European Journal of Medicinal Chemistry 46 (2011) 241–248

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# Synthesis and antimicrobial activity of thiosemicarbazides, *s*-triazoles and their Mannich bases bearing 3-chlorophenyl moiety

Tomasz Plech [a,*], Monika Wujec [a], Agata Siwek [a], Urszula Kosikowska [b], Anna Malm [b]

[a] Department of Organic Chemistry, Faculty of Pharmacy, Medical University, Staszica 6, 20-081 Lublin, Poland
[b] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Medical University, Chodzki 1, 20-093 Lublin, Poland

ARTICLE INFO

*Article history:*
Received 5 August 2010
Received in revised form
5 November 2010
Accepted 5 November 2010
Available online 3 December 2010

*Keywords:*
*s*-Triazoles
Mannich bases
Minimal inhibitory concentration (MIC)
Minimal bactericidal concentration (MBC)

ABSTRACT

A fast and efficient synthesis of some 1,4-disubstituted thiosemicarbazide derivatives is described. The reaction of 3-chlorobenzoic acid hydrazide with various aryl isothiocyanates gave thiosemicarbazide derivatives (**1**–**11**) in good yield. The cyclization of compounds (**1**–**11**) in the presence of 2% NaOH resulted in the formation of compounds (**12**–**22**) containing the 1,2,4-triazole ring. A series of new Mannich bases (**23**–**33**) related to the structure of 1,2,4-triazole has been also synthesized. All of these compounds were tested for their *in vitro* antibacterial activity against the reference strains of aerobic bacteria - 6 Gram-positive and 3 Gram-negative ones; 12 *Staphylococcus aureus* clinical isolates were also examined. An attempt was made to clarify the influence of the nature/position of substituents on antibacterial activity of compounds described.



## 1. Introduction

Since the introduction of the first antibiotic (penicillin, 1942) into medical practice, to date, there has been an ongoing "race" between scientists creating new drugs and pathogenic bacteria. This specific "arms race" causes that thousands of potentially active chemicals are synthesized in laboratories around the world every day. A literature survey reveals that 1,4-disubstituted thiosemicarbazides as well as 1,2,4-triazoles [1–3] are known as promising antimicrobial agents. Compounds of the said structure often exhibit higher activity than standard antibiotics: penicillin G [4], ampicillin [1], gentamicin [5]. On the other hand, many authors reveal that the introduction of halogen atoms into the pharmacophore structure can, in many cases, be beneficial for antimicrobial activity [6,7]. With this in mind, we have synthesized a number of compounds in which the nature of the halogen atom seems to influence their biological action. Moreover, according to some authors, the improvement of activity of 1,2,4-triazoles can be obtained through the introduction of substituents in N-2 nitrogen position of a 1,2,4-triazole ring [8–10]. The aminomethylation reaction is most commonly used for this purpose and it results in the creation of the so-called Mannich bases. Using the Mannich reaction we obtained a series of new derivatives containing a pyrrolidin-1-ylmethyl substituent coupled with a 1,2,4-triazole ring. The choice of pyrrolidine as one of the substrates in the Mannich reaction was dictated by its positive impact on antimicrobial activity in other groups of drugs [11].

Another issue taken up in this article is the modification of synthesis of 1,4-disubstituted derivatives of thiosemicarbazide allowing for maximum shortening of the synthesis duration and obtaining high reaction efficiency. The synthesis methods adapted by other researchers include, among others: (i) synthesis in dimethylacetamide (DMA) environment [12], (ii) solvent-free synthesis (solid-state synthesis) [13], (iii) synthesis in dimethylformamide (DMF)/ethanol mixture [1], and (iv) synthesis in ethanol environment [14–17]. The use of DMA as a solvent required 24-h heating of the reaction mixture, whilst the reaction yield reached 88%. Solvent-free synthesis lasted 12 h and as a result appropriate 1,4-disubstituted thiosemicarbazides with a yield of 85–95% were obtained. The lowest yield (36–63%) was obtained for the reaction of hydrazides with isothiocyanates in DMF/EtOH mixture (reaction time: 4 h). In turn, the said synthesis was carried out in ethanol environment most frequently. Reaction mixture was heated in this case for 2 h (66,5–82,5% yield), 3 h (74–96%), 4 h (85–90%). Since the fact of the sensitivity of isothiocyanates to water is well known, we used only anhydrous ethanol (contrary to the previously cited authors) in the experiments the results of which are presented in this article.

* Corresponding author. Tel.: +48 081 532 05 19; fax: +48 081 532 45 46.
*E-mail address:* tomasz.plech@umlub.pl (T. Plech).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.11.010

## 2. Results and discussion

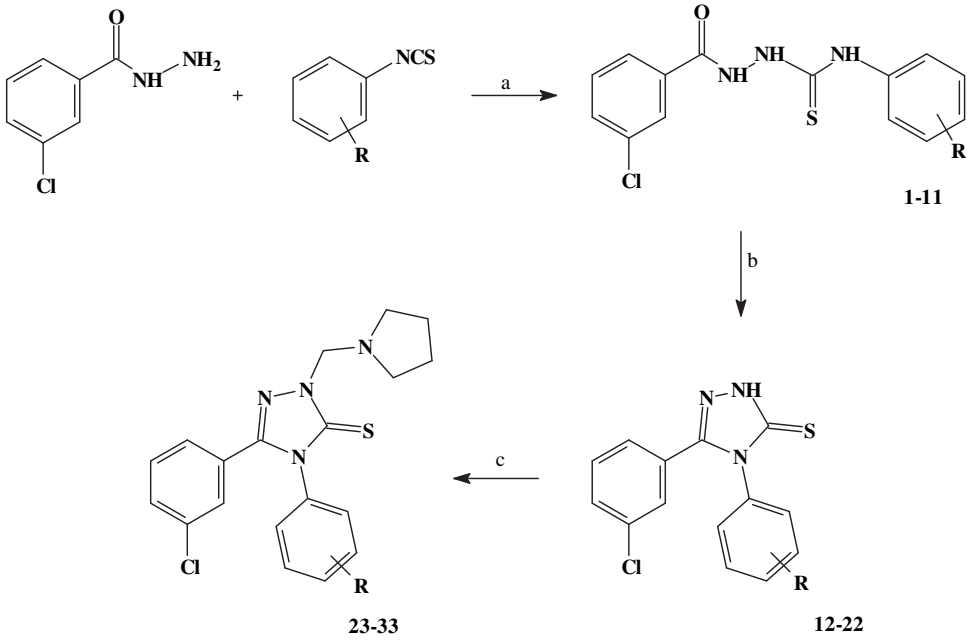
### 2.1. Chemistry

The synthesis pathway leading to the title compounds is depicted in Scheme 1. In order to select the optimum synthesis method, various experimental parameters, including solvents (ethanol, dimethylacetamide, the lack of solvent) and temperature conditions were examined (data not shown). 3-Chlorobenzoic acid hydrazide (commercially available) was added to appropriate aryl isothiocyanates, and 1-[(3-chlorophenyl)carbonyl]-4-substituted thiosemicarbazides (**1**–**11**) were obtained with a short reaction time (from 2 to 5 min, details in the Experimental section). The reaction medium, in all cases, was anhydrous ethanol and the syntheses were performed at boiling point temperature. Good to excellent yields (up to 96% in the case of compound **6**) were obtained.

The test results indicate that the method of synthesis used is definitely quicker and more efficient than the solid-state synthesis method [13,18–20], and even faster than reactions executed in similar conditions described by other authors [8,14–17]. Taking into consideration time and efficiency, this method of the synthesis of thiosemicarbazides may be treated as competitive to the microwave-assisted method. The currently executed computational studies aim to explain the differences in the length of reaction time depending on the used dissolvent (or the lack of such an agent). The results shall be presented shortly.

