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Synthesis of some new biheterocyclic triazole derivatives and evaluation of their antimicrobial activity

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2-{3-(4-Substitutedbenzyl)-4-[2-(1H-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}-N′-(arylmethylene)acetohydrazides (5a-g), 4-amino-2-{3-(4-substitutedbenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}-N′-(arylmethylene)acetohydrazides (6a,b), and 4-[2-(1H-indol-3-yl)ethyl]-5-(4-substitutedbenzyl)-2-{[5-(phenylamino)-1,3,4-thiadiazol-2-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-ones (8a,b) were synthesized starting from 4-alkyl-5-(4-substitutedbenzyl)-2,4-dihydro-3H-1,2,4-triazol-3-ones (2a-c) by several steps and their structures 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 obtained antimicrobial activity results revealed that 12 among the 24 compounds tested displayed variable growth inhibition effects on the tested gram-positive and gram-negative bacterial strains. None of the compounds showed antifungal activity against yeast-like fungi.

Key Words: 1,2,4-Triazole, 1*H*-indole, 1,3,4-thiadiazole, Schiff base, antimicrobial activity.

During the past decades, the incidence of microbial infection has increased to alarming levels all over the world as a result of antimicrobial resistance. The growing number of immuno-compromised patients as a result of cancer chemotherapy, organ transplantation, and HIV infection is a major factor contributing to this increase. For instance, tuberculosis (TB) causes approximately three million deaths worldwide every year. According to the World Health Organization (WHO), about 30 million people will be infected within the next 20 years. Due to this reason, new classes of antibacterial agents with novel mechanisms are crucial to combat multidrug resistant infections.[1−5]

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In a wide variety of heterocyclic structures, the indole nucleus occupies a position of major importance, and many indole derivatives constitute the basis of a range of pharmaceuticals. Biological properties of 1*H*-indole-2,3-dione include a range of actions in the brain and offer protection against certain types of infections. Methisazone (Figure 1) plays an important role as prophylactic agent against several viral diseases. In recent years, some 1*H*-indole derivatives including also Schiff and Mannich base structures have been reported to exhibit broad spectrum chemotherapeutic properties such as antiviral, anti-tuberculosis, antifungal, and antibacterial activity.[6] Due to the high level of activity of 1*H*-indole derivatives, a number of efforts have been devoted to the design and synthesis of new indole-based medicinal agents.[7−11] In addition, heterocyclic compounds bearing 1,2,4-triazole have long been the focus of synthetic organic chemistry due to their broad spectrum of applications in biological, pharmacological, and material areas.[12−21] The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents. Conazoles, such as Fluconazole, Itraconazole, and Posaconazole, have been used for the treatment of fungal infections in the current regimen (Figure 1).[22−25] Ribavirin (antiviral), Rizatriptan (antimigraine), Alprazolam (anxiolytic), and the antitumor drugs Vorozole, Letrozole, and Anastrozole are some other examples of drugs containing 1,2,4-triazole moiety (Figure 2).[26−28] In recent years, some Schiff bases containing 1,2,4-triazole nucleus have been reported as antimicrobial agents.[29−33]

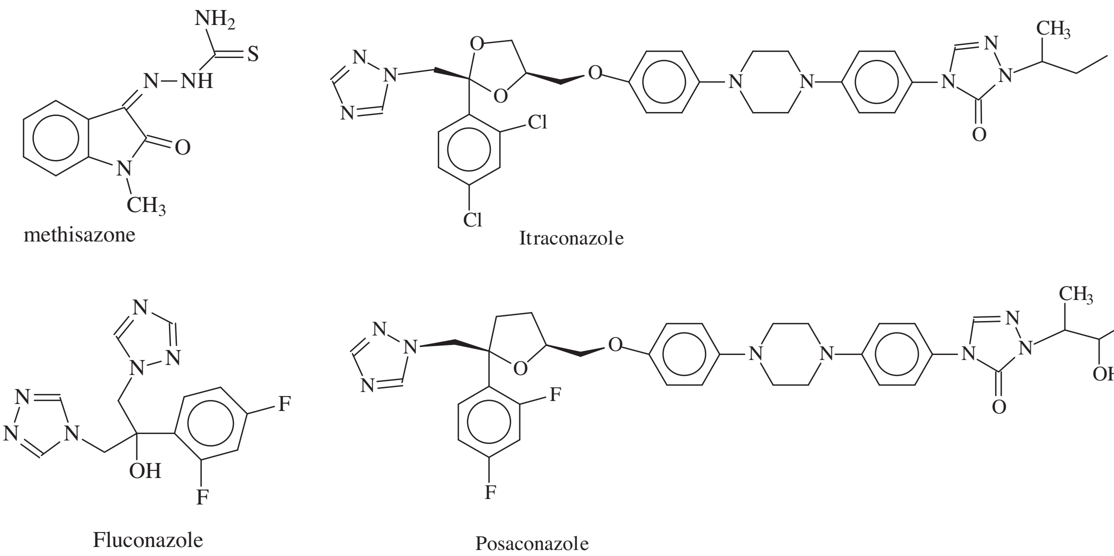


Figure 1.

In the design of new bioactive agents, the development of hybrid molecules through the combination of different pharmacophores in the same structure may lead to compounds having more efficiency in biological activity.[11]

In view of these facts, the aim of the present study was to obtain 1,2,4-triazole derivatives, some of which contain 1*H*-indole and/or 1,3,4-thiadiazole ring beside a Schiff base structure as possible antimicrobial agents.

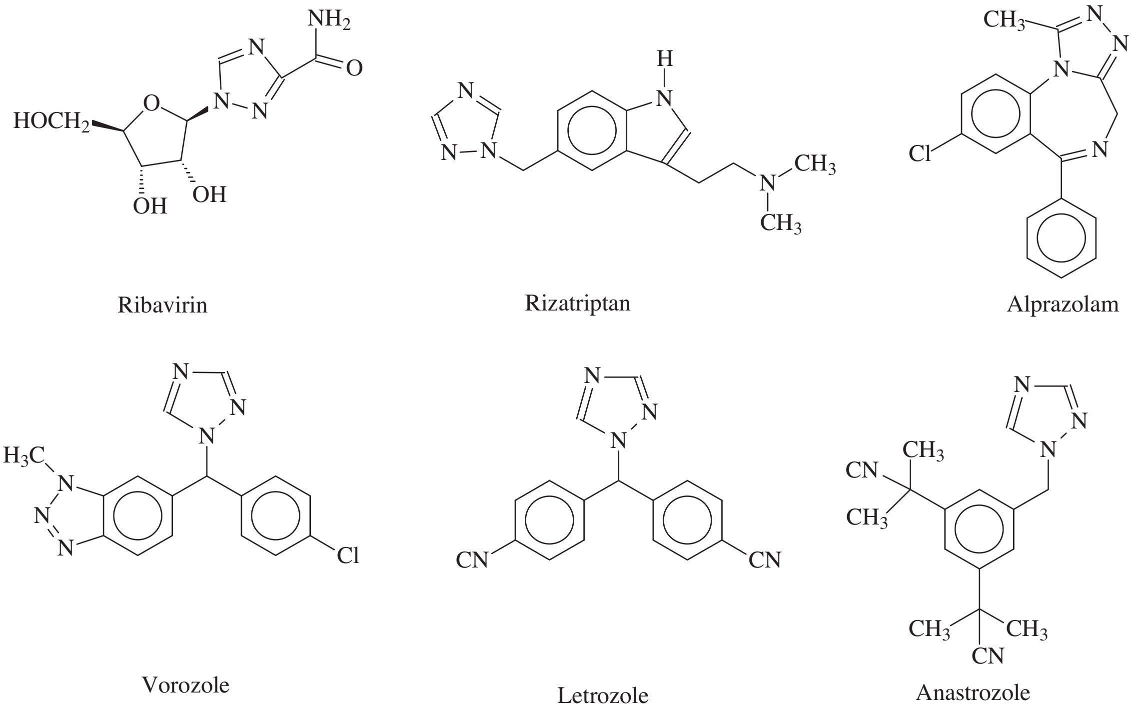


Figure 2.

Experimental

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 **2c, 3c, 4c, 6a, 6b, 7c,** and **8c**). Elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer (except compounds **5e, 5f, 7a, 7b, 8a,** and **8b**). All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland).

General method for the synthesis of compounds 2a,b

A mixture of the corresponding compound **1** (10 mmol) and tryptamine (10 mmol) was heated in an oil bath at 120-125 °C for 2 h. On cooling it to room temperature a solid was obtained. This crude product was recrystallized dimethyl sulfoxide-water (1:1) to obtain the desired product.

