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Synthesis and Antimicrobial Activities of Some New 1,2,4-Triazole Derivatives

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A series of acylhydrazones (**2a-f**) were synthesized by the condensation of iminoester hydrochlorides (**1a-f**) with acyl hydrazines. 2,5-Dialkyl 1,3,4-oxadiazoles (**3a**,**c**,**e**) were obtained in the same reaction media. The treatment of acylhydrazones with hydrazine hydrate afforded 4-amino-3,5-dialkyl-1,2,4-triazoles **(4a-c).** The acetylation of 4-amino-3,5-dialkyl-1,2,4-triazoles produced *N*-[3,5-dialkyl-4*H*-1,2,4-triazol-4-yl] acetamides (**5-7**). The treatment of compounds **4a and 4c** with various aromatic aldehydes resulted in the formation of 4-arylidenamino-3,5-dialkyl-1,2,4-triazoles (**8a**,**d**, **e**, and **10a-e**). Sodium borohydride reduction of 4-arylidenamino derivatives afforded 4-alkylamino-3,5-dialkyl-1,2,4-triazoles (**9a**,**d**, **e**, and **11a-e**).

Compounds **4b**, **4c**, **7**, **8d**, and **11c** showed good antifungal activity only against yeast-like fungi, while compounds **3e**, **6**, **8e**, and **9e** showed antimicrobial activity against bacteria and yeast-like fungi.

Key Words: Acyl hydrazone, 1,2,4-triazole, 1,3,4-oxadiazole, Schiff Base, reduction, acetylation, antimicrobial activity.

Introduction

There is an increasing demand for the preparation of new antimicrobial agents due to the developing resistance towards conventional antibiotics.[1−3] The synthesis of 1,2,4-triazole derivatives has attracted widespread attention due to their diverse biological activities, including antimicrobial, anti-inflammatory, analgesic, and antitumoral.[4−11] Therefore, we have synthesized some 1,2,4-triazole derivatives possessing antimicrobial activity.[9−11]

Small molecules are suitable as precursors for the preparation of novel compounds that can possess some biological properties. For instance, iminoester hydrochlorides (**1**) have been used intensively as starting material for the preparation of 1,2,4-triazole derivatives in our laboratories.[12−15] It is known that compounds **1** can react easily with the compounds bearing an amino group to form 1,2,4-triazole or 1,3,4-thiadiazole derivatives. In addition to this, the treatment of iminoester hydrochlorides with hydrazide-type compounds produces hydrazones, which are useful intermediates for further ring closure.[14−18]

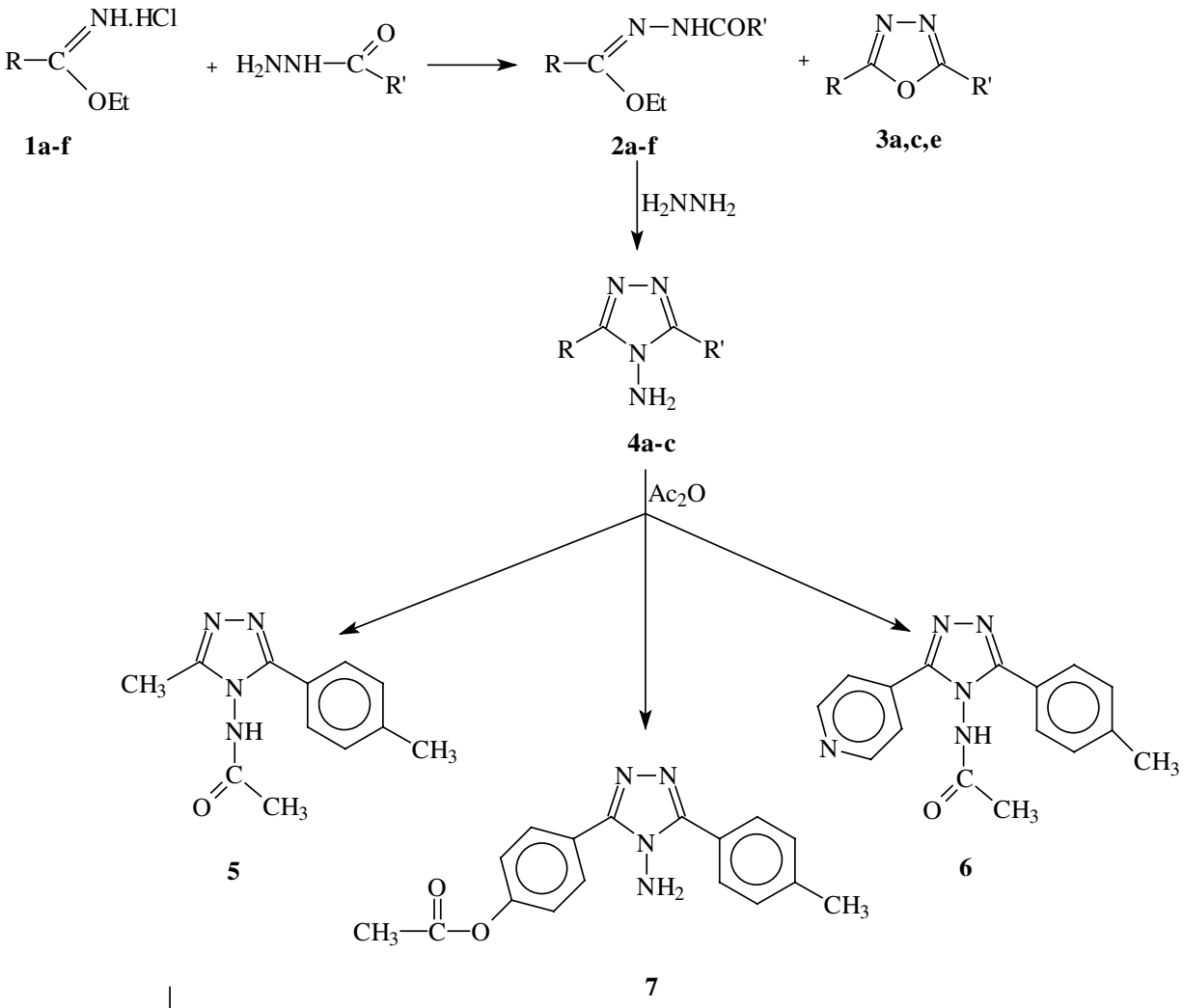
In view of these facts, we aimed to prepare novel 1,2,4-triazole derivatives with probable antimicrobial activity.

