

## Synthesis and biological activities of methylenebis-4*H*-1,2,4-triazole derivatives

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**Abstract:** 5,5'-Methylenebis(4-phenyl-4*H*-1,2,4-triazole-3-thiol) (**2**) was synthesized starting from hydrazinecarbothioamide compound (**1**). Treatment of compound **2** with ethyl bromoacetate produced diethyl 5,5'-[methylenebis(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)thio]diacetate (**3**), which was converted to the corresponding diacetohydrazide derivative (**4**) by treatment with hydrazine hydrate. The reaction of compound **4** with several aldehydes produced the corresponding arylidene hydrazides, **5a–d**. Syntheses of Mannich bases **6a–c** were carried out by the treatment of compound **2** with several amines in the presence of formaldehyde. (4{[5-({5-[(4-Amino-2-chlorophenyl)thio]-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl]thio}-3-chlorophenyl)amine (**8**) was prepared by reduction of 2 nitro groups of 3,3'-methylenebis{5-[(2-chloro-4-nitrophenyl)thio]-4-phenyl-4*H*-1,2,4-triazole} (**7**) that were obtained from the condensation of **2** with 3,4-dichloronitrobenzene.

The newly synthesized compounds were screened for their antimicrobial activities; some of them were found to be active towards the test microorganisms as the results demonstrated that the synthesized compounds exhibited a broad spectrum of activity with minimum inhibitory concentration (MIC) values of 31.3–500  $\mu\text{g/mL}$  against gram-positive and gram-negative bacteria, *Candida albicans* and *Saccharomyces cerevisiae*. All compounds displayed lower activity in this series against the microorganisms with MIC values of 31.3–500  $\mu\text{g/mL}$  than did the compared control drugs of ampicillin, streptomycin, and fluconazole.

**Key words:** 1,2,4-Triazole, morpholine, furan, Schiff base, Mannich base, antimicrobial activity

### 1. Introduction

1,2,4-Triazole moiety has been incorporated into a number of therapeutically important agents.<sup>1,2</sup> Itraconazole, fluconazole, voriconazole,<sup>3,4</sup> triazolam,<sup>5</sup> alprazolam,<sup>6</sup> etizolam and furacilin,<sup>7</sup> ribavirin,<sup>8</sup> hexaconazole,<sup>9</sup> triadimefon,<sup>10</sup> myclobutanil,<sup>11</sup> rizatriptan,<sup>12</sup> and fluotrimazole<sup>13</sup> are known drugs containing 1,2,4-triazole rings.

It is well known that one of the main aims of therapeutic treatment is the control of serious infections, along with the prevention and treatment of some infectious complications due to other therapeutic procedures such as cancer chemotherapy and surgery. In some cases such as tuberculosis, cancer, or AIDS, and in cases of organ transplantation, fungal infections became an important complication and a major cause of morbidity and mortality in immune-compromised individuals.<sup>14</sup> However, in recent years, much attention has been focused on the treatment of infections caused by multidrug-resistant bacteria and fungi resulting from the widespread use and misuse of the known antimicrobial agents.<sup>15</sup> This serious global health problem requires efforts toward the design and synthesis of new antimicrobial agents that are effective against pathogenic microorganisms resistant to currently available drugs.

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In our recent studies, we obtained some bitriazolyl compounds containing another heterocyclic group responsible for biological activity such as morpholine, thiomorpholine, or a furan nucleus.<sup>16</sup> Moreover, some 4-arylideneamino-1,2,4-triazoles including morpholine or a piperazine nucleus synthesized in our laboratory were found to possess antimicrobial activity.<sup>17</sup>

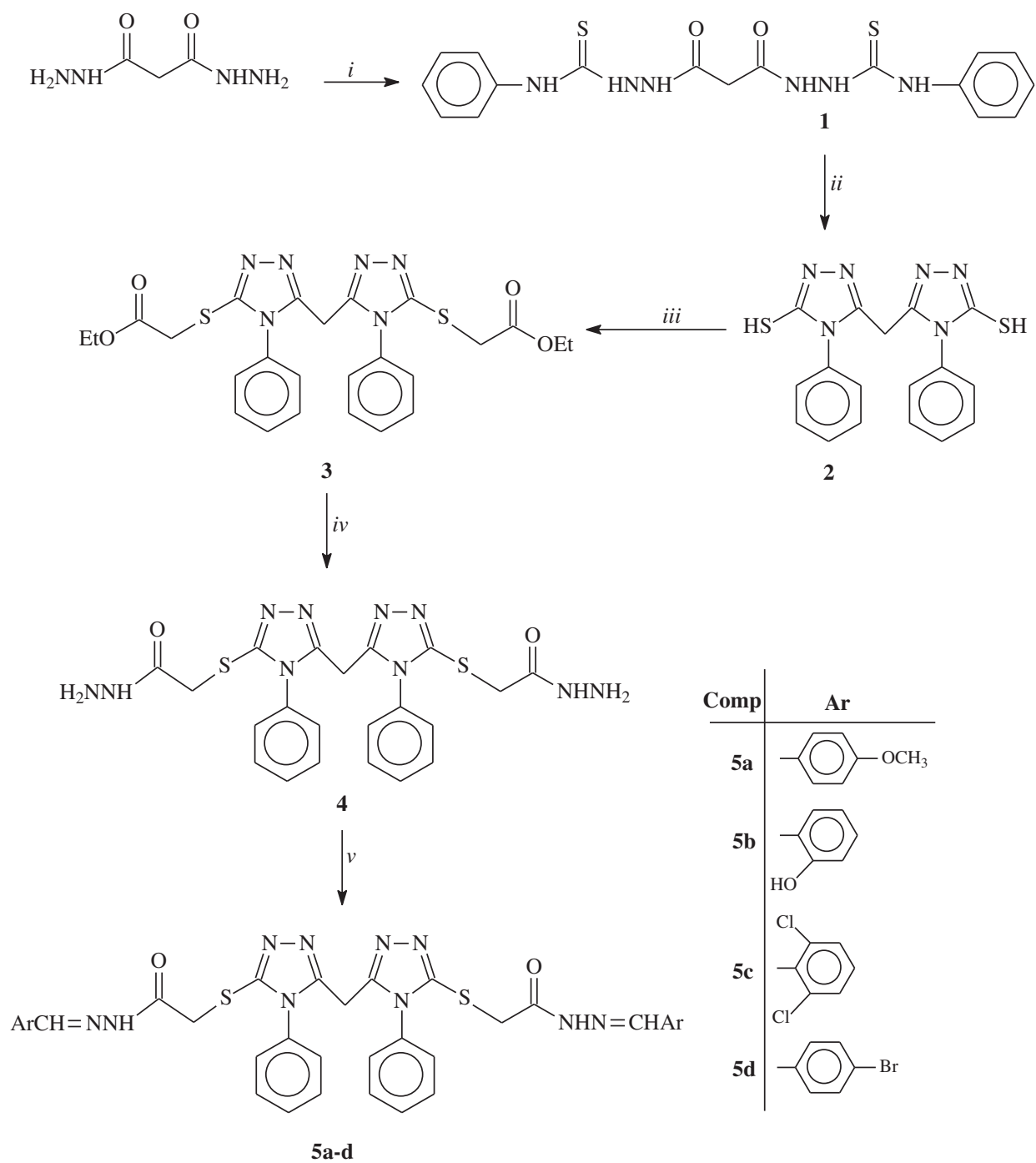
In organic syntheses, the compounds incorporating amino and mercapto groups together have been accepted as useful intermediates for further reactions. In this connection, the synthesis of some triazolothiadiazoles or triazolothiadiazines having antimicrobial activity has been performed starting from 4-amino-5-mercapto-1,2,4-triazoles in our laboratories. The amino and mercapto groups, which can also react with electrophiles, are considered as ready-made nucleophilic centers for the synthesis of condensed heterocyclic rings. Some 1,2,4-triazole derivatives containing *N*-methylpiperazine moiety, which were obtained by Mannich reactions, have been reported as antimicrobial agents. More recently, some Mannich bases possessing antimicrobial activity have been synthesized in our laboratory between 4-arylideneamino-1,2,4-triazoles and methyl piperazine or morpholine.<sup>18</sup>

