European Journal of Medicinal Chemistry 44 (2009) 2896–2903

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis of some new 1,3,4-thiadiazol-2-ylmethyl-1,2,4-triazole derivatives and investigation of their antimicrobial activities

Ahmet Demirbas [a,*], Deniz Sahin [a], Neslihan Demirbas [a], Sengül Alpay Karaoglu [b]

[a] Department of Chemistry, Karadeniz Technical University, 61080 Trabzon, Turkey
[b] Department of Biology, Rize University, 53100 Rize, Turkey

ARTICLE INFO

Article history:
Received 3 July 2008
Received in revised form
10 November 2008
Accepted 8 December 2008
Available online 16 December 2008

Keywords:
1,2,4-Triazole
1,3,4-Thiadiazole
Mannich base
Schiff base
Antimicrobial activity

ABSTRACT

4-Amino-2-[(5-arylamino-4,5-dihydro-1,3,4-thiadiazol-2-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**3a–c**) were obtained in acidic media via the formation of 2-[(4-amino-3-aryl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)acetyl]-*N*-arylhydrazinecarbothioamides (**2a–c**), and then, compound **3b** was converted to methylated derivative, **4**. The basic treatment of carbothioamide derivatives, **2a–c**, afforded 4-amino-2-[(4-aryl-5-sulphanyl-4*H*-1,2,4-triazol-3-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**5a–c**). The alkylation reactions of compounds 4*H*-1,2,4-triazol-3-ylmethyl-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one derivatives (**5a–c**) were performed by using methyl iodide or ethyl bromide in the presence of sodium ethoxide, while the treatment of the same intermediates, **5a–c**, with aromatic aldehydes produced 2-{[4-(4-aryl)-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl}-4-(arylmethylene)amino-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**8a–d**). The synthesis of 4-amino-(or arylideneamino)-5-(4-methylphenyl)-2-{[(4-methylpiperazin-1-yl or morpholin-4-ylethyl)methyl]-4-aryl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl}methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**7a**, **b** and **9**) was performed by a one pot three-component Mannich reaction involving the corresponding compounds, 4-(substituted)amino-4*H*-1,2,4-triazol-3-ylmethyl-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one derivatives **5a**, **b** and **8a**, methylpiperazine or 2-(4-morpholino)ethylamine and formaldehyde.
The newly synthesized compounds were well characterized by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. They were also screened for their microbial activities. The antimicrobial activity study revealed that some of which **2a**, **c**, **3c**, **5a–c**, **8a–d** showed good activity against a variety of microorganisms.

1. Introduction

In the past decades, the problem of multi-drug resistant microorganisms has reached on alarming level around the world. For the treatment of microbial infections, the synthesis of new anti-infectious compounds has become an urgent need. For this purpose, several compounds that contain a piperazine or morpholine nucleus possessing antimicrobial activity have been synthesized; some of which contains an azole ring as well [1–6]. For instance, while eperezolid, which are the members of oxazolidinone class antibiotics, consist of morpholine and oxazolidinone rings linked each other via a fluorophenilene linkage, another antibiotic, Linezolid, contains a piperazine ring instead of morpholine [7,8]. On the other hand, Itraconazole, posaconazole and ketoconazole that are being used for the treatment of fungal infections, contains a piperazine and one or more azole ring in their structures [9] (Fig. 1, Chart 1).

The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents. Ribavirin (antiviral), Rizatriptan (antimigraine), Alprazolam (anxiolytic), Vorozole, Letrozole and Anastrozole (antitumoral) are some examples of drugs containing 1,2,4-triazole moiety [10–14] (Chart 2).

1,2,4-Thiadiazole derivatives are another important class of heterocycles due to their biological activities. Although the only commercially available 1,2,4-thiadiazole drug is the antibiotic cefozopram (Chart 3), there are a number of thiadiazole derivatives possessing a broad range of biological activities. For instance, KC 12291 has been found to possess a cardioprotective activity, while another thiadiazole derivative, SCH-202676, has been reported as allosteric modulator of G-protein coupled receptors [15]. Other thiadiazolidinones (TDZD) have been described as non-ATP competitive glycogen synthase kinase 3ß inhibitors [15] (Chart 3).

Multicomponent reactions are a major part of synthetic organic chemistry with advantages ranging from lower reaction times and

* Corresponding author. Tel.: +90 462 3772600; fax: +90 4623253196.
E-mail address: ndemirbas651@mynet.com (A. Demirbas).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.12.005

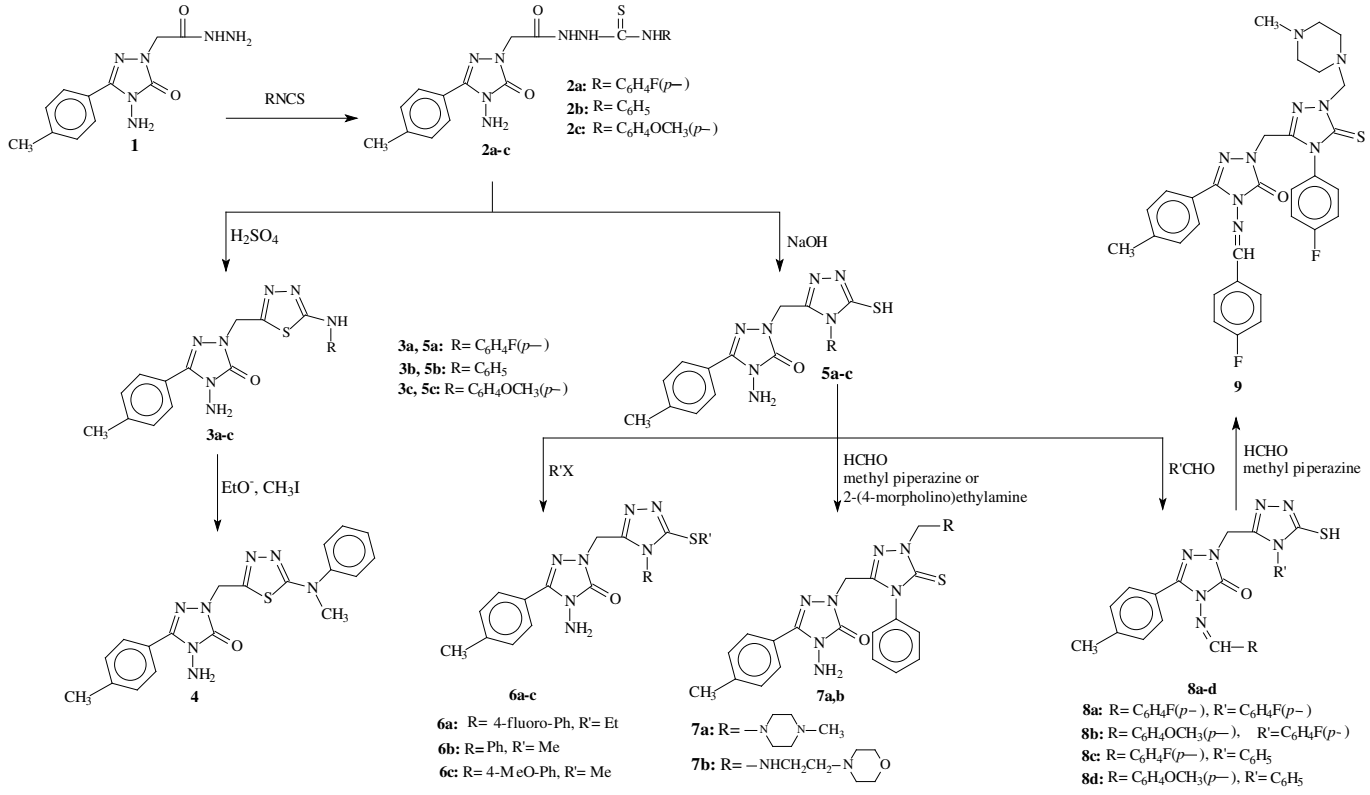
RNCS
2a: R= C6H4F(p–)
2b: R= C6H5
2c: R= C6H4OCH3(p–)
H2SO4
NaOH
3a, 5a: R= C6H4F(p–)
3b, 5b: R= C6H5
3c, 5c: R= C6H4OCH3(p–)
EtO-, CH3I
R'X
HCHO
methyl piperazine or
2-(4-morpholino)ethylamine
R'CHO
HCHO
methyl piperazine
6a: R= 4-fluoro-Ph, R'= Et
6b: R= Ph, R'= Me
6c: R= 4-MeO-Ph, R'= Me
7a: R= –N N–CH3
7b: R= –NHCH2CH2–N O
8a: R= C6H4F(p–), R'= C6H4F(p–)
8b: R= C6H4OCH3(p–), R'=C6H4F(p–)
8c: R= C6H4F(p–), R'= C6H5
8d: R= C6H4OCH3(p–), R'= C6H5

Fig. 1.