4-[2-(1*H*-Indol-3-yl)ethyl]-5-(4-chlorobenzyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (2a): Yield 70%, mp 224-225 °C; *Anal.* Calcd. (%) for: $C_{19}H_{17}N_4OCl$: C, 64.68, H, 4.86, N, 15.88, Found; C, 64.62, H, 4.78, N, 15.80; IR (KBr, ν, cm^{-1}): 3322, 3173 (2NH), 1705 (C=O), 1603 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.81 (t, 2H, tryp-CH_2, J=6.4 Hz), 3.51 (s, 2H, benzyl-CH_2), 3.66 (t, 2H, trp-CH_2, J=6.4 Hz), 6.95-7.13 (m, 5H, ar-H), 7.32-7.41 (m, 4H, ar-H), 10.93 (s, 1H, tryp-NH), 11.56 (s,1H, triazole-NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 24.82 (tryp-CH_2), 32.06 (benzyl-CH_2), 43.03 (tryp-CH_2), ar-C:[111.94 (C), 113.11 (C), 119.57

(C), 120.09 (C), 122.73 (C), 124.80 (C), 128.51 (2C), 130.02 (2C), 132.09 (C), 133.11 (C), 135.82 (C), 137.74 (C)], 147.58 (triazole C-3), 156.66 (triazole C-5); MS (ESI): m/z (%) 353 (M+1, 6), 254 (20), 222 (22), 153 (189), 144 (100).

4-[2-(1*H*-Indol-3-yl)ethyl]-5-(4-methylbenzyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (2b): Yield 80%, mp 222-223 °C; *Anal.* Calcd. (%) for: $C_{20}H_{20}N_4O$: C, 72.27, H, 6.06, N, 16.85, Found; C, 72.18, H, 6.12, N, 16.80; IR (KBr, ν, cm^{-1}): 3301 (2NH), 1719 (C=O), 1587 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.25 (s, 3H, CH_3), 2.75 (t, 2H, trp-CH_2), 3.46 (s, 2H, benzyl-CH_2), 3.60 (t, 2H, tryp-CH_2), 6.89-6.98 (m, 3H, ar-H), 7.04-7.12 (m, 4H, ar-H), 7.35 (m, 2H, ar-H), 10.90 (s,1H, tryp-NH), 11.52 (s, 1H, triazole-NH);^{13}C-NMR (DMSO-d_6)δ (ppm): 21.29 (CH_3), 24.78 (tryp-CH_2), 31.62 (benzyl-CH_2), 42.12 (tryp-CH_2), ar-C:[111.10 (C), 112.22 (C), 118.76 (C), 119.16 (C), 121.85 (C), 123.86 (C), 127.64 (C), 129.06 (2C), 129.86 (2C), 132.87 (C), 136.66 (C), 136.89 (C)], 147.11 (triazole C-3), 155.87 (triazole C-5); MS (ESI): m/z (%) 333 (M+1, 20), 355 (98), 229 (28), 144 (100).

General method for the synthesis of compounds 3a-c

The corresponding compound **2** (10 mmol) was refluxed with an equivalent amount of sodium in absolute ethanol for 2 h. Then ethyl bromoacetate (10 mmol) was added and the mixture refluxed for an additional 8 h. After evaporating the solvent under reduced pressure, a solid appeared. This was recrystallized from ethanol/water (1:2) (for **3c**) or ethanol (for **3a,b**) to afford the desired compound.

Ethyl {4-[2-(1*H*-indol-3-yl)ethyl]-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl} acetate (3a): Yield 85%, mp 129-130 °C; *Anal.* Calcd. (%) for: $C_{23}H_{23}N_4O_3Cl$: C, 62.94, H, 5.28, N, 12.76, Found; C, 62.89, H, 5.25, N, 12.78; IR (KBr, ν, cm^{-1}): 3390 (NH), 1736 (ester C=O), 1716 (triazole C=O); ^{1}H-NMR (DMSO-d_6)δ (ppm): 1.21 (t, 3H, $CH_2\underline{CH}_3$, J=6.8 Hz), 2.82 (t, 2H, tryp-CH_2, J=7.2 Hz), 3.45 (s, 2H, benzyl-CH_2), 3.69 (t, 2H, tryp-CH_2, J=7.2 Hz), 4.14 (q, 2H, $\underline{CH}_2CH_3$, J=6.8 Hz), 4.55 (s, 2H, CH_2), 6.94-7.13 (m, 5H, ar-H), 7.32-7.42 (m, 4H, ar-H), 10.94 (s, 1H, tryp-NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 14.72 (CH_3), 24.60 (trp-CH_2), 30.84 (benzyl-CH_2), 42.82 (tryp-CH_2), 46.89 (NCH_2), 61.82 (CH_2), ar-C: [110.83 (C), 112.23 (C), 118.74 (C), 119.29 (C), 121.91 (C), 124.07 (C), 127.55 (C), 129.18 (2C), 131.19 (2C), 132.36 (C), 134.46 (C), 136.88 (C)], 146.25 (triazole C-3), 154.39 (triazole C-5), 168.66 (C=O); MS (ESI): m/z (%) 439 (M+1, 20), 461 (M+Na, 98), 357 (22), 188 (32), 148 (68), 129 (58)121 (40).

Ethyl {4-[2-(1*H*-indol-3-yl)ethyl]-3-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}acetate (3b): Yield 84%, mp 127-128 °C; *Anal.* Calcd. (%) for: $C_{24}H_{26}N_4O_3$: C, 68.88, H, 6.26, N, 13.39, Found; C, 68.82, H, 6.30, N, 13.32; IR (KBr, ν, cm^{-1}): 3391 (NH), 2989, 2923 (CH_2), 1735 (ester C=O), 1716 (triazole C=O); ^{1}H-NMR (DMSO-d_6)δ (ppm): 1.22 (t, 3H, $CH_2\underline{CH}_3$, J=6.8 Hz), 2.25 (s, 3H, CH_3), 2.78 (t, 2H, tryp-CH_2), 3.40 (s, 2H, benzyl-CH_2), 3.66 (t, 2H, tryp-CH_2, J=6.2 Hz), 4.17 (q, 2H, $\underline{CH}_2CH_3$, J= 6.8 Hz), 4.57(s, 2H, CH_2), 6.85-6.88 (m, 2H, ar-H), 6.99-7.11 (m, 5H, ar-H), 7.34-7.43 (m, 2H, ar-H), 10.93 (s, 1H, tryp-NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 14.72 (CH_3), 21.28 (CH_3), 24.58 (trp-CH_2), 31.25 (benzyl-CH_2), 42.77 (tryp-CH_2), 46.88 (NCH_2), 61.81 (CH_2), ar-C:[110.85 (C), 112.22 (C), 118.77 (C), 119.23 (C), 121.89 (C), 124.02 (C), 127.55 (C), 129.05 (2C), 129.87 (2C), 132.41 (C), 136.78 (C), 136.90 (C)], 146.63 (triazole C-3), 154.49 (triazole C-5), 168.71 (C=O); MS (ESI): m/z (%) 419 (M, 20), 420 (M+1, 10), 441 (32), 276 (14), 144 (100).

Ethyl [4-benzylidenamino-3-(4-nitrobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl] acetate (3c): Yield 94%, mp 155-156 °C; *Anal.* Calcd. (%) for: $C_{13}H_{15}N_5O_5$: C, 48.60, H, 4.71, N, 21.80, Found; C, 48.65, H, 4.70, N, 21.78; IR (KBr, ν, cm^{-1}): 3210-3112 (NH_2), 1746 (ester-C=O), 1711 (triazole-C=O), 1583 (C=N), 1215 (C-O); ^{1}H-NMR (DMSO-d_6)δ (ppm): 1.16-1.23 (m, 3H, CH_3), 4.10-4.24 (m, 2H, $\underline{CH_2}CH_3$), 4.31 (s, 2H, benzyl-CH_2), 4.64 (s, 2H, NCH_2), 5.25 (s, 2H, NH_2), 7.603-7.638 (d, 2H, ar-H, J=7.0 Hz), 8.18-8.22 (d, 2H, ar-H, J=7.4 Hz); ^{13}C-NMR (DMSO-d_6)δ (ppm): 15.77 (CH_3), 32.48 (CH_2), 48.35 (CH_2), 63.06 (CH_2), arC: [125.34 (C), 130.80 (2C), 131.38 (2C), 134.90 (C)], 151.57 (triazole C-3), 158.45 (triazole C-5), 169.27 (C=O).

General Method for the Synthesis of Compounds 4a-c

A solution of the corresponding compound **3** (10 mmol) in *n*-butanol was refluxed with hydrazine hydrate (25 mmol) for 4 h. After cooling it to room temperature, a white solid appeared. This was recrystallized from ethanol-water (1:2) (for **4a,b**) or dimethyl sulfoxide-water (1:1) (for **4c**) to obtain the desired compound.