Alkaline cyclization of compounds (**1**–**11**) using 2% solution of sodium hydroxide afforded the corresponding 5-(3-chlorophenyl)-4-substituted-2,4-dihydro-3H-1,2,4-triazole-3-thiones (**12**–**22**). The structures and purity of these compounds were characterized using the $^1$H NMR, IR and MS methods together with elemental analysis. The $^1$H NMR spectra of the compounds showed the signals of aromatic protons in the range of 6.89–8.01 ppm. For the thiosemicarbazides, the signals of the proton linked to N1-N2 and N3 nitrogens were shown at 9.25–10.79 and 8.14–9.87 ppm, respectively. In the obtained 1,2,4-triazoles, the signal of the NH proton was observed in the range of 12.42–14.31 ppm. It is important to note that all synthesized 1,2,4-triazoles are present in thione form (–C=S) as indicated by their IR (presence of absorption maxima at 1328–1360 cm$^{-1}$ characteristic for the C=S group) and NMR spectra (absence of a signal of the proton of the SH group).

Mannich bases (**23**–**33**) were synthesized in the reaction of suitable 4,5-disubstituted derivatives of 1,2,4-triazolino-3-thione (**12**–**22**) with pyrrolidine and formaldehyde (37%), in ethanol (96%) environment. The occurrence of the aminomethylation reaction is shown by the loss of signal for the proton at the N-2 nitrogen atom of the 1,2,4-triazole ring. Moreover, in $^1$H NMR spectra a sharp




| | R | | R |
|---|---|---|---|
| **1, 12, 23** | H | **7, 18, 29** | 2-I |
| **2, 13, 24** | 4-$CH_3$ | **8, 19, 30** | 4-Cl |
| **3, 14, 25** | 4-$OCH_3$ | **9, 20, 31** | 4-F |
| **4, 15, 26** | 4-Br | **10, 21, 32** | 2,5-diF |
| **5, 16, 27** | 2-Br | **11, 22, 33** | 2,6-diF |
| **6, 17, 28** | 4-I | | |

**Scheme 1.** Reagents and conditions: (a) EtOH, 2–5 min; (b) 2% NaOH, 2 h; (c) HCOH, pyrrolidine, EtOH, 1 h.

**Table 1**
Screening for antimicrobial activity of the compounds.

| Compounds | Microorganisms/MIC (μg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* ATCC 25923 | *S. aureus* ATCC 6538 | *S. epidermidis* ATCC 12228 | *B. subtilis* ATCC 6633 | *B. cereus* ATCC 10876 | *M. luteus* ATCC 10240 |
| **1** | 125 | 125 | 125 | 125 | 125 | 125 |
| **2** | 62.5 | 62.5 | [a] | [a] | [a] | [a] |
| **3** | 62.5 | 125 | 125 | 125 | 125 | 62.5 |
| **4** | **15.63** | **15.63** | **15.63** | 31.25 | **15.63** | **15.63** |
| **5** | 62.5 | 31.25 | 125 | 125 | 62.5 | 62.5 |
| **6** | 31.25 | 31.25 | 31.25 | 31.25 | **15.63** | 31.25 |
| **7** | 31.25 | 31.25 | 31.25 | **15.63** | 31.25 | 31.25 |
| **8** | 31.25 | 31.25 | 31.25 | 31.25 | 62.5 | 31.25 |
| **9** | [a] | [a] | [a] | [a] | [a] | [a] |
| **10** | 62.5 | 62.5 | 125 | 125 | 125 | 62.5 |
| **11** | 125 | 250 | 250 | 250 | 250 | 250 |
| **12** | [a] | [a] | [a] | 125 | [a] | [a] |
| **13** | [a] | [a] | [a] | [a] | [a] | [a] |
| **14** | [a] | [a] | [a] | [a] | [a] | [a] |
| **15** | 31.25 | 31.25 | 31.25 | **15.63** | **15.63** | 31.25 |
| **16** | [a] | [a] | 125 | 1000 | 1000 | [a] |
| **17** | 250 | 500 | 250 | 31.25 | 250 | [a] |
| **18** | [a] | [a] | [a] | [a] | [a] | [a] |
| **19** | 125 | 125 | 125 | 125 | 125 | 125 |
| **20** | [a] | [a] | [a] | [a] | [a] | [a] |
| **21** | [a] | [a] | 250 | 1000 | 1000 | 1000 |
| **22** | 250 | 250 | 250 | 500 | 250 | 125 |
| **Ampicillin** | 2 | 3 | 6 | 3 | **62.5** | 6 |

[a] - MIC value higher than 1000 μg/mL.

singlet corresponding to the methylene group, linking the triazole ring with the pyrrolidine ring, occurs in the range of 5.25–5.38 ppm. The signal of the four protons of pyrrolidine deriving from the fragment $-(CH_2)_2N-$ becomes evident in the form of a triplet at 2.90–3.01 ppm. The signal of the remaining four protons of pyrrolidine is shown as a multiplet (1.69–1.86 ppm). In turn, in the IR spectra, absorption bands characteristic for fragments: C=S, C=N and $-CH_2-$, are visible in the ranges of: 1321–1338 $cm^{-1}$, 1581–1599 $cm^{-1}$ and 2833–2971 $cm^{-1}$, respectively.

Among the above-mentioned compounds there are both newly-synthesized compounds as well as earlier known derivatives (details in the Experimental section). An evaluation of their antimicrobial activities was performed and is described here for the first time.

### 2.2. Antimicrobial evaluation

The *in vitro* antimicrobial activity of the synthesized compounds (**1**–**33**) was evaluated using the broth microdilution method against the reference strains of Gram-negative and Gram-positive bacterial species (Table 1, Table 2).

Among the Gram-positive species, the most sensitive to all of the assayed compounds were *Staphylococcus aureus* ATCC 25923 (MIC from 15.63 to >1000 μg/mL) and *S. aureus* ATCC 6538 (MIC from 15.63 to >1000 μg/mL). It was found that the most effective against Gram-positive species was compound (**4**) (MIC = 15.63 μg/mL; for *Bacillus subtilis* ATCC 6633 MIC = 31.25 μg/mL). Compounds (**6**) and (**7**) (MIC = 15.63–31.25 μg/mL) as well as compound (**8**) (MIC = 31.25–62.5 μg/mL) were also characterized by good activity against the tested Gram-positive bacteria. Among the investigated triazoles, the most active was compound (**15**) (MIC = 15.63–31.25 μg/mL). As can be seen from Table 1, compounds (**4**), (**6**), (**15**) appeared to be four-fold more effective against *Bacillus cereus* ATCC 10876 than ampicillin in the same *in vitro* test. Compounds (**1**–**22**) were found to be inactive against Gram-negative bacteria.

The novel Mannich bases (**23**–**33**) had differential activity against Gram-positive bacteria, with MIC values ranged from 31.25

**Table 2**
Screening for antimicrobial activity of the compounds.

| Species | Sa 25923 | | Sa 6538 | | Se 12228 | | Bs 6638 | | Bc 10876 | | Ml 10240 | | Ec 25922 | | Kp 13883 | | Pm 12453 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compounds | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| **23** | 125 | 500 | 125 | 500 | 125 | 1000 | 125 | 500 | 125 | [a] | 125 | 1000 | 500 | 500 | 250 | 500 | 500 | [a] |
| **24** | 31.25 | [a] | 62.5 | [a] | 125 | [a] | 31.25 | 1000 | 125 | [a] | 62.5 | [a] | [a] | [a] | [a] | [a] | [a] | [a] |
| **25** | 125 | 500 | 250 | 500 | 250 | 1000 | 250 | 500 | 125 | [a] | 125 | 1000 | 250 | 500 | 250 | 500 | 500 | [a] |
| **26** | 31.25 | [a] | 62.5 | [a] | 250 | [a] | 62.5 | 500 | **62.5** | [a] | 62.5 | [a] | [a] | [a] | [a] | [a] | [a] | [a] |
| **27** | 250 | [a] | 250 | [a] | 500 | [a] | [a] | [a] | [a] | [a] | 500 | [a] | [a] | [a] | [a] | [a] | [a] | [a] |
| **28** | 125 | 1000 | 250 | 1000 | 250 | [a] | 125 | 1000 | 125 | [a] | 125 | [a] | [a] | [a] | 1000 | [a] | [a] | [a] |
| **29** | 500 | [a] | 500 | [a] | 500 | [a] | 250 | [a] | 500 | [a] | 500 | [a] | [a] | [a] | [a] | [a] | [a] | [a] |
| **30** | 125 | 125 | [a] | [a] | 1000 | 1000 | 125 | [a] | 125 | [a] | 1000 | 1000 | 1000 | 1000 | 500 | 1000 | [a] | [a] |
| **31** | 125 | 250 | 125 | 250 | 125 | 500 | 125 | 250 | 250 | [a] | 125 | 500 | 500 | 500 | 500 | 500 | 500 | 1000 |
| **32** | 125 | 500 | 125 | 500 | 125 | 1000 | 125 | 1000 | 125 | [a] | 125 | 1000 | 500 | [a] | 250 | [a] | 500 | [a] |
| **33** | 125 | 500 | 125 | 500 | 125 | 1000 | 125 | 500 | 125 | 1000 | 125 | 1000 | 500 | 1000 | 1000 | 1000 | 1000 | 1000 |
| **Amp.** | 2 | nd | 3 | nd | 6 | nd | 3 | nd | **62.5** | nd | 6 | nd | 7.81 | nd | 62.5 | nd | 0.5 | nd |