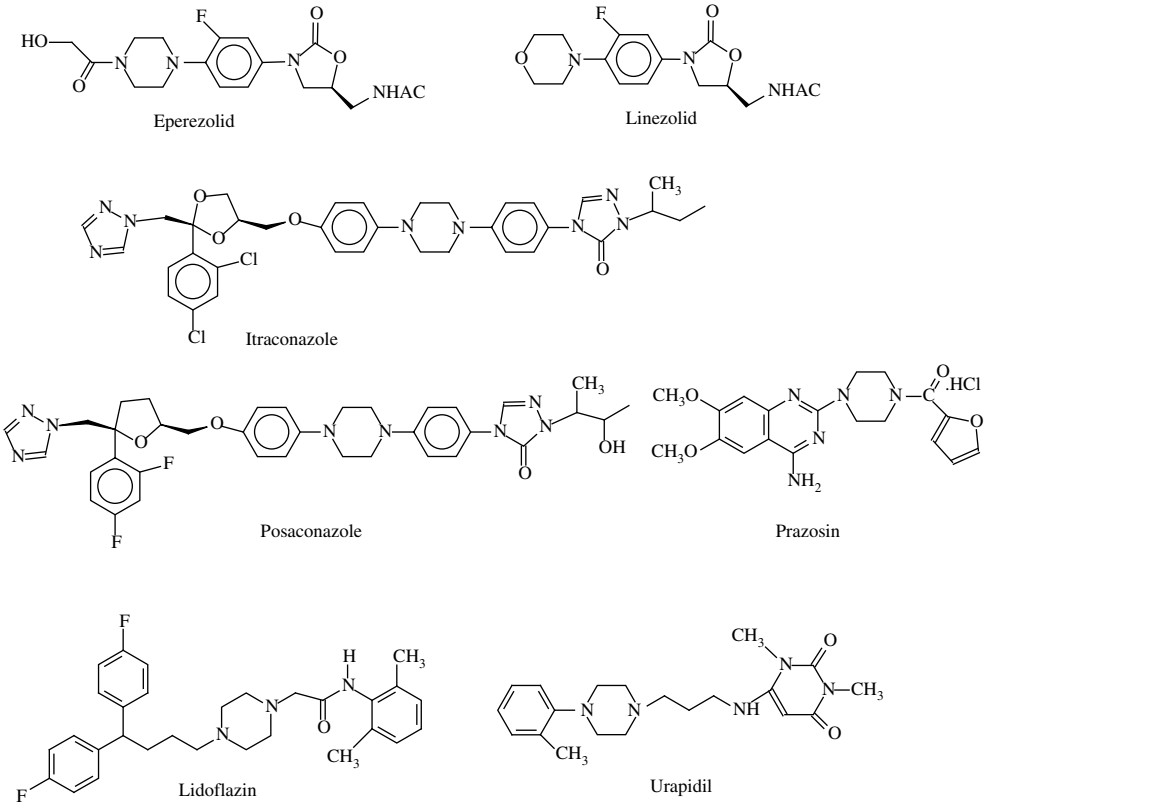
Eperezolid
Linezolid
Itraconazole
Posaconazole
Prazosin
Lidoflazin
Urapidil

Chart 1.

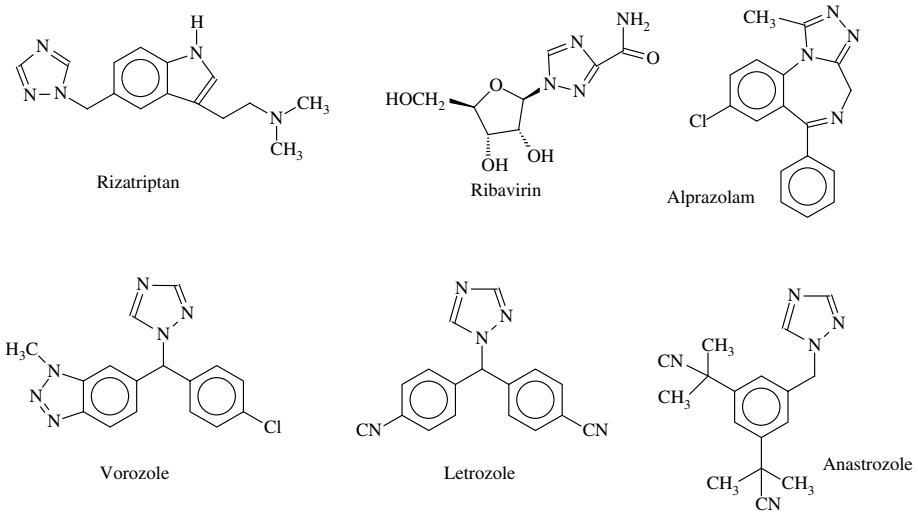

Chart 2.

temperatures to higher yields. Mannich reactions are three-component condensation reactions involving carbonyl compounds, which exist as enol–keto tautomeric forms, formaldehyde and a primary or secondary amine. The amino alkylation of aromatic compounds by Mannich reaction has been reported to have a considerably importance for designing efficient bioactive molecules [16]. A number of Mannich base has been reported as antitubercular, antimalarial, vasorelaxing, anticancer and analgesic drugs [16–19]. Mannich bases of 1,2,4-triazole derivatives containing *N*-methylpiperazine or morpholine moiety have been reported to possess antibacterial activity. Prazosin [20], Lidoflazine [21] and Urapidil [22], that are efficient cardiovascular drugs used in current treatment, are containing a piperazine nucleus in their structures (Chart 1). Moreover, organic compounds including a fluorine atom in their structures have found application in the pharmaceutical field owing to their diverse biological properties [23].

In recent years, various antitumor drugs have been developed for the treatment of cancer. Among these, we prepared some 1,2,4 triazole derivatives incorporating Schiff base structure as antitumor agents. [24–27]. However, cancer is still a major health problem because of the insufficiency of the conventional methods. In addition, some 1,2,4-triazole derivatives have been found to possess antimicrobial activity [27–32].

As a continuation of our studies on obtaining bioactive molecules, we have herein synthesized some 1,3,4-thiadiazolyl-1,2,4-triazoles and Mannich bases of 1,2,4-triazolyl-1,2,4-triazole derivatives containing piperazine or morpholine ring beside schiff bases, some of which contains fluorophenyl moiety in their structures.

2. Chemistry

2-{[4-Amino-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]acetyl}-*N*-arylhydrazinecarbothioamides (**2a–c**) were obtained from the reaction of compound 2-[4-amino-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]acetohydrazide (**1**) with 4-fluorophenyl-(for compound **2a**), phenyl-(for **2b**) or 4-methoxyphenyl (for **2c**) isothiocyanate. The cyclization of compounds **2a–c** in the presence of sodium hydroxide resulted in the formation of **5a–c**. The alkylations of compounds **5a** and **5b** were performed by the reaction with methyl iodide or ethyl bromide in basic media, thus compounds **6a** and **6b** were obtained. On the other hand, the reactions of the same precursors **5a**, **5b** with methyl piperazine or 2-(4-morpholino)ethylamine in the presence of formaldehyde solution afforded the corresponding Mannich base derivatives **7a**, **b**. The treatment of 4-amino-2-([4-aryl-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl)-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**5a–c**) with 4-fluoro- or 4-methoxy

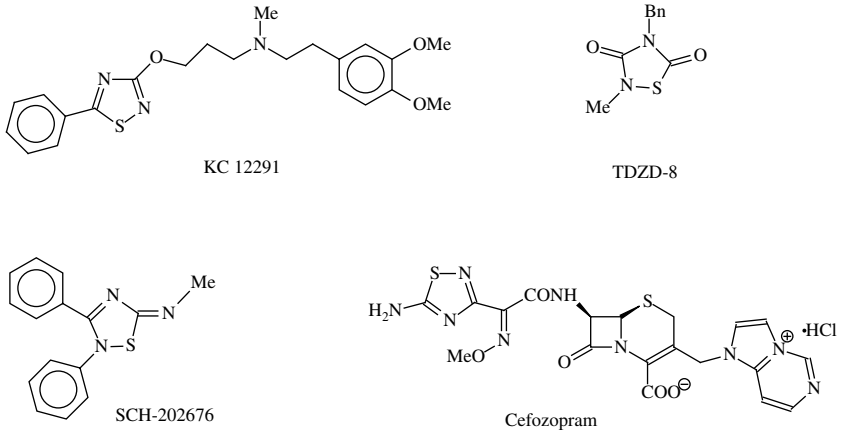

Chart 3.

benzaldehyde produced 2-([4-aryl-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl)-4-arylmethyleneamino-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**8a–d**). The treatment of compound **8a** with methyl piperazine in the presence of formaldehyde resulted in the formation of compound **9**.