2-{3-(4-Chlorobenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl} acetohydrazide (4a): Yield 76%, mp 169-170 °C; *Anal.* Calcd. (%) for: $C_{21}H_{21}N_6O_2$Cl: C, 59.36, H, 4.98, N, 19.78, Found; C, 59.32, H, 4.90, N, 19.80; IR (KBr, ν, cm^{-1}): 3298, 3181 (2NH + NH_2), 1704 (triazole-C=O), 1677 (hydrazide-C=O); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.82 (t, 2H, CH_2, J=6.4 Hz), 3.48 (s, 2H, CH_2), 3.67 (t, 2H, CH_2, J=6.4 Hz), 4.26 (s, 2H, CH_2), 4.30 (s, 2H, NH_2), 6.99-7.09 (m, 5H, ar-H), 7.32-7.41 (m, 4H, ar-H), 9.23 (s, 1H, NH), 10.93 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 23.86 (tryp-CH_2), 30.19 (benzyl-CH_2), 42.04 (tryp-CH_2), 46.05 (NCH_2), ar-C:[110.14 (C), 111.48 (C), 118.98 (C), 118.53 (C), 121.14 (C), 123.34 (C), 126.81 (C), 128.39 (2C), 130.49 (2C), 131.57 (C), 133.81 (C), 136.10 (C)], 145.11 (triazole C-3), 153.82 (triazole C-5), 166.05 (C=O); MS (ESI): m/z (%) 425 (M+1, 12), 188 (20), 144 (100).

2-{3-(4-Methylbenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl} acetohydrazide (4b): Yield 65%, mp 170-171 °C; *Anal.* Calcd. (%) for: $C_{22}H_{24}N_6O_2$: C, 65.33, H, 5.98, N, 20.78, Found; C, 65.28, H, 5.92, N, 20.80; IR (KBr, ν, cm^{-1}): 3345 and 3193 (2NH + NH_2), 1704 (triazole-C=O), 1692 (hydrazide-C=O); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.25 (s, 3H, CH_3), 2.78 (t, 2H, CH_2,J=6.6 Hz), 3.43 (s, 2H, CH_2), 3.63 (t, 2H, CH_2,J=6.6 Hz), 4.27 (s, 2H, CH_2), 4.31 (s, 2H, NH_2), 6.87-6.91 (m, 2H, ar-H), 6.99-7.12 (m, 5H, ar-H), 7.34-7.41 (m, 2H, ar-H), 9.25 (s, 1H, NH), 10.92 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 21.31 (CH_3), 24.62 (tryp-CH_2), 31.38 (benzyl-CH_2), 42.77 (tryp-CH_2), 46.79 (NCH_2), ar-C: [110.93 (C), 112.22 (C), 118.78 (C), 119.21 (C), 121.87 (C), 124.05 (C), 127.57 (C), 129.14 (2C), 129.85 (2C), 132.52 (C), 136.73 (C), 136.88 (C)], 146.25 (triazole C-3), 154.64 (triazole C-5), 166.84 (C=O); MS (ESI): m/z (%) 405 (M+1, 32), 428 (M+Na, 30), 357 (18), 229 (80). MS (ESI): m/z (%) 405 (M+1, 32), 428 (M+Na, 30), 357 (18), 229 (80).

2-{3-(4-Nitrobenzyl)-4-amino-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}acetohyd-razide (4c): Yield 98%, mp 149-150 °C; *Anal.* Calcd. (%) for: $C_{11}H_{13}N_7O_4$: C, 43.00, H, 4.26, N, 31.91, Found; C, 43.05, H, 4.25, N, 31.90; IR (KBr, ν, cm^{-1}): 3302-3208 (NH+2NH_2), 1720 (triazole C=O), 1665 (hydrazide-C=O), 1606 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 4.04 (s, benzyl-CH_2), 4.22-4.33 (bs, 4H, $NH\underline{NH_2}$+ NCH_2), 5.30 (s, 2H, NH_2), 7.52-7.65 (m, 2H, ar-H), 8.15-8.19 (m, 2H, ar-H), 9.19 (s,1H, $\underline{NH}NH_2$); ^{13}C NMR (DMSO-d_6)δ (ppm): 32.08 (CH_2), 48.37 (CH_2), arC: [125.12 (C), 129.58 (C), 130.79 (C), 131.93 (C), 132.03 (C), 145.67 (C)], 148.30 (triazole C-3), 155.13 (triazole C-5), 167.62 (C=O).

General Method For The Synthesis of Compounds 5a-g and 6a,b

A solution of the corresponding compound **4** (10 mmol) in absolute ethanol was refluxed with appropriate aldehyde (10 mmol) for 3 h. After cooling the mixture to room temperature, a white solid appeared. This crude product was recrystallized from dimethyl sulfoxide/water (1:2) to yield the target product.

2-{3-(4-Chlorobenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(phenylmethylene)acetohydrazide (5a): Yield 70%, mp 245-246 °C; *Anal.* Calcd. (%) for: $C_{28}H_{25}N_6O_2Cl$: C, 65.56, H, 4.91, N, 16.38, Found; C, 65.51, H, 4.95, N, 16.42; IR (KBr, ν, cm^{-1}): 3345, 3186 (NH), 1707 (triazole C=O), 1690 (hydrazide C=O), 1619 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.84 (bs, 2H, tryp-CH_2), 3.46 (s, 2H, benzyl-CH_2), 3.70 (bs, 2H, tryp-CH_2), 4.47 and 4.88 (s, 2H, CH_2, *trans and cis* conformers, *trans/cis* ratio 72/28), 6.96-7.13 (m, 5H, ar-H), 7.31-7.45 (m, 7H, ar-H), 7.71-7.73 (m, 2H, ar-H), 8.02 and 8.21 (s, 1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 70/30), 10.95 (s, 1H, tryp-NH), 11.68 (bs, 1H, NH);^{13}C-NMR (DMSO-d_6)δ (ppm): 23.84 (tryp-CH_2), 30.06 (benzyl-CH_2), 41.95 (tryp-CH_2), 46.16 and 46.84 (NCH_2, *trans/cis*), ar-C: [110.06 (C), 111.40 (C), 117.94 (C), 118.46 (C), 121.08 (C), 123.31 (C), 126.75 (C), 126.86 (2C), 128.34 (2C), 128.67 (2C), 129.86 (2C), 130.36 (C), 131.48 (C), 133.82 (2C), 136.04.87 (C)], 143.92 and 144.12 (N=CH, *trans/cis*), 145.02 (triazole C-3), 154.01 (triazole C-5), 167.98 (C=O); MS (ESI): *m/z* (%) 513 (M, 38), 535 (M+Na, 100), 357 (20), 229 (16), 144 (34).

2-{3-(4-Chlorobenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(2,6-dichlorophenylmethylene)acetohydrazide (5b): Yield 84%, mp 114 °C; *Anal.* Calcd. (%) for: $C_{28}H_{23}N_6O_2Cl_3$: C, 57.80, H, 3.98, N, 14.44, Found; C, 57.78, H, 3.95, N, 14.46; IR (KBr, ν, cm^{-1}): 3357, 3205 (NH), 1710 (triazole C=O), 1684 (hydrazide C=O), 1618 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.83 (bs, 2H, tryp-CH_2), 3.47 (s, 2H, benzyl-CH_2), 3.69 (bs, 2H, tryp-CH_2), 4.51 and 4.80 (s, 2H, CH_2, *trans and cis* conformers, *trans/cis* ratio 78/22), 6.98-7.13 (m, 5H, ar-H), 7.32-7.66 (m, 7H, ar-H), 8.29 and 8.39 (s, 1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 79/21), 10.94 (s, 1H, tryp-NH), 11.96 (bs, 1H, NH);^{13}C-NMR (DMSO-d_6)δ (ppm): 23.86 (tryp-CH_2), 30.11 (benzyl-CH_2), 41.91 (tryp-CH_2), 46.49 and 47.06 (NCH_2, *trans/cis*), ar-C: [110.01 (C), 111.37 (C), 117.92 (C), 118.41 (C), 121.04 (C), 123.28 (C), 126.72 (C), 128.39 (2C), 129.07 (2C), 129.29 (2C), 130.36 (2C), 130.96 (C), 131.50 (C), 133.78 (2C), 136.03 (C)], 142.12 and 142.57 (N=CH, *trans/cis*), 145.04 (triazole C-3), 154.03 (triazole C-5), 168.21 (C=O); MS (ESI): *m/z* (%) 581 (M, 10), 419 (14), 254 (32), 188 (48), 144 (100).

2-{3-(4-Chlorobenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(2-hydroxyphenylmethylene)acetohydrazide (5c): Yield 71%, mp 250-251 °C; *Anal.* Calcd. (%) for: $C_{28}H_{25}N_6O_3Cl$: C, 63.57, H, 4.76, N, 15.89, Found; C, 63.52, H, 4.74, N, 15.90; IR (KBr, ν, cm^{-1}): 3399 (OH), 3203 (NH), 1713 (triazole C=O), 1683 (hydrazide-C=O), 1620 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.84 (bs, 2H, tryp-CH_2), 3.46 (s, 2H, benzyl-CH_2), 3.70 (bs, 2H, tryp-CH_2), 4.50 and 4.85 (s, 2H, CH_2, *trans and cis* conformers, *trans/cis* ratio 54/46), 6.81-7.13 (m, 6H, ar-H), 7.20-7.44 (m, 5H, ar-H), 7.54, 7.58 (d, 1H, ar-H, J=8.2 Hz), 7.74-7.77 (d, 1H, ar-H, J=7.8 Hz), 8.33 and 8.43 (s, 1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 54/46), 10.06 (s, 1H, OH), 10.97 (s, 1H, tryp-NH), 11.60 and 11.90 (s, 1H, NH, *trans and cis* conformers, *trans/cis* ratio 54/46);^{13}C-NMR (DMSOd$_6$)δ (ppm): 23.88 (tryp-CH_2), 30.16 (benzyl-CH_2), 41.88 (tryp-CH_2), 45.16 and 46.12 (NCH_2, *trans/cis*), ar-C:[110.11 (C), 111.40 (C), 116.54 (C), 118.47 (C), 121.23 (C), 122.76 (C), 126.25 (C), 126.86 (2C), 128.64 (2C), 128.98 (2C), 129.27 (2C), 130.36 (C), 131.71 (C), 132.82 (2C), 137.37 (C)], 144.35 and 144.86 (N=CH, *trans/cis*), 145.48 (triazole C-3), 153.67 (triazole C-5), 167.95 (C=O). MS (ESI): m/z (%) 529 (M, 22), 531 (M+2, 100), 551 (78), 357 (18), 189 (24), 144 (30).