Abbreviations: Sa25923 - *Staphylococcus aureus* ATCC 25923, Sa6538 - *Staphylococcus aureus* ATCC 6538, Se12228 - *Staphylococcus epidermidis* ATCC 12228, Bs6638 - *Bacillus subtilis* ATCC 6638, Bc10876 - *Bacillus cereus* ATCC 10876, Ml10240 - *Micrococcus luteus* ATCC 10240, Ec25922 - *Escherichia coli* ATCC 25922, Kp13883 - *Klebsiella pneumoniae* ATCC 13883, Pm12453 - *Proteus mirabilis* ATCC 12453, Amp. – ampicillin, nd – not determined.

[a] MIC or MBC value higher than 1000 μg/mL.

to >1000 μg/mL. On the basis of MBC/MIC ratio, the tested compounds are considered to have bactericidal (MBC/MIC ≤ 4) or bacteriostatic (MBC/MIC > 4) effect on the tested strains [21,22]. As opposed to compounds (**1**–**22**), some of the synthesized Mannich bases were also active towards Gram-negative bacteria. The MIC values for compounds (**23**), (**25**), (**31**–**33**) were in the range of 250–1000 μg/mL. The MBC values were in the similar range, suggesting bactericidal activity.

Moreover, the most active (against the reference strains) compounds (**4**–**8**, **10**, **15**, **24**, **26**) were examined against twelve clinical isolates of *S. aureus*. It was found that most of the tested compounds inhibited the growth of these bacterial isolates with MIC ranging from 15.63 to 31.25 μg/mL (Table 3); the mode MIC value was 15.63 μg/mL. Compounds (**6**), (**7**) and (**8**) had the highest activity, with MIC = 15.63 μg/mL for all of the tested clinical isolates of *S. aureus*. Only derivatives (**15**), (**24**) and (**26**) showed MIC values higher than 31.25 μg/ml. The difference of sensitivity of referential strains and clinical strains on the studied substances is clearly noticeable. For example, compound (**4**) inhibited the growth of referential strains (*S. aureus* ATCC 25923, *S. aureus* ATCC 6538) in the concentration of 15.63 μg/mL, while stopping the growth of the majority of clinical strains (8 out of 12) took place in a concentration that was twice as high (31.25 μg/mL). A contrary situation is observed in the case of iodine and chlorine derivatives of thiosemicarbazide (**6**,**7**,**8**). The mentioned compounds were characterized by the values of MIC = 31.25 μg/mL for *S. aureus* ATCC 25923 and *S. aureus* ATCC 6538, while the concentration inhibiting the growth of all twelve clinical strains was twice as low (15.63 μg/mL). From the microbiological point of view, the difference written above in the sensitivity of both types of strains of *S. aureus* is easy to explain. A similar situation is described by Lopes et al. [23], who investigated different enterococci strains. Clinical strains, which were used in the studies, were isolated from patients at various ages, treated due to various diseases, treated by various drugs. Therefore, there was a great probability of a mutation taking place in the bacterial genome, which caused a different sensitivity to the studied substances. Also due to this, conclusions concerning structure-activity dependencies were formulated on the basis of results obtained for referential strains.

Results obtained in this work clearly indicate that among the described compounds, thiosemicarbazide derivatives exhibit much stronger antibacterial activity compared to related 1,2,4-triazoles and their Mannich bases. Only compound (**15**) (MIC = 15.63–31.25 μg/mL), based on the 1,2,4-triazole scaffold, displays activity similar to that of the initial thiosemicarbazide. The antibacterial activity of the title compounds distinctly depends on the nature of substituents and their location. In order to analyze the relationship: nature/position of the substituent–the activity of compounds (**1**–**33**), the values of the following parameters: surface area (*SA*), volume (*V*), hydration energy (*HE*), logarithm of the partition coefficient (*logP*), molecular refractivity (*MR*), polarizability ($\alpha$), dipole moment ($\mu$), energy of the highest occupied molecular orbital ($E_{HOMO}$), energy of the lowest unoccupied molecular orbital ($E_{LUMO}$) were determined. Compounds (**1**), (**12**), (**23**), containing an unsubstituted phenyl ring, demonstrate weak activity against Gram-positive bacteria. Among other compounds there were derivatives with electron-donating or electron-withdrawing substituents connected to the phenyl ring. The introduction of the halogen atom in general increased antibacterial activity. Moreover, the advantageous position for the electron-withdrawing substituents is the *para* position in the phenyl ring. It is clearly visible in the case of the corresponding bromine and iodine derivatives. *Para* isomers of these compounds are characterized by higher antibacterial activity than their *ortho* isomers. Unexpectedly, compounds (**9**) and (**20**), containing a 4-fluorophenyl fragment, were deprived of any activity directed towards the tested microorganisms. Furthermore, it should be noted that the compounds (**1**–**22**) were not active against Gram-negative bacteria. Next, comparing the activity of 1,2,4-triazoles (**12**–**22**) with their respective N-2-(pyrrolidin-1-ylmethyl) derivatives (**23**–**33**), it is clear that the substitution of hydrogen at nitrogen N-2 causes a significant increase in antibacterial activity mainly for those compounds, which had the electron-donating substituents ($CH_3$, $OCH_3$) at the phenyl ring. For example, compound (**13**), which was inactive against all tested bacterial strains, after moving it in the appropriate Mannich base (**24**), acquires relatively substantial activity. Moreover, the introduction of the substituent in N-2 position extends the spectrum of activity of certain compounds also on the Gram-negative bacteria. According to the data listed in Table S.1 (in the Supplementary data section), it can be hypothesize that lipophilicity (*logP*) has no decisive influence on antibacterial activity for these compounds. Therefore, it is necessary to think that the structural or electronic parameters may have a greater impact on the activity. Investigation of the dependency between the dipole moments ($\mu$) and antimicrobial activity for analogs with bromo and iodo substitution (**4**-**7**, **15**-**18**, **26**–**29**) revealed that 4-bromo (**4**, **15**, **26**) and 4-iodo (**6**, **17**, **28**) analogs, with lower dipole moments than their 2-bromo (**5**, **16**, **27**) and 2-iodo (**7**, **18**, **29**) isomers, were more biologically active.

**Table 3**
Antibacterial activity of the tested compounds against twelve clinical isolates of *Staphylococcus aureus*.

| Compound | MIC (μg/mL) | |
|---|---|---|
| | 15.63 | 31.25 |
| | Number (%) of isolates | |
| **4** | 4 (33.3) | 8 (66.7) |
| **5** | 7 (58.3) | 5 (41.7) |
| **6** | 12 (100) | 0 (0) |
| **7** | 12 (100) | 0 (0) |
| **8** | 12 (100) | 0 (0) |
| **10** | 0 (0) | 12 (100) |

## 3. Conclusions

We describe a fast (several minute long), facile and efficient method for the preparation of 1,4-disubstituted thiosemicarbazide derivatives. A variety of novel s-triazoles and their Mannich bases were prepared as well. Some derivatives showed promising antimicrobial activity, especially against Gram-positive bacteria. Compounds (**4**), (**6**), (**15**) appeared to be four-fold more effective against *B. cereus* ATCC 10876 than ampicillin. We think that our results should be of value for further studies, especially for searching new derivatives showing better antimicrobial activity against both opportunistic (e.g., *Bacillus* spp., *Staphylococcus epidermidis* or *Escherichia coli* and *Proteus mirabilis*) or pathogenic (e.g., *S. aureus*, *Klebsiella pneumoniae*) bacteria.