Compounds **3a–c** were prepared by the treatment of carbothioamide derivatives **2a–c** with concentrated sulfuric acid. Then, compound **3b** was converted to its methylated derivative, **4** by treatment with methyl iodide in the presence of sodium ethoxide.

The structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data (except **3b**, **6c**, **7b**, **8b** and **9**), and elemental analyses.

3. Antimicrobial activity

3.1. Antimicrobial activity assessment

All bacterial and yeast strain were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 709 ROMA, *Candida tropicalis* ATCC 13803 and *Candida albicans* ATCC 60193. All the newly synthesized compounds were dissolved in dimethylsulphoxide (DMSO) and ethanol to prepare chemicals stock solution of 10 mg/mL.

3.1.1. Agar-well diffusion method

Simple susceptibility screening test using agar-well diffusion method [34] as adapted earlier [35] was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately 10^6 colony forming units (cfu) per mL. They were "flood-inoculated" onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI) and then dried. For *C. albicans* and *C. tropicalis*, SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 μl of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 μg) and Fluconazole (5 μg) were standard drugs. Dimethylsulphoxide was used as solved control. The antimicrobial activity results were summarized in Table 1.

Table 1
Screening for antimicrobial activity of the compounds (mm).

Comp. No.	E. coli	Y. ps.	P. aur.	E. fec.	S. au.	B. cer.	C. trop.	C. alb
2a	34	28	35	30	30	22	–	–
2b	–	–	–	–	6	–	–	–
2c	24	25	35	28	28	22	–	–
3a	–	–	–	–	–	–	–	–
3b	–	–	–	–	–	–	–	–
3c	30	22	>30	20	28	23	–	–
4	–	–	–	–	–	7	6	–
5a	30	26	35	30	26	25	–	–
5b	25	22	30	20	24	20	–	–
5c	28	22	30	25	30	25	–	–
6a	–	–	–	–	8	9	6	6
6b	–	–	–	–	–	–	–	–
6c	–	–	–	–	–	–	–	–
7a	–	–	–	–	–	–	–	–
7b	–	–	–	–	–	–	–	–
8a	25	22	30	20	24	20	–	–
8b	30	30	35	24	26	22	–	–
8c	25	25	30	20	28	20	–	–
8d	28	25	30	25	30	25	–	–
9	–	–	–	–	–	8	–	–
DMSO	–	–	–	–	–	–	–	–
Amp.	10	18	18	10	35	15		
Flu.							25	25

Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 27853, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bc: *Bacillus cereus* 702 Roma, Ct: *Candida tropicalis* ATCC 13803, Ca: *Candida albicans* ATCC 60193. Amp.: Ampicillin, Flu.: Fluconazole, (–): no activity, solvent is DMSO.

4. Results and discussion

It is known that the $-NH_2$ group on 4,5-dihydro-1*H*-1,2,4-triazol-5-ones resonate between 5.20 and 5.50 ppm in the ^{1}H NMR spectrum [5,8,11–16]. As expected, the ^{1}H NMR spectra of compounds **2a–c** displayed a signal belonging to $-NH_2$ group at 5.54–5.57 ppm. Moreover, three –NH– signals recorded between 9.62 and 10.38 ppm (controlled by changed with D_2O) in the ^{1}H NMR spectra of compounds **2a–c**. Moreover, the stretching bands derived from NH + NH_2 groups were seen in the FT-IR spectra of compounds **2a–c**. The elemental analyses data of these compounds are consistent with the assigned structures. A stable molecular ion peak was seen for **2b**, while compounds **2a** and **2c** gave peaks derived from M + 2 (for **2a**) or M + K (for **2c**) in the Mass spectra. Among compounds **2a–c**, **2a** and **2c**, which are containing a 4-substituted phenyl ring in the carbothioamide structure, displayed moderate activity against the test microorganisms except *C. tropicalis* and *C. albicans*.

The signal observed at 13.91–14 ppm in the ^{1}H NMR spectra of compounds **5a–c** was attributed to –SH group. This group was observed at 2760–2768 cm^{-1} in the FT-IR spectra of compounds **5a–c**. The elemental analyses data are consistent with the assigned structures of compounds **5a–c**. Furthermore, **5a–c** gave stable molecular ion peak (**5a** and **5c**) or M + Na peak (for **5b**) in the mass spectra. Compounds **5a–c** displayed moderate activity towards the microbial strain used in this study except *C. tropicalis* and *C. albicans*. It is known that sulphanyl triazoles exist as thioxo-sulphanyl tautomeric forms. This sulphanyl group in type **5** compounds is acidic enough for further reactions; for example, alkylation reactions could be achieved [33]. The reaction of compound **5a–c** with methyl iodide (for compounds **6b** and **6c**) or ethyl bromide (for compound **6a**) in the presence of sodium hydroxide produced 4-amino-2-{[5-(alkylthio)-4-aryl-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]methyl}-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**6a–c**). In the ^{1}H NMR spectra of compounds **6b** and **6c** there is no peak derived from –NH– or –SH group. Instead, new signals due to methyl group appeared at 2.53 ppm, while ethyl substituent of compound **6a** resonated at 1.28 (CH_3CH_2) and 3.08 ppm (CH_3CH_2). In addition, the FT-IR spectra of compounds **6a** and **6b** do not contain a peak due to –NH– or –SH group. M^+ (for **6a**) or M + 1 (for **6b**) peak exists in the mass spectra of compound **6a**, **b**. Furthermore, compounds **6a**, **b** gave good elemental analysis results.

The Mannich base derivatives of compound **5b** were obtained in relatively mild conditions. The ^{1}H and ^{13}C NMR spectra of compounds **7a**, **b** displayed additional signals originated from methyl piperazine or morpholinoethylamine residue and methylene linkage, while the –NH– signal belonging to 5-thioxo-1,2,4-triazole ring was disappeared. Due to less solubility in any NMR solvent, ^{13}C NMR spectra of compounds **7a** and **7b** were not recorded. A peak representing a stable molecular ion was present in the mass spectra of **7a**. Moreover, the elemental analysis data of **7a**, **b** are consistent with their structures.

In the ^{1}H NMR spectra of compounds **8a–d**, the $-NH_2$ signal disappeared, while additional signals belonging to aldehyde residue appeared at aromatic region. The ^{1}H NMR spectra of compound **9** displayed new peaks owing to methylene linkage and methyl piperazine moiety at 5.14 and 2.31–2.50 ppm. Due to less solubility in any deuterated solvent, a satisfactory ^{13}C NMR spectrum was not taken for compound **9**. Compounds **8a–d** were found to possess moderate activity against the bacterial strain used in the study except *C. tropicalis* and *C. albicans*.

The acidic treatment of compounds **2a–c** afforded 4-amino-2-({5-[(4-fluorophenyl)amino]-1,3,4-thiadiazol-2-yl}methyl)-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (**3a–c**). Compound **3b** was ethylated by using ethyl bromide in the presence of sodium hydride. Compounds **3a–c** and **4** gave satisfactory spectroscopic and elemental analysis data. Among compounds **3a–c**, antimicrobial activity was showed for **3c**.

5. Conclusion

This study reports the successful synthesis of some new 5-sulphanyl-4*H*-1,2,4-triazol-2-ylmethyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ones, 1,3,4-thiadiazol-2-ylmethyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ones, and conversion some of them into the corresponding Shiff and Mannich bases and alkylated derivatives. 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. The presence of *N*-methylpiperazine or morpholine moiety is also instrumental in contributing to the net biological activity of a system. Also we already reported antimicrobial activities of some biheterocyclic compounds incorporating 1,2,4-triazole and 1,3,4-thiadiazole rings, in addition to some alkylated derivatives of 1,2,4-triazole compounds. Hence herein we combined all these three potential units, namely 1,2,4-triazole and 1,3,4-thiadiazole nucleus (or second 1,2,4-triazole moiety), methyl piperazine/morpholine ring. The antimicrobial screening suggests that among the newly synthesized compounds, **2a–c**, **3c**, **5a–c** and **8a–d** exhibited moderate activity against all the tested microorganisms except *C. tropicalis* and *C. albicans*. On the contrary to expected, the introduction of third heterocyclic ring (methyl piperazine or morpholine) to the structure of compounds **5a–c** by a Mannich reaction caused no antimicrobial activity. But, it might be speculated that these results were obtained probably due to slight solubility of compounds **7a**, **b** and **9** in known organic solvents.