2-{3-(4-Methylbenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(phenylmethylene)acetohydrazide (5d): Yield 87%, mp 270-271 °C; *Anal.* Calcd. (%) for: $C_{29}H_{28}N_6O_2$: C, 70.71, H, 5.73, N, 17.06, Found; C, 70.75, H, 5.70, N, 17.10; IR (KBr, ν, cm^{-1}): 3340, 3193 (NH), 1705 (triazole C=O), 1691 (hydrazide C=O), 1615 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.46 (s, 3H, CH_3), 2.80 (t, 2H, tryp-CH_2), 3.41 (s, 2H, benzyl-CH_2), 3.66 (t, 2H, tryp-CH_2, J=6.4 Hz), 4.49 and 4.89 (s, 2H, CH_2, *trans and cis* conformers, *trans/cis* ratio 75/25), 6.88 (d, 2H, ar-H), 6.97-7.14 (m, 6H, ar-H), 7.35-7.44 (m, 4H, ar-H), 7.16-7.74 (m, 2H, ar-H), 8.03 and 8.22 (s,1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 73/27), 10.95 (s, 1H, tryp-NH), 11.69 and 11.72 s, 1H, NH, *trans/cis* ratio 70/30); ^{13}C-NMR (DMSO-d_6)δ (ppm): 20.49 (CH_3), 23.83 (tryp-CH_2), 30.49 (benzyl-CH_2), 41.87 (tryp-CH_2), 46.13 and 46.24 (NCH_2, *trans/cis*), ar-C:[110.07 (C), 111.37 (C), 117.96 (C), 118.37(C), 121.04 (C), 123.25 (C), 126.72 (C), 126.85(2C), 128.23 (2C), 128.67 (2C), 129.02 (2C), 129.88 (C), 131.74 (C), 133.80 (C), 135.87 (C), 143.89 (C)], 145.36 and 145.59 (N=CH, *trans/cis*), 154.05 (triazole C-3), 163.12 (triazole C-5), 167.99 (C=O); MS (ESI): m/z (%) 493 (M+1, 28), 515 (M+Na, 100), 357 (18), 229 (24), 144 (24).

2-{3-(4-Methylbenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(2,6-dichlorophenylmethylene)acetohydrazide (5e): Yield 75%, mp 248-249 °C; IR (KBr, ν, cm^{-1}): 3332 (NH), 1710 (C=O), 1688 (C=O), 1600 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.26 (s, 3H, -CH_3), 2.80 (t, 2H, CH_2), 3.42 (s, 2H, CH_2), 3.66 (bs, 2H, CH_2), 4.53 and 4.83 (s, 2H, CH_2, *trans and cis* conformers, *trans/cis* ratio 79/21), 6.9 (d, 2H, ar-H, J=6.0 Hz), 6.97-7.12 (m, 5H, ar-H), 7.35-7.48 (m, 3H, ar-H), 7.58 (d, 2H, ar-H), 8.30 and 8.41 (s, 1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 78/22), 10.95 (s, 1H, NH), 11.98 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 20.52 (CH_3), 23.78 (CH_2), 30.49 (CH_2), 41.91 (CH_2), 46.04 and 46.52 (NCH_2, *trans/cis*), ar-C: [110.07 (C), 111.40 (C), 117.99 (C), 118.40(C), 121.08 (C), 123.31 (C), 126.75 (C), 128.30 (2C), 128.93 (2C), 129.25 (2C), 129.37 (2C), 131.07 (C), 131.75 (C), 133.86 (C), 135.94 (C), 139.09 (C)], 145.49 and 145.56 (N=CH, *trans/cis*), 154.14 (triazole C-3), 163.44 (triazole C-5), 168.29 (C=O); MS (ESI): m/z (%) 561 (M^+, 52), 583 (100), 357 (21), 144 (38).

2-{3-(4-Methylbenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(3-fluorophenylmethylene)acetohydrazide (5f): Yield 78%, mp 286-287 °C; IR (KBr, ν, cm^{-1}): 3345, 3193 (NH), 1704 (C=O), 1692 (C=O), 1581(C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.25 (s, 3H, CH_3), 2.80 (t, 2H, CH_2), 3.41 (s, 2H, CH_2), 3.66 (bs, 2H, CH_2), 4.51 and 4.91 (s, 2H, CH_2, *trans and cis* conformers, *trans/cis* ratio 73/27), 6.88 (d, 2H, ar-H, J=8.2 Hz), 7.01-7.07 (m, 5H, ar-H), 7.26-7.66 (m, 6H, ar-H), 8.02 and 8.23 (s, 1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 68/32), 10.94 (s, 1H, NH), 11.79 and 11.89 (s, 1H, NH, *trans and cis* conformers, *trans/cis* ratio 76/24); ^{13}C-NMR (DMSO-d_6)δ (ppm): 20.49 (CH_3), 23.78 (tryp-CH_2), 30.46 (CH_2), 41.88 (CH_2), 46.23 and 46.98 (NCH_2, *trans/cis*), ar-C:[110.07 (C), 111.37 (C), 112.44 (C), 117.96 (C), 118.37 (C), 121.04 (C), 123.26 (C), 123.51 (C), 126.72 (C), 128.23 (2C), 129.02 (2C), 130.71 (C), 131.74 (C), 135.88 (C), 136.03 (C), 136.32 (C), 136.48 (C), 142.52 (C)], 145.40 and 145.62 (N=CH, *trans/cis*), 154.03 (triazole C-3), 163.32 (triazole C-5), 168.21 (C=O); MS (ESI): m/z (%) 511 (M+1, 26), 533 (M+Na, 42), 229 (100), 129 (18).

2-{3-(4-Methylbenzyl)-4-[2-(1*H*-indol-3-yl)ethyl]-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(2-hydroxyphenylmethylene)acetohydrazide (5g): Yield 85%, mp 273-274 °C; *Anal.* Calcd. (%)

for: $C_{29}H_{28}N_6O_3$ C, 68.49, H, 5.55, N, 16.52, Found; C, 68.51, H, 5.58, N, 16.50; IR (KBr, ν, cm^{-1}): 3396 (OH), 3198 (NH), 1710 (triazole C=O), 1684 (hydrazide-C=O), 1618 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.25 (s, 3H, CH_3), 2.81 (t, 2H, tryp-CH_2, J=6.6 Hz), 3.40 (s, 2H, benzyl-CH_2), 3.66 (t, 2H, tryp-CH_2, J=6.6 Hz), 4.52 and 4.87 (s, 2H, CH_2, *trans and cis* conformers, *trans/cis* ratio 55/45), 6.82-7.10 (m, 9H, ar-H), 7.14-7.45 (m, 3H, ar-H), 7.77 (s, 1H, ar-H), 8.34 and 8.45 (s, 1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 54/46), 10.07 (s, 1H, OH), 10.97 (s, 1H, tryp-NH), 11.62 and 11.94 (s, 1H, NH, *trans and cis* conformers, *trans/cis* ratio 56/44); ^{13}C-NMR (DMSO-d_6)δ (ppm): 20.49 (CH_3), 23.80 (tryp-CH_2), 30.49 (benzyl-CH_2), 41.90 (tryp-CH_2), 46.43 and 48.34 (NCH_2, *trans/cis*), ar-C:[110.08 (C), 111.36 (C), 112.48 (C), 117.95(C), 118.37 (C), 119.25 (C), 121.01 (C), 123.25 (C), 126.15 (C), 126.72(C), 128.28 (2C), 129.00 (2C), 130.75 (C), 131.62 (C), 131.74 (C), 135.84 (C), 136.02 (C), 157.14 (C)], 145.28 and 147.34 (N=CH, *trans/cis*), 154.08 (triazole C-3), 163.35 (triazole C-5), 167.64 (C=O). MS (ESI): m/z (%) 509 (M+1, 48), 531 (M+Na, 100), 357 (28), 229 (100).