## 4. Experimental

### 4.1. Chemistry

#### 4.1.1. General comments

All reagents were purchased from Lancaster and Merck Co. and used without further purification. Melting points were determined by using Fischer-Johns apparatus (Sanyo, Japan) and are uncorrected. The $^1$H NMR spectra were recorded on a Bruker Avance 250 MHz instrument using DMSO-$d_6$ or $CDCl_3$ as solvents and TMS as an internal standard. Chemical shifts are expressed as $\delta$ (ppm). The IR spectra were recorded in KBr discs using a Perkin–Elmer 1725X

FTIR spectrometer. The mass spectra were obtained on a Finnigan Trace DSQ spectrometer operating at 70 eV. The purity of the compounds was checked by TLC on plates precoated with silica gel Si 60 $F_{254}$, produced by Merck Co. (Darmstadt, Germany). The spots were detected by exposure to UV-lamp at $\lambda = 254$ nm. Elemental analyses were performed on AMZ 851 CHX analyser and the results were within ±0.2% of the theoretical value.

#### 4.1.2. General procedure for the synthesis of 1-[(3-chlorophenyl) carbonyl]-4-substituted-thiosemicarbazides (**1**–**11**)

A solution of 0.01 mol of 3-chlorobenzoic acid hydrazide and equimolar amount of appropriate isothiocyanate in 25 ml of anhydrous EtOH was heated under reflux for 2 min (for compounds **2**, **6**, **8**–**10**) or for 5 min (in the case of compounds **1**, **3**–**5**, **7**, **11**). Next, the solution was cooled and the solid formed was filtered off, washed with diethyl ether, dried, and crystallized from EtOH.

*1*-[(*3-Chlorophenyl*)*carbonyl*]-*4-phenylthiosemicarbazide* (**1**) Yield: 81%, spectral and physicochemical data consistent with [24].

*1*-[*(3-Chlorophenyl)carbonyl*]-*4*-(*4-methylphenyl*)*thiosemicarbazide* (**2**) Yield 93%, CAS Registry Number: 891082-71-6.

*1-[(3-Chlorophenyl)carbonyl]-4-(4-methoxyphenyl)thiosemicarbazide* (**3**) Yield 84%, CAS Registry Number: 905262-18-2.

*4-(4-Bromophenyl)-1-[(3-chlorophenyl)carbonyl]thiosemicarbazide* (**4**) Yield 88%, spectral and physicochemical data consistent with [25].

*4*-(*2-Bromophenyl*)-*1*-[(*3-chlorophenyl*)*carbonyl*]*thiosemicarbazide* (**5**) Yield 86%, m.p. 150–152 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.15–7.23 (m, 1H, Ar–H), 7.33–7.42 (m, 2H, Ar–H), 7.49–7.68 (m, 3H, Ar–H), 7.85–7.90 (m, 1H, Ar–H), 8.00 (s, 1H, Ar–H), 9.68, 9.85, 10.72 (3s, 3H, 3NH, exch. $D_2O$). IR (KBr, $\nu$, cm$^{-1}$): 3359 (NH), 3027 ($CH_{arom.}$), 1671 (C=O), 1338 (C=S). EI-MS *m*/*z* (%): 384 ($M^+$, 0.1), 213 (50), 170 (19.2), 139 (100), 111 (52.9), 90 (13.5), 75 (47.1), 63 (11.5), 50 (20.2). Anal. Calc. for $C_{14}H_{11}BrClN_3OS$ (%): C 43.71, H 2.88, N 10.92. Found: C 43.87, H 2.71, N 10.99.

*1*-[(*3-Chlorophenyl*)*carbonyl*]-*4*-(*4-iodophenyl*)*thiosemicarbazide* (**6**) Yield 96%, m.p. 180–182 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.27–7.32 (m, 2H, Ar–H), 7.50–7.57 (m, 1H, Ar–H), 7.63–7.68 (m, 3H, Ar–H), 7.84–7.89 (m, 1H, Ar–H), 7.98 (s, 1H, Ar–H), 9.84 (s, 2H, 2NH, exch. $D_2O$), 10.67 (s, 1H, NH, exch. $D_2O$). IR (KBr, $\nu$, cm$^{-1}$): 3320 (NH), 3023 ($CH_{arom.}$), 1669 (C=O), 1345 (C=S). EI-MS *m*/*z* (%): 431 ($M^+$, 0.13), 261 (93.2), 219 (27.8), 212 (12.5), 170 (19), 139 (100), 111 (50), 90 (24), 75 (36.2), 50 (27.5). Anal. Calc. for $C_{14}H_{11}ClIN_3OS$ (%): C 38.95, H 2.57, N 9.73. Found: C 38.87, H 2.52, N 9.83.

*1*-[(*3-Chlorophenyl*)*carbonyl*]-*4*-(*2-iodophenyl*)*thiosemicarbazide* (**7**) Yield 80%, m.p. 160–162 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.00–7.06 (m, 1H, Ar–H), 7.26–7.40 (m, 2H, Ar–H), 7.49–7.56 (m, 1H, Ar–H), 7.62–7.67 (m, 1H, Ar–H), 7.83–7.91 (m, 2H, Ar–H), 8.02 (s, 1H, Ar–H), 9.67, 9.80, 10.71 (3s, 3H, 3NH, exch. $D_2O$). IR (KBr, $\nu$, cm$^{-1}$): 3345 (NH), 3003 ($CH_{arom.}$), 1670 (C=O), 1337 (C=S). EI-MS *m*/*z* (%): 303 (9), 261 (68.2), 219 (5), 170 (15.3), 139 (100), 111 (52), 90 (15.4), 75 (28.8), 63 (10), 50 (18.2). Anal. Calc. for $C_{14}H_{11}ClIN_3OS$ (%): C 38.95, H 2.57, N 9.73. Found: C 38.81, H 2.50, N 9.83.

*4*-(*4-Chlorophenyl*)-*1*-[(*3-chlorophenyl*)*carbonyl*]*thiosemicarbazide* (**8**) Yield 88%, spectral and physicochemical data consistent with [25].

*1*-[(*3-Chlorophenyl*)*carbonyl*]-*4*-(*4-fluorophenyl*)*thiosemicarbazide* (**9**) Yield 90%, CAS Registry Number: 316151-86-7.

*1*-[(*3-Chlorophenyl*)*carbonyl*]-*4*-(*2,5-difluorophenyl*)*thiosemicarbazide* (**10**) Yield 91%, m.p. 178–180 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.08–7.22 (m, 2H, Ar–H), 7.28–7.37 (m, 1H, Ar–H), 7.49–7.70 (m, 2H, Ar–H), 7.83–8.00 (m, 2H, Ar–H), 9.70, 10.05, 10.77 (3s, 3H, 3NH, exch. $D_2O$). IR (KBr, $\nu$, cm$^{-1}$): 3320 (NH), 3009 ($CH_{arom.}$), 1633 (C=O), 1352 (C=S). EI-MS *m*/*z* (%): 341 ($M^+$, 0.5), 212 (6), 171 (51), 152 (11), 139 (100), 129 (26), 111 (32), 75 (19), 63 (10), 50 (7). Anal. Calc. for $C_{14}H_{10}ClF_2N_3OS$ (%): C 49.20, H 2.95, N 12.30. Found: C 49.12, H 2.87, N 12.32.

