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Synthesis and Antibacterial Activity of Some Novel N2-Hydroxymethyl and N2-Aminomethyl Derivatives of 4-aryl-5-(3-Chlorophenyl)-2,4-Dihydro-3*H*-1,2,4-Triazole-3-Thione

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ABSTRACT: *In this investigation, several novel N2-hydroxymethyl and N2-aminomethyl derivatives of 5-(3-chlorophenyl)-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and 4-(4-bromophenyl)-5-(3-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione were prepared. All synthesized compounds were screened for their antibacterial activity against six Gram-positive and four Gram-negative bacterial strains.* © 2011 Wiley Periodicals, Inc. Heteroatom Chem 22:737–743, 2011; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.20737

INTRODUCTION

The triazole ring is considered to be an important element of many pharmacophore structures. It determines antifungal [1,2], antibacterial [3,4], antiviral [5], antiepileptic [6], anticancer [7–9], anxiolytic [10], and antimigraine [11] activities, and others. In our previous study [12] we described, among others, the antibacterial activity of aminomethyl (strictly: pyrrolidin-1-ylmethyl) derivatives of 4-aryl-5-(3-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione. Some authors report that the aminomethylation of 1,2,4-triazoles permits them to obtain more active compounds [13–15], but the results of studies by other researchers do not confirm this thesis [16–18]. Using the results obtained by us it has been discovered that, for the described compounds, the Mannich reaction gave more active derivatives when in the N-4 position a phenyl ring with electron-donating substituents (CH_3, OCH_3) was present. On the other hand, for the 1,2,4-triazole derivatives with electron-withdrawing substituents in the phenyl ring (in the N-4 position), no increased antibacterial activity was found after transforming these compounds into respective Mannich bases. As in the cited work, only pyrrolidin-1-ylmethyl derivatives of 4-aryl-5-(3-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione were described; it seemed useful to check how the antibacterial activity of the compounds will change after the introduction of different substituents in the N-2 position. Figure 1 shows the parent compounds (**A, B**) that were used as the substrates in the Mannich reaction. The choice of these compounds resulted from their completely different microbiological and physicochemical properties (**A**—microbiologically inactive

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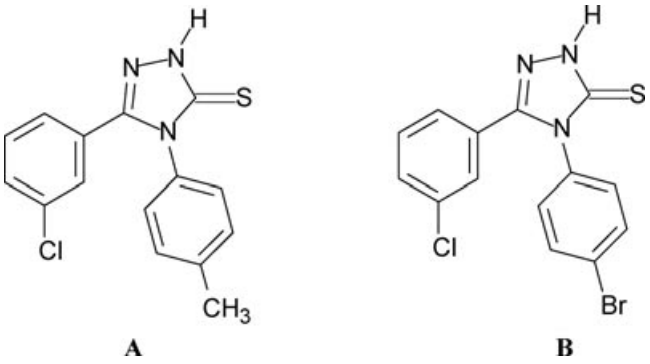


FIGURE 1 Structure of the parent compounds (**A**—inactive, MIC >1000 μg/mL), (**B**—active, MIC = 15.63–31.25 μg/mL).

compound with electron-donating substituent in the *para* position of the phenyl ring; **B**—microbiologically active compound with electron-withdrawing substituent in this position).

RESULTS AND DISCUSSION

Chemistry

The parent compounds (**A, B**) were synthesized according to the method reported in [12]. The synthesis of compounds (**1–14**) has been carried out as depicted in Fig. 2.