6. Experimental

6.1. Chemistry

Melting points were determined on a Büchi B-540 melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer. The IR spectra were measured as potassium bromide pellets using a Perkin–Elmer 1600 series FT-IR spectrometer. Mass spectra were obtained at a Quattro LC-MS (ESI, 70 eV) Instrument (except compounds **3b**, **6c**, **7b**, **8b** and **9**). Combustion analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compound **1** was prepared by the way reported earlier [27].

6.1.1. General method for the synthesis of compounds *2a–c*

A mixture of compound **1** (10 mmol) and 4-fluorophenyl isothiocyanate (1.53 g, 10 mmol) (for compound **2a**), phenyl isothiocyanate (1.35 g, 10 mmol) (for compound **2b**) or 4-methoxyphenyl isocyanate (1.65 g, 10 mmol) (for compound **2c**) was refluxed in ethanol for 3 h. Then, the solution was cooled to room temperature and a white solid appeared. This was filtered and recrystallized from ethanol to afford the desired compound.

6.1.2. 2-{[4-Amino-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-N-(4-fluorophenyl)hydrazinecarbothioamide (*2a*)

Yield 96%, m.p. 224–225 °C. Anal. Calcd. (%) for: $C_{18}H_{18}FN_7O_2S$: C, 52.04; H, 4.37, N, 23.60. Found; C, 52.12; H, 4.38; N, 23.57; IR (KBr, ν, cm^{-1}): 3335, 3274 and 3159 ($NH_2 + 3NH$), 1693 (C=O), 1611 (C=N), 1191 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.35 (3H, s, CH_3), 4.60 (2H, s, CH_2), 5.54 (2H, s, NH_2), 7.13–7.46 (6H, m, arH), 7.88 (2H, d, arH, $J = 8.0$ Hz), 9.68 (H, s, NH), 9.80 (H, s, NH), 10.36 (H, s, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 20.85 (CH_3), 46.82 (CH_2), arC: [114.45 (2CH), 114.88 (2CH), 123.64 (C), 127.32 (2CH), 128.79 (2CH), 135.19 (2C), 139.57 (C)], 144.84 (triazole C-3), 153.74 (triazole C-5), 166.30 (C=O), 180.97 (C=S); MS (ESI): *m*/*z* (%) 413.28 (M + 2) (16), 384.12 (18), 331.12 (19), 301.08 (16), 273.99 (21), 252.90 (25), 251.90 (33), 232.94 (37), 231.94 (27), 202.84 (54), 190.83 (44), 163.80 (30), 162.80 (94), 145.71 (88), 144.78 (50), 120.81 (100).

6.1.3. 2-{[4-Amino-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-N-phenylhydrazinecarbothioamide (*2b*)

Yield 76%, m.p. 190–191 °C. Anal. Calcd. (%) for: $C_{18}H_{19}N_7O_2S$: C, 54.39; H, 4.82, N, 24.67. Found; C, 54.47; H, 4.90; N, 24.53; IR (KBr, ν, cm^{-1}): 3311, 3210 ($3NH + NH_2$), 1685 and 1718 (2C=O), 1597 (C=N), 1202 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.36 (3H, s, CH_3), 4.61 (2H, s, CH_2), 5.57 (2H, s, NH_2), 7.18 (2H, t, arH, $J = 7.0$ Hz), 7.32 (3H, t, arH, $J = 8.4$ Hz), 7.39–7.45 (2H, m, arH), 7.89 (2H, d, arH, $J = 8.0$ Hz), 9.70 (H, s, NH), 9.79 (H, s, NH), 10.38 (H, s, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 21.03 (CH_3) 47.07 (CH_2), arC: [123.82 (C), 125.07 (CH), 127.44 (3CH), 127.96 (2CH), 128.75 (3CH), 138.97 (C), 139.53 (C)], 144.98 (triazole C-3), 153.89 (triazole C-5), 166.35 (C=O), 180.77 (C=S); MS (ESI): *m*/*z* (%) 398.06 (M^+, 26), 277.98 (15), 241.00 (14), 202.84 (44), 190.82 (15), 161.79 (23), 156.72 (100), 144.77 (59), 132.76 (17), 117.74 (21).

6.1.4. 2-{[4-Amino-3-(4-methylphenyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl}-N-(4-methoxyphenyl) hydrazinecarbothioamide (*2c*)

Yield 74%, m.p. 196–199 °C. Anal. Calcd. (%) for: $C_{19}H_{21}N_7O_3S$: C, 53.38; H, 4.95, N, 22.94. Found; C, 53.51; H, 5.03; N, 22.90; IR (KBr, ν, cm^{-1}): 3292, 3153 ($3NH + NH_2$), 1695 (2C=O), 1598 (C=N), 1188 (C–S); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.29 (3H, s, CH_3), 2.36 (3H, s, OCH_3), 4.61 (2H, s, CH_2), 5.56 (2H, s, NH_2), 7.14 (2H, d, arH, $J = 8.4$ Hz), 7.29 (4H, d, arH, $J = 8.8$ Hz), 7.89 (2H, d, arH, $J = 8.2$ Hz), 9.62 (H, s, NH), 9.72 (H, s, NH), 10.34 (H, s, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 20.47 (CH_3), 20.88 (OCH_3), 46.84 (CH_2); arC: [123.71 (C), 125.61 (CH), 127.34 (2CH), 128.49 (3CH), 128.82 (2CH), 134.35 (C), 136.30 (C), 139.60 (C)], 144.84 (triazole C-3), 153.76 (triazole C-5), 166.33 (C=O), 180.69 (C=S); MS (ESI): *m*/*z* (%) 466.11 (M + K, 9), 434.14 (16), 412.12 (14), 300.92 (11), 262.97 (11), 202.87 (20), 193.81 (79), 192.80 (100), 178.83 (56), 166.94 (18), 156.74 (38), 144.78 (19), 118.67 (68).

6.2. General method for the synthesis of compounds *3a–c*

A mixture of corresponding carbothioamide **2** (10 mmol) in cold concentrated sulfuric acid (28 mL) was stirred for 10 min then, the mixture was allowed to reach room temperature. After stirring for an additional 30 min, the resulting solution was poured into ice-cold water and made alkaline to pH 8 with ammonia. The precipitated product was filtered, washed with water and recrystallized from ethanol to afford pure compounds.

6.2.1. 4-Amino-2-({5-[(4-fluorophenyl)amino]-1,3,4-thiadiazol-2-yl}methyl)-5-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (*3a*)

Yield 79%, m.p. 234–235 °C. Anal. Calcd. (%) for: $C_{18}H_{16}FN_7OS$: C, 54.40; H, 4.06, N, 24.67. Found; C, 54.54; H, 4.12; N, 24.61; IR (KBr, ν, cm^{-1}): 3275 and 3156 ($NH + NH_2$), 1725 (C=O), 1619, 1550 and 1503 (3C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.23 (3H, s, CH_3), 5.14 (2H, s, CH_2), 5.56 (2H,s, NH_2), 7.18 (2H, t, arH, $J = 8.6$ Hz), 7.30 (2H, d, arH, $J = 8.0$ Hz), 7.62 (2H, q, arH, $J = 4.6$ Hz), 7.89 (2H, d, arH, $J = 8.0$ Hz) 10.37 (H, s, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.20

(CH_3), 45.42 (CH_2), arC: [116.80 (CH), 117.24 (CH),120.48 (CH), 124.88 (C), 128.83 (CH), 128.99 (2CH), 130.32 (2CH), 138.24 (C), 141.32 (2C)], 146.92 (triazole C-3), 154.46 (triazole C-5), 155.72 (thiadiazole C-2), 166.85 (thiadiazole C-5); MS (ESI): *m*/*z* (%) 420.08 (M + Na, 18), 394.18 (6), 335.05 (26), 316.96 (12), 294.06 (12), 293.00(41), 278.05 (14), 196.70 (35), 193.76 (84), 179.75 (30), 156.72(100), 148.71(16), 118.74 (54), 116.62 (16).