*1*-[(*3-Chlorophenyl*)*carbonyl*]-*4*-(*2,6-difluorophenyl*)*thiosemicarbazide* (**11**) Yield 92%, m.p. 152–154 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.10–7.17 (m, 2H, Ar–H), 7.32–7.40 (m, 1H, Ar–H), 7.49–7.57 (m, 1H, Ar–H), 7.62–7.67 (m, 1H, Ar–H), 7.85–7.90 (m, 1H, Ar–H), 8.00 (s, 1H, Ar–H), 9.46, 10.08, 10.79 (3s, 3H, 3NH, exch. $D_2O$). IR (KBr, $\nu$, cm$^{-1}$): 3324 (NH), 3017 ($CH_{arom.}$), 1688 (C=O), 1353 (C=S). EI-MS *m*/*z* (%): 341 ($M^+$, 3), 212 (12.5), 171 (42.3), 139 (100), 129 (61.5), 111 (30.7), 75 (21), 63 (9.6), 50 (6.7). Anal. Calc. for $C_{14}H_{10}ClF_2N_3OS$ (%): C 49.20, H 2.95, N 12.30. Found: C 49.12, H 2.87, N 12.32.

#### 4.1.3. General procedure for the synthesis of 5-(3-chlorophenyl)-4-substituted-2,4-dihydro-3H-1,2,4-triazole-3-thiones (**12**–**22**)

Suitable substituted thiosemicarbazides (**1**–**11**) (0.01 mol) were dissolved in 2% solution of sodium hydroxide and the resulting solution was heated under reflux for 2 h. After cooling, the mixture was neutralized with 3M hydrochloric acid. The precipitate formed was filtered and washed several times with distilled water. The compounds were crystallized from EtOH.

*5*-(*3-Chlorophenyl*)-*4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**12**) Yield: 87%, spectral and other physicochemical data consistent with [26].

*5*-(*3-Chlorophenyl*)-*4*-(*4-methylphenyl*)-*2,4-dihydro-3H-1,2,4-triazole-3-thione* (**13**) Yield 73%, CAS Registry Number: 893725-08-1.

*5-(3-Chlorophenyl)-4-(4-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**14**) Yield 89%, CAS Registry Number: 714214-94-5.

*4*-(*4-Bromophenyl*)-*5*-(*3-chlorophenyl*)-*2,4-dihydro-3H-1,2,4-triazole-3-thione* (**15**) Yield 95%, m.p. 224–226 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.22–7.25 (m, 1H, Ar–H), 7.36 (dd, 2H, $J = 8.70$ Hz, 1.90 Hz, Ar–H, p-substituted ring), 7.39–7.45 (m, 2H, Ar–H), 7.51–7.55 (m, 1H, Ar–H), 7.72 (dd, 2H, $J = 8.73$ Hz, 1.90 Hz, Ar–H, p-substituted ring), 14.24 (s, 1H, NH, exch. $D_2O$). IR (KBr, $\nu$, cm$^{-1}$): 3437 (NH), 3062 ($CH_{arom.}$), 1578 (C=N), 1328 (C=S). EI-MS *m*/*z* (%): 370 ($[M+4]^+$, 27), 368 ($[M+2]^+$, 94), 366 ($M^+$, 100), 308 (10.5), 285 (9.6), 227 (13.5), 169 (15.5), 152 (44.2), 143 (25), 137 (38.4), 125 (75), 111 (26), 90 (42.3), 75 (60.5), 63 (30), 50 (23). Anal. Calc. for $C_{14}H_9BrClN_3S$ (%): C 45.86, H 2.47, N 11.46. Found: C 45.72, H 2.42, N 11.50.

*4-(2-Bromophenyl)-5-(3-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**16**) Yield 85%, m.p. 228–230 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.25–7.43 (m, 3H, Ar–H), 7.47–7.53 (m, 2H, Ar–H), 7.56–7.64 (m, 1H, Ar–H), 7.71–7.82 (m, 2H, Ar–H), 14.31 (s, 1H, NH, exch. $D_2O$). IR (KBr, $\nu$, cm$^{-1}$): 3445 (NH), 3101 ($CH_{arom.}$), 1577 (C=N), 1335 (C=S). EI-MS *m*/*z* (%): 367 ($[M+1]^+$, 4), 286 (65.4), 149 (100), 137 (8.6), 125 (11.5), 105 (13.5), 90 (8.6), 75 (13.5), 63 (5), 50 (5). Anal. Calc. for $C_{14}H_9BrClN_3S$ (%): C 45.86, H 2.47, N 11.46. Found: C 45.70, H 2.36, N 11.49.

*5*-(*3-Chlorophenyl*)-*4*-(*4-iodophenyl*)-*2,4-dihydro-3H-1,2,4-triazole-3-thione* (**17**) Yield 90%, m.p. 236–238 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.19 (dd, 2H, $J = 8.49$ Hz, 2.00 Hz, Ar–H, p-substituted ring), 7.22–7.25 (m, 1H, Ar–H), 7.35–7.42 (m, 2H, Ar–H), 7.48–7.57 (m, 1H, Ar–H), 7.86 (dd, 2H, $J = 8.50$ Hz, 1.95 Hz, Ar–H, p-substituted ring), 14.22 (s, 1H, NH, exch. $D_2O$). IR (KBr, $\nu$, cm$^{-1}$): 3435 (NH), 3101 ($CH_{arom.}$), 1578 (C=N), 1333 (C=S). EI-MS *m*/*z* (%): 415 ($[M+2]^+$, 32.1), 413 ($M^+$, 100), 286 (9.6), 149 (21), 122 (11.5), 108 (18.2), 90 (25), 76 (34.6), 63 (18.2), 50 (16.3). Anal. Calc. for $C_{14}H_9ClIN_3S$ (%): C 40.65, H 2.19, N 10.16. Found: C 40.62, H 2.22, N 10.20.

*5-(3-Chlorophenyl)-4-(2-iodophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**18**) Yield 65%, m.p. 260–261 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) $\delta$ (ppm): 7.25–7.43 (m, 4H, Ar–H), 7.48–7.69 (m, 3H, Ar–H), 7.95–8.00 (m, 1H, Ar–H), 14.28 (s, 1H, NH, exch. $D_2O$). IR

(KBr, ν, $cm^{-1}$): 3434 (NH), 3099 ($CH_{arom.}$), 1574 (C=N), 1334 (C=S). EI-MS *m/z* (%): 413 ($M^+$, 7.6), 286 (71), 149 (100), 122 (13.4), 105 (16.3), 90 (9.6), 76 (17.3), 63 (6.7), 50 (7.6). Anal. Calc. for $C_{14}H_9ClIN_3S$ (%): C 40.65, H 2.19, N 10.16. Found: C 40.60, H 2.11, N 10.23.

*5-(3-Chlorophenyl)-4-(4-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**19**) Yield 85%, CAS Registry Number: 879074-80-3.

5-(3-Chlorophenyl)-4-(4-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**20**) Yield 90%, CAS Registry Number: 720667-75-4.

*5-(3-Chlorophenyl)-4-(2,5-difluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**21**) Yield 79%, m.p. 138–140 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 7.16–7.28 (m, 5H, Ar–H), 7.39–7.46 (m, 2H, Ar–H), 12.42 (s, 1H, NH, exch. $D_2O$). IR (KBr, ν, $cm^{-1}$): 3431 (NH), 3092 ($CH_{arom.}$), 1581 (C=N), 1360 (C=S). EI-MS *m/z* (%): 325 ($[M+2]^+$, 32.3), 323 ($M^+$, 100), 304 (67), 288 (7), 250 (11), 186 (12), 152 (37), 137 (22.3), 111 (21), 90 (13), 75 (27), 50 (9). Anal. Calc. for $C_{14}H_8ClF_2N_3S$ (%): C 51.94, H 2.49, N 12.98. Found: C 52.11, H 2.36, N 13.13.