In the present study, as an extension of our previous studies on the synthesis of nitrogenated heterocycles with potential chemotherapeutic activities, synthesis and antimicrobial activity screening studies of some new 1,2,4-triazole derivatives have been performed.

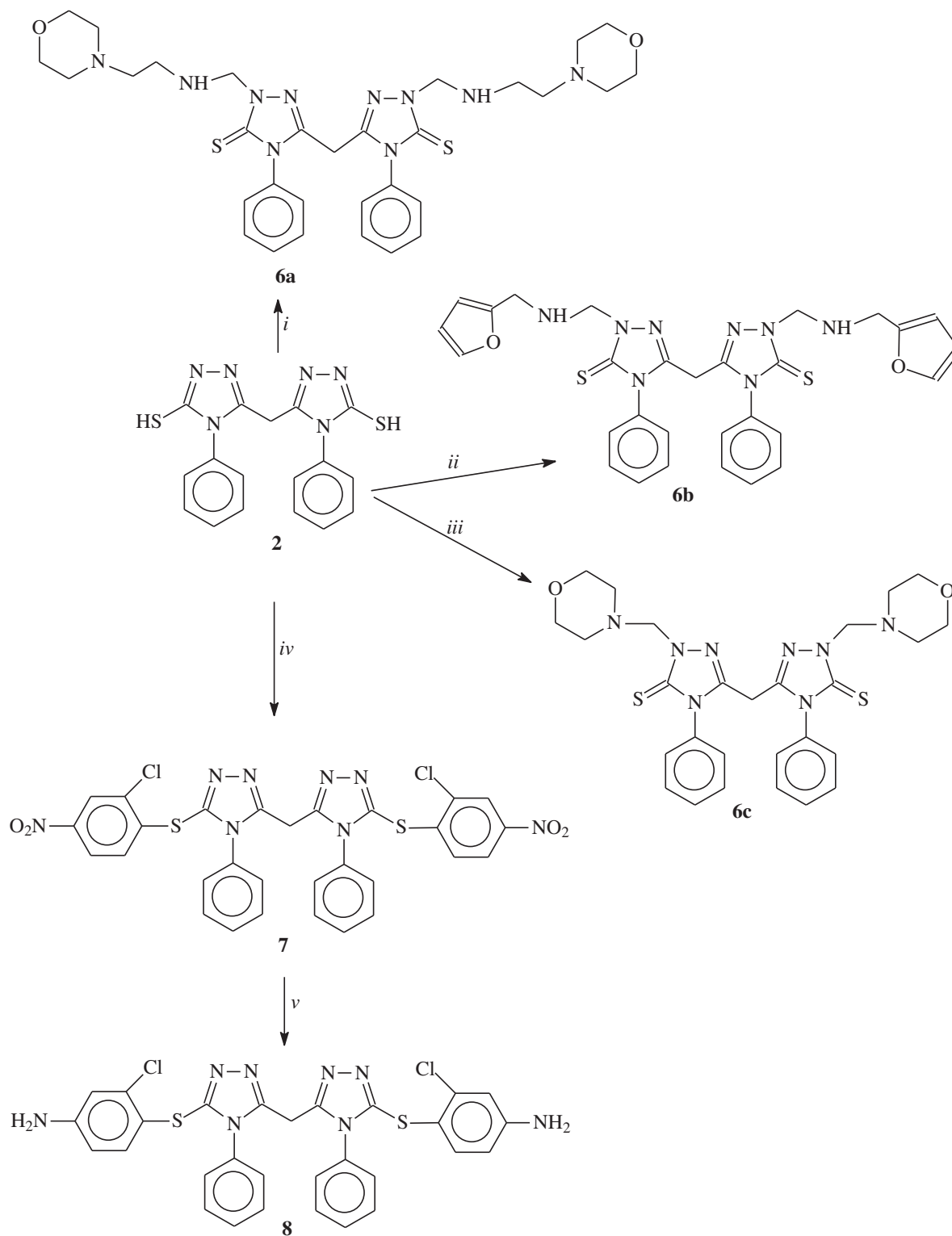
## 2. Results and discussion

The synthetic route of the compounds is shown in Figures 1 and 2. Synthesis of 5,5'-methylenebis(4-phenyl-4*H*-1,2,4-triazole-3-thiol) (**2**) was performed by cyclocondensation of 2,2'-(1,3-dioxopropane-1,3-diyl)bis(*N*-phenylhydrazinecarbothioamide) (**1**) in basic media. In the IR and NMR spectra of compound **2**, the absence of any signals pointing toward a carbonyl group supported the ring cyclization. In addition, in the <sup>1</sup>H NMR spectrum, the presence of the signal pointing toward 2 -SH groups that was observed at 13.89 ppm as a D<sub>2</sub>O exchangeable singlet integrating 2 protons pointed toward a cyclic form for this compound. The reaction of **2** with ethyl bromoacetate in the presence of sodium ethoxide produced diethyl 5,5'-{methylenebis[(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)thio]}diacetate (**3**). In the <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound **3**, additional signals originating from ester moiety were recorded at the related chemical shift values, while the signal due to the -SH function disappeared. Compound **3** was converted to a hydrazide derivative with hydrazine hydrate in good yield. The IR and <sup>1</sup>H NMR spectra of the hydrazide compound (**4**) exhibited signals belonging to the -NHNH<sub>2</sub> function. Several aldehydes were treated with compound **4** to give the corresponding Schiff bases (**5a-d**) in good yields. In the <sup>1</sup>H NMR spectra of compounds **5a-d**, additional signals originating from arylidene moiety were observed at the aromatic region between 8.04 and 8.34 ppm. The signal originating from the -NHNH<sub>2</sub> group of parent compound **4** disappeared in the <sup>1</sup>H NMR and IR spectra of compounds **5a-d**.

It was reported that arylidene hydrazides may exist as *E/Z* geometrical isomers about the -N=CH double bond and as *cis/trans* amide conformers.<sup>18-20</sup> A literature survey<sup>19</sup> revealed that dimethyl-*d*<sub>6</sub> sulfoxide solution causes the emergence of the geometrical *E* isomer in higher percentages, whereas less polar solvents support the *Z* isomer by an intramolecular hydrogen bond. In the present study, the NMR spectral data were taken in dimethyl-*d*<sub>6</sub> sulfoxide solution and no signals pointing to the *Z* isomer were present. However, due to the *cis/trans* conformers of the *E* geometrical isomer of compounds **5a-d**, certain signals were recorded as 2 sets in the NMR spectra. The signals observed as 2 sets at 7.96–8.36 ppm and 10.08–12.05 ppm were attributed to the -N=CH bond and 2 -NH functions of *cis*- and *trans*-conformers, respectively. The upfield lines of -N=CH and 2NH protons were attributed to the *cis*-conformer of the amide structure and downfield



**Figure 1.** Synthetic pathway for the preparation of compounds 1–5. *i*:  $\text{PhNCS}$ ; *ii*:  $\text{NaOH}$ ; *iii*:  $\text{Na}_{(k)}$ ,  $\text{EtOH}$ ,  $\text{BrCH}_2\text{COOEt}$ ; *iv*:  $\text{H}_2\text{NNH}_2$ ; *v*:  $\text{ArCHO}$ .



**Figure 2.** Reaction and conditions for the synthesis of compounds **6a–c**, **7**, and **8**. *i*: 2-morpholinoethanamine, HCHO; *ii*: furan-2-ylmethanamine, HCHO; *iii*: morpholine, HCHO; *iv*: Na(k), EtOH, 3,4-dichloronitrobenzene; *v*: H<sub>2</sub>NNH<sub>2</sub>, Pd-C.

lines of the protons of the same group to the *trans*-conformer.