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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Preparation and antimicrobial activity evaluation of some new bi- and triheterocyclic azoles

Serpil Demirci · Serap Basoglu · Arif Bozdereci · Neslihan Demirbas

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Abstract Synthesis of the carbothioamides (**5**, **13**, **22**) was performed starting from 3*H*-1,2,4-triazol-3-ones (**2**, **17**) by several steps, and then, these carbothioamides was converted to triheterocyclic compounds incorporating 1,2,4-triazole, imidazole and 1,3-thiazol(idinone) moieties. The reaction of compound **2** with 3,4-difluoronitrobenzene afforded the 2-(2-fluoro-4-nitrophenyl) derivative, **10**. Compound **10** was converted to the arylideneamino derivatives (**12a**, **b**) via the reduction of nitro group. On the other hand, the treatment of the hydrazide (**20**) that was obtained starting from **17**, with several aromatic aldehydes generated the corresponding arylidenhydrazides (**21a–c**). Mannich reaction between compound **2** and a suitable heterocyclic amine resulted in the N-alkylation of **2**. All newly synthesized compounds were screened for their antimicrobial activities. In general, most compounds except **22** were Found (%) to be active against *Mycobacterium smegmatis*, *Candida albicans* and/or *Saccharomyces cerevisiae*. Furthermore, **9a** and **b**, which are Mannich bases incorporating morpholine or piperazine nucleus, exhibited excellent antimicrobial activity on test microorganisms. In addition, the hydrazide, **4**, was Found (%) to have activity towards Ec and Yp.

Keywords 1,2,4-Triazole · 1,3-Thiazole · 1,3-Thiazolidinone · Arylidenhydrazide · Mannich base · Antimicrobial activity

S. Demirci · S. Basoglu · N. Demirbas (✉)
Department of Chemistry, Faculty of Art and Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey
e-mail: neslihan@ktu.edu.tr

A. Bozdereci
Department of Biology, Recep Tayyip Erdoğan University, 53100 Rize, Turkey

Introduction

The limitations in the use of the existing antibacterial drugs due to various reasons such as drug resistance, the serious side effects and/or lack of efficacy made infectious diseases a vicious cycle. In addition, the treatment of resistant strains requires a prolonged therapy containing the use of more toxic drugs and increases the financial burden. One of the infections leading to death is tuberculosis (TB). According to the survey reported by Global Alliances, there are 8–10 million new active cases of TB with approximately 3 million deaths each year (Mallikarjuna *et al*., 2009; Kumar *et al*., 2010; Silverman, 2004; Pablos-Mendez *et al*., 1998).

Therefore, it is inevitable to discover and develop new drugs for the treatment of dreadful infections, which have spread worldwide now. To search and synthesize of combinational chemotherapeutic drugs with different mechanisms of action and low side effects constitutes an important part of the methods aiming to overcome the antimicrobial resistance. Beside the exploitation of new targets, there is another approach that contains to combine two different groups responsible for biological activity in one molecule. According to the literature data, more efficacious antibacterial compounds can be designed by joining two or more biologically active heterocyclic nuclei in a single molecular framework (Thomas *et al*., 2011; Kumar *et al*., 2010; Solomon *et al*., 2010; Hu *et al*., 2010; Patil *et al*., 2010; Bayrak *et al*., 2010). These two pharmacophores, by addressing the active site of two different targets, offer the possibility to overcome the current resistance and in addition to reduce the appearance of new resistant strains (Chandra *et al*., 2006; Yu and Huiyuan, 2002; Phillips *et al*., 2007; Bonde and Gaikwad, 2004; Dixit *et al*., 2006; Hubschwerlen *et al*., 2003). Nevertheless, the majority of the efforts on the discovery new antibacterial

agents are based on analogue-based approach and that their market value represents two-thirds of all drug sales (Vicini *et al*., 2008; Basoglu *et al*., 2012).

Triazoles have been shown to possess a number of desirable features in the context of medicinal chemistry such as stability to acidic and basic hydrolysis, reductive/oxidative conditions and also resistance to metabolic degradation. Their varied biological activities such as antibacterial (Genin *et al*., 2000), antifungal (Xu *et al*., 2011), antiallergic (Buckle *et al*., 1986), anti-HIV (Alvarez *et al*., 1994), anticonvulsant (Deng *et al*., 2011), anti-inflammatory (Abdel-Megeed *et al*., 2009) and β-lactamase inhibition properties (Faridoon *et al*., 2012) made 1,2,4-triazole derivatives important tools in the medicinal chemistry. Tazobactam, a β-lactamase inhibitor is the best known examples of triazole containing structures with the broad-spectrum antibiotic piperacillin (Whiting *et al*., 2006; Tornoe *et al*., 2004). Favourable properties and enhanced biological activities of triazole nucleus have been attributed to its dipole character, hydrogen bonding capability, rigidity and stability under in vivo conditions (Kategaonkar *et al*., 2010; Eswaran *et al*., 2009).

Thiazolidinone derivatives have been further reported to possess diverse pharmacological properties, such as antibacterial, antifungal, anticonvulsant, anticancer, anti-TB and anti-human immunodeficiency virus type 1 (HIV-1) activities. Thiazolidinones are novel inhibitors of the bacterial enzyme MurB, a precursor acting during the biosynthesis of peptidoglycan as an essential component of the cell wall of both gram-positive and -negative bacteria (El-Gaby *et al*., 2009; Kucukguzel *et al*., 2002; Aridoss *et al*., 2007; Basoglu *et al*., 2012).

Substituted piperazines are important molecule portions of a number of important drugs such as crixivan, an HIV protease inhibitor, piperazinyl-linked ciprofloxacin dimers, which are potent antibacterial agents and an oxazolidinone antibiotic eperezolid (Yolal *et al*., 2012). The drugs prazosin, lidoflazine and urapidil, which contain a piperazine nucleus in their structures, have been used as cardiovascular agents (Karthikeyan *et al*., 2006; Ridley *et al*., 2004).

Another important group responsible for biological activity, morpholine is incorporated in a wide variety of therapeutically important drugs, one of which is linezolid that belongs to the oxazolidinone class of antibiotics and used for the treatment of infections caused by gram-positive bacteria (Sahin *et al*., 2012; Wyrzykiewicz *et al*., 2006; Dixit *et al*., 2005; Panneerselvam *et al*., 2005). Furthermore, 4-phenylmorpholine derivatives have been reported to possess antimicrobial, anti-inflammatory and central nervous system activities (Dixit *et al*., 2006).

The Mannich reaction is a three-component condensation reaction involving an active hydrogen containing compound, formaldehyde and a secondary amine. The aminomethylation of aromatic substrates by the Mannich reaction has a considerable importance for the synthesis and modification of biologically active compounds (Demirbas *et al*., 2010). Several Mannich bases have been Found (%) to possess antibacterial, antifungal, anticancer, antitubercular, analgesic and anti-inflammatory properties (Almajan *et al*., 2009).

Certain small heterocyclic molecules act as highly functional scaffolds and they are known as important molecule parts of a number of biologically active and medicinally useful molecules (Nandhakumar *et al*., 2007).

Motivated by these findings and in continuation of our ongoing efforts endowed with the discovery of nitrogenated heterocycles with potential chemotherapeutic activities, we would like to report here the synthesis and investigation of antimicrobial activities of new 1,2,4-triazole derivatives incorporating various heterocyclic rings responsible for biological activity in a single structure such as 1,2,4-triazole, 1,3-thiazole 1,3-thiazolidinone, morpholine piperazine or 1,3-thiazole moieties.

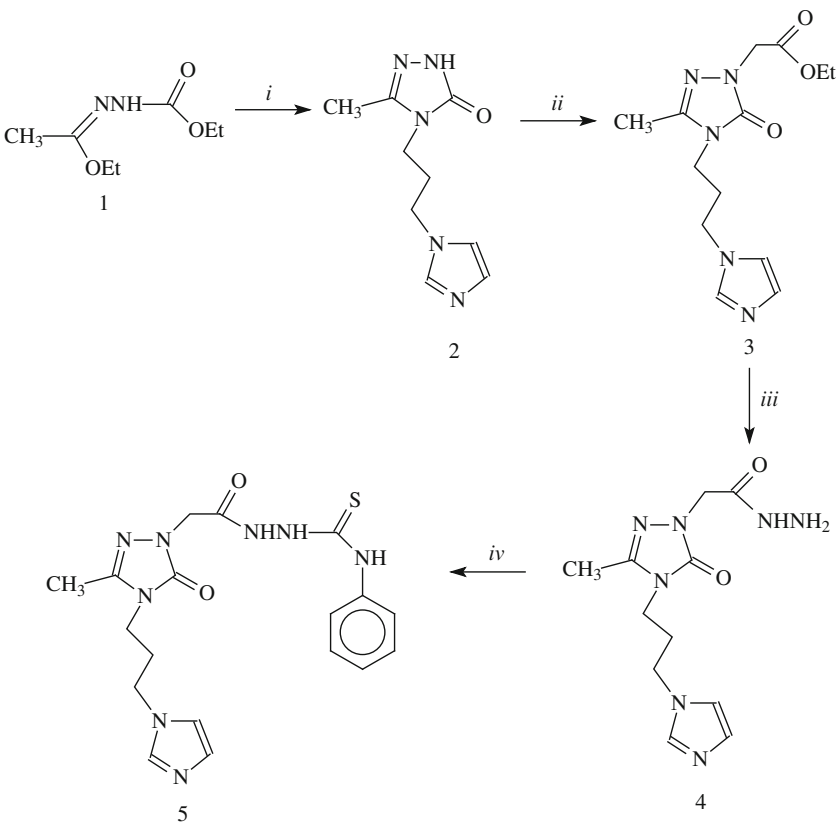
Results and discussion

The synthetic route for the newly synthesized compounds is illustrated and outlined in Schemes 1, 2, 3, 4, 5 and 6.

The synthesis of compound **2** was performed from the reaction of compound **1** with 3-(1*H*-imidazol-1-yl)-propanamine. Then, compound **2** was converted to hydrazide (**4**) via the formation of the corresponding ester (**3**). Other ester, compound **20**, was obtained starting from compound **17** via the formation of its Schiff base derivative, **19**. The IR and ^{1}H NMR spectra of compounds **4** and **20** displayed signals pointing the presence of hydrazide function, whereas the signals due to ester group disappeared in the NMR spectra of these compounds. The treatment of hydrazides (**4** and **20)** with phenylisothiocyanate produced the corresponding carbothioamides (**5** and **22**). In the ^{1}H NMR spectra of these carbothioamides, the signal derived from NH_2 group disappeared, instead, additional signals originated from phenyl moiety and three NH function were recorded at the related chemical Schiff values. Moreover, compounds **5** and **22** exhibited EI MS spectra and elemental analysis data consistent with the proposed structure.