4-Amino-2-{3-(4-Nitrobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(2,6-dichloro-phenylmethylene)acetohydrazide (6a): Yield 76%, mp 256-257 °C; *Anal.* Calcd. (%) for: $C_{18}H_{15}N_7O_4Cl_2$: C, 46.57, H, 3.26, N, 21.12, Found; C, 46.53, H, 3.25, N, 21.10; IR (KBr, ν, cm^{-1}): 3290,3188 (NH+NH_2), 1720, 1707 (C=O), 1518 (C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.15 (s, benzyl-CH_2), 4.72 and 4.82 (s, 2H, NCH_2, *trans and cis* conformers, *trans/cis* ratio 75/25), 5.42 (s, 2H, NH_2), 7.24-7.38 (m, 2H, ar-H), 7.40-7.56 (m, 2H, ar-H), 7.78-8.12 (m, 3H, ar-H), 8.15 and 8.22 (s, 1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 78/22), 11.92 and 11.98 (s, 1H, NH, *trans and cis* conformers, *trans/cis* ratio 73/27);^{13}C-NMR (DMSO-d_6)δ (ppm): 32.16 (CH_2), 41.56 and 41.84 (CH_2, *trans/cis*), arC: [116.52 (2C), 118.18 (C), 124.27 (C), 127.19 (2C), 129.78 (2C), 131.95 (2C), 137.57 (C), 145.78 (C)], 144.80 and 144.34 (N=CH, *trans/cis*), 147.86 (triazole C-3), 148.54 (triazole C-5), 169.92 (C=O).

4-Amino-2-{3-(4-Nitrobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(2-chloro-4-fluorophenylmethylene)acetohydrazide (6b): Yield 85%, mp 269-270 °C; *Anal.* Calcd. (%) for: $C_{18}H_{15}N_7O_4ClF$: C, 48.28, H, 3.38, N, 21.89, Found; C, 48.25, H, 3.35, N, 21.88; IR (KBr, ν, cm^{-1}): 3292, 3197, 3107 (NH+NH_2), 1704 (C=O), 1520 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 4.10 (s, benzyl-CH_2), 4.81 and 4.98 (s, 2H, NCH_2, *trans and cis* conformers, *trans/cis* ratio 78/22), 5.39 (s, 2H, NH_2), 7.34-7.42 (m, 1H, ar-H), 7.44-7.60 (m, 3H, ar-H), 8.18-8.22 (m, 3H, ar-H), 8.26 and 8.34 (s, 1H, N=CH, *trans and cis* conformers, *trans/cis* ratio 65/35), 11.89 and 11.96 (s, 1H, NH, *trans and cis* conformers, *trans/cis* ratio 70/30);^{13}C-NMR (DMSO-d_6)δ (ppm): 32.06 (CH_2), 42.61 and 43.20 (CH_2, *trans/cis*), arC: [117.55 (2C), 118.15 (C), 125.27 (C), 128.13 (2C), 129.18 (2C), 131.92 (2C), 138.77 (C), 145.74 (C)], 146.14 and 147.12 (N=CH, *trans/cis*), 148.80 (triazole C-3), 149.24 (triazole C-5), 169.90 (C=O).

General method for the synthesis of compounds 7a-c

A mixture of corresponding compound **4** (10 mmol) and phenylisothiocyanate was refluxed in ethanol for 4 h. Then the solution was cooled to room temperature and a white solid appeared. This product was filtered and recrystallized from an appropriate solvent to obtain the desired compound.

2-{4-[2-(1*H*-Indol-3-yl)ethyl]-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(2-phenylethanethioyl)acetohydrazide (7a): Recrystallized from ethanol-water (1:2). Yield 90%, mp

172-173 °C; IR (KBr, ν, cm^{-1}): 3213, 3115 (NH), 1694 (C=O), 1593 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 2.83 (bs, 2H, CH_2), 3.46 (s, 2H, CH_2), 3.70 (bs, 2H, CH_2), 4.52 (s, 2H, CH_2), 6.96-7.21 (m, 5H, ar-H), 7.29-7.46 (m, 6H, ar-H), 7.52-7.55 (d, 3H, ar-H, J=7.4 Hz), 9.73 (s, 1H, NH), 9.91 (s, 1H, NH), 10.34 (s, 1H, NH), 10.95 (s, 1H, NH);^{13}C-NMR (DMSO-d_6)δ (ppm): 23.81 (tryp-CH_2), 30.07 (benzyl-CH_2), 42.01 (tryp-CH_2), 44.14 (NCH_2), ar-C:[110.04 (C), 111.45 (C), 115.19 (C), 116.68 (C), 117.48 (C), 118.53 (C), 120.95 (C), 121.13 (C), 123.36 (2C), 124.33 (2C), 126.77 (2C), 128.06 (2C), 129.08 (C), 131.56 (C), 136.08 (C), 138.89 (C)], 145.28 (triazole C-3), 153.45 (triazole C-5), 166.61 (C=O), 178.61 (C=S); MS (ESI): m/z (%) 560 (M^+, 54), 582 (58), 362 (40), 210 (40), 129 (100).

2-{4-[2-(1*H*-Indol-3-yl)ethyl]-3-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}-*N*'-(2-phenylethanethioyl)acetohydrazide (7b): Recrystallized from dimethyl sulfoxide-water (1:2). Yield 95%, mp 182-183 °C; IR (KBr, ν, cm^{-1}): 3272 (NH), 1697 (C=O), 1618 (C=N); ^{1}H-NMR (DMSO-d$_6$)δ (ppm): 2.24 (s, 3H, CH_3), 2.79 (bs, 2H, CH_2), 3.39 (s, 2H, CH_2), 3.64 (bs, 2H, CH_2), 4.53 (s, 2H, CH_2), 6.85-6.8 (m, 3H, ar-H), 6.94-7.21 (m, 5H, ar-H), 7.30-7.46 (m, 6H, ar-H), 9.69 (s, 1H, NH), 9.77 (s, 1H, NH), 10.34 (s, 1H, NH), 10.93 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 20.53 (CH_3), 23.78 (tryp-CH_2), 30.52 (benzyl-CH_2), 42.03 (tryp-CH_2), 46.19 (NCH_2), ar-C:[110.06 (C), 111.44 (C), 117.97 (C), 118.45 (C), 121.11 (C), 123.31 (C), 125.30 (C), 125.88 (C), 126.76 (2C), 128.05 (2C), 128.38 (2C), 129.07 (2C), 131.62 (C), 135.97 (C), 136.08 (C), 138.91 (C)], 145.68 (triazole C-3), 153.99 (triazole C-5), 166.73 (C=O), 180.22 (C=S); MS (ESI): m/z (%) 540 (M+1, 80), 562 (M+Na, 100), 427 (28), 146 (62), 129 (86).

2-[4-Amino-3-(4-nitrobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]-*N*'-(2-phenylethanethioyl)acetohydrazide (7c): Recrystallized from ethanol. Yield 67%, mp 139-140 °C; *Anal.* Calcd. (%) for: $C_{18}H_{18}N_8O_4S$: C, 48.86, H, 4.10, N, 25.33, S, 7.25, Found; C, 48.90, H, 4.08, N, 25.30, S, 7.22; IR (KBr, ν, cm^{-1}): 3208 (3NH+NH_2), 1702 (triazol-C=O), 1680 (exocyclic-C=O), 1598 (C=N), 1347 (C=S); ^{1}H-NMR (DMSO-d_6)δ (ppm): 4.07 (s, 2H, benzyl-CH_2), 4.49 (s, 2H, NCH_2), 5.37 (s, 2H, NH_2), 7.14-7.21 (m, 1H, ar-H), 7.30-7.44 (m, 4H, ar-H), 7.53-7.57 (m, 2H, ar-H), 8.16-8.20 (m, 2H, ar-H), 9.65 (s, 1H, NH), 9.73 (s, 1H, NH), 10.28 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 31.05 (CH_2), 46.72 (CH_2), arC: [118.39 (2C), 121.95 (C), 123.22 (2C), 130.85 (2C), 131.76 (2C), 144.32 (C), 145.48 (C), 148.47 (C)], 148.75 (triazole C-3), 155.54 (triazole C-5), 165.45 (C=O), 178.35 (C=S).

General method for the synthesis of compounds 8a-c

A mixture of the corresponding **7** (10 mmol) in cold concentrated sulfuric acid (28 mL) was stirred for 10 min. Then the mixture was allowed to cool to 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 and recrystallized from ethanol to afford the desired product.

4-[2-(1*H*-Indol-3-yl)ethyl]-5-(4-chlorobenzyl)-2-{[5-(phenylamino)-1,3,4-thiadiazol-2-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (8a): Recrystallized from acetone-water (1:2)] Yield 61%, mp 224-225 °C; IR (KBr, ν, cm^{-1}): 3274 (NH), 1695 (C=O), 1605 (C=N); ^{1}H-NMR (DMSO-d$_6$)δ (ppm): 2.84 (bs, 2H, CH_2), 3.46 (s, 2H, CH_2), 3.72 (bs, 2H, CH_2), 5.17 (s, 2H, CH_2), 6.94-7.10 (m, 6H, ar-H), 7.31-7.39 (m, 6H, ar-H), 7.58-7.62 (d, 2H, ar-H, J=8.2 Hz), 10.40 (s, 1H, NH), 10.93 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 23.75 (tryp-CH_2), 31.12 (benzyl-CH_2), 43.43 (tryp-CH_2), 54.40 (NCH_2), ar-C:[110.11 (C), 111.43

(C), 117.33 (C), 117.78 (C), 118.46 (C), 121.09 (C), 121.92 (C), 123.23 (C), 126.78 (2C), 128.37 (2C), 129.05 (2C), 130.47 (2C), 131.53 (C), 133.54 (C), 136.02 (C), 140.38 (C)], 145.96 (triazole C-3), 152.93 (triazole C-5), 154.48 (thiadiazole C-2), 165.39 (thiadiazole C-5); MS (ESI): m/z (%) 542 (M, 36), 564 (98), 357 (28), 229 (38), 144 (28).