<sup>21</sup> Furthermore, the -OH group of compound **5b** was seen as 2 different singlets due to the existence of *cis/trans*-amide conformers. The treatment of **2** with several primary and secondary amines in the presence of formaldehyde afforded the corresponding Mannich bases (**6a–c**). With this conversion, additional signals derived from amine moiety and methylene linkage were observed at the related chemical shift values in the <sup>1</sup>H- and <sup>13</sup>C NMR spectra of compounds **6a–c**, while no signal pointing toward the -SH function is present. Treatment of compound **2** with 3,4-difluoronitrobenzene resulted in the formation of 3,3'-methylenebis{5-[(2-chloro-4-nitrophenyl)thio]-4-phenyl-4*H*-1,2,4-triazole} (**7**), and in the NMR spectra there are additional signals of aromatic protons instead of the -SH protons. The nitro groups of this compound were then reduced to amine groups using hydrazine hydrate as the hydrogen source in the presence of Pd/C catalyst. In the <sup>1</sup>H NMR spectrum of compound **8**, the presence of a D<sub>2</sub>O exchangeable signal at 5.78 ppm integrating 4 protons confirmed the reduction. The absorption bands due to amino groups were observed at 3337 and 3210 cm<sup>-1</sup> in the IR spectrum of **8**.

All of the newly synthesized compounds gave elemental analysis results consistent with the proposed structures. In addition, molecular masses of the newly synthesized compounds were confirmed by the appearance of [M]<sup>+</sup>, [M + 1]<sup>+</sup>, [M + 2]<sup>+</sup>, or [M + Na]<sup>+</sup> ion peaks at corresponding *m/z* values in the mass spectra of these compounds.

All of the newly synthesized compounds were tested for their antimicrobial activities, and only the positive results are presented in the Table. According to the results obtained, compound **1** displayed better activity against *Staphylococcus aureus* and *Enterococcus faecalis*, which are gram-positive cocci, and *Bacillus cereus*, which is a gram-positive spore bacillus, than against enteric bacteria *Escherichia coli* and *Yersinia pseudotuberculosis* and gram-negative bacillus *Pseudomonas aeruginosa*. This compound (**1**) displayed complete inactivity towards *Mycobacterium smegmatis*, an atypical tuberculosis factor, and *Candida albicans* and *Saccharomyces cerevisiae*, which are yeast-like fungi. According to the obtained results, it can be concluded that the conversion of carbothioamide moiety into the 1,2,4-triazole nucleus in compound **2** and the further substitution reactions leading to the formation of ester (**3**) and hydrazide (**4**) derivatives resulted in the inactivity against the test microorganisms. Schiff base derivatives (**5a–c**) of compound **4** exhibited antibacterial activity against the test microorganisms with minimum inhibitory concentration (MIC) values varying between 62.5 and 250 μg/mL. Moreover, marginal antifungal activities were observed for compounds **5a–c** against *Candida albicans* and *Saccharomyces cerevisiae*. Among the Mannich bases (**6a–c**), compound **6c** was found to have the most potent antibacterial activity against the test microorganisms, except for *Mycobacterium smegmatis*, with MIC values between 62.5 and 125 μg/mL. Furthermore, compound **6c** displayed slight antifungal activity against *Saccharomyces cerevisiae* with a MIC value of 250 μg/mL.

Motivated by these findings and in continuation of our ongoing efforts, we would like to report here the synthesis and investigation of biological activities of new bitriazolyl derivatives incorporating various heterocyclic rings responsible for biological activity in a single structure.

