causes the changes of the electronic structure of the nitrogen atom N4 [25, 26]. The nitrogen atom N4 may appear as the amine-type, the pyrrole-type or the pyridine-type. In case of the electron-accepting pyridine-type nitrogen N4, allyl radical acquires partial positive charge and the nitrogen atom N4 partial negative charge; it increases the negative electrostatic potential of the molecule and determines the chemical behaviour similar to those in the case of the carbonyl fragment.

The application of amidrazones in this reaction seems to be interesting due to the presence of R-C-NH-R' or R-C=NR' instead of R–C=O group in the molecule. In the series of N^1-(cinnamyl/allyl-thiocarbamyl) amidrazones, compounds (**11**) and (**12**) show activities against Gram-positive bacteria. Due to the presence of the pyridine ring in the molecule (compounds **11**, **12**) the ionic resonance structures are possible. Based on the data regarding the activity of (**6**, **9**), compound (**6**) shows no inhibitory effect on Gram-positive bacteria used and (**11**, **12**) one can state that different ionic and tautomeric forms of the derivatives are required to show biological activity. The searching of the new derivatives of the presented linear compounds showing better antimicrobial activity may be successful.

In case of 3-substituted-4-allyl-1,2,4-triazole-5-thione and 1,3,4-thiadiazole[3,2-a]pyrimidine derivatives the influence of the substituent at positions 3 and 2, respectively on the antibacterial activity is observed. The presence of phenyl substituent in both series of the derivatives (compounds **20**, **37**) increases the antibacterial activity. The phenyl substituent on the contrary to the pyridine-2-yl one increases the negative electrostatic potential of the 1,2,4-triazole and 1,3,4-thiadiazole rings, respectively of the discussed compounds (**20**, **37**).

The antibacterial activity of the studied 1-(o-hydroxybenzoil)-4-allyl-thiosemicarbazide (**9**), N^1-allyl-thiocarbamyl-N^3-phenyl-(pyridin-2-yl)amidrazone (**12**) and the corresponding cyclic derivatives of 3-substituted-4-allyl-1,2,4-triazole-5-thiones is more pronounced in case of the linear derivatives.