The synthesis of 1,3-thiazole derivatives (**6** and **24**) was carried out by the cyclocondensation reaction between 4-chlorophenacyl bromide and the corresponding carbothioamide (**5** or **22**). On the other hand, the treatment of compound **5** with ethyl bromoacetate resulted in the formation of 1,3-thiazolidin-2-ylidene]acetohydrazide derivative (**7**). The structures of these compounds were confirmed on the basis of FT-IR, EI MS, ^{1}H-, ^{13}C NMR spectroscopic methods and elemental analysis.

The aminoalkylation of compound **2** with several amines namely morpholine, piperidine, phenylpiperazine,



Scheme 1 Synthetic pathway for the preparation of compounds **2–5**. *i* 3-(1*H*-imidazol-1-yl) propanamine, *ii* ethyl bromoacetate, *iii* hydrazine hydrate, *iv* phenylisothiocyanate

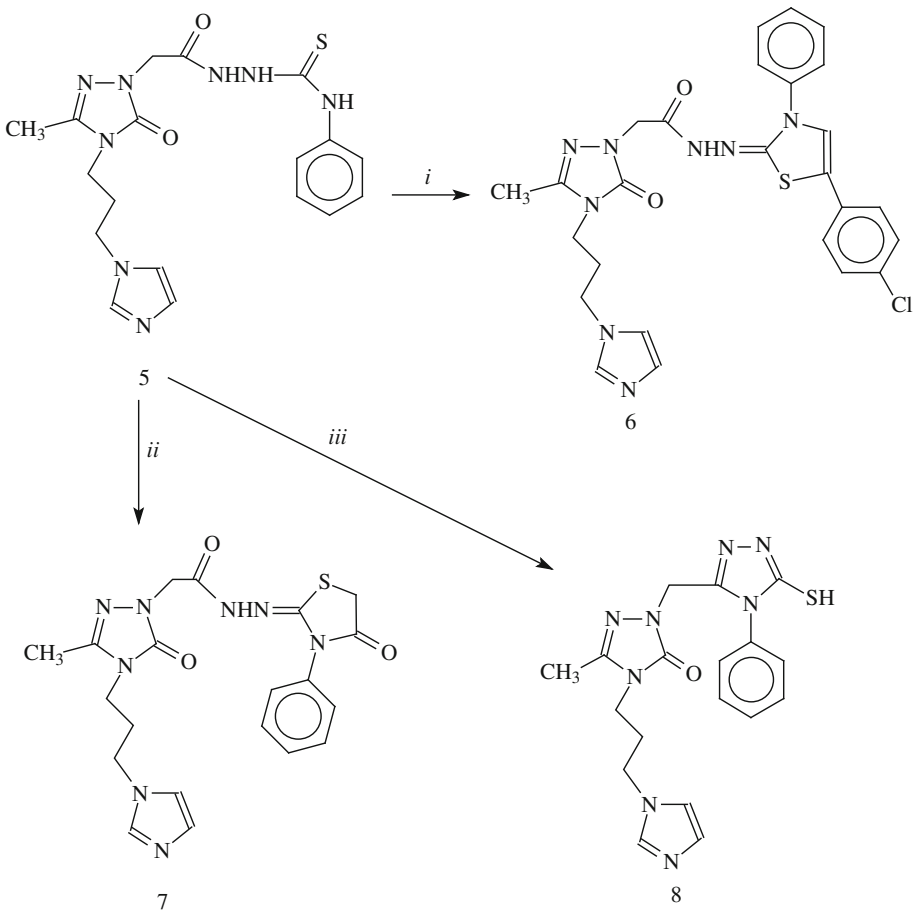
furan-2-ylmethanamine, 2-(piperazin-1-yl)ethanamine and 6-aminopenicillanic acid in the presence of formaldehyde afforded the corresponding Mannich bases, (**9a–c**). In NMR spectra of compounds **9a–c**, the presence of the peaks belonging to amine moiety used in this reaction confirmed the condensation. Moreover, these compounds exhibited elemental analysis data consistent with the proposed structures.

The basic treatment of intermediates **5** and **22** afforded [(5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one derivatives (**8** and **23**). In the ^{1}H NMR spectra of compounds **8** and **23**, the signal due to SH group was recorded at 10.11 and 13.55 ppm, respectively, as an evidence of intramolecular cyclisation. This group was seen at 2,857 cm^{-1} in the FT-IR spectra. In the ^{13}C NMR spectra of compounds **8** and **23**, the C-3 and C-5 carbon atoms belonging to 1,2,4-triazole nucleus resonated at the chemical Schiff values consistent with the literature findings (Buckle *et al*., 1986; Vicini *et al*., 2008; Xu *et al*., 2011]. Furthermore, $[M]^{+}$ ion peaks were observed at the related *m*/*z* value supporting the proposed structure, and these compound gave reasonable elemental analysis data.

The treatment of compound **2** with 3,4-difluoronitrobenzene generated 4-[3-(1*H*-imidazol-1-yl)propyl]-5-methyl-2-(4-nitrobenzyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**10**). Then, this compound (**10**) was converted to the corresponding amine (**11**) by the treatment with hydrazine hydrate in the presence of Pd–C catalyst. The IR spectra of **10** displayed two sharp signals at 1,334 and 1,495 cm^{-1} due to nitro group, while IR spectra of **11** exhibited NH_2 absorption at 3,387 and 3,487 cm^{-1}. The latter group resonated at 4.88 ppm in the ^{1}H NMR spectrum of compound **11** as D_2O exchangeable singlet. Moreover, additional signals originated from phenyl moiety recorded at aromatic region in the ^{1}H- and ^{13}C NMR spectra of compounds **10** and **11**.

The reaction of amine, **11** with aromatic aldehydes namely 4-bromobenzaldehyde and 2,6-dichlorobenzaldehyde produced the corresponding arylmethylenamino compounds **12a** and **b**. On the other hand, the reaction of hydrazide **20** with several aromatic aldehydes afforded the corresponding arylidenhydrazides (**21a–c**). In the ^{1}H NMR spectra of these compounds, the signal derived from NH_2 group disappeared, instead, new signals originated from imine bond and aldehyde moiety were recorded at the related chemical shift values. Moreover, these compounds (**12a**, **b** and **21a–c**) exhibited EI MS and elemental analysis data supporting the proposed structures.

The synthesis of 2-{5-[(3-benzyl-4-oxo-1,3-thiazolidin-2-ylidene)amino]-2-fluorophenyl}-4-[3-(1*H*-imidazol-1-yl) propyl]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**14**) was performed from the cyclocondensation reaction between ethyl bromoacetate and compound **13** that was obtained by the treatment of amine **11** with benzylisothiocyanate. On the other hand, the treatment of the same



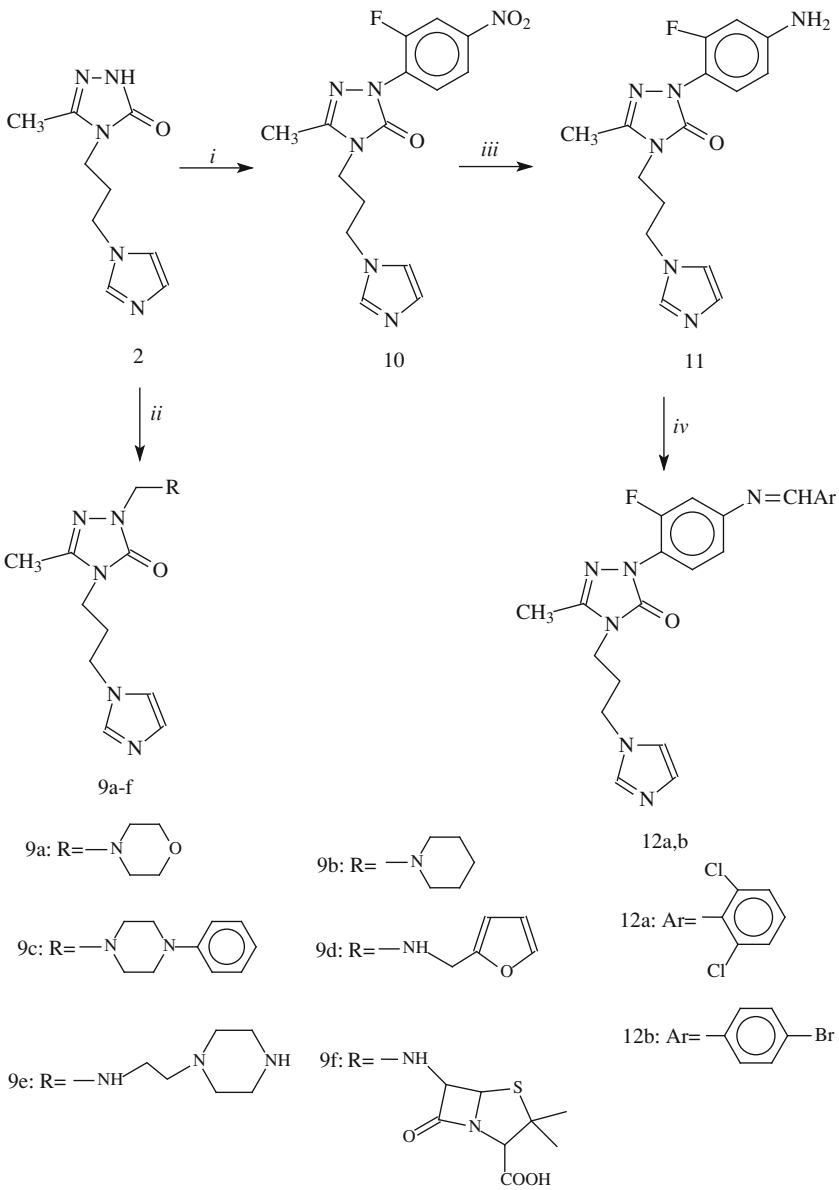
Scheme 2 Reactions and conditions leading to the formation of compounds **6–9**. *i* 4-chlorophenacyl bromide, *ii* ethyl bromoacetate, *iii* NaOH

intermediate **11** with 4-chlorophenacyl bromide produced 2-(4-amino-2-fluorophenyl)-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**15**). In this reaction, the expected product **16** could not be obtained as a result of hydrolysis. The structures of compounds **14** and **15** were confirmed on the basis of FT-IR, EI MS, ^{1}H-, ^{13}C NMR spectroscopic methods and elemental analysis. An evidence for the formation of **15** is the absence of any signals due to imidazol-1-ylpropyl and two phenyl moieties in the ^{1}H- and ^{13}C NMR spectra, and the presence of D_2O exchangeable signals observed at 5.52 and 8.26 ppm, which were attributed to NH_2 and NH functions, respectively. In the EI MS spectrum of compound **15**, the presence of $[M + H_2O]^+$ and $[M]^+$ ion peaks constitutes another evidence for the formation of hydrolysis product, **15**.