### 3. Experimental

All of the chemicals were purchased from Fluka Chemie AG (Buchs, Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets. The mobile phase was acetone and diethyl ether (1:2), and detection was done using UV light. IR spectra were recorded as potassium bromide pellets using a PerkinElmer

**Table.** Screening for antimicrobial activity of the compounds **1**, **5a–d**, and **6a–c**.

Comp. no.	Minimal inhibition concentration values ( $\mu\text{g/mL}$ )								
	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
<b>1</b>	62.5	62.5	62.5	31.3	31.3	31.3	-	-	-
<b>5a</b>	62.5	125	62.5	125	31.3	62.5	62.5	500	500
<b>5b</b>	62.5	125	62.5	125	31.3	62.5	62.5	500	500
<b>5c</b>	62.5	125	62.5	125	31.3	62.5	62.5	250	250
<b>5d</b>	250	250	-	125	125	250	125	500	500
<b>6a</b>	250	250	500	500	500	250	-	-	-
<b>6b</b>	250	250	500	500	500	250	-	-	-
<b>6c</b>	62.5	62.5	62.5	125	62.5	62.5	-	-	250
Amp.	2	32	> 128	2	2	< 1			
Strep.							4		
Flu.								< 8	< 8

Ec: *E. coli* ATCC 35218, Yp: *Y. pseudotuberculosis* ATCC 911, Pa: *P. aeruginosa* ATCC 10145, Sa: *S. aureus* ATCC 25923, Ef: *E. faecalis* ATCC 29212, Bc: *B. cereus* 709 Roma, Ms: *M. smegmatis* ATCC607, Ca: *C. albicans* ATCC 60193, *S. cerevisiae* RSKK 251, Amp.: ampicillin, Strep.: streptomycin, Flu.: fluconazole, (-): no activity at test concentrations.

1600 series FT-IR spectrometer.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a BRUKER AVANCE II 400 MHz NMR spectrometer (chemical shift in ppm downfield from TMS as an internal reference). The mass spectra were obtained with a Quattro LC-MS (70 eV) instrument. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All compounds gave C, H, and N analysis values within  $\pm 0.4\%$  of the theoretical values.

### 3.1. 2,2'-(1,3-Dioxopropane-1,3-diyl)bis(*N*-phenylhydrazinecarbothioamide) (**1**)

The mixture of malonohydrazide (10 mmol) and phenyl isothiocyanate (20 mmol) in absolute ethanol was refluxed for 3 h. On cooling the reaction content to room temperature, a white solid formed. This crude product was filtered off and recrystallized from ethanol to afford the desired compound. Yield 91%, mp 181–182 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3220 (6NH), 3120 (Ar CH), 1675 (C=O), 1352 (2C=S), 1521 (Ar C=C), Anal. Calcd. (%) for  $\text{C}_{17}\text{H}_{18}\text{O}_2\text{S}_2\text{N}_6$ : C, 50.73; N, 20.88; H, 4.51. Found: C, 50.77; N, 20.84; H, 4.47.  $^1\text{H}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 2.86 (2H, s,  $\text{CH}_2$ ), 6.52–6.74 (2H, brs, arH), 6.84–7.11 (8H, m, arH), 9.20 (2H, s, 2NH), 9.40 (2H, s, 2NH), 9.91 (2H, s, 2NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 44.33 ( $\text{CH}_2$ ), arC: [125.60 (4CH), 129.30 (4CH), 136.11 (2CH), 137.07 (2C)], 167.12 (2C=O), 169.22 (2C=S); EI MS  $m/z$  (%): 101.13 (31), 168.11 (25), 403.39 ( $[\text{M} + 1]^+$ , 100), 404.33 ( $[\text{M} + 2]^+$ , 19), 425.36 ( $[\text{M} + \text{Na}]^+$ , 18).

### 3.2. 5,5'-Methylenebis(4-phenyl-4*H*-1,2,4-triazole-3-thiol) (**2**)

A solution of compound **1** (10 mmol) in water was refluxed in the presence of 2 N NaOH (20 mmol) for 2 h; the resulting solution was then cooled to room temperature and acidified to pH 4 with 37% HCl. The precipitate formed was filtered off, washed with water, and recrystallized from ethanol to afford the desired compound. Yield 91%, mp 82–83 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3054 (Ar CH), 2727 (SH), 1574 and 1590 (C=N), 1442 (Ar C=C). Anal. Calcd. (%) for  $\text{C}_{17}\text{H}_{14}\text{N}_6\text{S}_2$ : C, 55.72; N, 22.93; H, 3.85. Found: C, 55.70; N, 22.96; H, 3.80.  $^1\text{H}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 3.87 (2H, s,  $\text{CH}_2$ ), 7.06 (4H, d,  $J = 4.8$  Hz, arH), 7.5 (6H, d,  $J = 4.8$  Hz, arH),

13.89 (2H, s, 2SH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 24.29 ( $\text{CH}_2$ ), arC: [128.54 (4CH), 130.10 (4CH), 130.38 (2CH), 133.57 (2C)], 147.48 (triazole 2C-3), 168.50 (triazole 2C-5); EI MS  $m/z$  (%): 118.87 (50), 145.90 (22), 160.98 (25), 176.93 (23), 335.17 (39), 367.21 ( $[\text{M} + 1]^+$ , 100), 368.21 ( $[\text{M} + 2]^+$ , 25), 405.19 ( $[\text{M} + \text{K}]^+$ , 18).

### 3.3. Diethyl 5,5'-{methylenebis[(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)thio]}diacetate (3)

Metallic sodium (20 mmol) was added to a solution of compound **2** (10 mmol) in absolute ethanol, and the mixture was refluxed for 2 h. After cooling the reaction content to room temperature, ethyl bromoacetate (20 mmol) was added and this mixture was refluxed for another 3 h (the progress of the reaction was monitored by TLC). The precipitated salt was removed by adding water and a white solid appeared. This crude product was recrystallized from ethanol and water (1:2) to afford the desired product. Yield 93%, mp 133–134 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3068 (Ar CH), 1747 and 1735 (2C=O), 1596 (2C=N), 1439 and 1496 (Ar C=C). Anal. Calcd. (%) for  $\text{C}_{25}\text{H}_{26}\text{N}_6\text{O}_4\text{S}_2$ : C, 55.75; N, 15.60; H, 4.87. Found: C, 55.71; N, 15.65; H, 4.83.  $^1\text{H}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 1.15 (6H, t,  $J = 7.1$  Hz,  $2\text{CH}_3$ ), 4.01 (6H, s,  $3\text{CH}_2$ ), 4.12 (4H, q,  $2\text{CH}_2$ ), 7.13 (4H, d,  $J = 2.4$  Hz, arH), 7.55 (6H, s, arH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 14.66 ( $2\text{CH}_3$ ), 22.66 ( $2\text{CH}_2$ ), 34.51 ( $2\text{CH}_2$ ), 61.89 ( $2\text{CH}_2$ ), arC: [127.62 (2CH), 128.86 (2CH), 130.38 (2CH), 130.60 (2CH), 130.89 (2CH), 132.85 (2C)], 151.80 (triazole 2C-3), 154.70 (triazole 2C-5), 168.68 (2C=O); EI MS  $m/z$  (%): 409.27 (72), 410.21 (16), 539.30 ( $[\text{M} + 1]^+$ , 100), 540.36 ( $[\text{M} + 2]^+$ , 29), 541.36 (12).