Hydroxymethyl derivatives (**1, 2**) were obtained from the reaction of compounds (**A, B**, respectively) with formaldehyde while the compounds (**3–14**) were synthesized using the so-called Mannich reaction. Its mechanism was extensively described by Almajan et al. [18]. For all the described substances the reaction time was 1 h, while the reaction yields were from 74 to 90% (Table 1). In the ^{1}H NMR spectra, the signal of the two protons of the CH_2 group (bound to the N-2 nitrogen atom) was visible in the range of 5.19–5.74 ppm. For the compounds (**1, 2**), the signal of the hydroxyl group proton was registered at 7.09 ppm and 7.14 ppm, respectively. The chemical shifts of the aromatic protons were in the range of 6.85–7.76 ppm. Proof that the reaction is completed can also be found in the disappearance of the peak of the proton bound to the N-2 nitrogen atom in the initial compounds (**A** and **B**), observed earlier at 14.01 ppm and 14.24 ppm, respectively. In the IR spectra, characteristic bands are visible: for the C=S group in the range of 1317–1348 cm^{-1}, for aromatic rings in the range of 3009–3112 cm^{-1}, and for aliphatic C-H bonds—the vibrations in the range of 2809–2991 cm^{-1}. Furthermore, the N2-hydroxymethyl derivatives (**1, 2**) have characteristic wide bands of stretching vibrations at 3437 cm^{-1} and at 3401 cm^{-1}, respectively.

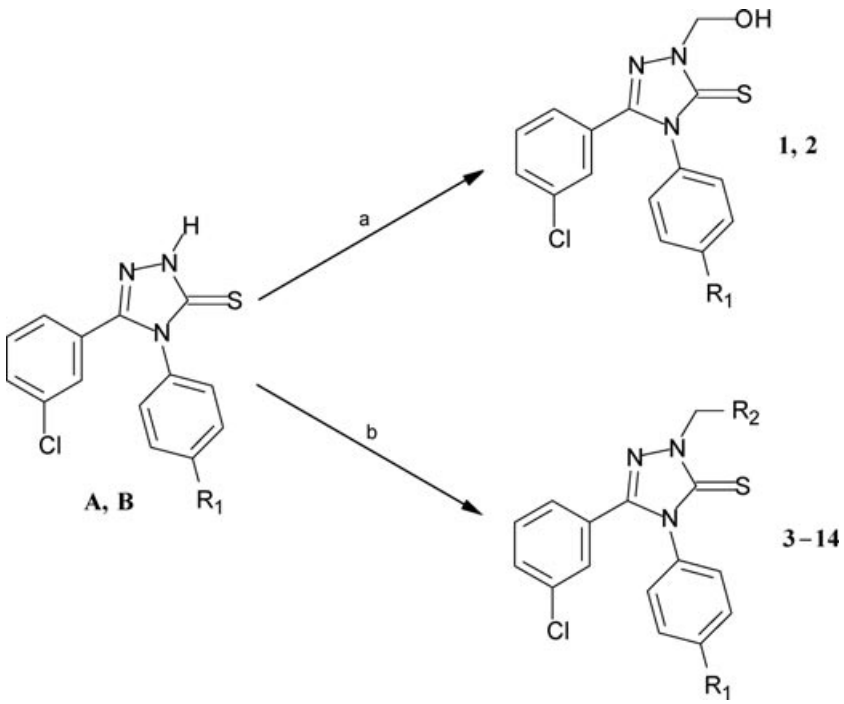
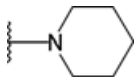
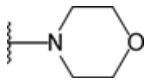
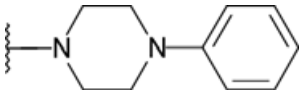
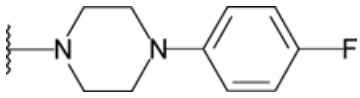
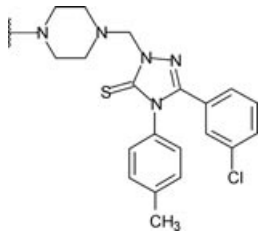
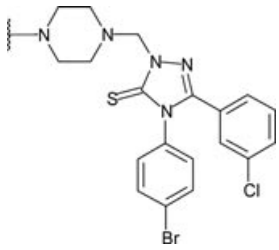


FIGURE 2 Synthesis of compounds **1–14**. Reagents and conditions: (a) HCOH, EtOH, rt, 1 h; (b) HCOH, amine, EtOH, rt, 1 h.