4-[2-(1*H*-Indol-3-yl)ethyl]-5-(4-methylbenzyl)-2-{[5-(phenylamino)-1,3,4-thiadiazol-2-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (8b): Recrystallized from ethanol-water (1:2)] Yield 82%, mp 220-221 °C; IR (KBr, ν, cm^{-1}): 3291 (NH), 1697 (C=O), 1618 (C=N); ^{1}H-NMR (DMSO-d$_6$)δ (ppm): 2.27 (s, 3H, CH_3), 2.50 (bs, 2H, CH_2), 3.43 (s, 2H, CH_2), 3.47 (bs, 2H, CH_2), 4.90 (s, 2H, CH_2), 6.87-7.15 (m, 7H, arom-H), 7.28-7.46 (m, 7H, arom-H), 10.89 (s, 1H, NH), 13.99 (s, 1H, SH); ^{13}C-NMR (DMSO-d_6)δ (ppm): 20.45 (CH_3), 23.85 (tryp-CH_2), 30.22 (benzyl-CH_2), 44.45 (tryp-CH_2), 55.63 (NCH_2), ar-C:[110.14 (C), 111.43 (C), 117.67 (C), 117.83 (C), 118.16 (C), 119.09 (C), 121.95 (C), 123.23 (C), 126.65 (2C), 128.33 (2C), 129.23 (2C), 130.44 (2C), 131.55 (C), 133.54 (C), 136.12 (C), 139.38 (C)], 146.92 (triazole C-3), 153.93 (triazole C-5), 155.42 (thiadiazole C-2), 164.39 (thiadiazole C-5); MS (ESI): m/z (%) 522 (M+1, 62), 544 (M+Na, 100), 357 (28), 229 (40), 129 (54).

4-Amino-5-(4-nitrobenzyl)-2-{[5-(phenylamino)-1,3,4-thiadiazol-2-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (8c): Recrystallized from dimethyl sulfoxide-water (1:2). Yield 80%, mp 234-235 °C; *Anal.* Calcd. (%) for: $C_{18}H_{16}N_8O_3S$: C, 50.94, H, 3.80, N, 26.40, S, 7.55, Found; C, 50.90, H, 3.82, N, 26.43, S, 7.54; IR (KBr, ν, cm^{-1}): 3352-3137 (NH+NH_2), 1720 (C=O), 1570 (C=N), 1515 (C=N); ^{1}H-NMR (DMSO-d_6)δ (ppm): 4.07 (s, 2H, benzyl-CH_2), 5.14 (s, 2H, NCH_2), 5.38 (s, 2H, NH_2), 7.00 (t, 1H, ar-H, J= 7.2 Hz), 7.33 (t, 2H, ar-H, J=7.4 Hz), 7.53 (m, 4H, ar-H), 8.16 (d, 2H, ar-H, J=8.4 Hz), 10.36 (s, 1H, NH);^{13}C-NMR (DMSO-d_6) δ (ppm): 32.08 (CH_2), 45.83 (CH_2), arC: [119.39 (2C), 123.97 (C), 125.25 (2C), 130.85 (2C), 131.92 (2C), 142.65 (C), 145.47 (C), 148.34 (C)], 148.73 (triazole C-3), 154.51 (triazole C-5), 156.18 (thiadiazole C-2), 167.37 (thiadiazole C-5).

Antimicrobial activity

All test microorganisms were obtained from the Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC35218, *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC13883, *Yersinia pseudotuberculosis (Y. pseudotuberculosis*) ATCC911, *Enterobacter aerogenes* (*E. aerogenes*) ATCC13048, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC10145, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Bacillus cereus* (*B. cereus)* 709 Roma, *Candida tropicalis* (*C. tropicalis*) ATCC13803, *Candida glabrata* ATCC66032, and *Candida albicans* ATCC60193. All the newly synthesized compounds were weighed and dissolved in dimethylsulfoxide to prepare extract stock solution of 10,000 μg/mL.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (μg/mL) were determined.[34] The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI, USA) at pH 7.3 and buffered Yeast Nitrogen Base (Difco) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35 °C. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10 μg) and fluconazole (5 μg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide with a dilution of 1:10 was used as solvent control. The results are shown in the Table.

Table. Antimicrobial activity of the newly synthesized compounds (μg/mL).

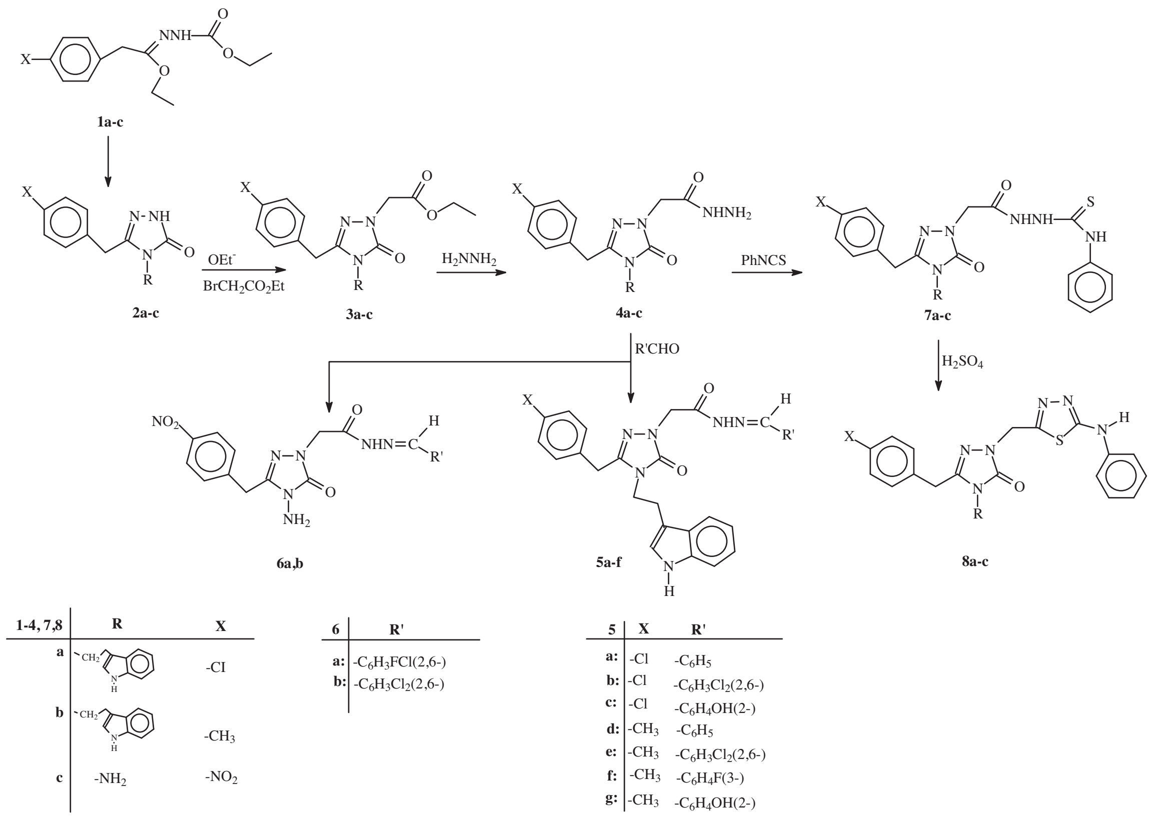
Compounds	Microorganisms and minimal inhibition concentration							
no.	Ec.	Kp.	Yp.	En.	Pa.	Sa.	Ef.	Bc.
2a	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
2b	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
2c	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
3a	250	> 500	> 500	> 500	> 500	62.5	62.5	250
3b	250	> 500	> 500	> 500	> 500	250	250	250
3c	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
4a	62.5	125	62.5	62.5	62.5	62.5	125	125
4b	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
4c	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
5a	125	125	> 500	> 500	> 500	> 500	> 500	> 500
5b	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
5c	125	250	> 500	> 500	> 500	> 500	> 500	> 500
5d	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
5e	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
5f	62,5	62.5	> 500	> 500	> 500	125	125	125
5g	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
6a	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
6b	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
7a	> 500	> 500	> 500	62.5	> 500	3.90	62.5	62.5
7b	62.5	> 500	> 500	> 500	> 500	62.5	62.5	62.5
7c	62.5	62.5	> 500	> 500	> 500	125	125	62.5
8a	125	> 500	> 500	> 500	> 500	125	125	125
8b	125	> 500	> 500	> 500	> 500	125	125	125
8c	> 500	> 500	> 500	> 500	> 500	125	125	125
Amp.	10	> 128	18	> 128	18	35	10	15

Ec.: *Escherichia coli* ATCC 35218, Kp.: *Klebsiella pneumoniae* ATCC 13883, Yp.: *Yersinia pseudotuberculosis* ATCC 911, En.: *Enterobacter aerogenes* ATCC 13048, Pa.: *Pseudomonas aeruginosa* ATCC 10145, Sa.: *Staphylococcus aureus* ATCC 25923, Ef.: *Enterococcus faecalis* ATCC 29212, Bc.: *Bacillus cereus* 709 Roma, Amp.: Ampicillin.