### 3.4. 2,2'-{Methylenebis[(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)thio]}diacetohydrazide (4)

Hydrazine hydrate (60 mmol) was added to a solution of compound **3** (10 mmol) in absolute ethanol, and the mixture was allowed to reflux for 3 h. On cooling the reaction mixture to room temperature, a white solid appeared. The crude product was filtered off and recrystallized from ethanol and water (1:2) to give the desired compound **4**. Yield 50%, mp 210–211 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3048 (Ar CH), 1665 (C=O), 1623 and 1597 (C=N), 1541 and 1498 (Ar C=C). Anal. Calcd. (%) for  $\text{C}_{21}\text{H}_{22}\text{N}_{10}\text{O}_2\text{S}_2$ : C, 49.40; N, 27.43; H, 4.34. Found: C, 49.37; N, 27.44; H, 4.30.  $^1\text{H}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 3.80 (4H, s,  $2\text{CH}_2$ ), 3.96 (2H, s,  $\text{CH}_2$ ), 4.28 (4H, s,  $2\text{NH}_2$ ), 7.11 (4H, d,  $J = 7.1$  Hz, arH), 7.52 (6H, d,  $J = 7.0$  Hz, arH), 9.31 (2H, s,  $2\text{NH}$ );  $^{13}\text{C}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 34.50 ( $\text{CH}_2$ ), 38.08–40.62 (DMSO +  $2\text{CH}_2$ ), arC: [127.47 (4CH), 130.69 (4CH), 131.15 (2CH), 132.32 (2C)], 151.13 (triazole 2C-3), 151.66 (triazole 2C-5), 167.15 (2C=O); EI MS  $m/z$  (%): 479.25 (95), 480.32 (25), 533.31 ( $[\text{M} + \text{Na}]^+$ , 18), 551.30 (50), 573.33 (100), 574.40 (30), 575.28 (24).

### 3.5. General method for the synthesis of compounds 5a–d

A mixture of compound **4** (10 mmol) and an appropriate aldehyde (20 mmol) in glacial acetic acid was stirred at room temperature for 15 min. The mixture was then refluxed for 3 h, controlled by TLC. After the solvent was evaporated under reduced pressure, a solid was obtained. This crude product was recrystallized from an appropriate solvent.

#### 3.5.1. 2,2'-{Methylenebis[(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)thio]}bis{*N'*-[(4-methoxy phenyl)methylene]acetohydrazide} (5a)

Recrystallized from ethyl acetate. Yield 82%, mp 204–205 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3192 (NH), 3115 (Ar CH), 1687 and 1667 (C=O), 1606 and 1572 (C=N), 1512 and 1497 (Ar C=C), 1251 (C-O). Anal. Calcd. (%) for

$C_{37}H_{34}N_{10}O_4S_2$ : C, 59.50; N, 18.75; H, 8.57. Found: C, 59.53; N, 18.76; H, 8.53.  $^1H$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 3.77 (6H, s,  $2CH_3$ ), 4.00 (4H, s,  $2CH_2$ ), 4.40 (2H, s,  $CH_2$ ), 6.98 (2H, d,  $J = 7.8$  Hz, arH), 7.15 (6H, s, arH), 7.57 (10H, m, arH), 8.11 and 7.93 (2H, s,  $2N=CH$ , *cis/trans* conformers), 11.58 and 11.63 (2H, s,  $2NH$  *cis/trans* conformers);  $^{13}C$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 22.57 ( $CH_2$ ), 35.61 ( $2CH_2$ ), 55.99 ( $2CH_3$ ), arC: [115.02 ( $2CH$ ), 127.19 ( $2C$ ), 127.20 ( $3CH$ ), 127.72 ( $3CH$ ), 129.15 ( $3CH$ ), 129.46 ( $2CH$ ), 130.57 ( $3CH$ ), 130.81 ( $2CH$ ), 133.02 ( $2C$ ), 150.75 ( $2C$ )], 144.43 ( $2CH$ ), 147.69 ( $2CH$ ), 161.44 (triazole  $2C-3$ ), 163.68 (triazole  $2C-5$ ), 168.83 ( $2C = O$ ); EI MS  $m/z$  (%): 335.17 (39), 686.66 (55), 687.72 (100), 701.66 (58), 702.66 (28), 708.73 (20), 721.73 (19), 722.79 (15), 743.80 (35), 744.74 ( $[M - 2]^+$ , 22).

### 3.5.2. 2,2'-{Methylenebis[(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)thio]}bis{*N'*-[(2-hydroxy phenyl)methylene]acetohydrazide} (5b)

Recrystallized from ethanol. Yield 80%, mp 193–194 °C. IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3400 (OH), 3200 (NH), 3051 (Ar CH), 1681 (C=O), 1621 and 1596 (C=N), 1497 (C=C). Anal. Calcd. (%) for  $C_{35}H_{30}N_{10}O_4S_2$ : C, 58.48; N, 19.49; H, 4.21. Found: C, 58.47; N, 19.40; H, 4.22.  $^1H$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 4.01 (4H, s,  $2CH_2$ ), 4.40 (2H, s,  $CH_2$ ), 6.90 (4H, d,  $J = 7.2$  Hz, arH), 7.19 (6H, d,  $J = 7.4$  Hz, arH), 7.60 (8H, t,  $J = 7.6$  Hz, arH), 8.29 and 8.39 (2H, s,  $2N=CH$ , *cis/trans* conformers), 10.08 and 10.97 (2H, s,  $2NH$  *cis/trans* conformers), 11.60 and 11.97 (2H, s,  $2OH$ , *cis/trans* conformers);  $^{13}C$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 22.74 ( $CH_2$ ), 35.31 ( $CH_2$ ), 56.74 ( $2CH_2$ ), arC: [116.85 (CH), 117.05 (CH), 120.10 ( $3CH$ ), 127.69 ( $3CH$ ), 126.96 ( $2CH$ ), 129.87 ( $2CH$ ), 130.87 ( $3CH$ ), 131.98 ( $3CH$ ), 132.86 ( $2C$ ), 150.67 ( $2C$ ), 151.66 ( $2C$ )], 142.03 ( $2CH$ ), 147.93 ( $2CH$ ), 157.97 (triazole  $2C-3$ ), 163.86 (triazole  $2C-5$ ), 168.76 ( $2C=O$ ); EI MS  $m/z$  (%): 717.44 (28), 719.39 ( $[M + 1]^+$ , 89), 720.39 (38), 741.35 (100), 742.36 (38), 757.42 (42).