TABLE 1 Physical Data of the Compounds **1–14**

No.	R_1	R_2	*M.p. (°C)*	*Yield (%)*	*M.w*
1	$-CH_3$	–	142–144	86	331.82
2	–Br	–	130–131	74	396.69
3	$-CH_3$	$-N(C_2H_5)_2$	112–114	75	386.94
4	–Br	$-N(C_2H_5)_2$	96–98	76	451.81
5	$-CH_3$		118–120	83	398.95
6	–Br		106–108	77	463.82
7	$-CH_3$		146–148	79	400.92
8	–Br		138–140	82	465.79
9	$-CH_3$		120–122	83	476.04
10	–Br		78–80	80	540.90
11	$-CH_3$		150–152	90	494.03
12	–Br		88–90	88	558.90
13	$-CH_3$		252–254	90	713.74
14	–Br		212–214	79	843.48

Microbiological Part

The determination of the antibacterial activity of the newly synthesized compounds was made with the use of the broth microdilution method. The microbiological studies were performed for six strains of Gram-positive and 4 strains of Gram-negative bacteria. The activity was expressed as the minimum concentration that inhibits the growth of the bacteria (MIC—minimal inhibitory concentration); for some substances the values of minimal bactericidal concentration (MBC) were also determined. Cefuroxime and ampicillin were used as reference antibiotics. The values of MIC (and/or MBC) are given in Table 2. The activities of the substances (**1–14**) against Gram-negative bacteria were insignificant (MIC $\geq$500), which is why in Table 2 only the values of MIC (and/or MBC) for the strains of Gram-positive bacteria are presented.

The N2-substituted derivatives of 4-(4-bromophenyl)-5-(3-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**B**) completely inhibited the growth of the bacteria at the concentration of 31.25–125°C μg/mL, with the only exception being the inactive compound (**14**). The most promising activity was that of the N2-hydroxymethyl derivative (**2**), which acted against *B. cereus* ATCC 10876 twice as strongly as ampicillin. Moreover, four other derivatives (**4, 6, 8, 10**) of the initial compound (**B**) demonstrated an antibacterial activity equal to that

TABLE 2 In vitro Antibacterial Screening of the Compounds **1–14** (MIC and MBC Values Are Given in μg/mL)

	Microorganisms											
	S. aureus *ATCC 25923*		***S. aureus*** *ATCC 6538*		***S. epidermidis*** *ATCC 12228*		***B. subtilis*** *ATCC 6633*		***B. cereus*** *ATCC 10876*		***M. luteus*** *ATCC 10240*	
Compounds	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
A[a]	>1000	nd	>1000	nd	>1000	nd	>1000	nd	>1000	nd	>1000	nd
B[a]	31.25	nd	31.25	nd	31.25	nd	15.63	nd	15.63	nd	31.25	nd
1	62.5	nd	125	nd	125	nd	62.5	nd	**62.5**	nd	125	nd
2	31.25	nd	62.5	nd	62.5	nd	31.25	nd	**31.25**	nd	62.5	nd
3	62.5	>1000	62.5	>1000	125	>1000	125	500	**62.5**	>1000	62.5	1000
4	31.25	>1000	62.5	>1000	62.5	>1000	62.5	500	**62.5**	>1000	31.25	500
5	62.5	>1000	125	1000	125	>1000	125	500	125	>1000	62.5	>1000
6	31.25	>1000	62.5	>1000	62.5	>1000	62.5	1000	**62.5**	1000	31.25	>1000
7	125	>1000	125	>1000	125	>1000	125	1000	125	>1000	62.5	>1000
8	31.25	>1000	125	>1000	125	>1000	62.5	1000	**62.5**	>1000	31.25	500
9	250	nd	250	nd	250	nd	250	nd	250	nd	250	nd
10	62.5	nd	125	nd	125	nd	62.5	nd	**62.5**	nd	62.5	nd
11	125	nd	250	nd	250	nd	62.5	nd	125	nd	250	nd
12	125	nd	125	nd	125	nd	125	nd	125	nd	125	nd
13	>1000	nd	>1000	nd	>1000	nd	>1000	nd	>1000	nd	>1000	nd
14	>1000	nd	>1000	nd	>1000	nd	>1000	nd	>1000	nd	>1000	nd
Ampicillin	nd	nd	nd	nd	nd	nd	nd	nd	**62.5**	nd	nd	nd
Cefuroxime	0.49	nd	0.98	nd	0.24	nd	15.63	nd	**31.25**	nd	0.98	nd