Results and discussion

The main aim of the present study was to synthesize and investigate the antimicrobial activity of new triazole-containing compounds.

Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Scheme 1. The starting compounds ethyl 2-(2-arylmethyl-1-ethoxyethylidene)hydrazinecarboxylates (**1a-c**) and 4-amino-5-(4-chlorobenzyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**2c**) were prepared following a previously reported literature procedure.[35] Ethoxycarbonylmethylation of 2,4-dihydro-3*H*-1,2,4-triazol-3-one derivatives (**2a-c**) with ethyl bromoacetate by refluxing in absolute ethanol in the presence of sodium ethoxide afforded the ethyl acetate derivatives (**3a-c**) in good yields. The ^{1}H- and ^{13}C-NMR spectra of compounds **3a-c** exhibited additional signals derived from the $-CH_2CO_2Et$ group at the related chemical shift values. The IR spectra of acid hydrazides (**4a-c**) showed an additional peak at 1665-1692 cm^{-1} due to exocyclic-carbonyl function derived from the ester structure beside the endocyclic-carbonyl peak at position 3 of the 1,2,4-triazole ring. Moreover, compounds **3a** and **3b** gave a stable M+1 ion peak.



Scheme 1. Synthetic pathway for the preparation of compounds **2-8**.

The ethoxy group on compounds **3a-c** is an easy leaving group for further nucleophilic substitution. The treatment of compounds **3a-c** resulted in the formation of hydrazide derivatives (**4a-c**) in good yields, which were employed as key intermediates for synthesis of the target compounds. The ^{1}H-NMR spectra of compounds **4a-c** displayed no signals belonging to the $-OCH_2CH_3$ group; instead, new signals derived from the hydrazide structure appeared at 4.22-4.33 ppm (-NH$\underline{NH_2}$) and 9.19-10.93 ppm (-$\underline{NH}$NH$_2$) integrating for 2 protons and 1 proton, respectively (controlled by changing with D_2O). Furthermore, compounds **4a** and **4b** gave a relatively stable M+1 ion peak and all compounds **4** gave reasonable elemental analysis data.

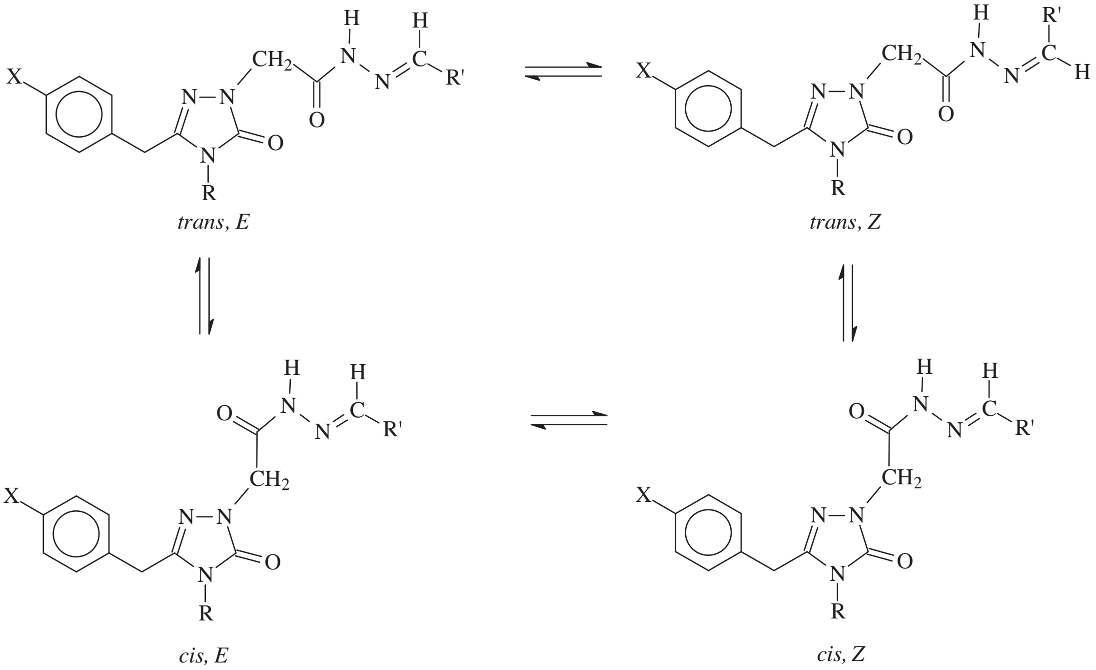
The condensation of acetohydrazides (**4a-c**) with several aromatic aldehydes in ethanol afforded the corresponding Schiff bases (**5a-f** and **6a,b**). In general, the 4-amino group on the 1,2,4-triazole ring is observed at about 5.30 ppm, while hydrazide $-NH_2$ is recorded at about 4.30 ppm.[36−38] In the ^{1}H-NMR spectra of compounds **6a** and **6b**, a signal at 5.39 (for **6a**) and 5.42 (for **6b**) was present. On the other hand, no signal was observed at about 4.30 ppm. This observation showed that only hydrazide $-NH_2$ of compound **4c** was reacted to aldehydes in the reaction conditions, although this compound includes 2 $-NH_2$ groups in the structure. Moreover, ^{1}H- and ^{13}C-NMR spectra of compounds **5a-f** and **6a,b** displayed the appearance of signals corresponding to arylidene moiety. Our literature survey revealed that compounds having the arylidene-hydrazide structure may exist as E/Z geometrical isomers about the C=N double bond and *cis/trans* amide conformers at the CO-NH single bond.[39−42] (Scheme 2). According to the literature,[39−42] compounds containing an imine bond are present in higher percentages in dimethyl-d_6 sulfoxide solution in the form of geometrical E isomer about the –C=N double bond. The Z isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In this respect, the ^{1}H-NMR spectra (in DMSO-d_6) of these Schiff bases confirmed their existence as E geometrical isomers, which exhibited ^{1}H-NMR data consistent with the literature findings for analogous compounds containing the imine functionality. On the other hand, further interpretation of their ^{1}H-NMR spectra revealed presence of 2 sets of signals at 8.02-8.30 and 8.21-8.41 ppm belonging to the imine bond of the *cis* and *trans* conformers, respectively. According to the literature, the upfield lines of the -N=CH proton were assigned to the *cis* conformer of the amide structure, while the downfield lines were attributed to the *trans* conformer of compounds **5** and **6** and the *trans/cis* conformer ratios in each case were calculated by using ^{1}H-NMR and ^{13}C-NMR data. When D_2O was added to the DMSO-d_6 solution of compounds **5** and **6**, the *trans/cis* ratio reversed. This change is evidence of the existence of *trans/cis* conformers, not E/Z geometrical isomers, since E/Z isomers are rigid structures.

The NH signal was observed as 2 sets due to *trans/cis* amide conformers at 11.60-11.62 ppm and 11.90-11.94 ppm for compounds **5c** and **5g**. Furthermore, compounds **5a-f** and **6a,b** gave mass spectra and elemental analysis data consistent with the assigned structures.

The synthesis of carbothioamide derivatives (**7a-c**) was performed from the reaction of **4a-c** with phenylisothiocyanate in ethanol. The structures of these compounds were confirmed using spectroscopic methods and elemental analysis.

Finally, the intramolecular cyclization of carbothioamides (**7a-c**) in acidic media produced 1,3,4-thiadiazol-2-yl]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-one derivatives (**8a-c**). The ^{1}H-NMR spectra of compounds **8a-c** exhibited a complete absence of NH signals relevant to a carbothioamide structure. Moreover, an obvious change in the chemical shifts between acyclic carbothioamide structure and 1,3,4-thiadiazole nucleus was observed in the ^{13}C-NMR spectra. As a comparison, the exocyclic -C=O and -C=S groups resonated at 165.45-166.73 and 178.35-180.22 ppm, respectively, in the ^{13}C-NMR spectra of compounds **7a-c**, whereas the corresponding carbons were recorded at a lower field, at 154.48-156.18 and 164.39-167.37 ppm, respectively, in the ^{13}C-NMR spectra of compounds **8a-c**. In addition, the absence of a -C=S stretching band in the IR spectra of compounds **8a-c** confirmed the conversion of the carbothioamide structure into a 1,3,4-thiadiazole ring. Furthermore, compounds **8a-c** gave satisfactory elemental analysis data and mass spectra (**8a** and **8b**).