The newly synthesized compounds **2–24** were evaluated in vitro for their antimicrobial activities and the results are presented in the Table 1. Among the compounds tested, compounds **9a–f** which contain different heterocyclic moieties with the Mannich base structure were Found (%) to be most active against all the test microorganisms. Furthermore, the activities of compounds **9a–c** appear to be better than the standard drug, ampicillin with the minimal inhibition concentration (MIC) values varying between 0.12 and 62.5 μg/mL. Moreover, these compounds exhibited good activity towards *Mycobacterium smegmatis* (Ms), a non-pigmented, rapidly growing mycobacterium and an atypical TB factor leading to morbidity and mortality, beside *Candida albicans* (Ca) and *Saccharomyces cerevisiae* (Sc) which are yeast like fungy. Other compounds possessing activity on Ms displayed MIC values between 0.24 and 250 μg/mL. except compounds **3**, **10**, **19**, **21–24** which were Found (%) to be inactive against Ms. Among the compounds having Ms activity, the activity of **12a** is better than standard drug streptomycin with the MIC value 0.24 μg/mL while compounds **9b** and **12a** displayed as anti-Ms activity as the standard drug, streptomycin. In general, all compounds, except **4**, **7**, **12b** and **22**, demonstrated activity on yeast like fungy, Ca and/or Sc with the MIC values varying 3.9–1,000 μg/mL. Among these compounds, the ones having the most activity are compounds **9a** and **b**.

Conclusion

This study reports the synthesis of some new 1,2,4-triazole derivatives incorporating several other heterocyclic moieties having importance for biological activity in a single



Scheme 3 Synthetic pathway for the preparation of compounds **10–12**. *i* 3,4-difluoronitrobenzene, *ii* HCHO and amine, *iii* 2,6-dichlorobenzaldehyde or 4-bromobenzaldehyde

structure. Hence, herein, we combined all these potential chemotherapeutic units, namely 1,2,4-triazole, imidazole, pyridine, 1,3-thiazole, 1,3-thiazolidinone, piperazine, morpholine, piperidine and penicillic acid moieties. The antimicrobial screening studies were also performed in the study. 1,2,4-Triazole nucleus is one of the active components present in many standard drugs and it is known to increase the pharmacological activity of the molecules. Other heterocyclic groups getting involved in the structures of the new synthesized compounds in the study are known to have an important role for contributing to the net biological activity of a system. The structures of new compounds were confirmed by IR, ^{1}H-, ^{13}C NMR, mass spectroscopic and elemental analysis techniques. In addition, the newly synthesized compounds were screened for their antimicrobial activities.

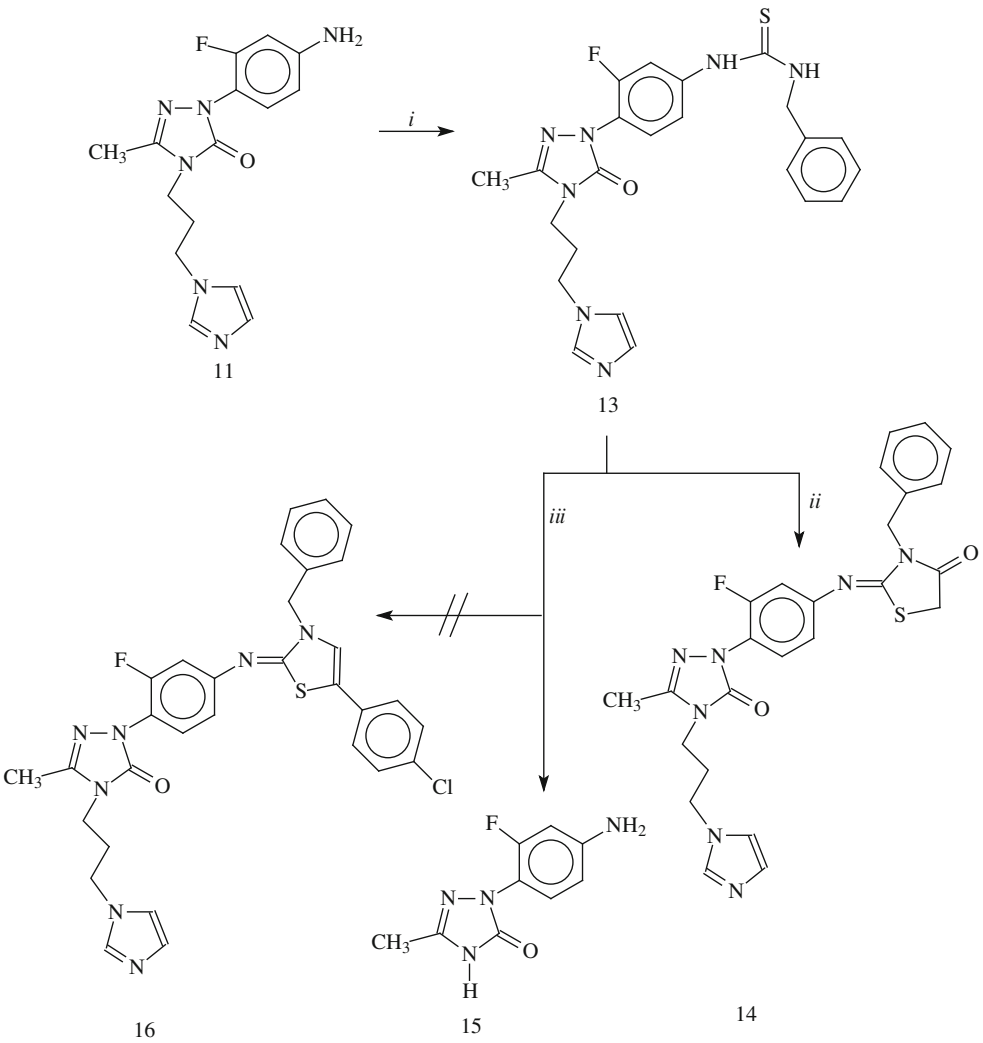
The antimicrobial screening suggests that among the newly synthesized compounds, **9a–f** exhibited excellent activities against most of the test microorganisms. Other new compounds were Found (%) to possess activity especially on Ca and Sc.

Experimental

Chemistry

General information for chemicals

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were



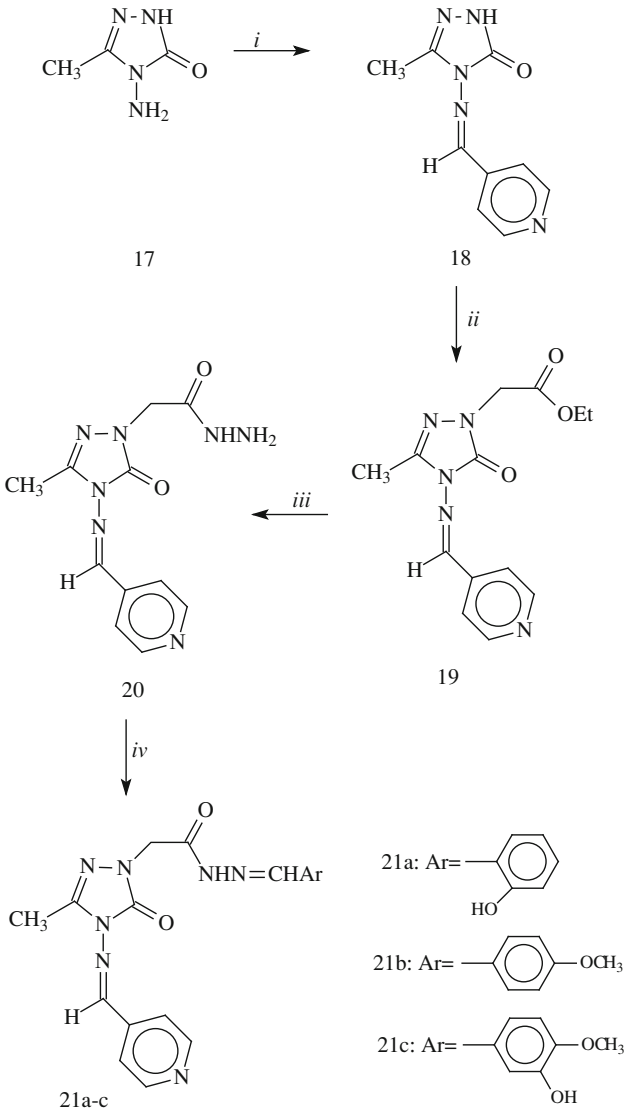
Scheme 4 Reaction and conditions for the synthesis of compounds **14–16**. *i* PhCH2NCS, *ii* ethyl bromoacetate, *iii* 4-chlorophenacyl bromide

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 aluminium sheets. The mobile phase was ethanol:ethyl acetate, 1:1, and detection was made using UV light. FT-IR spectra were recorded as potassium bromide pellets using a *Perkin Elmer* 1600 series FT-IR spectrometer. ^{1}H- and ^{13}C NMR spectra were registered in dimethylsulfoxide (DMSO)-d_6 on a *BRUKER AVENE II* 400 MHz NMR Spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference, J values are given in Hz. The elemental analysis was performed on *a Costech Elemental Combustion System* CHNS–O elemental analyser. All the compounds gave C, H and N analysis within ±0.4 % of the theoretical values. The mass spectra were obtained on a *Quattro LC–MS* (70 eV) Instrument. Compounds **1**, **2** and **17** were synthesized by the way reported previously (Bektas *et al*., 2010a, b).

*4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**2**)* A mixture of the corresponding compound **1** (10 mmol) and imidazole (10 mmol) was heated in an oil bath at 135 °C for 3 h. On cooling it to room temperature, a solid was obtained. This crude product was recrystallized from ethyl acetate to give the pure compound. Yield: 1.69 g, 82 %, mp 161 °C. IR (KBr, υ, cm^{-1}): 3192 (NH), 3020 (ArCH), 1597 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.96–2.10 (m, 5H, CH_3 + CH_2), 3.49 (s, 2H, CH_2), 3.97 (t, 2H, CH_2, J = 6.0 Hz), 6.89 (s, 1H, ArH), 7.23 (s, 1H, ArH), 7.68 (s, 1H, ArH), 11.46 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 12.03 (CH_3), 30.83 (CH_2), 40.89 (CH_2), 44.13 (CH_2), ArC: [120.00 (CH), 129.08 (2CH), 145.09 (C), 155.76 (C=O)]. EI MS *m/z* (%): 230.00 ($[M + Na]^+$, 9), 209.04 (18), 207.98 ($[M]^+$, 95), 140.96 (40), 139.83 (100), 116.92 (15).