nd—not determined. [a]—data derived from [12].

of ampicillin against this strain. Unfortunately, none of the introduced substituents caused an intensification of the action of the initial compound (**B**). On the other hand, some very interesting results have been obtained for the derivatives of the antibacterially inactive 5-(3-chlorophenyl)-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**A**). With the exception of compound (**13**), which was inactive, the remaining N2-substituted derivatives showed the activity on the level of concentrations 62.5–250 μg/mL. The N2-hydroxymethyl derivative (**1**) and the N2-(diethylamino)methyl derivative (**3**) affected the *B. cereus* ATCC 10876 strain as strongly as the reference antibiotic (ampicillin). When compared to the inactive initial compound (**A**), it is a significant, positive change. Interesting conclusions are also provided by the comparison of the MIC and MBC values for the tested substances. A high value of the MBC/MIC ratio suggests that these compounds display a bacteriostatic mechanism of action.

Despite a large number of scientific reports on the antibacterial activity of the derivatives of 1,2,4-triazole, the mechanism of action of these substances has not been found yet. Knowing this mechanism would help in fighting an increasing amount of common drug-resistant strains of pathogen bacteria, such as *S. aureus*. For most of the studied bacterial strains, and particularly for the *S. aureus* ATCC 25923, a significant dependence structure/activity is visible. The introduction of new substituents into the active compound (**B**) does not improve its activity in any way. The parent compound (**B**) and its derivatives (**2, 4, 6, 8**) are characterized by the same MIC values. Only after the introduction of large-volume substituents (**10, 12, 14**) does the efficiency of these derivatives decrease gradually. It could be supposed that a substituent in the N-2 position could be a steric hindrance, which impedes (**10, 12**) or totally prevents (**14**) the binding with the potential drug-target sites. Another situation is observed in the case of substance (**A**), initially inactive, which after the transformation into N2-substituted derivatives starts to act as a significant antibacterial agent. In summary, it is possible to propose the following hypotheses: (1) a substituent in the N-4 position determines the antibacterial activity to a much larger extent than a substituent in the N-2 position; (2) the presence of an electron-withdrawing substituent (-Br) at the phenyl ring is important—probably because the electron characteristic of this substituent determines the possibility of binding the molecule with the potential drug-target site; (3) the interaction of the 4-bromophenyl moiety with the active site is strong enough for the influence of the presence of a substituent in the N-2 position to be unnoticeable (but only until this substituent starts to be a steric hindrance, impeding the binding of the

whole molecule); and (4) the influence of the N2-substituted fragment becomes visible in the case of substances with electron-donating substituents bound to the phenyl ring in the N-4 position of the 1,2,4-triazole. Due to the relatively small number of compounds, the proposed hypotheses are only preliminary and need to be completed. This will be done successively.

EXPERIMENTAL

Chemistry

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 δ (ppm). The IR spectra were recorded in KBr discs using a Perkin-Elmer 1725X FTIR spectrometer. 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 the exposure to UV-lamp at $\lambda = 254$ nm. Elemental analyses were performed on AMZ 851 CHX analyzer and the results were within $\pm 0.4\%$ of the theoretical value.