### 3.5.3. 2,2'-{Methylenebis[(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)thio]}bis{*N'*-[(2,6-dichloro phenyl)methylene]acetohydrazide} (5c)

Recrystallized from ethanol and water (1:2). Yield 75%, mp 187–188 °C. IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3202 (NH), 3064 (Ar CH), 1685 (C=O), 1556 and 1582 (C=N), 1498 (C=C). Anal. Calcd. (%) for  $C_{35}H_{26}N_{10}O_2S_2Cl_2$ : C, 50.98; N, 17.20; H, 3.18. Found: C, 51.00; N, 17.22; H, 3.15.  $^1H$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 3.95 (4H, brs,  $2CH_2$ ), 4.35 (2H, s,  $CH_2$ ), 7.13 (4H, s, arH), 7.42 (12H, q, arH), 8.22 and 8.36 (2H, s,  $2N=CH$ , *cis/trans* conformers), 11.90 and 12.05 (2H, s,  $2NH$ , *cis/trans* conformers);  $^{13}C$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 22.74 ( $CH_2$ ), 38.19–40.71 ( $2CH_2 + DMSO$ ), arC: [127.47 ( $2CH$ ), 129.89 ( $2CH$ ), 128.94 ( $2CH$ ), 130.58 ( $2CH$ ), 131.02 ( $2CH$ ), 131.89 ( $2CH$ ), 132.43 ( $2CH$ ), 132.55 ( $2CH$ ), 133.53 ( $4C$ ), 134.57 ( $4C$ ), 139.97 ( $2CH$ )], 150.92 (triazole  $2C-3$ ), 164.46 (triazole  $2C-5$ ), 169.59 ( $2C=O$ ); EI MS  $m/z$  (%): 118.80 (31), 301.23 (44), 413.37 (53), 845.24 (69), 847.20 ( $[M + Na]^+$ , 100), 849.21 ( $[M + Na + 2]^+$ , 60).

### 3.5.4. 2,2'-{Methylenebis[(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)thio]}bis{*N'*-[(4-bromo phenyl)methylene]acetohydrazide} (5d)

Recrystallized from dimethyl sulfoxide and water (1:3). Yield 78%, mp 196–197 °C. IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3200 (NH), 1692 (C=O), 1610 and 1591 (C=N), 1497 (Ar C=C). Anal. Calcd. (%) for  $C_{35}H_{28}N_{10}O_2S_2Br_2$ : C, 47.77; N, 16.58; H, 3.34. Found: C, 49.79; N, 16.55; H, 3.33.  $^1H$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 4.01 (4H, d,  $J =$



4.6 Hz, 2CH<sub>2</sub>), 4.41 (2H, s, CH<sub>2</sub>), 7.16 (4H, s, arH), 7.58 (14H, d,  $J = 16.2$  Hz, arH), 7.96 and 8.15 (2H, s, 2N=CH, *cis/trans* conformers), 11.71 and 11.81 (2H, s, 2NH, *cis/trans* conformers); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 22.73 (CH<sub>2</sub>), 35.52 (2CH<sub>2</sub>), arC: [124.92 (2C), 127.68 (2CH), 129.42 (2CH), 129.65 (2CH), 130.58 (2CH), 130.83 (2CH), 132.52 (4CH), 132.97 (C), 134.01 (C), 143.34 (2CH), 146.51 (2CH), 150.94 (2C)], 151.68 (triazole 2C-3), 164.09 (triazole 2C-5), 169.16 (2C=O); EI MS  $m/z$  (%): 701.20 (23), 803.77 (100), 804.72 (53), 845.18 ([M]<sup>+</sup>, 37).

### 3.6. General method for the synthesis of compounds 6a–c

The mixture of compound **2** (10 mmol) and the corresponding amine (20 mmol) in DMF was stirred in the presence of formaldehyde (37%, 10 mmol) at room temperature for 2.5 h (the progress of the reaction was monitored by TLC). Water was added and this mixture was kept overnight in cold conditions. The solid that separated was collected by filtration and recrystallized from DMSO and water (1:3) to yield the target compound.

#### 3.6.1. 5,5'-Methylenebis(2-[(2-morpholin-4-ylethyl)amino]methyl}-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione) (6a)

Recrystallized from dimethyl sulfoxide and water (1:3). Yield 83%, mp 193–194 °C. IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3049 (Ar CH), 1591 and 1575 (Ar C=C), 1641 (C=N), 1114 (C=S). Anal. Calcd. (%) for C<sub>31</sub>H<sub>42</sub>N<sub>10</sub>O<sub>2</sub>S<sub>2</sub>: C, 57.21; N, 21.52; H, 6.50. Found: C, 57.17; N, 21.50; H, 6.47. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 2.32–2.42 (16H, m, 8CH<sub>2</sub>), 2.98 (4H, brs, 2CH<sub>2</sub>), 3.52 (4H, s, 2CH<sub>2</sub>), 4.01 (2H, brs, CH<sub>2</sub>), 5.33 (4H, brs, 2CH<sub>2</sub>), 7.17 (4H, s, arH), 7.52 (6H, s, arH), 11.24 (2H, s, 2NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 38.92–41.42 (6CH<sub>2</sub>), 54.13 (2NCH<sub>2</sub>), 66.94 (2NCH<sub>2</sub> + OCH<sub>2</sub>), 66.93 (NCH<sub>2</sub>N), arC: [128.61 (4CH), 130.29 (6CH), 133.87 (2C)], 146.33 (triazole 2C-3), 168.41 (triazole 2C-5); EI MS  $m/z$  (%): 335.17 (39), 533.32 (30), 561.43 (23), 585.54 (37), 587.68 (100), 588.56 (100), 596.68 (25), 603.60 (37), 603.72 (21), 604.60 (15), 663.63 (48), 664.64 (22).