*6.2.2. 4-Amino-2-[(5-anilino-1,3,4-thiadiazol-2-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**3b**)*

Yield 98%, m.p. 212–213 °C. Anal. Calcd. (%) for: $C_{18}H_{17}N_7OS$: C, 56.98; H, 4.52; N, 25.84. Found; C, 57.18; H, 4.55; N, 24.73; IR (KBr, ν, cm^{-1}): 3317–3201 (NH_2), 1704 (C═O), 3137 (NH), 1602, 1574 and 1504 (3C═N); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.36 (3H, s, CH_3), 5.26 (2H, s, CH_2), 5.56 (2H, s, NH_2), 6.98 (1H, t, arH, $J = 7.2$ Hz), 7.21–7.36 (4H, m, arH), 7.59 (2H, d, arH, $J = 7.60$ Hz), 7.91 (2H, d, arH, $J = 8.4$ Hz), 10.36 (H, s, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 20.96 (CH_3), 44.06 (CH_2), arC: [117.33 (2CH), 121.84 (CH), 123.53 (C), 127.47 (2CH), 128.80 (2CH), 128.95 (2CH), 139.77 (C), 140.39 (C)], 153.07 (triazole C-3), 154.14 (triazole C-5, thiadiazole C-2), 165.39 (thiadiazole C-5).

*6.2.3. 4-Amino-2-({5-[(4-methoxyphenyl)amino]-1,3,4-thiadiazol-2-yl}methyl)-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**3c**)*

Yield: 81%, m.p. 231–232 °C. Anal. Calcd. (%) for: $C_{19}H_{19}N_7O_2S$: C, 55.73; H, 4.68; N, 23.95. Found; C, 55.86; H, 4.75; N, 23.88; IR (KBr, ν, cm^{-1}): 3329, 3267 (NH_2 + NH), 1713 (C═O), 1618, 1543 and 1501 (3C═N); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.22 (3H, s , CH_3), 2.33 (3H, s, OCH_3), 5.23 (2H, s, CH_2), 5.56 (2H, s, NH_2), 7.12 (2H, d, arH, $J = 8.4$ Hz), 7.28 (2H, d, arH, $J = 8.4$ Hz), 7.44 (2H, d, arH, $J = 8.4$ Hz), 7.87 (2H, d, arH, $J = 8.0$ Hz), 10.26 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 20.23 (CH_3), 20.87 (OCH_3), 44.01 (CH_2), arC: [117.44 (2CH), 123.44 (C), 127.42 (2CH), 128.83 (2CH), 129.37 (2CH), 130.87 (C), 137.95 (C), 139.82 (C)], 145.41 (triazole C-3), 152.99 (thiadiazole C-2), 153.82 (triazole C-5), 165.54 (thiadiazole C-5); MS (ESI): *m*/*z* (%) 407.21 (M^+, 26), 395.38 (16), 394.24 (49), 317.03 (33), 301.86 (20), 292.99 (36), 291.98(31), 279.21 (25), 277.95 (100), 270.96 (41), 264.86 (28), 240.82 (88), 214.95 (25), 203.81 (33). 193.81 (83), 178.77(53), 156.68 (39).

*6.3. General method for the synthesis of compounds **5a–c***

A solution of corresponding carbothioamide **2a–c** (10 mmol) in equivalent amount of 2 N NaOH solution was refluxed for 3 h. The resulting solution was cooled to room temperature and acidified to pH 3–4 with 37% HCl. The precipitate formed was filtered, washed with water and recrystallized from dimethyl sulfoxide/water (1:1) to afford the desired compound.

*6.3.1. 4-Amino-2-{[4-(4-fluorophenyl)-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl}-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**5a**)*

Yield 84%, m.p. 254–256 °C. Anal. Calcd. (%) for: $C_{18}H_{16}FN_7OS$: C, 54.40; H, 4.06; N, 24.67. Found; C, 54.57; H, 4.18; N, 24.62; IR (KBr, ν, cm^{-1}): 3316, 3145 (NH_2), 2763 (SH), 1682 (C═O), 1583, 1508 and 1505 (C═N), 1224 (C═S); ^{1}H NMR (DMSO-d_6) δ ppm: 2.35 (3H, s, CH_3), 4.95 (2H, s, CH_2), 5.36 (2H, s, NH_2), 7.26–7.43 (6H, m, arH), 7.75 (2H, d, arH, $J = 8.4$ Hz), 13.97 (H, s, SH); ^{13}C NMR (DMSO-d_6) δ ppm: 20.77 (CH_3), 38.09–40.69 (NCH_2 + DMSO-d_6), arC: [115.82 (CH), 116.29 (CH), 123.21 (C), 127.31 (2CH), 128.68 (2CH), 129.09 (C), 129.91 (CH), 130.09 (CH),139.63 (C), 145.00 (C)], 147.37 (triazole C-3), 152.50 (triazole C-3), 159.62 (triazole C-5), 164.52 (triazole C-3), 168.45 (triazole C-5); MS (ESI): *m*/*z* (%) 397.99 (M^+, 23), 394.30 (31), 293.06 (18), 277.86 (28), 271.91 (100), 270.91 (67), 269.91 (19), 253.83 (19), 242.88 (16), 240.88 (34); 194.83 (16), 193.83 (100), 192.83 (65), 178.81 (28), 156.72 (34).

*6.3.2. 4-Amino-2-[(5-sulphanyl-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl]-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**5b**)*

Yield 91%, m.p. 277–278 °C. Anal. Calcd. (%) for: $C_{18}H_{17}N_7OS$: C, 56.98; H, 4.52; N, 25.84 Found; C, 57.23; H, 4.55; N, 25.77; IR (KBr, ν, cm^{-1}): 3274 and 3199 (NH_2), 1702 (C═O), 2768 (SH), 1615, 1578 and 1495 (3C═N); Anal. Calcd. (%) for: $C_{18}H_{17}N_7OS$: C, 56.98; H, 4.52, N, 25.84. Found; C, 57.06; H, 4.56; N, 25.67; ^{1}H NMR (DMSO-d_6) δ ppm: 2.35 (3H, s, CH_3), 4.95 (2H, s, NCH_2), 5.318 (2H, s, NH_2), 7.26–7.34 (4H, m, arH), 7.43–7.50 (3H, m, arH), 7.74 (2H, d, arH, $J = 8.4$ Hz), 14.00 (s, SH); ^{13}C NMR (DMSO-d_6) δ ppm: 20.87 (CH_3), 38.09–40.70 (DMSO-d_6 + CH_2), arC: [123.30 (C), 132.79 (C), 139.69 (C), 127.56 (2CH), 127.41 (2CH), 128.77 (2CH), 129.42 (CH), 129.09 (2CH)], 145.057 (triazole C-3), 152.59 (triazole C-5), 147.50 (sulphanyl triazole C-2), 168.25 (sulphanyl triazole C-5); MS (ESI): *m*/*z* (%) 403.06 (M^+ Na, 32), 380.08 (M + 1), 277.96 (23), 240.90 (20), 190.89 (26), 181.64 (20), 156.72 (86), 148.67 (22), 134.70 (22), 118.78 (94), 103.62(100).

*6.3.3. 4-Amino-2-{[4-(4-methoxyphenyl)-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl}-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**5c**)*

Yield 92%, m.p. 268–269 °C. Anal. Calcd. (%) for: $C_{19}H_{19}N_7O_2S$: C, 55.73; H, 4.68; N, 23.95. Found; C, 55.79; H, 4.71; N, 23.88; IR (KBr, ν, cm^{-1}): 3275 and 3176 (NH_2), 2760 (SH), 1698 (C═O), 1629, 1568 and 1516 (3C═N); ^{1}H NMR (DMSO-d_6) δ ppm: 2.27(3H, s, CH_3), 2.33 (3H, s, OCH_3), 4.91 (2H, s, CH_2), 5.33 (2H, s, NH_2), 7.14–7.28 (6H, m, arH), 7.70 (2H, d, arH, $J = 8.4$ Hz), 13.91 (H, s, SH); ^{13}C NMR (DMSO-d_6) δ ppm: 20.63 (CH_3), 20.87 (CH_3), 38.03–40.94 (CH_2 + DMSO-d_6), arC: [123.34 (C), 127.37 (3CH), 128.74 (3CH), 129.59 (2CH), 130.26 (C), 139.06 (C), 139.64 (C)], 144.88 (triazole C-3), 147.53 (triazole C-3), 152.65 (triazole C-3), 168.35 (triazole C-5); MS (ESI): *m*/*z* (%) 409.25 (M^+, 41), 409.13 (100), 409.00 (32), 393.62 (19), 365.16 (17), 334.90 (19), 304.19(78), 241.85(22), 189.70 (19).