*5-(3-Chlorophenyl)-4-(2,6-difluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**22**) Yield 77%, m.p. 158–160 °C, $^1$H NMR (250 MHz) (DMSO-$d_6$) δ (ppm): 7.25–7.28 (m, 1H, Ar–H), 7.36–7.48 (m, 5H, Ar–H), 7.56–7.62 (m, 1H, Ar–H), 14.21 (s, 1H, NH, exch. $D_2O$). IR (KBr, ν, $cm^{-1}$): 3432 (NH), 3080 ($CH_{arom.}$), 1570 (C=N), 1332 (C=S). EI-MS *m/z* (%): 325 ($[M+2]^+$, 31.8), 323 ($M^+$, 100), 304 (90), 250 (8.6), 167 (24), 152 (34.6), 137 (27.9), 125 (25.2), 90 (11.5), 75 (30), 63 (16), 50 (7). Anal. Calc. for $C_{14}H_8ClF_2N_3S$ (%): C 51.94, H 2.49, N 12.98. Found: C 52.00, H 2.41, N 13.03.

#### 4.1.4. General procedure for the synthesis of Mannich bases (23–33)

To a solution of corresponding compounds (**12**–**22**) (1 mmol) in 96% ethanol, pyrrolidine (1 mmol) and formaldehyde (37%, 0.2 mL) were added and the mixture was stirred at room temperature for 1 h. Next, distilled water was added and the precipitate formed was filtered off, washed several times with distilled water, and crystallized from ethanol.

*5-(3-Chlorophenyl)-4-phenyl-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**23**) Yield 85%, m.p. 102–104 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.70–1.77 (m, 4H, 2× $CH_2$), 2.94 (t, 4H, 2× $CH_2$, $J$ = 6.60 Hz), 5.28 (s, 2H, $CH_2$), 6.99–7.14 (m, 2H, Ar–H), 7.18–7.30 (m, 3H, Ar–H), 7.33–7.36 (m, 1H, Ar–H), 7.41–7.47 (m, 3H, Ar–H). IR (KBr, ν, $cm^{-1}$): 3045 ($CH_{arom.}$), 2911, 2878 ($CH_{aliph.}$), 1581 (C=N), 1326 (C=S). EI-MS *m/z* (%): 370 ($M^+$, 1), 286 (3), 84 (100), 77 (4.8), 55 (3.8), 42 (4.8). Anal. calc. for $C_{19}H_{19}ClN_4S$ (%): C 61.53, H 5.16, N 15.11. Found: C 61.55, H 5.21, N 15.05.

*5-(3-Chlorophenyl)-4-(4-methylphenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**24**) Yield 72%, m.p. 104–106 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.81–1.86 (m, 4H, 2 × $CH_2$), 2.46 (s, 3H, $CH_3$), 3.01 (t, 4H, 2× $CH_2$, $J$ = 6.50 Hz), 5.38 (s, 2H, $CH_2$), 7.12–7.16 (m, 1H, Ar–H), 7.20 (dd, 2H, $J$ = 8.33 Hz, 2.00 Hz, Ar–H, p-substituted ring), 7.30 (dd, 2H, $J$ = 8.30 Hz, 2.00 Hz, Ar–H, p-substituted ring), 7.34–7.41 (m, 2H, Ar–H), 7.49 (t, 1H, $J$ = 1.90 Hz, Ar–H). IR (KBr, ν, $cm^{-1}$): 3040 ($CH_{arom.}$), 2964, 2833 ($CH_{aliph.}$),1599 (C=N), 1327 (C=S). EI-MS *m/z* (%): 384 ($M^+$, 0.6), 300 (3.4), 163 (1), 138 (1.4), 84 (100), 55 (3.8), 42 (4.2). Anal. calc. for $C_{20}H_{21}ClN_4S$ (%): C 62.41, H 5.50, N 14.56. Found: C 62.58, H 5.61, N 14.49.

*5-(3-Chlorophenyl)-4-(4-methoxyphenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**25**) Yield 69%, m.p. 102–104 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.70–1.76 (m, 4H, 2× $CH_2$), 2.98 (t, 4H, 2× $CH_2$, $J$ = 6.60 Hz), 3.78 (s, 3H, $OCH_3$), 5.27 (s, 2H, $CH_2$), 6.92 (dd, 2H, $J$ = 8.80 Hz, 2.20 Hz, Ar–H, p-substituted ring), 7.01–7.06 (m, 1H, Ar–H), 7.14 (dd, 2H, $J$ = 8.80 Hz, 2.30 Hz, Ar–H, p-substituted ring), 7.19 (s, 1H, Ar–H), 7.25–7.30 (m, 1H, Ar–H), 7.40 (t, 1H, $J$ = 1.90 Hz, Ar–H). IR (KBr, ν, $cm^{-1}$): 3056 ($CH_{arom.}$), 2945, 2888 ($CH_{aliph.}$), 1590 (C=N), 1338 (C=S). EI-MS *m/z* (%): 400 ($M^+$, 1.1), 317 (1.9), 138 (1.4), 84 (100), 55 (3), 42 (2.8). Anal. calc. for $C_{20}H_{21}ClN_4OS$ (%): C 59.91, H 5.28, N 13.97. Found: C 59.98, H 5.20, N 13.85.

*4-(4-Bromophenyl)-5-(3-chlorophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**26**) Yield 80%, m.p. 136–138 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.84–1.86 (m, 4H, 2× $CH_2$), 3.01 (t, 4H, 2× $CH_2$, $J$ = 6.75 Hz), 5.37 (s, 2H, $CH_2$), 7.05–7.10 (m, 1H, Ar–H), 7.21 (dd, 2H, $J$ = 8.78 Hz, 2.05 Hz, Ar–H, p-substituted ring), 7.25–7.30 (m, 1H, Ar–H), 7.39–7.44 (m, 1H, Ar–H), 7.51 (t, 1H, $J$ = 1.90 Hz, Ar–H), 7.67 (dd, 2H, $J$ = 8.80 Hz, 2.05 Hz, Ar–H, p-substituted ring). IR (KBr, ν, $cm^{-1}$): 3047 ($CH_{arom.}$), 2961, 2876 ($CH_{aliph.}$), 1584 (C=N), 1326 (C=S). EI-MS *m/z* (%): 450 ($[M+1]^+$, 0.3), 366 (2.1), 155 (1.5), 138 (1.3), 84 (100), 55 (3.8), 42 (5.1). Anal. calc. for $C_{19}H_{18}BrClN_4S$ (%): C 50.73, H 4.03, N 12.46. Found: C 50.66, H 4.18, N 12.41.

*4-(2-Bromophenyl)-5-(3-chlorophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**27**) Yield 86%, m.p. 172–174 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.69–1.77 (m, 4H, 2× $CH_2$), 2.95 (t, 4H, 2× $CH_2$, $J$ = 6.60 Hz), 5.27 (s, 2H, $CH_2$), 7.05–7.21 (m, 2H, Ar–H), 7.26–7.50 (m, 5H, Ar–H), 7.64–7.69 (m, 1H, Ar–H). IR (KBr, ν, $cm^{-1}$): 3053 ($CH_{arom.}$), 2961, 2843 ($CH_{aliph.}$), 1589 (C=N), 1321 (C=S). EI-MS *m/z* (%): 450 ($[M+1]^+$, 0.6), 286 (2.4), 149 (2.9), 84 (100), 55 (3.2), 42 (2.5). Anal. calc. for $C_{19}H_{18}BrClN_4S$ (%): C 50.73, H 4.03, N 12.46. Found: C 50.81, H 4.10, N 12.49.

*5-(3-Chlorophenyl)-4-(4-iodophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**28**) Yield 83%, m.p. 143–144 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.70–1.76 (m, 4H, 2× $CH_2$), 2.90 (t, 4H, 2× $CH_2$, $J$ = 6.60 Hz), 5.26 (s, 2H, $CH_2$), 6.97 (dd, 2H, $J$ = 8.80 Hz, 2.10 Hz, Ar–H, p-substituted ring), 7.11–7.19 (m, 2H, Ar–H), 7.28–7.34 (m, 1H, Ar–H), 7.41 (t, 1H, $J$ = 1.88 Hz, Ar–H), 7.71 (dd, 2H, $J$ = 8.80 Hz, 2.10 Hz, Ar–H, p-substituted ring). IR (KBr, ν, $cm^{-1}$): 3062 ($CH_{arom.}$), 2958, 2846 ($CH_{aliph.}$), 1590 (C=N), 1329 (C=S). EI-MS *m/z* (%): 496 ($M^+$, 0.6), 413 (3.2), 203 (1.9), 138 (2.5), 84 (100), 63 (1.2), 55 (6.7), 42 (9.6). Anal. calc. for $C_{19}H_{18}ClIN_4S$ (%): C 45.94, H 3.65, N 11.28. Found: C 45.87, H 3.59, N 11.35.