Elemental analysis for $C_9H_{13}N_5O$, Calculated (%), C: 52.16; H: 6.32; N: 33.79.

Found (%), C: 52.29; H: 6.29; N: 34.01.



Scheme 5 Synthesis of compounds **18**–**21a**–**c**. *i* pyridine-4-carbaldehyde, *ii* ethyl bromoacetate, *iii* hydrazine hydrate, *vi* suitable aldehyde

*Ethyl {4-[3-(1H-imidazol-1-yl)propyl]-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}acetate (**3**)* Ethylbromoacetate (10 mmol) was added to the mixture of compound **2** (10 mmol) in absolute ethanol, and the reaction mixture was refluxed for 10 h (the progress of the reaction was monitored by TLC). Then, the solvent was removed under reduced pressure, 1 mL of H_2O was added and the organic phase was extracted with CH_2Cl_2 (3 × 40 mL). Combined organic phases were washed with water (2 × 5 mL) and dried on Na_2SO_4. The solvent was evaporated under reduced pressure and the obtained solid was recrystallized from butylacetate:diethylether (1:2) to afford the desired product. Yield 54 %, mp 109–110 °C. IR (KBr, υ, cm^{-1}): 1729 (C=O), 1117 (C–O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.18 (s, 3H, CH_3), 2.15 (brs, 5H, CH_3 + CH_2), 3.55 (dd, 2H, CH_2, $J_{2,3}$ = 6.4 Hz, $J_{1,3}$ = 8.2 Hz), 3.97 (t, 2H, CH_2, J = 6.3 Hz), 4.11 (dd, 2H, CH_2, $J_{1,2}$ = 6.6 Hz, $J_{1,3}$ = 7.8 Hz), 4.49 (s, 2H, CH_2), 6.89 (s, 1H, ArH), 1.23 (s, 1H, ArH), 7.66 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.06 (CH_3), 10.94 (CH_3), 26.94 (CH_2), 37.53 (CH_2), 40.17 (CH_2), 42.99 (CH_2), 58.04 (CH_2), 116.20 (CH), 125.29 (2CH), 140.92 (triazole C-5), 150.65 (triazole C-3), 164.89 (C=O). EI MS *m/z* (%): 332.30 ($[M - 1 + K]^+$, 10), 316.26 ($[M - 1 + Na]^+$, 18), 295.26 ($[M + 1]^+$, 12), 294.31 ($[M]^+$, 78), 226.13 (100), 136.07 (32).

Elemental analysis for $C_{13}H_{19}N_5O_3$, Calculated (%), C: 53.23; H: 6.53; N: 23.88.

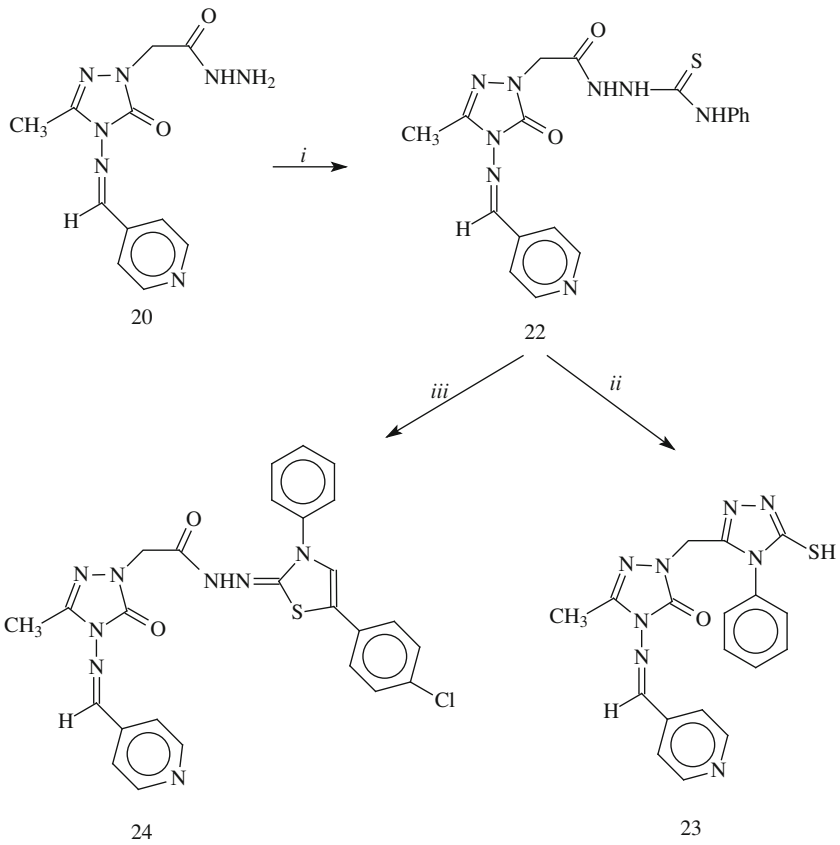
Found (%), C: 53.59; H: 6.67; N: 23.71.

*2-(4-(3-(1H-imidazol-1-yl)propyl)-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl) acetohydrazide (**4**)* Hydrazine hydrate (25 mmol) was added to the solution of compound **3** (10 mmol) in absolute ethanol, and the mixture was heated under reflux for 10 h. On cooling the reaction mixture to room temperature, a white solid appeared. The crude product was filtered off and recrystallized from butylacetate:diethylether (1:2) to afford the desired compound. Yield 33 %, mp 172–173 °C. IR (KBr, υ, cm^{-1}): 3339 (NH_2), 3339 (NH), 1653 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 4.27 (s, 3H, CH_3), 5.70 (brs, 2H, CH_2), 5.95 (brs, 4H, 2CH_2), 6.37 (s, 2H, CH_2), 6.50 (brs, 2H, NH_2 exch. D_2O), 9.06 (s, 1H, ArH), 9.40 (s, 1H, ArH), 9.86 (s, 1H, ArH), 9.22 (s, 1H, NH exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 11.71 (CH_3), 30.54 (2CH_2), 44.10 (CH_2), 46.63 (CH_2), 120.16 (CH), 128.63 (2CH), 144.69 (triazole C-5), 154.63 (triazole C-3), 166.87 (C=O). EI MS *m/z* (%): 317.27 ($[M - 1 + K]^+$, 10), 316.26 ($[M - 2 + K]^+$, 22), 228.02 (29), 148.96 (78), 123.93 (100).

Elemental analysis for $C_{11}H_{17}N_7O_2$, Calculated (%), C: 47.30; H: 6.14; N: 35.10.

Found (%), C: 47.62; H: 6.33; N: 35.47.

*2-({4-[3-(1H-imidazol-1-yl)propyl]-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}acetyl)-N-phenylhydrazinecarbothioamide (**5**)* The mixture of compound **4** (10 mmol) and phenylisothiocyanate (10 mmol) in absolute ethanol was refluxed for 5 h. Upon cooling the reaction content to room temperature, a white solid formed. This crude product was filtered and recrystallized from ethanol to afford the desired compound. Yield 54 %, mp 112–113 °C. IR (KBr, υ, cm^{-1}): 3331 (NH), 3164 (2NH), 1690 (C=O), 1226 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.14 (brs, 3H, CH_3), 3.42 (d, 4H, 2CH_2, J = 4.2 Hz), 3.86 (brs, 4H, 2CH_2), 4.46 (s, 2H, CH_2), 6.98 (s, 1H, ArH), 7.35 (brs, 6H, ArH), 7.82 (s, 1H, ArH), 9.66–9.77 (m, 2H, NH exch. D_2O), 10.33 (s, 1H, NH exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 10.32 (CH_3), 24.53 (CH_2), 41.09 (CH_2), 42.79 (CH_2), 48.54 (CH_2), 120.24 (CH), 124.69 (2CH), 125.08 (2CH), 130.59 (C), 134.21 (2CH), 138.21 (C), 144.23 (triazole C-5), 148.67 (C=O), 161.79 (triazole C-3),



Scheme 6 *i* PhCH2NCS, *ii* ethyl bromoacetate, *iii* 4-chlorophenacyl bromide

180.51 (C=S). EI MS *m/z* (%): 453.21 ($[M + K]^+$, 10), 417.39 ($[M + 1]^+$, 10), 416.39 ($[M]^+$, 12), 415.39 ($[M - 1]^+$, 46), 398.37 ($[M - 1 - H_2O]^+$, 53), 231.06 (31), 226.18 (34), 152.91 (100), 139.95 (16).

Elemental analysis for $C_{18}H_{22}N_8O_2S$, Calculated (%), C: 52.16; H: 5.35; N: 27.03.

Found (%), C: 52.45; H: 5.37; N: 27.28.

*N′-[5-(4-chlorophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]-2-{4-[3-(1H-imidazol-1-yl)propyl]-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}acetohydrazide (**6**)* 4-Chloro phenacylbromide (10 mmol) and dried sodium acetate (16.4 g, 200 mmol) was added to the solution of compound **5** in absolute ethanol, and the reaction mixture was refluxed for 14 h. Then, it was cooled to room temperature, poured into ice-cold water while stirring and left overnight in cold. The formed solid was filtered, washed with water three times and recrystallized from acetone to afford compound **6**. Yield 27 %, mp 143–144 °C. IR (KBr, υ, cm^{-1}): 3360 (NH), 3063 (aromatic CH), 1736 and 1749 (2C=O), 1453 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.85 (s, 3H, CH_3), 2.98 (s, 2H, CH_2), 4.53 (brs, 4H, $2CH_2$), 5.21 (brs, 2H, CH_2), 7.21 (brs, 4H, ArH), 8.09 (brs, 8H, ArH), 9.70 (s, 1H, NH exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 7.56 (CH_3), 39.70 (CH_2), 40.23 ($2CH_2$), 42.26 (CH_2), 102.65 (CH), 102.97 (C) 103.42 (CH), 118.42 (2CH), 137.36 (triazole C-5), 141.14 (triazole C-3), 149.78 (CH), 149.91 (2CH), 150.27 (CH), 155.32 (CH), 156.84 (C), 162.85 (C), 263.78 (C), 164.78 (C=O), 168.30 (C=O). EI MS *m/z* (%): 585.32 ($[M - 2 + K]^+$, 21), 546.22 ($[M - 2]^+$, 18), 357.56 (68), 234.21 (100), 123.65 (43).

Elemental analysis for $C_{26}H_{25}ClN_8O_2S$, Calculated (%), C: 56.88; H: 4.59; N: 20.41.

Found (%), C: 57.21; H: 4.64; N: 20.13.