*6.4. General method for the synthesis of compounds **8a–d***

An equivalent amount of 4-fluorobenzaldehyde (for compounds **8a** and **8c**) or 4-methoxy benzaldehyde (for compounds **8b**, **8d**) was added to a solution of corresponding compounds **5a** and **5b** (10 mmol) (for compounds **8a–d**) in absolute ethanol, and the mixture was heated until a clear solution was obtained. Then, a few drops of concentrated sulfuric acid were added as a catalyst and the solution was allowed to reflux for 3–4 h. On cooling the reaction content to room temperature, a solid appeared. The product was filtered-off and recrystallized from dimethyl sulfoxide/water (1:1) (for **8a**, **8b**, **8d**) or ethanol (for **8c**) to yield the desired compound.

*6.4.1. 2-{[4-(4-Fluorophenyl)-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl}-4-{[(4-fluorophenyl)-methylene]amino}-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**8a**)*

Yield 90%, m.p. 240–242 °C. Anal. Calcd. (%) for: $C_{25}H_{19}F_2N_7OS$: C, 59.63; H, 3.80; N, 19.47 Found; C, 59.77; H, 3.92; N, 19.48; IR (KBr, ν, cm^{-1}): 2920 (SH), 3168 (NH), 1725 (C═O), 1602 (C═N), 1508 (C═N), 1223 (C═S); ^{1}H NMR (DMSO-d_6) δ ppm: 2.38 (3H, s, CH_3), 5.09 (2H, s, CH_2), 7.27–7.44 (6H, m, arH), 7.66 (2H, d, arH, $J = 8.0$ Hz), 7.83 (2H, dd, arH, $J = 8.6$ Hz), 7.87 (2H, dd, arH, $J = 5.4$ Hz), 9.35 (H, s, N═CH), 14.05 (H, s, SH); ^{13}C NMR (DMSO-d_6) δ ppm: 20.79 (CH_3), 38.08–40.60 (CH_2 + DMSO-d_6), 96.41 (N═CH), arC: [115.82 (CH), 116.34 (CH), 122.52 (C), 127.86 (CH), 128.98 (2CH), 129.10 (2CH), 129.42 (C), 129.72 (CH), 129.90 (CH) 130.11 (CH), 130.28 (CH), 140.25 (C), 143.93 (C), 147.22 (C), 148.51 (C), 155.48 (CH), 159.10 (triazole

C-3), 161.52 (triazole C-3), 164.55 (triazole C-5), 166.50 (triazole C-5); MS (ESI): *m/z* (%) 503.13 (M^+, 38), 502.38 (28), 442.68(60), 405.64(29), 400.14(66), 381.99(100), 364.72(25), 343.19(69), 309.59 (49), 309.40 (39), 276.12 (27), 222.18 (24), 182.88 (33), 167.05 (29), 126.88 (31), 112.43 (64).

*6.4.2. 2-{[4-(4-Methoxyphenyl)-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl}-4-{[(4-fluorophenyl)-methylene]amino}-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**8b**)*

Yield 87%, m.p. 237–239 °C. Anal. Calcd. (%) for: $C_{26}H_{22}F\,N_7OS$: C, 60.57; H, 4.30; N, 19.02. Found; C, 60.68; H, 4.33; N, 18.84; IR (KBr, *ν*, cm^{-1}): 3164 (NH), 1726 (C=O), 1599, 1508 (C=N), 1260 (C–O), 1222 (C=S); ^{1}H NMR: 2.39 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 5.04 (2H, s, NCH_2), 7.03 (2H, d, arH, $J = 8.8$ Hz), 7.27–7.37 (6H, m, arH) 7.69 (4H, t, arH, $J_1 = 8.0$ Hz, $J_2 = 8.8$ Hz), 9.19 (1H, s, N=CH), 14.00 (1H, s, SH); ^{13}C NMR (DMSO-d_6) *δ* ppm: 20.77 (CH_3), 20.93 (OCH_3), 44.27 (CH_2), 96.88 (N=CH), arC: [117.15 (CH), 119.41 (CH), 122.57 (C), 125.49 (CH), 128.31 (2CH), 129.11 (2CH), 129.76 (C), 129.82 (CH), 129.94 (CH) 132.32 (CH), 133.43 (CH), 141.61 (C), 144.07 (C), 145.80 (C), 148.51 (C), 147.34 (CH)], 148.33 (triazole C-3), 157.84 (triazole C-3), 164.45 and 164.50 (2triazole C-5).

*6.4.3. 2-{[4-(4-Fluorophenyl)-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl}-4-[(phenyl-methylene)-amino]-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**8c**)*

Yield 92%, m.p. 210–211 °C. Anal. Calcd. (%) for: $C_{25}H_{20}\,N_7OS$: C, 61.84; H, 4.15; N, 20.19 Found; C, 62.05; H, 4.22; N, 20.07; IR (KBr, *ν*, cm^{-1}): 2746 (SH), 1721 (C=O), 1567, 1600 and 1503 (3C=N); ^{1}H NMR (DMSO-d_6) *δ* (ppm): 2.38 (3H, s, CH_3), 5.09 (2H, s, NCH_2), 7.32–7.36 (5H, m, arH), 7.38–7.51 (4H, m, arH), 7.64 (2H, d, arH, $J = 8.2$ Hz), 7.83-7.90 (2H, m, arH), 9.31 (1H, s, N=CH), 14.05 (1H, s, SH); ^{13}C NMR (DMSO-d_6) *δ* ppm: 21.03 (CH_3), 38.06–40.67 (CH_2 + DMSO-d_6), 98.27 (N=CH), arC: [117.56 (CH), 118.52 (CH), 121.66 (C), 127.83 (CH), 128.12 (2CH), 129.19 (2CH), 129.56 (C), 130.63 (CH), 130.85 (CH) 130.11 (2CH), 130.48 (CH), 135.80 (CH), 140.27 (C), 143.67 (C), 147.20 (C)], 155.13 (triazole C-3), 160.91 (triazole C-3), 164.62 (triazole C-5), 166.89 (triazole C-5); MS (ESI): *m/z* (%) 486.11 (M + 1, 16), 274.04 (16), 261.01 (16), 231.94 (18), 162.78 (15), 161.90 (46), 156.74 (100), 144.78 (43), 132.76 (30), 118.67 (56).

*6.4.4. 2-{[4-(4-Methoxyphenyl)-5-sulphanyl-4*H*-1,2,4-triazol-3-yl]methyl}-4-[(methylene)-amino]-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**8d**)*

Yield 86%, m.p. 245–246 °C. Anal. Calcd. (%) for: $C_{26}H_{23}N_7O_2S$: C, 62.76; H, 4.66; N, 19.71 Found; C, 62.78; H, 4.79; N, 62.75; IR (KBr, *ν*, cm^{-1}): 2835 (SH), 1722 (C=O), 1257 (C=S); ^{1}H NMR (DMSO-d_6) *δ* (ppm): 2.36 (3H, s, CH_3), 2.50 (3H, s, OCH_3), 3.83 (2H, s, CH_2), 7.07 (2H, d, arH, $J = 8.6$ Hz), 7.32 (2H, d, arH, $J = 8.2$ Hz), 7.43–7.47 (4H, m, arH), 7.62-7.76 (5H, m, arH), 9.14 (1H, s, N=CH), 13.97 (1H, s, SH); ^{13}C NMR (DMSO-d_6) *δ* ppm: 21.62 (CH_3), 22.45 (OCH_3), 44.88 (CH_2), 101.11 (N=CH), arC: [120.34 (CH), 121.28 (CH), 122.87 (C), 125.19 (2CH), 127.46 (CH), 129.68 (CH), 128.94 (C), 129.66 (2CH), 129.47 (CH) 133.08 (CH), 133.61 (2CH), 138.17 (CH), 141.66 (C), 144.43 (C), 144.96 (C), 147.85 (C)], 148.33 (triazole C-3), 157.84 (triazole C-3), 164.50 (triazole C-5), 164.45 (triazole C-5); MS (ESI): *m/z* (%) 498.08 (M + 1, 6), 394.24 (9), 194.88 (12), 193.87 (80), 192.80 (100), 178.77 (54), 156.74 (38), 151.77 (16), 118.60 (53).

*6.5. General method for the synthesis of compounds **7a–b** and **9***

Methyl piperazine (for compounds **7a** and **9**) (10 mmol) or 2-(4-morpholinoethylamine) (for compound **7b**) (10 mmol) and formaldehyde (40%, 1.5 mL) were added to a solution of corresponding compound **5b** or **8a** (10 mmol) in dimethyl formamide, and the mixture was stirred at room temperature for 2 h. Then, distilled water was added and kept overnight in cold. The solid separated was collected by filtration and recrystallized from dimethyl sulfoxide to yield the target compounds.