*General Procedure for the Synthesis of N2-hydroxymethyl Derivatives (***1, 2***).* A mixture of 10 mmol of compounds (**A**) or (**B**) and formaldehyde (37%, 15 mmol) in ethanol medium (20 ml) was stirred at room temperature for 1 h. The obtained products were filtered off, dried, and crystallized from ethanol.

*5-(3-Chlorophenyl)-2-(hydroxymethyl)-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***1***)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 2.43 (s, 3H, CH_3), 5.74 (s, 2H, CH_2), 7.09 (s, 1H, OH), 7.18–7.46 (m, 8H, Ar-H). IR (KBr, ν, cm^{-1}): 3437, 3091, 2962, 2903, 1588, 1519, 1481, 1333, 709. Anal. Calc. for $C_{16}H_{14}ClN_3OS$ (%): C 57.91, H 4.25, N 12.66. Found: C 58.02, H 4.19, N 12.75.

*4-(4-Bromophenyl)-5-(3-chlorophenyl)-2-(hydroxymethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***2***)* ^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 5.62 (d, 2H, CH_2, $J = 7.6$ Hz), 7.14 (t, 1H, OH, $J = 7.3$ Hz), 7.28–7.81 (m, 8H, Ar-H). IR (KBr, ν, cm^{-1}): 3401, 3055, 2972, 2899, 1583, 1529, 1491, 1333, 714. Anal. Calc. for $C_{15}H_{11}BrClN_3OS$ (%): C 45.42, H 2.79, N 10.59. Found: C 45.30, H 2.97, N 10.41.

*General Procedure for the Synthesis of N2-aminomethyl Derivatives (***3–14***).* 10 mmol of the starting compound (**A** or **B**) was dissolved in 20 mL of anhydrous ethanol and then equimolar amounts of appropriate secondary amine and formaldehyde (37%) were added to this solution. In order to obtain the compounds (**13**) and (**14**), a double excess of the triazole derivative (**A** or **B**, respectively), in relation to the quantity of piperazine, was used. The obtained 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)-2-[(diethylamino)methyl]-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***3***)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.24 (t, 6H, 2 × CH_3, $J = 7.2$ Hz), 2.46 (s, 3H, CH_3), 2.94 (q, 4H, 2 × CH_2, $J = 7.1$ Hz), 5.35 (s, 2H, CH_2), 7.11–7.54 (m, 8H, Ar-H). IR (KBr, ν, cm^{-1}): 3042, 3020, 2991, 2906, 1601, 1524, 1491, 1319, 701. Anal. Calc. for $C_{20}H_{23}ClN_4S$ (%): C 62.08, H 5.99, N 14.48. Found: C 62.00, H 6.11, N 14.32.

*4-(4-Bromophenyl)-5-(3-chlorophenyl)-2-[(diethylamino)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (***4***)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.23 (t, 6H, 2 × CH_3, $J = 7.1$ Hz), 2.93 (q, 4H, 2 × CH_2, $J = 7.1$ Hz), 5.34 (s, 2H, CH_2), 7.06–7.68 (m, 8H, Ar-H). IR (KBr, ν, cm^{-1}): 3050, 3026, 2967, 2843, 1574, 1539, 1492, 1412, 1330, 809. Anal. Calc. for $C_{19}H_{20}BrClN_4S$ (%): C 50.51, H 4.46, N 12.40. Found: C 50.56, H 4.38, N 12.43.

*5-(3-Chlorophenyl)-4-(4-methylphenyl)-2-(piperidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***5***)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.41–1.50 (m, 2H, CH_2), 1.61–1.69 (m, 4H, 2 × CH_2), 2.46 (s, 3H, CH_3), 2.90 (t, 4H, 2 × CH_2, $J = 5.4$ Hz), 5.27 (s, 2H, CH_2), 7.12–7.50 (m, 8H, Ar-H). IR (KBr, ν, cm^{-1}): 3067, 2983, 2871, 1590, 1520, 1482, 1341, 714. Anal. Calc. for $C_{21}H_{23}ClN_4S$ (%): C 63.22, H 5.81, N 14.04. Found: C 63.36, H 5.88, N 14.11.