*2-{4-[3-(1H-imidazol-1-yl)propyl]-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}-N′-(4-oxo-3-phenyl-1,3-thiazolidin-2-ylidene)acetohydrazide (**7**)* Ethyl bromoacetate was added to the solution of compound **5** in absolute ethanol (10 mmol) and the mixture was refluxed in the presence of dried sodium acetate (16.4 g, 200 mmol) for 10 h. Then, the mixture was cooled to room temperature, poured into ice-cold water while stirring and left overnight in cold. The formed solid was filtered, washed with water three times and recrystallized from diethylether to afford the pure compound. Yield 36 %, mp 132–133 °C. IR (KBr, υ, cm^{-1}): 3361 (NH), 3065 (aromatic CH), 1709, 1747 and 1751 (3C=O), 1450 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.19 (s, 3H, CH_3), 2.48 (s, 2H, CH_2), 4.13 (brs, 4H, $2CH_2$), 4.59 (brs, 4H, $2CH_2$), 7.79 (brs, 5H, ArH), 8.72 (brs, 3H, ArH), 9.72 (s, 1H, NH exch. D_2O). ^{13}C NMR (DMSO-d_6, δ

Table 1 Antimicrobial activity of the compounds (μg/mL)

Compound numbers	Microorganisms and MIC									
	Ec	Yp	Pa	Lm	Ef	Sa	Bc	Ms	Ca	Sc
2	–	–	–	–	–	–		125	500	1000
3	–	–	–	–	–	–	–	–	250	–
4	125	125	–	–	–	–	–	125	–	–
5	–	–	–	–	–	–	–	31.3	250	62.5
6	–	–	–	–	–	–		39.9	250	1000
7	–	–	–	–	–	–	–	62.5	–	–
8	–	–	–	–	–	–	–	125	500	500
9a	0.97	0.48	0.48	0.24	0.97	0.48	0.24	15.6	31.25	7.81
9b	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	3.9	15.6	3.9
9c	–	0.7	31.3	–	–	62.5	1.2	–	–	125
9d	–	–	–	–	–	–	125	125	500	–
9e	1.2	62.5	–	–	–	125	–	500	125	
9f	–	125	125	–	–	62.5	125	31.3	–	–
10	–	–	–	–	–	–	–	–	500	500
11	–	–	–	–	–	–	–	31.3	250	62.5
12a		–	–	–	–	–	–	3.9	250	–
12b	–	–	–	–	–	–	–	0.24	–	–
13	–	–	–	–	–	–		250	500	500
14	–	–	–	–	–	–	–	125	500	500
15	–	–	–	–	–	–	–	31.3	125	500
18	–	–	–	–	–	–	–	125	125	500
19	–	–	–	–	–	–	–	–	250	–
20	–	–	–	–	–	–	–	250	500	–
21a	–	–	–	–	–	–	–	–	250	–
21b	–	–	–	–	–	–	–	–	250	–
21c	–	–	–	–	–	–	–	–	125	–
22	–	–	–	–	–	–	–	–	–	–
23	–	–	–	–	–	–	–	–	250	–
24	–	–	–	–	–	125	125	–	125	250
Amp.	2	32	>128	8	2	2	<1			
Strep.								4		
Flu.									<8	<8

Ec, *E. coli* ATCC 35218; Yp, *Y. pseudotuberculosis* ATCC 911; *Pa*, *P. aeruginosa* ATCC 10145; Lm, *L. monositogenes* ATCC 43251; Ef, *E. faecalis* ATCC 29212; Sa, *S. aureus* ATCC 25923; Bc, *B. cereus* 709 Roma; Ms, *M. smegmatis* ATCC 607; Ca, *C. albicans* ATCC 60193; Sc, *S. cerevisiae* RSKK 251, Amp., ampicillin; Strep., streptomycin; Flu., fluconazole; –, no activity of test concentrations

ppm): 7.86 (CH_3), 39.30 (CH_2), 40.17 (CH_2), 40.57 (CH_2), 43.23 (CH_2), 58.18 (CH_2), 101.55 (2CH), 118.42 (2CH), 137.36 (triazole C-5), 141.14 (triazole C-3), 147.44 (CH), 148.54 (2CH), 151.21 (CH), 156.84 (C), 162.85 (C=O), 164.78 (C=O), 168.30 (C=O). EI MS *m/z* (%): 506.32 (100), 492.56 (68), 491.49 ($[M - 2 + K]^+$, 21), 475.28 ($[M - 2 + Na]^+$, 13), 44.18 (62), 434.49 ($[M - 2 - H_2O]$, 63), 429.48 ($[M - 2 - Na]^+$, 41), 413.65 ($[M - 2 - K]^+$, 12).

Elemental analysis for $C_{20}H_{22}N_8O_3S$, Calculated (%), C: 52.85; H: 4.88; N: 24.65.

Found (%), C: 52.89; H: 4.77; N: 24.71.

*4-[3-(1H-imidazol-1-yl)propyl]-2-[(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)methyl]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**8**)* A solution of compound **5** (10 mmol) in ethanol:water (1:1) was refluxed in the presence of 2 N NaOH for 3 h, then, the resulting solution was cooled to room temperature and acidified to pH 4 with 37 % HCl. The precipitate formed was filtered off, washed with water and recrystallized from acetone to afford the desired compound. Yield 44 %, mp 141–142 °C. IR (KBr, υ, cm^{-1}): 3352 (SH), 3078 (aromatic CH), 1709 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.18 (s, 3H, CH_3), 2.67 (s, 2H, CH_2), 4.54 (brs, 4H, $2CH_2$), 4.59 (brs, 2H, CH_2), 7.52 (brs, 5H, ArH), 8.58 (brs,

3H, ArH), 10.11 (s, 1H, SH exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 7.51 (CH_3), 32.36 (CH_2), 41.67 (CH_2), 42.47 (CH_2), 43.73 (CH_2), 102.53 (2CH), 117.71 (2CH), 136.71 (triazole C-5), 142.34 (triazole C-3), 146.84 (CH), 149.74 (2CH), 150.27 (CH), 157.44 (C), 161.95 (C), 169.80 (C=O). EI MS *m/z* (%): 396.45 ($[M]^+$, 10), 395.25 ($[M - 1]^+$, 19), 291.43 (100), 158.23 (37).

Elemental analysis for $C_{18}H_{20}N_8OS$, Calculated (%), C: 54.53; H: 5.08; N: 28.26.

Found (%), C: 54.59; H: 5.13; N: 28.17.

*General method for the synthesis of compounds **9a–c***

The suitable amine was added to a solution of compound **2** (10 mmol) in tetrahydrofuran and the mixture was stirred at room temperature in the presence of formaldehyde (37 %, 7.4 mL) for 4 h. Then, water was added and kept overnight in cold conditions. The solid separated was collected by filtration and recrystallized from ethyl acetate–petroleum ether (1:2) to yield the target compounds.

*4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**9a**)* Yield: 0.53 g, 52 %, mp 123 °C. IR (KBr, υ, cm^{-1}): 1712 (C=O), 1117 (C–O). ^1H NMR (DMSO-d_6, δ ppm): 2.13 (s, 5H, $CH_3 + CH_2$), 2.48 (brs, 4H, $2CH_2$), 3.23 (brs, 6H, $3CH_2$), 3.97 (t, 2H, CH_2, $J = 6.5$ Hz), 4.66 (s, 2H, CH_2), 6.88 (s, 1H, ArH), 7.23 (s, 1H, ArH), 7.67 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.85 (CH_3), 30.69 (CH_2), 44.08 (CH_2), 49.90 (CH_2), 50.61 (CH_2), 66.16 (CH_2), 66.91 (CH_2), 82.59 (CH_2), 84.61 (CH_2), ArC: [119.97 (CH), 129.11 (CH), 138.95 (CH), 143.97 (C), 154.86 (C=O)]. EI MS *m/z* (%): 407.09 (54), 306.23 ($[M]^+$, 59), 215.30 (100).

Elemental analysis for $C_{14}H_{22}N_6O_2$, Calculated (%), C: 54.89; H: 7.24; N: 27.43.

Found (%), C: 55.22; H: 7.46; N: 27.21.

*4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2-(piperidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**9b**)* Yield: 0.81 g, 78 %, mp 110 °C. IR (KBr, υ, cm^{-1}): 3027 (ArCH), 1725 (C=O). ^1H NMR (DMSO-d_6, δ ppm): 1.41 (s, 5H, $CH_3 + CH_2$), 2.12 (s, 4H, $2CH_2$), 2.45 (brs, 4H, $2CH_2$), 3.94 (brs, 4H, $2CH_2$), 3.97 (t, 2H, CH_2, $J = 6.8$ Hz), 4.67 (s, 4H, $2CH_2$), 6.88 (s, 1H, ArH), 7.22 (s, 1H, ArH), 7.67 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.79 (CH_3), 24.15 (CH_2), 26.19 (CH_2), 30.69 (CH_2), 44.18 (CH_2), 50.04 (CH_2), 51.48 (CH_2), 66.98 (CH_2), 82.57 (CH_2), 84.64 (CH_2), ArC: [119.98 (CH), 129.00 (CH), 139.20 (CH), 143.70 (C), 154.85 (C=O)]. EI MS *m/z* (%): 524.12 (68), 303.40 ($[M - 1]^+$, 23), 297.48 (35), 227.45 (100).

Elemental analysis for $C_{15}H_{24}N_6O$, Calculated (%), C: 59.19; H: 7.95; N: 27.61.

Found (%), C: 59.43; H: 7.67; N: 27.42.

*4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (**9c**)* Yield: 1.00 g, 80 %, mp 102 °C. IR (KBr, υ, cm^{-1}): 3027 (ArCH); 1725 (C=O). ^1H NMR (DMSO-d_6, δ ppm): 2.13 (s, 5H, $CH_3 + CH_2$), 2.66 (brs, 4H, $2CH_2$), 3.08 (s, 4H, $2CH_2$), 3.42 (s, 2H, CH_2), 4.50 (s, 4H, $2CH_2$), 6.89 (s, 2H, ArH), 7.22 (s, 3H, ArH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.86 (CH_3), 30.71 (CH_2), 44.09 (CH_2), 49.03 (CH_2), 50.21 (CH_2), 65.97 (CH_2), 67.37 (CH_2), 82.60 (CH_2), 84.63 (CH_2), ArC: [116.09 (CH), 116.25 (CH), 119.38 (CH), 119.59 (CH), 119.98 (CH), 129.02 (CH), 129.12 (CH), 129.59 (CH), 151.72 (C), 153.79 (C), 154.86 (C=O)]. EI MS *m/z* (%): 382.35 ($[M + 1]^+$, 10), 175.99 (100), 131.88 (13).

Elemental analysis for $C_{20}H_{27}N_7O$, Calculated (%), C: 62.79; H: 7.13; N: 25.70.

Found (%), C: 63.11; H: 7.27; N: 25.46.