*6.5.1. 4-Amino-5-(4-methylphenyl)-2-({4-methyl-1-[(4-methylpiperazin-1-yl)methyl]-4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl}methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**7a**)*

Yield 42%, m.p. 247–248 °C. Anal. Calcd. (%) for: $C_{24}H_{29}\,N_9OS$: C, 58.63; H, 5.95; N, 25.64. Found; C, 58.67; H, 6.05; N, 25.61; IR (KBr, *ν*, cm^{-1}): 3455 and 3324 (NH_2), 1717 (C=O), 1613 and 1580 (C=N), 1326 (C=S); ^{1}H NMR: 2.32 (3H, s, CH_3), 2.49 (3H, s, CH_3), 3.28 (8H, m, 4CH_2-piperazine), 4.55 (2H, s, NCH_2), 5.12 (2H, s, NCH_2N), 5.45 (2H, s, NH_2), 6.85–6.97 (2H, m, arH), 7.20–7.70 (5H, m, arH), 7.98 (2H, d, arH, $J = 8.2$ Hz); MS (ESI): *m/z* (%) 491.04 (M^+, 26), 451.70 (31), 449.69 (11), 437.68 (20), 421.79 (18), 420.72 (58), 414.09 (19), 395.19 (19), 394.32 (53), 392.00 (100), 386.68 (27), 381.86 (42), 381.68 (36), 376.86 (22), 365.85 (28), 365.03 (50), 358.77 (44), 349.76 (29), 348.76 (34), 335.00 (24), 332.93 (30), 320.79 (31), 317.98 (42), 316.85 (64), 305.84 (25), 304.90 (56), 301.83 (66).

*6.5.2. 4-Amino-5-(4-methylphenyl)-2-{[4-methyl-1-(morpholin-4-ylmethyl)-4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl] methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**7b**)*

Yield: 42%, m.p. 228–230 °C; Anal. Calcd. (%) for: $C_{25}H_{31}\,N_9O_2S$: C, 57.56; H, 5.99; N, 24.17; Found; C, 57.68; H, 5.93; N, 24.11; IR (KBr, *ν*, cm^{-1}): 3457 and 3320 (NH_2), 1717 (C=O), 1616 and 1581 (C=N), 1331 (C=S); ^{1}H NMR (DMSO-d_6) *δ* (ppm): 2.46 (3H, s, CH_3), 3.40 (8H, bs, 4CH_2), 4.24 (2H, bs, NCH_2), 4.71–5.10 (4H, m, 2CH_2), 5.43 (4H, bs, $NCH_2N + NH_2$), 7.01 (2H, bs, arH), 7.31 (2H, d, arH, $J = 7.6$ Hz), 7.42–7.75 (3H, m, arH), 8.00 (2H, d, arH, $J = 7.8$ Hz).

*6.5.3. 4-[(4-Fluorobenzylidene)amino]-5-(4-methylphenyl)-2-({4-methyl-1-[(4-methylpiperazin-1-yl)methyl]-4-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl}methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**9**)*

Yield 55% m.p. 166–168 °C, Anal. Calcd. (%) for: $C_{31}H_{31}F_2N_9OS$: C, 60.47; H, 5.07; N, 20.47 Found; C, 60.61; H, 5.16; N, 20.36; IR (KBr, *ν*, cm^{-1}): 1713 (C=O), 1600 and 1509 (2C=N), 1224 (C=S), ^{1}H NMR (DMSO-d_6) *δ* (ppm): 2.14 (3H, s, CH_3), 2.31 (4H, bs, 2CH_2), 2.37 (3H, s, CH_3), 2.50 (4H, bs, 2CH_2), 5.10 (2H, s, CH_2), 5.14 (2H, s, NCH_2N), 9.34 (1H, s, N=CH), 7.27–7.46 (6H, m, arH), 7.65 (3H, d, arH, $J = 6.4$ Hz), 7.85 (3H, q, arH, $J = 5.8$ Hz).

*6.6. General method for the synthesis of compounds **4** and **6a–c***

To a solution of corresponding compound **3b** or **5a**, **b** (10 mmol) in absolute ethanol, equivalent amount of methyl iodide (for compounds **4** and **6a**) or ethyl bromide (for compound **6b**) was added and the reaction content was refluxed in the presence of sodium ethoxide (10 mmol) for 3–4 h (completion of the reaction was controlled by TLC). After removing of the reaction solvent under reduced pressure, a solid was obtained. This was recrystallized from DMSO:water (1:1) to afford the desired compound.

*6.6.1. 4-Amino-5-(4-methylphenyl)-2-({5-[methyl(phenyl)amino]-4,5-dihydro-1,3,4-thiadiazol-2-yl}methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**4**)*

Yield 68%, m.p. 173–175 °C. Anal. Calcd. (%) for: $C_{19}H_{19}N_7OS$: C, 58.00; H, 4.87; N, 24.92. Found; C, 58.23; H, 4.89; N, 24.90; IR (KBr, *ν*, cm^{-1}): 3298, 3199 (NH_2), 1705 (C=O), 1618, 1575 and 1505 (3C=N); ^{1}H NMR (DMSO-d_6) *δ* (ppm): 2.37 (3H, s, CH_3), 3.22 (3H, s, NCH_3), 5.21 (2H, s, NCH_2), 5.45 (2H, s, NH_2), 6.93 (1H, q, arH, $J_1 = 7.4$ Hz, $J_2 = 7.8$ Hz), 7.21–7.27 (4H, m, arH), 7.32 (1H, bs, arH), 7.41 (1H, d, arH, $J = 7.8$ Hz), 7.89 (2H, t, arH, $J_1 = 8.4$, Hz, $J_2 = 8.4$ Hz);

^{13}C NMR (DMSO-d_6) δ (ppm): 20.96 (CH_3), 22.68 (CH_3), 44.48 (CH_2), arC: [120.41 (CH), 121.64 (CH), 125.53 (3CH), 127.06 (C), 128.17 (2CH), 128.63 (2CH), 137.84 (C), 139.11 (C)], 154.66 (triazole C-3), 156.07 (triazole C-5), 157.32 (thiadiazole C-2), 167.47 (thiadiazole C-5); MS (ESI): *m*/*z* (%) 416.02 (M + Na, 26), 394.12 (M + 1, 51), 252.90 (34), 251.89 (47), 240.88 (23), 204.76 (22), 203.81 (100), 190.79 (25), 164.80 (18), 117.72 (54).

*6.6.2. 4-Amino-2-{[5-(ethylthio)-4-(4-fluorophenyl)-4*H*-1,2,4-triazol-3-yl]methyl}-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**6a**)*

Yield 68%, m.p. 188–189 °C. Anal. Calcd. (%) for: $C_{20}H_{20}FN_7OS$: C, 56.46; H, 4.74; N, 23.04. Found; C, 56.53; H, 4.77; N, 22.94; IR (KBr, ν, cm^{-1}): 3314 and 3198 (NH_2), 1706 (C═O), 1638, 1603 and 1513 (C═N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.28 (3H, t, CH_3, $J = 7.4$ Hz), 2.35 (3H, s, CH_3), 3.08 (2H, q, CH_2, $J = 7.4$ Hz), 5.04 (2H, s, CH_2), 5.35 (2H, s, NH_2), 7.27 (2H, d, arH, $J = 8.2$ Hz), 7.32–7.48 (4H, m, arH), 7.76 (2H, d, arH, $J = 8$ Hz); ^{13}C NMR (DMSO-d_6) δ (ppm): 14.57 (CH_3), 20.75 (CH_3), 26.34 (SCH_2), 38.08-40.59 (NCH_2 + DMSO-d_6) arC: [116.19 (CH), 116.65 (CH), 123.30 (C), 127.28 (2CH), 128.60 (C) 128.65 (2CH), 129.23 (CH), 129.41 (CH), 139.55 (C), 144.75 (C)], 150.90 (triazole C-3), 152.52 (triazole C-3), 159.83 (triazole C-5), 164.76 (triazole C-5); MS (ESI): *m*/*z* (%) 449.11 (M + Na, 29), 426.09 (M + 1, 100), 235.81 (20).