*4-(4-Bromophenyl)-5-(3-chlorophenyl)-2-(piperidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***6***)* Yield 77%, m.p. 106–108°C. ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 1.46–1.50 (m, 2H, CH_2), 1.61–1.69 (m, 4H, 2 × CH_2), 2.89 (t, 4H, 2 × CH_2, $J = 5.4$ Hz), 5.26 (s, 2H, CH_2), 7.07–7.72 (m, 8H, Ar-H). IR (KBr, ν, cm^{-1}): 3061, 2982, 2860, 1591, 1541, 1417, 1329, 764. Anal. Calc. for $C_{20}H_{20}BrClN_4S$ (%): C 51.79, H 4.35, N 12.08. Found: C 51.85, H 4.21, N 12.18.

*5-(3-Chlorophenyl)-4-(4-methylphenyl)-2-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (***7***)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 2.46 (s, 3H, CH_3), 2.96 (t, 4H, 2 × CH_2, $J = 4.7$ Hz), 3.77 (t, 4H, 2 × CH_2, $J = 4.6$ Hz), 5.28 (s, 2H, CH_2),

7.11–7.56 (m, 8H, Ar-H). IR (KBr, ν, cm^{-1}): 3051, 2980, 2899, 1583, 1513, 1478, 1331, 777. Anal. Calc. for $C_{20}H_{21}ClN_4OS$ (%): C 59.91, H 5.28, N 13.97. Found: C 59.82, H 5.20, N 13.80.

*4-(4-Bromophenyl)-5-(3-chlorophenyl)-2-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**8**)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 2.95 (t, 4H, 2 × CH_2, $J = 4.6$ Hz), 3.77 (t, 4H, 2 × CH_2, $J = 4.6$ Hz), 5.27 (s, 2H, CH_2), 7.05–7.73 (m, 8H, Ar-H). IR (KBr, ν, cm^{-1}): 3052, 2935, 2909, 2851, 1575, 1552, 1489, 1436, 1327, 774. Anal. Calc. for $C_{19}H_{18}BrClN_4OS$ (%): C 48.99, H 3.90, N 12.03. Found: C 48.82, H 3.95, N 12.00.

*5-(3-Chlorophenyl)-4-(4-methylphenyl)-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**9**)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 2.46 (s, 3H, CH_3), 3.15–3.28 (m, 8H, 4 × CH_2), 5.29 (s, 2H, CH_2), 7.18–7.50 (m, 13H, Ar-H). IR (KBr, ν, cm^{-1}): 3052, 3009, 2927, 2838, 1598, 1527, 1478, 1345, 801. Anal. Calc. for $C_{26}H_{26}ClN_5S$ (%): C 65.60, H 5.51, N 14.71. Found: C 65.50, H 5.63, N 14.85.

*4-(4-Bromophenyl)-5-(3-chlorophenyl)-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (**10**)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 3.10–3.16 (m, 4H, 2 × CH_2), 3.23–3.30 (m, 4H, 2 × CH_2), 5.37 (s, 2H, CH_2), 6.85–7.72 (m, 13H, Ar-H). IR (KBr, ν, cm^{-1}): 3061, 3039, 2985, 2809, 1591, 1540, 1477, 1348, 740. Anal. Calc. for $C_{25}H_{23}BrClN_5S$ (%): C 55.51, H 4.29, N 12.95. Found: C 55.56, H 4.31, N 12.91.