*2-{[(2-Furylmethyl)amino]methyl}-4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**9d**)* Yield: 0.41 g, 85 %, mp 158 °C. IR (KBr, υ, cm^{-1}): 3258 (NH), 3025 (ArCH); 1724 (C=O). ^1H NMR (DMSO-d_6, δ ppm): 2.10 (brs, 5H, $CH_2 + CH_3$), 3.41–3.52 (m, 4H, $2CH_2$), 3.39 (brs, 2H, CH_2), 6.88 (s, 2H, ArH), 7.24 (s, 2H, ArH), 7.67 (s, 2H, ArH), 11.42 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 12.04 (CH_3), 30.72 (CH_2), 30.82 (CH_2), 44.10 ($2CH_2$), 67.36 (C), ArC: [102.23 (C), 119.96 (3CH), 129.09 (3CH)], 145.06 (C=O). EI MS *m/z* (%): 320.19 (53), 316,45 ($[M]^+$, 25), 119.30 (100).

Elemental analysis for $C_{15}H_{20}N_6O$, Calculated (%), C: 56.94; H: 6.37; N: 26.56.

Found (%), C: 57.27; H: 6.42; N: 26.38.

*4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2-{[(2-piperazin-1-ylethyl)amino]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one (**9e**)* Yield: 0.38 g, 75 %, mp 142 °C. IR (KBr, υ, cm^{-1}): 3258, 3174 (2NH), 3014 (ArCH), 1694 (C=O). ^1H NMR (DMSO-d_6, δ ppm): 1.75 (s, 3H, CH_3), 2.13 (s, 6H, $3CH_2$), 2.49 (brs, 2H, CH_2), 3.39 (brs, 10H, $5CH_2$), 3.97 (s, 2H, CH_2), 4.42 (brs, 2H, 2NH), 6.88 (s, 1H, Ar), 7.24 (s, 1H, ArH), 7.67 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.62 (CH_3), 30.46 ($2CH_2$), 42.38 ($2CH_2$), 44.15 ($2CH_2$), 49.84 (CH), 65.49 ($2CH_2$), 67.77 ($2CH_2$), 104.99 (C), ArC: [128.75 (3CH)], 154.93 (C=O). EI MS *m/z* (%): 350.76 ($[M + 2]^+$, 12), 350.19 (45), 257.43 (100).

Elemental analysis for $C_{16}H_{28}N_8O$, Calculated (%), C: 55.15; H: 8.10; N: 32.16.

Found (%), C: 55.11; H: 8.18; N: 32.45.

*6-[({4-[3-(1H-imidazol-1-yl)propyl]-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}methyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**9f**)* Yield: 0.41 g, 79 %, mp 185 °C. IR (KBr, υ, cm^{-1}): 3568

(OH), 3254 (NH), 3109 (ArCH), 1754, 1694 2(C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.13 (brs, 6H, 2CH_3), 1.49 (brs, 5H, CH_3 + CH_2), 2.12 (brs, 6H, 3CH_2), 6.98 (s, 1H, ArH), 7.27 (s, 1H, ArH), 7.85 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6, δ ppm): 9.09 (CH_3), 11.66 (CH_3), 11.81 (CH_3) 30.43 (CH_2), 44.38 (CH_2), 46.15 (CH_2), 56.74 (CH_2), 58.12 (C), 67.28 (C), 98.51 (CH), 101.89 (CH), 104.99 (CH), ArC: [120.31 (2CH), 128.04 (CH)], 144.70 (C=O), 145.50 (C=O), 153.91 (C=O). EI MS *m/z* (%): 458 ([M + Na]$^+$, 14), 452 (54), 436 ([M + 1]$^+$, 65), 230.43 (100).

Elemental analysis for $C_{18}H_{25}N_7O_4S$, Calculated (%), C: 49.65; H: 5.79; N: 22.51.

Found (%), C: 49.71; H: 5.87; N: 22.46.

*4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2-(2-fluoro-4-nitrophenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**10**)* A solution of compound **2** (1.71 g, 10 mmol) and metallic sodium (0.19 g, 10 mmol) in absolute ethanol (10 mL) was refluxed for 2 h. Then, the reaction mixture was allowed to cool to room temperature, 3,4-difluoronitrobenzene (10 mmol) was added into it refluxed for an additional 8 h (TLC controlled). The reaction content was poured into ice-water. The precipitated product was filtered off and recrystallized from ethanol. Yield: 1.63 g, 47 %, mp 83 °C. IR (KBr, υ, cm^{-1}): 1334 and 1495 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 1.33–1.40 (m, 5H, CH_3 + CH_2), 4.35 (q, 4H, 2CH_2, J = 6.3 Hz), 7.35 (t, 2H, ArH, J = 8.6 Hz), 8.08–8.12 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6, δ ppm): 14.92 (CH_3), 66.04 (3CH_2), ArC: [112.32 (CH), 112.78 (CH), 113.00 (CH), 114.56 (CH), 122.16 (2CH), 141.0 (C), 148.45 (2C), 153.00 (C), 153.20 (C), 153.37 (C=O)]. EI MS *m/z* (%): 348.21 ([M + 2]$^+$, 21), 347.21 ([M + 1]$^+$, 100), 279.09 (25).

Elemental analysis for $C_{15}H_{15}FN_6O_3$, Calculated (%), C: 52.02; H: 4.37; N: 24.27.

Found (%), C: 52.35; H: 7.64; N: 24.13.

*2-(4-Amino-2-fluorophenyl)-4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**11**)* Pd–C (5 mmol) catalyst was added to the solution of compound **11** (10 mmol) in butanol, and the mixture was refluxed in the presence of hydrazine hydrate (50 mmol) for 8 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the catalyst was separated by filtration and the reaction solvent was evaporated under reduced pressure. The crude product formed was recrystallized from ethanol. IR (KBr, υ, cm^{-1}): 3387, 3487 (NH_2), 3013 (ArCH). ^{1}H NMR (DMSO-d_6, δ ppm): 0.84 (brs, 3H, CH_3), 1.20–1.42 (m, 6H, 3CH_2), 4.88 (s, 2H, NH_2, D_2O exch.), 6.27–6.43 (m, 4H, ArH), 6.44–7.82 (t, 2H, ArH, J = 9.1 Hz). ^{13}C NMR (DMSO-d_6, δ ppm): 15.44 (CH_3), 19.32 (CH_2), 61.10 (CH_2), 66.21 (CH_2), ArC: [102.59 (CH), 103.01 (CH), 110.00 (CH), 110.05 (CH), 118.36 (CH), 118.43 (CH), 137.10 ve 137.33 (C), 144.40 (C), 144.61 (C), 151.33 (C), 156.12 (C=O)]. EI MS *m/z* (%): 340.49 ([M + 1 + Na]$^+$, 20), 339.49 ([M + Na]$^+$, 100), 261.40 (10), 249.39 (12), 198.27 (11).

Elemental analysis for $C_{15}H_{17}FN_6O$, Calculated (%), C: 56.95; H: 5.42; N: 26.57.

Found (%), C: 57.17; H: 5.75; N: 26.43.

*General method for the synthesis of compounds **12a** and **b***

A solution of compound **11** (10 mmol) in ethanol was refluxed with 2,6-dichlorobenzaldehyde (for **12a**) or 4-bromobenzaldehyde (for **12b**) for 6 h. On cooling the reaction mixture to room temperature, a solid appeared. This crude product was recrystallized from ethanol–water (1:2) (for **12a**) or ethanol (for **12b**) to afford the desired product.

*2-(4-{[(2,6-Dichlorophenyl)methylene]amino}-2-fluorophenyl)-4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**12a**)* Yield: 2.50 g, 53 %, mp 84 °C. IR (KBr, υ, cm^{-1}): 3018 (ArCH), 1714 (C=O), 1603 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.34 (s, 3H, CH_3), 5.03 (d, 2H, CH_2, J = 2.0 Hz), 4.06–4.16 (m, 4H, 2CH_2), 7.14–7.37 (m, 4H, ArH), 7.47–7.59 (m, 4H, ArH), 8.74 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 15.25 (CH_3), 65.21 (3CH_2), ArC: [109.26 (CH), 109.64 (CH), 115.65 (CH), 118.79 (CH), 118.85 (CH), 129.82 (2CH), 132.45 (2CH), 132.92 (C), 134.69 (C), 143.97 (C), 144.11 (C), 146.11 (C), 146.22 (C), 146.43 (C), 150.07 (C), 154.91 (C=O), 156.39 (N=CH)]. EI MS *m/z* (%): 473.33 ([M + 1]$^+$, 100), 476.67 (28).

Elemental analysis for $C_{22}H_{19}Cl_2FN_6O$, Calculated (%), C: 55.82; H: 4.05; N: 17.76.

Found (%), C: 56.11; H: 4.09; N: 17.84.

*2-(4-{[(4-Bromophenyl)methylene]amino}-2-fluorophenyl)-4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**12b**)* A solution of compound **12** (10 mmol) in ethanol was refluxed with 4-bromobenzaldehyde for 6 h. On cooling the reaction mixture to room temperature, a solid appeared. This crude product was recrystallized from ethanol to give the desired product. Yield: 2.79 g, 57 %, mp 89 °C. IR (KBr, υ, cm^{-1}): 3021 (ArCH), 1712 (C=O), 1610 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 3.51 (s, 5H, CH_3 + CH_2), 5.54 (s, 2H, CH_2), 6.26–6.29 (brs, 2H, CH_2), 9.31–9.50 (m, 4H, ArH), 9.89–10.02 (m, 6H, ArH), 10.81 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 15.29 (CH_3), 65.13 (3CH_2), ArC: [109.23 (CH), 109.61 (CH), 115.55 (CH), 119.04 (CH), 125.55 (C), 131.02 (CH), 131.93 (CH), 132.32 (CH), 132.56 (CH), 133.00 (CH), 135.86 (C), 144.52 (C), 145.70 (C), 145.91 (C), 150.03 (C), 154.90 (C=O), 159.29 (N=CH)]. EI MS *m/z* (%): 483.30 ([M$^+$], 22), 393.63 (18),

317.54 (29), 274.43 (25), 273.49 (100), 249.40 (70), 188.39 (74), 160.29 (80), 108.23 (40).

Elemental analysis for $C_{14}H_{22}N_6O_2$, Calculated (%), C: 54.89; H: 7.24; N: 27.43.

Found (%), C: 55.22; H: 7.46; N: 27.21.