*5-(3-Chlorophenyl)-4-(2-iodophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**29**) Yield 76%, m.p. 166–168 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.70–1.77 (m, 4H, 2× $CH_2$), 2.94 (t, 4H, 2× $CH_2$, $J$ = 6.60 Hz), 5.25 (s, 2H, $CH_2$), 7.06–7.22 (m, 3H, Ar–H), 7.26–7.53 (m, 4H, Ar–H), 7.88–7.93 (m, 1H, Ar–H). IR (KBr, ν, $cm^{-1}$): 3054 ($CH_{arom.}$), 2923, 2887 ($CH_{aliph.}$), 1585 (C=N), 1323 (C=S). EI-MS *m/z* (%): 496 ($M^+$, 0.9), 285 (4), 148 (11.5), 84 (100), 55 (4.2), 42 (4.7). Anal. calc. for $C_{19}H_{18}ClIN_4S$ (%): C 45.94, H 3.65, N 11.28. Found: C 45.98, H 3.71, N 11.20.

*4-(4-Chlorophenyl)-5-(3-chlorophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**30**) Yield 77%, m.p. 138–140 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.70–1.76 (m, 4H, 2× $CH_2$), 2.90 (t, 4H, 2× $CH_2$, $J$ = 6.60 Hz), 5.26 (s, 2H, $CH_2$), 6.95–7.01 (m, 1H, Ar–H), 7.15 (dd, 2H, $J$ = 8.10 Hz, 2.00 Hz, Ar–H, p-substituted ring), 7.19–7.21 (m, 1H, Ar–H), 7.28–7.33 (m, 1H, Ar–H), 7.40 (dd, 2H, $J$ = 8.10 Hz, 2.05 Hz, Ar–H, p-substituted ring), 7.41–7.44 (m, 1H, Ar–H). IR (KBr, ν, $cm^{-1}$): 3069 ($CH_{arom.}$), 2954, 2874 ($CH_{aliph.}$), 1589 (C=N), 1333 (C=S). EI-MS *m/z* (%): 406 ($[M+1]^+$, 0.5), 320 (3.5), 138 (4.7), 125 (5.4), 111 (20), 84 (100), 55 (9.6), 42 (8.6). Anal. calc. for $C_{19}H_{18}Cl_2N_4S$ (%): C 56.30, H 4.48, N 13.82. Found: C 56.38, H 4.35, N 13.91.

*5-(3-Chlorophenyl)-4-(4-fluorophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**31**) Yield 80%, m.p. 114–116 °C, $^1$H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.70–1.77 (m, 4H, 2× $CH_2$), 2.91 (t, 4H, 2× $CH_2$, $J$ = 6.62 Hz), 5.27 (s, 2H, $CH_2$), 6.98–7.04 (m, 1H, Ar–H), 7.14 (dd, 2H, $J$ = 8.05 Hz, 2.00 Hz, Ar–H, p-substituted ring) 7.21 (dd, 2H, $J$ = 8.05 Hz, 1.90 Hz, Ar–H, p-substituted ring), 7.23–7.26 (m, 1H, Ar–H), 7.28–7.33 (m, 1H, Ar–H), 7.36 (t, 1H, $J$ = 1.81 Hz, Ar–H). IR (KBr, ν, $cm^{-1}$): 3046 ($CH_{arom.}$), 2991, 2841 ($CH_{aliph.}$),

1581 (C=N), 1323 (C=S). EI-MS *m/z* (%): 388 ($M^+$, 0.6), 304 (2.4), 138 (1.5), 84 (100), 55 (3.8), 42 (4.8). Anal. calc. for $C_{19}H_{18}ClFN_4S$ (%): C 58.68, H 4.67, N 14.41. Found: C 58.71, H 4.58, N 14.40.

*5-(3-Chlorophenyl)-4-(2,5-difluorophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**32**) Yield 78%, m.p. 138–140 °C, $^1$H NMR (250 MHz) ($CDCl_3$) $\delta$ (ppm): 1.71–1.76 (m, 4H, 2× $CH_2$), 2.90 (t, 4H, 2× $CH_2$, $J = 6.60$ Hz), 5.27 (s, 2H, $CH_2$), 7.06–7.21 (m, 5H, Ar–H), 7.30–7.36 (m, 1H, Ar–H), 7.39 (t, 1H, $J = 1.82$ Hz, Ar–H). IR (KBr, ν, $cm^{-1}$): 3041 ($CH_{arom.}$), 2968, 2845 ($CH_{aliph.}$), 1583 (C=N), 1327 (C=S). EI-MS *m/z* (%): 406 ($M^+$, 0.8), 322 (1.2), 250 (1.6), 138 (1.5), 84 (100), 55 (3.6), 42 (5.1). Anal. calc. for $C_{19}H_{17}ClF_2N_4S$ (%): C 56.09, H 4.21, N 13.77. Found: C 56.15, H 4.31, N 13.72.

*5-(3-Chlorophenyl)-4-(2,6-difluorophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione* (**33**) Yield 70%, m.p. 94–96 °C, $^1$H NMR (250 MHz) ($CDCl_3$) $\delta$ (ppm): 1.70–1.77 (m, 4H, 2× $CH_2$), 2.90 (t, 4H, 2× $CH_2$, $J = 6.60$ Hz), 5.28 (s, 2H, $CH_2$), 6.98–7.07 (m, 2H, Ar–H), 7.12–7.22 (m, 2H, Ar–H), 7.30–7.36 (m, 1H, Ar–H), 7.39–7.51 (m, 2H, Ar–H). IR (KBr, ν, $cm^{-1}$): 3048 ($CH_{arom.}$), 2971, 2895 ($CH_{aliph.}$), 1593 (C=N), 1324 (C=S). EI-MS *m/z* (%): 406 ($M^+$, 0.9), 323 (1.6), 250 (1.7), 138 (1.7), 84 (100), 55 (3.9), 42 (4.7). Anal. calc. for $C_{19}H_{17}ClF_2N_4S$ (%): C 56.09, H 4.21, N 13.77. Found: C 56.10, H 4.27, N 13.79.

### 4.2. Microbiology

The antimicrobial activity of the compounds was tested on the Gram-positive strains (*S. aureus* ATCC 25923, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *B. subtilis* ATCC 6633, *B. cereus* ATCC 10876, *Micrococcus luteus* ATCC 10240) and on the Gram-negative strains ( *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *P. mirabilis* ATCC 12453). Besides, twelve clinical isolates of *S. aureus* from the collection of the Department of Pharmaceutical Microbiology of Medical University in Lublin were also used in our experiments. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of 0.5 McFarland standard – $150 \times 10^6$ CFU/mL (CFU – colony forming units). All stock solutions of the tested compounds were dissolved in dimethyl sulfoxide (DMSO). Mueller–Hinton medium was used with a series of two-fold dilutions of the tested substances in the range of final concentrations from 3.91 to 1000 μg/mL. It was dimethyl sulfoxide (DMSO) had no antimicrobial activity against any of the tested microorganisms. Ampicillin, antibiotic widely used in the treatment of infectious diseases, was used as control antimicrobial agent.

The *in vitro* antibacterial activity of the tested compounds were screened on the basis of MIC (minimal inhibitory concentration), usually defined as the lowest concentration of compound at which there was no visible growth of tested microorganisms. Determination of the MIC values was achieved by a broth microdilution method, according to CLSI recommendation [27]. MBC (minimal bactericidal concentration), defined as the lowest concentration of compound that resulted in >99.9% reduction in CFU of the initial inoculum, was assessed only for Mannich bases (**23**–**33**). MBC was determined by a broth microdilution technique by plating out the contents of wells (20 μL), that showed no visible growth of bacteria, onto Mueller–Hinton agar plates and incubating at 35 °C for 18 h. Both MIC and MBC values are given in μg/mL, according to CLSI reference [27].