The antimicrobial activity results presented in Table revealed that, among the tested compounds, compound **3a** exhibited activity against *S. aureus* and *E. faecalis*, while **4a**, which is the hydrazide derivative of



Scheme 2. *E/Z* geometrical isomers and *cis/trans* conformers of compounds **5a-g** and **6a,b**.

3a, displayed good activity against all the test microorganisms. Among the Schiff bases synthesized in the study, **5a, 5c,** and **5f,** which contain a 1*H*-indole ring bearing to position 4 of 1,2,4-triazol-3-one nucleus via an ethyl linkage and *N*'-phenyl-, 2-hydroxyphenyl- or 3-fluorophenylmethylene-acetohydrazide moiety in their structures, exhibited activity towards *E. coli* and *K. pneumoniae*; **5f** was found to be active towards *S. aureus*, *E. faecalis*, and *B. cereus* in addition to *E. coli* and *K. pneumoniae.* Compounds **7a-c**, possessing a carbothioamide structure, demonstrated good antimicrobial activity against *S. aureus*, *E. faecalis*, and *B. cereus.* In addition, good activity was exhibited by **7a** against *E. aerogenes*, by **7b** against *E. coli*, and by **7c** against *E. coli* and *K. pneumoniae*, as well. Among compounds **8a-c,** which were obtained from intramolecular cyclization of compounds **7a-c, 8a** and **8c** displayed moderate growth inhibition effects against *E. coli*, *S. aureus*, *E. faecalis*, and *B. cereus*, while **8b** was found to possess moderate activity against only gram-positive bacteria (*S. aureus*, *E. faecalis*, and *B. cereus*). As seen in the Table, some compounds showed antimicrobial activity against gram-positive and gram-negative bacteria, but no antifungal activity was observed against yeast-like fungi (data not shown).

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References

1. Ram, V. J. *J. Het. Chem.* **1998,** *2*, 253-256.
2. Yu, D.; Huiyuan, G. *Bioorg. Med. Chem. Lett.* **2002,** *12*, 857-859.
3. Bonde, C.; Gaikwad, N. J. *Bioorg. Med. Chem.* **2004,** *12*, 2151-2161.
4. Koca, M.; Servi, S.; Kirilmis, C.; Ahmedzade, M.; Kazaz, C.; Özbek, B.; Ötük, G. *Eur. J. Med. Chem.* **2005,** *40*, 1351-1358.
5. Sbardella, G.; Mai, A.; Artico, M.; Loddo, R.; Setzuc, M. G.; La Collac, P. *Bioorg. Med. Chem. Lett.* **2004,** *14*, 1537-1541.
6. Karalı, N.; Gürsoy, A.; Kandemirli, F.; Shvets, N., Kaynak, F. B.; Özbey, S.; Kovalishyn V.; Dimogloc, A. *Bioorg. Med. Chem.* **2007,** *15*, 5888-5904.
7. Leboho, T. C.; Michael, J. P.; van Otterlo, W. A. L.; van Vuuren, S. F.; de Koning, C. B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4948-4951.
8. Kutschy, P.; Mojmir, S.; Andreani, A.; Dzurilla, M.; Kovácik, V.; Alfödi J.; Rossi, M.; Gramatová, M. *Tetrahedron* **2002**, *58*, 9029-9039.
9. Ryu, C. K.; Lee, J. Y.; Park, R. E.; Ma, M. Y.; Nho, J. H. *Bioorg. Med. Chem. Lett.* **2007,** *17*, 127-131.
10. Samosorn, S.; Bremner, J. B.; Ball, A.; Lewis, K. *Bioorg. Med. Chem.* **2006,** *14*, 857-865.
11. Kaplancıklı, Z. A.; Turan-Zitouni, G.; Özdemir, A.; Revial, G. *Eur. J. Med. Chem.* **2008,** *43*, 155-159.
12. Küçükgüzel, S. G.; Rollas, S.; Erdeniz, H.; Kiraz, M. *Eur. J. Med. Chem.* **1999,** *34*, 153-160.
13. Yüksek, H.; Demirbaş, A.; Ikizler, A.; Johansson, C. B.; Çelik, C. Ikizler, A. A. *Arzn.-Forsh. Drug Res.* **1997,** *47*, 405-409.
14. Tozkoparan, B.; Gökhan, N.; Aktay, G.; Yeşilada, E.; Ertan, M. *Eur. J. Med. Chem.* **2000,** *35*, 743-750.
15. Ikizler, A. A.; Uzunali, E.; Demirbaş, A. *Indian J. Pharm. Sci.* **2000,** *5*, 289-292.
16. Demirbas, N.; Ugurluoglu, R.; Demirbas, A. *Bioorg. Med. Chem.* **2002,** *10*, 3717-3723.
17. Turan-Zitouni, G.; Sıvacı, M.; Kılıç, F. S.; Erol, K. *Eur. J. Med. Chem.* **2001,** *36*, 685-689.
18. Demirbas, A.; Johansson, C. B.; Duman, N.; Ikizler, A. *Acta Pol. Pharm.-Drug Res.* **1996,** *53*, 117-123.
19. Ikizler, A.; Demirbas, N.; Ikizler, A. A. *J. Het. Chem.* **1996,** *33*, 1765-1769.
20. Malbec, F.; Milcent, R.; Vicart ,P.; Bure, A. M. *J. Het. Chem.* **1984,** *21*, 1769-1774.
21. Zhang, J.; Chang, C-W. T. J. *Org. Chem.* **2009,** in press.
22. Goss, P. E. *Best Practice Res.* **2004,** *18*, 113-130.
23. Yu, L. T.; Ho, M. T.; Chang, C. Y. and Yang, T. K. *Tetrahedron: Asim.,* **2007**, *18*, 949-962.
24. Gupta, A.; Unadkat, J. D. and Mao, Q. *J. Pharm. Sci.* **2007**, *96*, 3226-3235.
25. Schiller, S. D. and Fung, H. B.; *Clinical Therapeutics* **2008**, *29*, 1862-1886.
26. Ashok, M.; Holla, B. S. and Poojary, B., *Eur. J. Med. Chem.* **2007**, *42*, 1095-1101.
27. Rao, B. M.; Sangaraju, S.; Srinivasu, M. K.; Madhavan, P.; Devi, M. L.; Kumar, P. R.; Candrasekhar, P.; Arpitha, Ch.; Balaji, T. S. *J. Pharm. Biomed. Anal.* **2006,** *41*, 1146-1151.
28. Hancu, G.; Gaspar, A.; Gyeresi, A. *J. Biochem. Biophys. Methods* **2007,** *69*, 251-259.

29. Bajetti, E.; Zilembo, N.; Bichisao, E.; Pozzi, P.; Toffolatti, T. *Critical Reviews in Oncology: Hematology* **2000,** *33*, 137-142.
30. Holla, B. S.; Shivananda, M. K.; Shenoy, M. S.; Antony, G. *Il Farmaco* **1998,** *53*, 531-535.
31. Holla, B. S.; Mahalinga, M.; Karthikeyan, M. S.; Poojary, B.; Akberali, P. M.; Kumari, N. S. *Eur. J. Med. Chem.* **2005,** *40*, 1173-1178.
32. Holla, B. S.; Rao, B. S.; Shridhara, K.; Akberali, P. M. *Il Farmaco* **2000,** *55*, 338-344.
33. Holla, B. S.; Veerendra, M. K. Shivananda, B. Poojary, B. *Eur. J. Med. Chem.* **2003,** *38*, 759-767.
34. Ashok, M.; Holla, B. S.; Poojary, B. *Eur. J. Med. Chem.* **2007,** *42*, 1095-1101.
35. National Committee for Clinical Laboratory Standards **1993.** NCCLS Document M7-A3, *13*, (25), Willanova, PA., USA.
36. Milcent, R.; Redeuilh, C. *J. Het. Chem.* **1979,** *16*, 403-407.
37. Demirbas, N.; Demirbas, A., Karaoglu, S. A.; Çelik, E. *Arkivoc* **2005,** (*i*), 75-91.
38. Bayrak, H.; Demirbas, A.; Demirbas, N.; Alpay-Karaoglu, S. *Eur. J. Med. Chem.* **2009,** *44*, 1057-1066.
39. Demirbas, A.; Sahin, D.; Demirbas, N.; Alpay-Karaoglu, S. *Eur. J. Med. Chem.* **2009**, *44*, 2896-2903.
40. Demirbas, N.; Karaoglu, S. A.; Demirbas, A.; Sancak, K. *Eur. J. Med. Chem.* **2004,** *39*, 793-804.
41. Demirbaş, A. *Turk. J. Chem.* **2004,** *28*, 311-323.
42. Galic, N.; Peric, B.; Kojic-Prodic, B.; Cimerman, Z. *J. Mol. Stuc.* **2001,** *559*, 187-194.
43. Wyrzykiewicz, E.; Prukah, D. *J. Het. Chem.* **1998,** *35*, 381-387.