*N-benzyl-N′-(3-fluoro-4-{4-[3-(1H-imidazol-1-yl)propyl]-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}phenyl) thiourea (**13**)* The mixture of compound **12** (10 mmol) and benzyl isothiocyanate (10 mmol) was allowed to reflux in absolute ethanol for 7 h. Then, the solution was cooled to room temperature and a solid appeared. This product was filtered off and recrystallized from ethanol. Yield: 2.9 g, 61 %, mp 193 °C. IR (KBr, υ, cm^{-1}): 3192, 3248 (NH), 1230 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.31 (s, 3H, CH_3), 3.34 (s, 2H, CH_2), 4.05 (q, 4H, $2CH_2$, $J = 3.0$ Hz), 4.71 (s, 2H, CH_2), 7.27–7.40 (m, 1H, ArH), 8.12 (s, 1H, NH, D_2O exch.), 9.53 (s, 1H, NH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 15.33 (CH_3), 47.86 ($2CH_2$), 65.13 ($2CH_2$), ArC: [113.03 (CH), 113.44 (CH), 115.33 (CH), 120.92 (CH), 127.53 (2CH), 128.05 (2CH), 128.94 (2CH), 132.59 (CH), 132.77 (C), 139.74 (C), 144.03 (C), 144.25 (C), 149.17 (C)], 154.00 (C=O), 181.61 (C=S). EI MS *m/z* (%): 466.34 ($[M + 1]^+$, 20), 343.13 (31), 324.17 (18), 306.15 (20), 305.15 (100), 291.13 (17), 223.93 (23), 176,88 (37), 116.81 (31).

Elemental analysis for $C_{23}H_{24}FN_7OS$, Calculated (%), C: 59.34; H: 5.20; N: 21.06.

Found (%), C: 59.42; H: 5.37; N: 21.33.

*2-{5-[(3-Benzyl-4-oxo-1,3-thiazolidin-2-ylidene)amino]-2-fluorophenyl}-4-[3-(1H-imidazol-1-yl)propyl]-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**14**)* A mixture of compound **14** (10 mmol) and ethyl bromoacetate in absolute ethanol was allowed to reflux in the presence of dried sodium acetate (50 mmol) for 10 h. Then, the mixture was poured into ice-water. The precipitated product was filtered off and recrystallized from acetone. Yield: 2.50 g, 50 %, mp 79 °C. IR (KBr, υ, cm^{-1}): 1726 (C=O), 1600 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.32 (brs, 5H, $CH_3 + CH_2$), 3.38 (s, 2H, CH_2), 4.03–4.10 (m, 4H, $2CH_2$), 4.89 (brs, 2H, CH_2), 6.66–6.83 (m, 3H, ArH), 7.06–7.10 (m, 2H, ArH), 7.12–7.34 (m, 6H, ArH). ^{13}C NMR (DMSO-d_6, δ ppm): 15.32 (CH_3), 33.24 (CH_2), 46.07 ($2CH_2$), 65.14 ($2CH_2$), ArC: [109.88 (CH), 110.27 (CH), 116.01 (CH), 116.07 (CH), 117.41 (CH), 117.48 (CH), 127.52 (CH), 128.07 (CH), 128.13 (CH), 128.41 (CH), 129.13 (CH), 136.81 (C), 141.69 (C), 143.71 (C), 149.90 (C),154.77 (C), 156.46 (C=N), 172.67 (C=O), 181.66 (C=O)]. EI MS *m/z* (%): 505.70 ($[M]^+$, 33), 529.60 ($[M + 1 + Na]^+$, 20), 543.55 ($[M + K]^+$, 16), 593.80 (30), 589.73 (34), 588.79 (100), 579.72 (27), 577.65 (42), 565.70 (22), 563.70 (32), 549.75 (33), 535.67 (23), 521.70 (20), 491.68 (25), 477.66 (23), 475.66 (32), 469.72 (34).

Elemental analysis for $C_{23}H_{24}FN_7OS$, Calculated (%), C: 59.39; H: 4.78; N: 19.37.

Found (%), C: 59.66; H: 4.84; N: 19.12.

*2-(4-Amino-2-fluorophenyl)-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (**15**)* A mixture of compound **14** (10 mmol) and 4-chloro phenacylbromide (20 mmol) in absolute ethanol was refluxed in the presence of dried sodium acetate (50 mmol) for 10 h. Then, the mixture was poured into ice-water. The precipitated product was filtered off and recrystallized from ethanol. Yield: 1.50 g, 62 %, mp 119 °C. IR (KBr, υ, cm^{-1}): 1450 (C=N), 1527 (NO_2). ^{1}H NMR (DMSO-d_6, δ ppm): 2.14 (s, 3H, CH_3), 5.52 (s, 2H, NH_2, D_2O exch.), 8.26 (q, 1H, NH, $J = 8.6$ Hz D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 20.98 (CH_3), 124.68 (2CH), 130.00 (CH), 139.11 (2C), 150.98 (C), 170.61 (C), 193.05 (C=O). EI MS *m/z* (%): 226.23 ($[M + H_2O]^+$, 13), 208.15 ($[M]^+$, 10), 169.16 (100), 108.20 (28).

Elemental analysis for $C_{23}H_{24}FN_7OS$, Calculated (%), C: 51.92; H: 4.36; N: 26.91.

Found (%), C: 52.35; H: 4.49; N: 26.64.

*5-Methyl-4-[(pyridin-4-ylmethylene)amino]-2,4-dihydro-3H-1,2,4-triazol-3-one (**18**)* The mixture of compound **17** (10 mmol) and 4-pyridinecarboxaldehyde (10 mmol) in absolute ethanol was irradiated with microwave (150 W, 160 °C) for 30 min. After completion of reaction (monitored by TLC), the formed solid was filtered and washed with diethylether. The obtained crude product was recrystallized from ethanol. Yield 99 %, mp 129–130 °C. IR (KBr, υ, cm^{-1}): 3166 (NH), 1721 (C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.26 (s, 3H, CH_3), 7.71 (s, 2H, ArH), 8.66 (s, 2H, ArH), 9.75 (s, 1H, N=CH), 11.91 (s, 1H, NH exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 11.24 (CH_3), 122.47 (2CH), 142.36 (triazole C-3), 144.56 (C), 151.198 (2CH), 161.15 (triazole C-5), 168.33 (N=CH). EI MS *m/z* (%): 226.13 ($[M + Na]^+$, 10), 204.03 ($[M + 1]^+$, 50), 181.07 ($[M + 1 - H_2O]^+$, 10), 164.05 (12), 148.97 (14), 135.95 (28), 105.03 (100).

Elemental analysis for $C_9H_9N_5O$, Calculated (%), C: 53.20; H: 4.46; N: 34.47.

Found (%), C: 53.47; H: 4.44; N: 34.15.

*Ethyl (3-methyl-5-oxo-4-[(pyridin-4-ylmethylene)amino]-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetate (**19**)* Ethylbromoacetate (10 mmol) was added to the solution of compound **18** (10 mmol) in absolute ethanol and the reaction mixture was refluxed for 10 h. After evaporating the solvent under reduced pressure, an oily product was obtained. 1 mL of H_2O was added into it and the formed solid was

separated by filtration. This crude product was washed with diethyl ether to afford the desired product. Yield 98 %, mp 135–136 °C. IR (KBr, υ, cm^{-1}): 1747 and 1709 (2C=O), 1592 (C=N), 1215 (C–O). ^{1}H NMR (DMSO-d_6, δ ppm): 1.21 (d, 3H, CH_3, $J = 6.2$ Hz), 2.35 (brs, 3H, CH_3), 4.16 (brs, 2H, CH_2), 4.62 (s, 2H, CH_2), 7.80 (s, 2H, ArH), 8.73 (brs, 2H, ArH), 9.74 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.57 (CH_3), 14.70 (CH_3), 46.97 (CH_2), 62.00 (CH_2), 122.17 (2CH), 141.31 (triazole C-3), 144.54 (C), 150.20 (2CH), 151.20 (triazole C-5), 152.18 (N=CH), 168.33 (C=O). EI MS *m/z* (%): 312.24 (25), 290.22 ($[M]^+$, 10), 105.00 (100).

Elemental analysis for $C_{13}H_{15}N_5O_3$, Calculated (%), C: 53.97; H: 5.23; N: 24.21.

Found (%), C: 54.24; H: 5.16; N: 24.48.

*2-(3-Methyl-5-oxo-4-[(pyridin-4-ylmethylene)amino]-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetohydrazide (**20**)* Hydrazine hydrate (25 mmol) was added to the solution of compound **19** (10 mmol) in absolute ethanol, and the mixture was reflux for 10 h. On cooling the reaction mixture to room temperature, a white solid appeared. The crude product was filtered off and recrystallized from ethanol to give the desired compound. Yield 43 %, mp 207–208 °C. IR (KBr, υ, cm^{-1}): 3300 and 3263 (NH_2 + NH), 1684 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.03 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 6.79 (brs, 2H, NH_2 exch. D_2O), 7.03 (s, 1H, ArH), 7.15 (brs, 3H, ArH), 7.56 (t, 1H, NH, exch. D_2O, $J = 16.0$ Hz), 8.07 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 8.76 (CH_3), 49.65 (CH_2), 111.58 (2CH), 141.14 (triazole C-3), 145.84 (C), 148.54 (2CH), 151.36 (triazole C-5), 162.85 (N=CH), 164.78 (C=O). EI MS *m/z* (%): 316.26 ($[M + 1 + K]^+$, 18), 228 (14), 148.96 (77), 123.93 (98), 113.93 (68), 105.00 (100).

Elemental analysis for $C_{11}H_{12}N_7O_2$, Calculated (%), C: 48.00; H: 4.76; N: 35.62.

Found (%), C: 48.28; H: 4.53; N: 35.61.

*General method for the synthesis of compounds **21a–c***

The mixture of compound **20** (10 mmol) and the suitable aldehyde in absolute ethanol was refluxed (10 mmol) for 4 h. Then, the reaction content was allowed to reach to room temperature, and a solid appeared. This crude product was filtered off and recrystallized from acetone to obtain the desired compound.

*N′-[(2-Hydroxyphenyl)methylene]-2-(3-methyl-5-oxo-4-[(pyridin-4-ylmethylene)amino]-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetohydrazide (**21a**)* Yield 43 %, mp 235–236 °C. IR (KBr, υ, cm^{-1}): 1701 and 1689 (2C=O), 1586 and 1583 (2C=N), 1250 (C–O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.35 (s, 3H, CH_3), 4.53 (s, 1H, OH D_2O exch.), 4.88 (s, 2H, CH_2), 7.32 (brs, 4H, ArH), 8.31 (s, 1H, NH D_2O exch.) 8.92 (s, 1H, N=CH), 9.93 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.51 (CH_3), 47.03 (CH_2), 122.43 (2CH), 112.21 (2CH), 112.79 (CH), 124.65 (2CH), 127.31 (CH), 142.21 (C), 144.15 (triazole C-3), 144.41 (C), 145.17 (N=CH), 147.39 (triazole C-5), 150.15 (C), 150.36 (C=O), 163.08 (N=CH), 167.87 (C=O). EI MS *m/z* (%): 380.35 ($[M + 1]^+$, 10), 263.15 (55), 241.98 (13), 148.86 (100), 124.00 (90).