*6.6.3. 4-Amino-5-(4-methylphenyl)-2-{[5-(methylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl] methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**6b**)*

Yield 66%, m.p. 257–259 °C. Anal. Calcd. (%) for: $C_{19}H_{19}N_7OS$: C, 58.00; H, 4.87; N, 24.92 Found; C, 58.16; H, 4.88; N, 24.87; IR (KBr, ν, cm^{-1}): 3312 and 3198 (NH_2), 1704 (C═O), 1642, 1599 and 1499 (C═N); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.53 (3H, t, CH_3), 2.32 (3H, s, CH_3), 5.12 (2H, s, CH_2), 5.33 (2H, s, NH_2), 7.23 (2H, d, arH, $J = 8.2$ Hz), 7.25–7.32 (5H, m, arH), 7.68 (2H, d, arH, $J = 8.0$ Hz); ^{13}C NMR (DMSO-d_6) δ (ppm): 17.41 (CH_3), 20.77 (CH_3), 47.12 (NCH_2), arC: [118.33 (CH), 118.62 (CH), 126.67 (C), 127.48 (2CH), 128.00 (CH) 128.62 (2CH), 128.83 (CH), 129.40 (CH), 135.55 (C), 142.81 (C)], 151.99 (triazole C-3), 154.44 (triazole C-3), 155.83 (triazole C-5), 162.11 (triazole C-5); MS (ESI): *m*/*z* (%) 416.08 (M + Na, 14), 394.05 (M + 1, 43), 303.95 (18), 283.99 (38), 269.91 (100), 268.91 (18), 253.96 (56), 252.89 (89), 251.89 (51), 240.88 (26), 193.83 (34), 179.87 (19), 164.73 (59), 148.84 (39), 120.68 (28), 104.85 (52).

*6.6.4. 4-Amino-2-{[4-(4-methoxyphenyl)-5-(methylthio)-4*H*-1,2,4-triazol-3-yl]methyl}-5-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**6c**)*

Yield 64 %, m.p. 247–249 °C. Anal. Calcd. (%) for: $C_{20}H_{21}N_7O_2S$: C, 56.72; H, 5.00; N, 23.15 Found; C, 56.80; H, 5.17; N, 23.05; IR (KBr, ν, cm^{-1}): 3298, 3204 (NH_2), 1713 (C═O), 1635, 1514, 1454 (3C═N), ^{1}H NMR (DMSO-d_6) δ (ppm): 2.32 (3H, s, CH_3), 2.35 (3H, s, OCH_3), 2.55 (3H, s, SCH_3), 5.02 (2H, s, CH_2) 5.34 (2H, s, NH_2), 7.21–7.32 (6H, m, arH), 7.74 (2H, d, arH, $J = 8$ Hz); ^{13}C NMR (DMSO-d_6) δ (ppm): 14.95 (SCH_3), 21.41 (CH_3), 21.66 (OCH_3), arC: [124.23 (C), 127.32 (2CH), 128.15 (2CH), 129.54 (2CH), 130.37(C), 130.82 (2CH), 140.38 (C), 140.51 (C)], 145.46 (triazole C-3), 151.81 (thiadiazole C-2), 153.05 (triazole C-5), 153.43 (thiadiazole C-5).

Acknowledgement

This project was supported by Karadeniz Technical University, BAP, Turkey (Ref. No. 2005.111.002.3) and is gratefully acknowledged.

References

[1] R.K. Rawal, Y.S. Phabhakar, S.B. Kati, E. De Clercq, Bioorg. Med. Chem. 13 (2005) 6771–6776.
[2] C.G. Bonde, N.J. Gaikwad, Bioorg. Med. Chem. 12 (2004) 2151–2161.
[3] R.K. Rawal, R. Tripathi, S.B. Kati, C. Pannecouque, E. De Clercq, Bioorg. Med. Chem. 15 (2007) 1725–1731.
[4] P.P. Dixit, V.J. Patil, P.S. Nair, S. Jain, N. Sinha, S.K. Arora, Eur. J. Med. Chem. 41 (2006) 423–428.
[5] Y. Cui, Y. Dang, Y. Yang, S. Zhang, R. Ji, Bioorg. Med. Chem. Lett. 40 (2005) 209–214.
[6] M. Weidinger-Wells, C.M. Boggs, B.D. Foleno, J. Melton, K. Bush, R.M. Goldschmitdt, D.J. Hlasta, Bioorg. Med. Chem. 10 (2002) 2345–2351.
[7] O.A. Philips, E.E. Udo, A.A.M. Ali, S.M. Samuel, Eur. J. Med. Chem. 42 (2007) 214–225.
[8] P.P. Dixit, P.S. Nair, V.J. Patil, S. Jain, S.K. Arora, N. Sinha, Bioorg. Med. Chem. Lett. 15 (2005) 3002–3005.
[9] A. Gupta, J.D. Unadkat, Q. Mao, Drug Discovery Interface 96 (2007) 3226–3235.
[10] S. Cai, Q.S. Li, R.T. Borchardt, K. Kuczera, R.L. Schowen, Bioorg. Med. Chem. 15 (2007) 7281–7287.
[11] B.M. Rao, S. Sangaraju, M.K. Srinivasu, P. Madhavan, M.L. Devi, P.R. Kumar, K.B. Candrasekhar, Ch. Arpitha, T.S. Balaji, J. Pharm. Biomed. Anal. 41 (2006) 1146–1151.
[12] G. Hancu, A. Gaspar, A. Gyeresi, J. Biochem. Biophys. Methods 69 (2007) 251–259.
[13] E. Bajetti, N. Zilembo, E. Bichisao, P. Pozzi, L. Toffolatti, Crit. Rev. Oncol. Hematol. 33 (2000) 137–142.
[14] A. Demirbas, S. Ceylan, N. Demirbas, J. Heterocycl. Chem. 44 (2007) 1271–1280.
[15] A. Castro, T. Castano, A. Encinas, W. Porcal, C. Gil, Bioorg. Med. Chem. 14 (2006) 1644–1652.
[16] M. Ashok, B.S. Holla, B. Poojary, Eur. J. Med. Chem. 42 (2007) 1095–1101.
[17] F. Lopes, R. Capela, L.O. Goncaves, P.N. Horton, B.M. Hursthouse, J. Iley, C.M. Casimiro, J. Bom, R. Moreira, Tetrahedron Lett. 45 (2004) 7663–7666.
[18] M.G. Ferlin, G. Chiarelotto, F. Antonucci, L. Caparrotta, G. Froldi, Eur. J. Med. Chem. 37 (2002) 427–434.
[19] B.S. Holla, B. Veerendra, M.K. Shivananda, B. Poorjary, Eur. J. Med. Chem. 38 (2003) 759–767.
[20] L. Liu, S. Zhu, Carbohydr. Polym. 68 (2007) 472–476.
[21] J.M. Ridley, P.C. Dooley, C.T. Milnes, H.J. Witchel, J.C. Hancox, J. Mol. Cell. Cardiol. 36 (2004) 701–705.
[22] Z. Li, S. Junfeng, Talanta 73 (2007) 943–947.
[23] B.S. Holla, M. Mahalinga, M.S. Karthikeyan, P.M. Akberali, N.S. Shetty, Bioorg. Med. Chem. 14 (2006) 2040–2047.
[24] N. Demirbas, R. Ugurluoglu, A. Demirbas, Bioorg. Med. Chem. 10 (2002) 3717–3723.
[25] N. Demirbaş, R. Uğurluoğlu, Turk. J. Chem. 28 (2004) 559–571.
[26] N. Demirbaş, R. Uğurluoğlu, Turk. J. Chem. 28 (2004) 679–690.
[27] N. Demirbas, S.A. Karaoglu, A. Demirbas, K. Sancak, Eur. J. Med. Chem. 39 (2004) 793–804.
[28] N. Demirbas, A. Demirbas, S.A. Karaoğlu, Russ. J. Bioorg. Chem. 31 (2005) 387–397.
[29] N. Demirbas, A. Demirbas, S.A. Karaoglu, E. Çelik, Arkivoc i (2005) 75–91.
[30] A. İkizler, N. Demirbaş, A.A. İkizler, J. Heterocycl. Chem. 33 (1996) 1765–1769.
[31] A. İkizler, N. Demirbaş, A. Demirbaş, A.A. İkizler, Polym. J. Chem. 70 (1996) 1114–1120.
[32] A.A. İkizler, F. Uçar, N. Demirbas, I. Yasa, A. Demirbas, T. Genzer, Indian J. Pharm. Sci. 61 (1999) 271–274.
[33] R. Smicius, V. Jakubkiene, M.M. Burbuliene, P. Vainilavicius, Monatsh. Chem. 133 (2002) 173–181.
[34] C. Perez, M. Pauli, P. Bazerque, Acta Bio. Med. Exp. 15 (1990) 113–115.
[35] I. Ahmad, Z. Mehmood, F. Mohammed, J. Ethnopharmacol. 62 (1998) 183–193.