#### 3.6.2. 5,5'-Methylenebis(2-[(2-furylmethyl)amino]methyl}-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione) (6b)

Recrystallized from dimethyl sulfoxide and water (1:3). Yield 95%, mp 191–192 °C. IR (KBr,  $\nu$ , cm<sup>-1</sup>): 3062 (Ar CH), 1146 (C=S), 1591 and 1574 (Ar C=C), 1667 (C=N). Anal. Calcd. (%) for C<sub>29</sub>H<sub>28</sub>N<sub>8</sub>O<sub>2</sub>S<sub>2</sub>: C, 59.57; N, 19.16; H, 4.83. Found: C, 59.60; N, 19.14; H, 4.80. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 3.39 (2H s, CH<sub>2</sub>), 4.04 (4H, brs, 2CH<sub>2</sub>), 5.33 (4H, brs, CH<sub>2</sub>), 6.20 (2H, s, furan-arH), 6.36 (4H, s, furan-arH), 7.17 (4H, a, arH), 7.51 (6H, s, arH), NH was not observed. <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 38.22–40.73 (DMSO + CH<sub>2</sub>), 47.20 (CH<sub>2</sub>), 72.50 (CH<sub>2</sub>), arC: [108.57 (CH), 110.55 (2CH), 128.15 (2CH), 129.79 (CH), 130.20 (CH), 133.33 (C), 142.52 (CH), 145.60 (C)], 151.98 (triazole 2C-3), 168.09 (triazole 2C-5); EI MS  $m/z$  (%): 539.35 (100), 540.35 (38), 540.48 (31), 541.42 (20), 547.59 (18), 553.63 (60), 561.37 (38), 569.74 (22), 585.66 ([M + 1]<sup>+</sup>, 31), 587.80 (30).

### 3.6.3. 5,5'-Methylenebis[2-(2-morpholin-4-ylmethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione] (6c)

Recrystallized from dimethyl sulfoxide and water (1:3). Yield 86%, mp 196–197 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3050 (Ar CH), 1592 and 1579 (C=N), 1498 (Ar C=C), 1113 (C=S). Anal. Calcd. (%) for  $\text{C}_{27}\text{H}_{32}\text{N}_8\text{O}_2\text{S}_2$ : C, 57.42; N, 19.84; H, 5.71. Found: C, 57.39; N, 19.81; H, 5.69.  $^1\text{H}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 2.64 (8H, s, 4CH<sub>2</sub>), 3.56 (8H, s, 4CH<sub>2</sub>), 4.01 (2H, s, CH<sub>2</sub>), 5.00 (4H, s, 2CH<sub>2</sub>), 7.12 (4H, d,  $J = 5.4$  Hz, arH), 7.51 (6H, s, arH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 24.01 (CH<sub>2</sub>), 50.74 (2CH<sub>2</sub>), 66.77 (4CH<sub>2</sub>), 69.31 (4CH<sub>2</sub>), arC: [128.59 (4CH), 130.17 (6CH), 133.89 (2C)], 146.02 (triazole 2C-3), 169.51 (triazole 2C-5); EI MS  $m/z$  (%): 367.28 (81), 466.46 (100), 467.46 (31), 565.52 ( $[\text{M} + 1]^+$ , 75), 566.58 ( $[\text{M} + 2]^+$ , 25).

### 3.7. 3,3'-Methylenebis{5-[(2-chloro-4-nitrophenyl)thio]-4-phenyl-4H-1,2,4-triazole} (7)

The mixture of compound **2** (10 mmol) and metallic sodium (20 mmol) in ethanol was refluxed for 2 h. 3,4-Dichloronitrobenzene (20 mmol) was then added and this mixture was refluxed for an additional 22 h (the progress of the reaction was monitored by TLC). The solvent was evaporated under reduced pressure, and the solid obtained by adding water was filtered off and recrystallized from ethyl acetate. Yield 71%, mp 77–78 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3096 (Ar CH), 1537, 1574, and 1598 (C=N), 1453 (C=C), 1520 and 1349 (NO<sub>2</sub>). Anal. Calcd. (%) for  $\text{C}_{29}\text{H}_{18}\text{N}_8\text{O}_4\text{S}_2\text{Cl}_2$ : C, 51.41; N, 16.54; H, 2.68. Found: C, 51.42; N, 16.55; H, 2.66.  $^1\text{H}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 4.27 (2H, s, CH<sub>2</sub>), 7.00 (2H, d,  $J = 8.6$  Hz, arH), 7.12 (4H, d,  $J = 6.6$  Hz, arH), 7.45 (7H, d,  $J = 6.6$  Hz, arH), 8.05 (2H, d,  $J = 9.0$  Hz, arH), 8.27 (1H, s, arH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 24.04 (CH<sub>2</sub>), arC: [123.53 (nitrophenyl 2CH), 125.30 (nitrophenyl 2CH), 127.74 (4CH), 128.88 (4CH), 130.88 (2CH), 131.06 (2CH), 132.76 (4C), 142.13 (2C), 144.94 (2C)], 147.12 (triazole 2C-3), 153.84 (triazole 2C-5); EI MS  $m/z$  (%): 553.43 (21), 569.68 (28), 587.63 (34), 601.63 (22), 607.57 ( $[\text{M} + 1 - 2\text{Cl}]^+$ , 20), 663.59 (79), 664.71 (44), 685.72 (100), 686.60 (50).