REFERENCES

1. Abdel-Rahman H.M., Hussein M.A.: Synthesis of β-hydroxypropanoic acid derivatives as potential antiinflammatory, analgesic and antimicrobial agents. *Arch. Pharm. Chem. Life Sci*., 339, 378, 2006.
2. Abdel-Wahab B.F., Abdel-Aziz H.A., Ahmed E.M.: Synthesis and antimicrobial evaluation of some (1,3-thiazole), 1,3,4-thiadiazole, 1,2,4-triazole and 1,2,4-triazolo[3,4-b]1,3,4-thiadiazine derivatives including a 5-(benzofuran-2-yl)-1-phenylpyrazole moiety. *Monatsh. Chem.*, 140, 601, 2009.
3. Bourgeois I. et al: Tolerance to the glycopeptides vancomycin and teicoplanin in coagulase-negative Staphylococci. Antimicrob. *Agents Chemother*., 51(2): 740, 2007.
4. Demirbas A. et al: Synthesis of some new 1,3,4-thiadiazol-2-ylmethyl-1,2,4-triazole derivatives and investigation of their antimicrobial activities. E*ur. J. Med. Chem*., 44, 2896, 2009.
5. Demirbas N., Ugurluoglu R. and Demirbas A.: Synthesis of 3-alkyl(aryl-4-alkylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones and 3-alkyl-4-alkylamino-4,5-dihydro-1H-1,2,4-triazol-5-ones as antitumor agents. *Bioorg. Med. Chem*., 10, 3717, 2002.
6. Galabov A.S., Galabov B.S., Neykova N.A.: Structure-activity relationship of diphenylthiourea antivirals. *J. Med. Chem*., 23, 1048, 1980.
7. Kadi A.A. et al: Synthesis, antimicrobial and antiinflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. *Eur. J. Med. Chem*., 42, 235, 2007.
8. Kerimow I. et al: Synthesis, antifungal and antioxidant screening of some novel benzimidazole derivatives. *J. Enzym. Inhibit. and Med. Chem*., 22, 696, 2007.
9. Keshk E.M. et al: Synthesis and reactions of some new quinoline thiosemicarbazide derivatives of potential biological activity. *Phosphorus Sulfur Silicon Relat. Elem*., 183, 1323, 2008.
10. Küçükgüzel İ. et al.: Synthesis of some novel thiourea derivatives obtained from 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones and evaluation as antiviral/antiHIV and antituberculosis agents. *Eur. J. Med. Chem*., 43, 381, 2008.
11. Olsson M.H.M., Mavri J., Warshel A.: Transition state theory can be used in studies of enzyme catalysis: lessons from simulations of tunneling and dynamical effects in lipoxygenase and other systems. *Phil.Trans. R. Soc*., B 361, 1417, 2006.
12. Rostom S.A.F. et al: Azole antimicrobial pharmacophpre-based tetrazoles: synthesis and biological evaluation as potential antimicrobial and anticonvulsant agents. *Bioorg. Med. Chem*., 17, 2410, 2009.
13. Rollas S., Büyüktimkin S., Çevikbas A.: N-[4-(3H-1,3,4-Oxadiazoline-2-thion-5- yl)phenyl]-N'-substituted thioureas: synthesis and antimicrobial activities. *Arch. Pharm.*, 324,189, 1991.
14. Salgín-Gökşen U. et al: 1-Acylthiosemicarbazides, 1,2,4-triazole -5(4H)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones: synthesis, analgesic-antiinflammatory and antimicrobial activities. *Bioorg. Med. Chem*., 15, 5738, 2007.
15. Shafiee A.: Synthesis and *in vitro* antimicrobial evaluation of 5-(1-methyl-5-nitro-2-imidazolyl)-4H-1,2,4-triazoles. *Arch. Pharm. Med. Chem.*, 10, 495, 2002.
16. Solak N., Rollas S.: Synthesis and antituberculosis activity of 2-(aryl/alkylamino)-5-(4-aminophenyl)-1,3,4-thiadiazoles and their Schiff bases. Arkivoc (xii), 173, 2006.
17. Strzemecka L.: Cyclization reaction of 1,4-disubstituted thiosemicarbazides. Part II. *Pol. J. Chem*., 63, 117, 1989.
18. Strzemecka L.: Cyclization of N^1-(cinnamyl-thiocarbamyl) amidrazones. Part II. *Pol. J. Chem*., 64, 557, 1990.
19. Strzemecka L.: Synthesis of 3,6-disubstituted 5,6-dihydrothiazolo-[2,3-c][1,2,4]triazole system. *Pol. J. Chem*., 57, 567, 1983.
20. Strzemecka L., Otto T.: Cyclization reaction of 1,4-disubstituted thiosemicarbazides. Part I. *Pol. J. Chem*., 62, 757, 1988.
21. Strzemecka L.: Cyclization of N^1-(cinnamyl-thiocarbamyl) amidrazones. Part I. *Pol. J. Chem*., 64, 157, 1990.
22. Strzemecka L.: Tautomerism of 1,3,4-thiadiazole. Part I. Ann. UMCS, Sectio AA, L/LI (7), 81, 1995/1996.
23. Strzemecka L.. Syntheses of allyl derivatives of 1,2,4-triazole and 1,3,4-thiadiazole. *Pol. J. Chem*., 57, 561, 1983.
24. Strzemecka L: Synthesis of 5H-3,7-disubstituted 6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine and 7H-2,5-disubstituted-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine systems. *Pol. J. Chem*., 57, 881, 1983.
25. Strzemecka L.: Tautomerism of 1,3,4-thiadiazole. Part II. Ann. UMCS, Sectio AA, LIV/LV (21), 363, 1999/2000.
26. Strzemecka L.: The electronic structure of the nitrogen atoms of allyl-[5-(pyridin-2-yl)-[1,3,4]-thiadiazol-2-yl]-amine. *Int. J. Mol. Sci*., 7, 231, 2006.
27. Strzemecka L.: The antiHIV, antitumor activity of allyl derivatives of 1,2,4-triazole-5-thione, 1,3,4-(thiadiazol-2-yl)-amine and derivatives of bicyclic systems: 1,2,4-triazole[3,4-b]1,3-thiazine, 1,2,4-triazole[3,2-b]1,3-thiazine, thiazolo[2,3-c][1,2,4] triazole. Curr. Issues Pharm. Med. Sci., 2012, 25 (1), 96-99 on-line www.umlub.pl/pharmacy, (formerly Annales UMCS, Sectio DDD, Pharmacia), (in press)
28. White E.L. et al: 2-Alkoxycarbonylaminopyridines: inhibitors of *Mycobacterium tuberculosis* FtsZ. J. Antimicrob. *Chemother*., 50, 111, 2002.