*5-(3-Chlorophenyl)-2-{[4-(4-fluorophenyl)piperazin-1-yl]methyl}-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**11**)* ^{1}H-NMR (250 MHz) ($CDCl_3$) δ (ppm): 2.46 (s, 3H, CH_3), 3.19 (br. s, 8H, 4 × CH_2), 5.38 (s, 2H, CH_2), 7.21–7.51 (m, 12H, Ar-H). IR (KBr, ν, cm^{-1}): 3069, 2941, 2861, 1584, 1531, 1510, 1470, 1317, 698. Anal. Calc. for $C_{26}H_{25}ClFN_5S$ (%): C 63.21, H 5.10, N 14.18. Found: C 63.32, H 5.00, N 14.08.

*4-(4-Bromophenyl)-5-(3-chlorophenyl)-2-{[4-(4-fluorophenyl)piperazin-1-yl]methyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione (**12**)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 3.13–3.19 (m, 8H, 4 × CH_2), 5.37 (s, 2H, CH_2), 6.93–7.70 (m, 12H, Ar-H). IR (KBr, ν, cm^{-1}): 3071, 3051, 2977, 2843, 1588, 1532, 1485, 1332, 763. Anal. Calc. for $C_{25}H_{22}BrClFN_5S$ (%): C 53.73, H 3.97, N 12.53. Found: C 53.79, H 4.08, N 12.44.

*5-(3-Chlorophenyl)-2-{[4-[[3-(3-chlorophenyl)-4-(4-methylphenyl)-5-thioxo-1,2,4-triazol-1-yl]methyl]piperazin-1-yl]methyl}-4-(4-methylphenyl)-1,2,4-triazole-3-thione (**13**)* ^{1}H NMR (250 MHz) ($CDCl_3$) δ (ppm): 2.46 (s, 6H, 2 × CH_3), 3.03 (s, 8H, 4 × CH_2), 5.29 (s, 4H, 2 × CH_2), 7.10–7.48 (m, 16H, Ar-H). IR (KBr, ν, cm^{-1}): 3112, 3046, 2961, 2915, 2862, 1594, 1505, 1466, 1348, 753. Anal. Calc. for $C_{36}H_{34}Cl_2N_8S_2$ (%): C 60.58, H 4.80, N 15.70. Found: C 60.66, H 5.01, N 15.83.

*4-(4-Bromophenyl)-2-{[4-[[4-(4-bromophenyl)-3-(3-chlorophenyl)-5-thioxo-1,2,4-triazol-1-yl]methyl]piperazin-1-yl]methyl}-5-(3-chlorophenyl)-1,2,4-triazole-3-thione (**14**)* ^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.89 (br. s, 8H, 4 × CH_2), 5.19 (s, 4H, 2 × CH_2), 7.33–7.76 (m, 16H, Ar-H). IR (KBr, ν, cm^{-1}): 3078, 3015, 2909, 2861, 2833, 1603, 1525, 1477, 1319, 750. Anal. Calc. for $C_{34}H_{28}Br_2Cl_2N_8S_2$ (%): C 48.41, H 3.35, N 13.28. Found: C 48.31, H 3.28, N 13.34.

Microbiology

The antimicrobial activity of the compounds was tested on the Gram-positive strains (*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 on the Gram-negative strains (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 9027). Ampicillin and cefuroxime, antibiotics widely used in the treatment of infectious diseases, were used as control antimicrobial agents. MIC, defined as the lowest concentration of compound at which there was no visible growth of tested microorganisms, was achieved by a broth microdilution method, according to the CLSI recommendation [19]. Briefly, microbial suspensions were prepared in sterile saline with an optical density of 0.5 McFarland standard -150×10^6 CFU/mL (CFU—colony forming unit). All stock solutions of the tested compounds were dissolved in dimethyl sulfoxide. Mueller–Hinton broth 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. 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. MBC was defined as the lowest concentration of compound that resulted in >99.9% reduction in CFU of the initial inoculum. All the tests were repeated three times and the results were averaged.

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