### 3.8. (4{[5-({5-[(4-amino-2-chlorophenyl)thio]-4-phenyl-4H-1,2,4-triazol-3-yl)methyl]-4-phenyl-4H-1,2,4-triazol-3-yl}thio}-3-chlorophenyl)amine (8)

Pd-C (20 mmol) catalyst was added to a solution of compound **7** (10 mmol) in butanol, and the mixture was allowed to reflux in the presence of hydrazine hydrate (60 mmol) for 6 h (the progress of the reaction was monitored by TLC). The catalyst was removed by filtration. After evaporating the solvent under reduced pressure, a white solid appeared. This crude product was recrystallized from ethyl acetate and ether (1:2) to afford the desired compound. Yield 31%, mp 111–113 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3337 and 3210 (NH<sub>2</sub>), 3100 (Ar CH), 1629 and 1596 (C=N), 1498 and 1478 (Ar C=C). Anal. Calcd. (%) for  $\text{C}_{29}\text{H}_{22}\text{N}_8\text{S}_2\text{Cl}_2$ : C, 56.40; N, 18.14; H, 3.59. Found: C, 56.42; N, 18.11; H, 3.62.  $^1\text{H}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 4.00 (2H, s, CH<sub>2</sub>), 5.78 (4H, s, 2NH<sub>2</sub>), 6.40 (2H, d,  $J = 8.4$  Hz, arH), 6.60 (2H, s, arH), 6.90 (6H, d,  $J = 10.06$  Hz, arH), 7.42 (6H, m, arH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ,  $\delta$  ppm): 24.07 (CH<sub>2</sub>), arC: [123.28 (nitrophenyl 2CH), 125.27 (nitrophenyl 2CH), 127.65 (4CH), 128.79 (4CH), 130.45 (2CH), 131.09 (2CH), 132.66 (4C), 142.09 (2C), 145.00 (2C)], 147.48 (triazole 2C-3), 153.87 (triazole 2C-5); EI MS  $m/z$  (%): 337.24 (31), 346.25 (75), 355.20 (62), 356.26 (47), 385.23 (100), 386.23 (81).

#### 4. Antimicrobial activity assessment

All test microorganisms were obtained from the Refik Saydam Hifzıssıhha Institute (Ankara, Turkey) and were as follows: *E. coli* ATCC35218, *Y. pseudotuberculosis* ATCC911, *P. aeruginosa* ATCC43288, *S. aureus* ATCC25923, *E. faecalis* ATCC29212, *B. cereus* 709 Roma, *M. smegmatis* ATCC607, *C. albicans* ATCC60193, and *S. cerevisiae* RSKK 251. All of the newly synthesized compounds were weighed and dissolved in dimethyl sulfoxide to prepare extract stock solutions of 5 mg/mL.

#### 5. MIC method

The antimicrobial activities of synthesized compounds were tested quantitatively in respective broth media by using double dilution and the MIC values ( $\mu\text{g/mL}$ ) were determined.<sup>22</sup> The antibacterial and antifungal assays were performed in Mueller-Hinton broth (Difco, Detroit, MI, USA) at pH 7.3 and in buffered yeast nitrogen base (Difco) at pH 7.0, respectively. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10  $\mu\text{g}$ ) and fluconazole (5  $\mu\text{g}$ ) were used as the standard antibacterial and antifungal drugs, respectively. Dimethyl sulfoxide at a dilution of 1:10 was used as the solvent control. The results are shown in the Table.

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#### References

1. Heindel, N. D.; Reid, I. R. *J. Heterocycl. Chem.* **1980**, *17*, 1087–1088.
2. Holla, B. S.; Kalluraya, B.; Sridhar, K. R.; Drake, E.; Thomas, L. M.; Bhandary, K. K.; Levine, M. S. *Eur. J. Med. Chem.* **1994**, *29*, 301–308.
3. Merk and Co. *The Merk Index*, 12th ed., Merk and Co. Inc., Whitehouse Station, NJ, USA, 1996.
4. Haber, J. *Cas. Lek. Cesk.* **2001**, *140*, 596–604.
5. Lee, S.; Kamide, T.; Tabata, H.; Takahashi, H.; Shiro, M.; Natsugari, H. *Bioorg. Med. Chem.* **2008**, *16*, 9519–9523.
6. Chaytor, A. *In xPharm: The Comprehensive Pharmacology Reference*; Enna, S. J.; Bylund, D. B., Eds.; Elsevier, Amsterdam, 2007.
7. Subrahmanya, B. K.; Poojary, B.; Jagadeesh P. D.; Naik, P.; Holla, B. S. *Eur. J. Med. Chem.* **2009**, *44*, 5066–5070.
8. Kumarapperuma, S. C.; Sun, Y.; Jeselnik, M.; Chung, K.; Parker, W. B.; Jonsson, C. B.; Arterburn, J. B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3203–3207.
9. Prasad, D. J.; Ashok, M.; Karegoudar, P.; Poojary, B.; Holla, B. S.; Kumari, N. S. *Eur. J. Med. Chem.* **2009**, *44*, 551–557.
10. Jin, Z.; Hu, Y.; Huo, A.; Tao, W.; Shao, L.; Liu, J.; Fang, J. *J. Organometallic Chem.* **2006**, *691*, 2340–2345.
11. Bhat, K. S.; Poojary, B.; Prasad, D. J.; Naik, P.; Holla, B. S. *Eur. J. Med. Chem.* **2009**, *44*, 5066–5070.
12. Dahlof, C. G. H.; Rapoport, A. M.; Sheftell, F. D.; Lines, C. R. *Clin. Ther.* **1999**, *21*, 1823–1836.
13. Faidallah, H. M.; Khan, K. A.; Asiri, A. M. *J. Fluorine Chem.* **2011**, *132*, 870–877.
14. Turan-Zitouni, G.; Kaplancikli, Z. A.; Yildiz, M. T.; Chevallet, P.; Kaya, D. *Eur. J. Med. Chem.* **2005**, *40*, 607–613.
15. Akbas, E.; Berber, I. *Eur. J. Med. Chem.* **2005**, *40*, 401–405.
16. Bayrak, H.; Demirbaş, A.; Belktaş, H.; Karaoğlu, Ş. A.; Demirbaş, N. *Turk. J. Chem.* **2010**, *34*, 835–846.

17. Bayrak, H.; Demirbas, A.; Demirbas, N.; Karaoglu, S. A. *Eur. J. Med. Chem.* **2009**, *44*, 4362–4366.
18. Bayrak, H.; Demirbas, A.; Karaoglu, S. A.; Demirbas, N. *Eur. J. Med. Chem.* **2009**, *44*, 1057–1066.
19. Wyrzykiewicz, E.; Prukala, D. *J. Heterocyclic Chem.* **1998**, *35*, 381–387.
20. Galic, N.; Peric, B.; Kojic-Prodic, B.; Cimerman, Z. *J. Mol. Struct.* **2001**, *559*, 187–194.
21. Rando, D.; Sato, D. N.; Siqueira, L.; Malvezzi, A.; Leite, C. Q. F.; Amaral, A. T.; Ferreira, E. I.; Tavares, L. C. *Bioorg. Med. Chem.* **2002**, *10*, 557–560.
22. NCCLS. NCCLS Document M7-A3, NCCLS, Villanova, PA, USA, 1993.