### 4.3. Computational part

The values of the following parameters: *SA*, *V*, *HE*, *logP*, *MR*, $\alpha$, $\mu$, $E_{HOMO}$, $E_{LUMO}$ were calculated using semi-empirical AM1 method as implemented in HyperChem [28]. In a first step, the molecular geometries of all compounds were fully optimized in the gas phase to gradients of 0.01 kcal/(mol A) and afterwards the molecular descriptors were determined.

## Appendix. Supplementary data

The supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2010.11.010.

## References


[1] U. Salgın-Gökşen, N. Gökhan-Kelekçi, Ö Göktaş, Y. Köysal, E. Kılıç, Ş Işık, G. Aktay, M. Özalp, 1-Acylthiosemicarbazides, 1,2,4-triazole-5(4H)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones: synthesis, analgesic-anti-inflammatory and antimicrobial activities, Bioorg. Med. Chem. 15 (2007) 5738–5751.

[2] S. Tehranchian, T. Akbarzadeh, M.R. Fazeli, H. Jamalifar, A. Shafiee, Synthesis and antibacterial activity of 1-[1,2,4-triazol-3-yl] and 1-[1,3,4-thiadiazol-2-yl]-3-methylthio-6,7-dihydro-benzo[c]thiophen-4(5H)-ones, Bioorg. Med. Chem. Lett. 15 (2005) 1023–1025.

[3] G. Turan-Zitouni, Z.A. Kaplancıklı, M.T. Yıldız, P. Chevallet, D. Kaya, Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[N-(2-thiazolyl)acetamido]-thio-4H-1,24-triazole derivatives, Eur. J. Med. Chem. 40 (2005) 607–613.

[4] H.M. Abdel-Rahman, M.A. Hussein, Synthesis of β-hydroxypropanoic acid derivatives as potential anti-inflammatory, analgesic and antimicrobial agents, Arch. Pharm. Chem. Life Sci. 339 (2006) 378–387.

[5] A. Shafiee, A. Sayadi, M.H. Roozbahani, A. Foroumadi, F. Kamal, Synthesis and in vitro antimicrobial evaluation of 5-(1-methyl-5-nitro-2-imidazolyl)-4H-1,2,4-triazoles, Arch. Pharm. Pharm, Med. Chem. 10 (2002) 495–499.

[6] H. Kitani, T. Kuroda, A. Moriguchi, H. Ao, F. Hirayama, Y. Ikeda, T. Kawakita, Synthesis and structural optimization of 7-(3,3-disubstituted-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acids as antibacterial agents, Bioorg. Med. Chem. Lett. 7 (1997) 515–520.

[7] Y. Tang, K.-X. Chen, H.-L. Jiang, R.-Y. Ji, QSAR/QSTR of fluoroquinolones: an example of simultaneous analysis of multiple biological activities using neural network method, Eur. J. Med. Chem. 33 (1998) 647–658.

[8] H. Bayrak, H. Demirbas, S.A. Karaoglu, N. Demirbas, Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities, Eur. J. Med. Chem. 44 (2009) 1057–1066.

[9] M. Ashok, B.M. Holla, B. Poojary, Convenient one pot synthesis and antimicrobial evaluation of some new Mannich bases carrying 4-methylthiobenzyl moiety, Eur. J. Med. Chem. 42 (2007) 1095–1101.

[10] M.S. Karthikeyan, D.J. Prasad, B. Poojary, K.S. Bhat, B.M. Holla, N.S. Kumari, Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety, Bioorg. Med. Chem. 14 (2006) 7482–7489.

[11] J.M. Domagala, Structure-activity and structure-side-effect relationships for the quinolone antibacterials, J. Antimicrob. Chemother. 33 (1994) 685–706.

[12] M. Dobosz, A. Pachuta-Stec, Synthesis of new thiourea derivatives of 1,2,4-triazoline-5-thione and 1,3,4-thiadiazole, Acta Polon. Pharm. 51 (1994) 457–465.

[13] A. Siwek, M. Wujec, M. Dobosz, E. Jagiełło-Wójtowicz, A. Chodkowska, A. Kleinrok, P. Paneth, Synthesis and pharmacological properties of 3-(2-methylfuran-3-yl)-4-substituted-$\Delta^2$-1,2,4-triazoline-5-thiones, Centr. Eur. J. Chem. 6 (2008) 47–53.

[14] H.N. Dogan, S. Rollas, H. Erdeniz, Synthesis, structure elucidation and antimicrobial activity of some 3-hydroxy-2-naphtoic acid hydrazide derivatives, Il Farmaco 53 (1998) 462–467.

[15] N. Demirbas, S.A. Karaoglu, A. Demirbas, K. Sancak, Synthesis and antimicrobial activities of some new 1-(5-phenylamino-[1,3,4]thiadiazol-2-yl)methyl-5-oxo-[1,2,4]triazole and 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-5-oxo-[1,2,4]triazole derivatives, Eur. J. Med. Chem. 39 (2004) 793–804.

[16] A. Demirbas, D. Sahin, N. Demirbas, S.A. Karaoglu, Synthesis of some new 1,3,4-thiadiazol-2-ylmethyl-1,2,4-triazole derivatives and investigation of their antimicrobial activities, Eur. J. Med. Chem. 44 (2009) 2896–2903.

[17] H. Bayrak, A. Demirbas, S.A. Karaoglu, N. Demirbas, Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities, Eur. J. Med. Chem. 44 (2009) 1057–1066.

[18] M. Wujec, U. Kosikowska, A. Siwek, A. Malm, New derivatives of thiosemicarbazide and 1,2,4-triazoline-5-thione with potential antimicrobial activity, Phosphorus Sulfur Silicon Relat. Elem. 184 (2009) 559–567.

[19] M. Wujec, U. Kosikowska, P. Paneth, A. Malm, Reaction of hydrazide of (tetrazol-5-yl)acetic acid with isothiocyanates and antimicrobial investigations of newly-obtained compounds, Heterocycles 71 (2007) 2617–2626.

[20] A. Siwek, M. Wujec, M. Dobosz, P. Paneth, Synthesis and theoretical characterization of some new 4-substituted-1,3-diphenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazoles with potential pharmacological activity, Heteroatom Chem. 19 (2008) 713–718.

[21] I. Bourgeois, M. Pestel-Caron, J.F. Lemeland, J.L. Pons, F. Caron, Tolerance to the glycopeptides vancomycin and teicoplanin in coagulase-negative Staphylococci, Antimicrob. Agents Chemother. 51 (2007) 740–743.

[22] E.L. White, W.J. Suling, L.J. Ross, L.E. Seitz, R.C. Reynolds, *2-Alkoxycarbonylaminopyridines: inhi*bitors of *Mycobacterium tuberculosis* FtsZ, J. Antimicrob. Chemother. 50 (2002) 111–114.

[23] M.F.S. Lopes, T. Ribeiro, M. Abrantes, J.J.F. Marques, R. Tenreiro, M.T.B. Crespo, Antimicrobial resistance profiles of dairy and clinical isolates and type strains of enterococci, Int. J. Food Microbiol. 103 (2005) 191–198.

[24] L. Nie, Z. Lie, J. Han, X. Zhang, R. Yang, W.-X. Liu, F.-Y. Wu, J.-W. Xie, Y.-F. Zhao, Y.-B. Jiang, Development of N-benzamidothioureas as a new generation of thiourea-based receptors for anion recognition and sensing, J. Org. Chem. 69 (2004) 6449–6454.
[25] J.-P. Li, Q.-F. Luo, Y.-L. Wang, H. Wang, An efficient solid-state method for the preparation of acylthiosemicarbazides, Synth. Commun. 31 (2001) 1793–1797.
[26] M.H. Shah, V.M. Patki, M.Y. Mhasalkar, 1,2,4-Triazole derivatives as possible diuretic agents, J. Sci. Ind. Res. 21C (1962) 76–78.
[27] CLSI, Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth International Supplement. CLSI Document M7-MIC. Clinical Laboratory Standards Institute, Wayne, PA, 2008.
[28] HyperChem 8.0.3. HyperCube Inc., Gainsville, FL, 2007.