Elemental analysis for $C_{18}H_{17}N_7O_3$, Calculated (%), C: 56.99; H: 4.52; N: 25.84.

Found (%), C: 57.17; H: 4.41; N: 25.62.

*N′-[(4-methoxyphenyl)methylene]-2-(3-methyl-5-oxo-4-[(pyridin-4-ylmethylene)amino]-4,5-dihydro-1H-1,2,4-triazol-1-yl) acetohydrazide (**21b**)* Yield 57 %, mp 224–225 °C. IR (KBr, υ, cm^{-1}): 3329 (NH), 3106–3040 (ArCH), 2977 (aliphatic-CH), 1716, 1696 (2C=O), 1416, 1477 (N=CH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.35 (s, 3H, CH_3), 3.85 (brs, 3H, OCH_3), 4.76 (s, 2H, CH_2), 6.97 (d, 2H, ArH, $J = 7.8$ Hz), 7.63 (d, 2H, ArH, $J = 8.2$ Hz), 7.91 (s, 3H, ArH), 8.12 (s, 1H, ArH), 8.72 (s, 1H, N=CH), 11.51 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.54 (CH_3), 47.12 (CH_2), 56.20 (OCH_3), 122.44 (2CH), 112.21 (2CH), 112.83 (CH), 124.65 (2CH), 127.26 (CH), 141.21 (C), 144.10 (triazole C-3), 144.10 (N=CH), 146.65 (triazole C-5), 151.42 (C), 151.46 (C=O), 162.18 (N=CH), 167.17 (C=O). EI MS *m/z* (%): 441.31 (13), 395.25 ($[M + 2]^+$, 18), 394.32 ($[M + 1]^+$, 24), 379.30 (46), 376.23 ($[M + 1 - H_2O]^+$, 10), 343.26 (84), 327.24 (69), 324.23 (100).

Elemental analysis for $C_{19}H_{19}N_7O_3$, Calculated (%), C: 58.01; H: 4.87; N: 24.92.

Found (%), C: 58.13; H: 4.89; N: 24.77.

*N′-[(3-Hydroxy-4-methoxyphenyl)methylene]-2-(3-methyl-5-oxo-4-[(pyridin-4-ylmethylene)amino]-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetohydrazide (**21c**)* Yield 57 %, mp 221–222 °C. IR (KBr, υ, cm^{-1}): 3217 (OH), 3095 (ArCH), 2982 (aliphatic-CH), 1722, 1684 (2C=O), 1411, 1478 (N=CH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.34 (s, 3H, CH_3), 3.79 (brs, 3H, OCH_3), 4.86 (s, 2H, CH_2), 6.89–6.99 (m, 3H, ArH), 7.17 (s, 1H, ArH), 7.84 (s, 1H, ArH), 8.30 (d, 1H, N=CH, $J = 6.2$ Hz), 8.94 (d, 2H, ArH, $J = 4.8$ Hz), 9.90 (s, 1H, NH D_2O exch.), 11.56 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.53 (CH_3), 47.05 (CH_2), 56.24 (OCH_3), 122.41 (2CH), 112.98 (2CH), 112.79 (CH), 124.65 (2CH), 137.31 (C), 144.15 (triazole C-3), 144.41 (C), 145.17 (N=CH), 147.39 (triazole C-5), 150.15 (C), 150.36 (C=O), 163.08 (N=CH), 167.87 (C=O). EI MS *m/z* (%): 411.32 ($[M + 2]^+$, 13), 410.32 ($[M + 1]^+$, 58), 306.20 (13), 214.91 (12), 167.05 (13).

Elemental analysis for $C_{19}H_{19}N_7O_4$, Calculated (%), C: 55.74; H: 4.68; N: 23.95.

Found (%), C: 55.67; H: 4.74; N: 24.12.

*2-[(3-Methyl-5-oxo-4-[(pyridin-4-ylmethylene)amino]-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl]-N-phenylhydrazine carbothioamide (**22**)* The mixture of compound **20** (10 mmol) and phenylisothiocyanate (10 mmol) in absolute ethanol was refluxed for 10 h. On cooling the reaction content to room temperature, a white solid formed. This crude product was filtered and recrystallized from ethanol to afford the desired compound. Yield 85 %, mp 211–212 °C. IR (KBr, υ, cm^{-1}): 3366 (2NH), 3323 (NH), 1682 and 1695 (C=O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.33 (s, 3H, CH_3), 4.54 (s, 2H, CH_2), 7.18 (s, 1H, ArH), 7.39 (brs, 4H, ArH), 7.39 (s, 2H, ArH), 8.72 (s, 2H, ArH), 9.74 (brs, 2H, NH exch. D_2O), 9.79 (s, 1H, NH exch. D_2O), 10.36 (s, 1H, N=CH). ^{13}C NMR (DMSO-d_6, δ ppm): 11.63 (CH_3), 47.03 (CH_2), 122.17 (CH), 126.10 (CH), 126.75 (CH), 128.88 (2CH), 139.65 (C), 141.19 (triazole C-3), 144.40 (C), 150.48 (3CH), 151.24 (triazole C-5), 151.96 (2CH), 166.93 (C=O), 181.43 (C=S). EI MS *m/z* (%): 412.43 ($[M + 2]^+$, 56), 392.04 ($[M - H_2O]^+$, 22), 327.41 (76), 119.32 (100).

Elemental analysis for $C_{18}H_{18}N_8O_2S$, Calculated (%), C: 52.67; H: 4.42; N: 27.30.

Found (%), C: 52.89; H: 4.26; N: 27.41.

*2-[(5-Mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)methyl]-5-methyl-4-[(pyridin-4-ylmethylene)amino]-2,4-dihydro-3H-1,2,4-triazol-3-one (**23**)* A solution of compound **22** (10 mmol) in ethanol:water (1:1) was refluxed in the presence of 2 N NaOH for 3 h, then, the resulting solution was cooled to room temperature and acidified to pH 4 with 37 % HCl. The precipitate formed was filtered off, washed with water and recrystallized from acetone to afford the desired compound. Yield 45 %, mp 201–202 °C. IR (KBr, υ, cm^{-1}): 2812 (SH), 1689 (C=O), 1604 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.22 (s, 3H, CH_3), 4.92 (s, 2H, CH_2), 7.30 (s, 1H, ArH), 7.41 (brs, 4H, ArH), 7.76 (s, 2H, ArH), 8.72 (s, 2H, ArH), 9.40 (s, 1H, N=CH), 13.55 (s, 1H, SH, D_2O exch.). ^{13}C NMR (DMSO-d_6, δ ppm): 11.42 (CH_3), 78.87 (CH_2), 122.02 (2CH), 128.16 (2CH), 129.81 (CH), 129.96 (2CH), 133.53 (C), 141.04 (triazole C-3), 143.71 (CH), 144.68 (triazole C-5), 147.86 (C), 149.00 (C=O), 151.08 (2CH), 151.64 (N=CH), 169.29 (C=S). EI MS *m/z* (%): 415.33 (27), 393.30 ($[M + 1]^+$, 90), 190.89 (27).

Elemental analysis for $C_{18}H_{16}N_8OS$, Calculated (%), C: 55.09; H: 4.11; N: 28.55.

Found (%), C: 55.22; H: 4.08; N: 28.38.

*N′-[5-(4-Chlorophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]-2-(3-methyl-5-oxo-4-[(pyridin-4-ylmethylene)amino]-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetohydrazide (**24**)* 4-Chlorophenacyl bromide (10 mmol) was added to the solution of compound **22** in absolute ethanol, and the reaction mixture was refluxed in the presence of dried sodium acetate (16.4 g, 200 mmol) for 14 h. Then, the mixture was cooled to room temperature, poured into ice-cold water while stirring and left overnight in cold. The formed solid was filtered, washed with water three times and recrystallized from acetone to afford compound **24**. Yield 71 %, mp 181–182 °C. IR (KBr, υ, cm^{-1}): 1743 and 1713 (2C=O), 1581 (C=N), 1215 (C–O). ^{1}H NMR (DMSO-d_6, δ ppm): 2.09 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 6.98 (s, 1H, ArH), 7.34 (brs, 4H, ArH), 7.53 (brs, 5H, ArH), 7.81 (s, 2H, ArH), 8.73 (s, 2H, ArH), 9.74 (s, 1H, N=CH), 10.61 (s, 1H, NH exch. D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 11.69 (CH_3), 46.23 (CH_2), 120.17 (2CH), 121.34 (C), 124.15 (CH), 126.45 (2CH), 127.23 (CH), 128.88 (2CH), 130.21 (2CH), 133.54 (C), 139.65 (2C), 140.43 (triazole C-3), 145.48 (C), 149.46 (2CH), 151.19 (triazole C-5), 158.64 (N=CH), 161.96 (N=CH), 168.93 (C=O). EI MS *m/z* (%): 584.59 ($[M + K]^+$, 10), 567.54 ($[M + Na]^+$, 11), 545.83 ($[M]^+$, 10), 399.52 (26), 385.55 (26), 301.48 (100), 217.21 (68).

Elemental analysis for $C_{26}H_{21}ClN_8O_2S$, Calculated (%), C: 57.30; H: 3.88; N: 20.58.

Found (%), C: 57.54; H: 3.93; N: 20.49.

Antimicrobial activity

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC 35218, *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*) ATCC 911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 43288, *Enterococcus faecalis* (*E. faecalis*) ATCC 29212, *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Bacillus cereus* (*B. cereus*) 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC 607, *Candida albicans* (*C. albicans*) ATCC 60193 and *Saccharomyces cerevisiae* (*S. cerevisiae*) RSKK 251. All the newly synthesized compounds were weighed and dissolved in hexane to prepare extract stock solution of 20.000 microgram/millilitre (μg/mL).

The antimicrobial effects of the substances were tested quantitatively in respective broth media using double microdilution and the MIC values (μg/mL) were determined (Willanova, 1993). The antibacterial and antifungal assays were performed in Mueller–Hinton broth (Difco, Detroit, MI) at pH 7.3 and buffered yeast nitrogen base (Difco, Detroit, MI) at pH 7.0, respectively. The microdilution test plates were incubated for 18–24 h at 35 °C. Brain heart infusion broth (BHI; Difco, Detriot, MI) was used for Ms, and incubated for 48–72 h at 35 °C (Woods *et al.*, 2003). Ampicillin (10 μg) and fluconazole (5 μg) were used as standard antibacterial and antifungal drugs, respectively. DMSO with dilution of 1:10 was used as solvent control. The results are presented in Table 1.

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