Results and Discussion

Compounds **2a-f** were synthesized from the reaction of corresponding iminoester hydrochlorides, which were obtained by a published method,[19] with various acyl hydrazines (acetyl hydrazine, 4-hydroxybenzoyl hydrazine, 4-chlorobenzoyl hydrazine, and isonicotinoyl hydrazine) and their structures were established by IR, ^{1}H-NMR, and ^{13}C-NMR techniques (Scheme 1). The formed acylhydrazones (**2**) underwent intramolecular cyclization partially; thus, 1,3,4-oxadiazoles (**3**) formed in the same reaction media. Compounds **3a-c**, and **e** gave the spectral data consistent with their structures. Compounds **4a-c** were obtained by treatment of compounds **2a-c** with hydrazine hydrate. The reaction was carried out in 1-propanol at refluxing temperature for 24 h and the desired 4-amino-3,5-dialkyl-1,2,4-triazoles (**4a-c**) were yielded. 4-Amino-1,2,4-triazoles (**4a-c**) were converted to their Schiff bases (**8a**,**d**, **e**, and **10a-e**) by refluxing with various aromatic aldehydes (4-chlorobenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 4-methyoxybenzaldehyde, 2,4,6-trimethoxybenzaldehyde, and 2-hydroxy1-naphthaldehyde) in acetic acid (Scheme 1). Previously, we obtained the Schiff bases of 1,2,4-triazole derivatives as antitumoral agents.[9,10−12,22−24] The synthesis of compounds **9** and **11** was performed by the reduction of only the exocyclic azomethine bond of the Schiff bases (**8** and **10)** (Scheme 2). These reduction reactions were conducted in considerably milder conditions (in methanol for 1 h) than those we studied earlier.[18−20] It was reported that Schiff bases can be obtained as their *E* and *Z*- geometrical isomers about the -C=N double bond.[25−28] The ratio of *E* isomer is generally higher than that of the other isomer, and polar solvents, such as dimethyl sulfoxide, augment the ratio of *E* isomer.[25,26]

When compounds **4a-c** were treated with acetic anhydride at refluxing temperature, compounds **4a** and **4c** produced N-acetylated derivatives (**5** and **6**), while compound **4b** gave O-acetylated product (**7**). The geometrical optimizations of compounds **4a** and **4b** were achieved by computer using the AM1 method and the most stable conformations were determined (Scheme 3). According to these results, either the -NH_2 group or -OH group on compounds **4a** and **4b** can be the nucleophilic center for an acetylation. On the other hand, the theoretical calculations showed that the distance between the -NH_2 and p-tolyl groups on the 1,2,4-triazole ring is 2.7 Å, while the distance between the -NH_2 and p-hydroxyphenyl groups is 2.98 Å. These results showed that the -NH_2group on compound **4b** was hindered by the p-tolyl and p-hydroxyphenyl groups located on position 3 and 5 of the 1,2,4-triazole ring. For this reason, acetylation took place only at the -OH group on compound **4b**. According to the theoretical measurements, the distance between the -NH_2 and the methyl groups on compound **4a** is 3.2 Å and this distance is sufficient for an acetylation at the -NH_2 group of these compounds.

Compounds **4b**, **4c**, **7**, **8d**, and **11c** showed good antifungal activity only against yeast-like fungi, while compounds **3e**, **6**, **8e**, and **9e** showed antimicrobial activity against bacteria and yeast-like fungi. Compounds **8a**, **10d**, and **11d** were only effective on the gram-positive bacteria, *S. aureus* ATCC 25923. Compound **11b** was effective on *P. aeruginosa* ATCC 10145. Compound **9a** was effective on both *P. aeruginosa* ATCC 10145 and *S. aureus* ATCC 25923.The best activity was observed against *Candida albicans* ATCC 60193 by compound **8e**.



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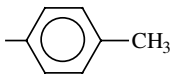
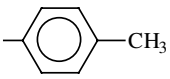
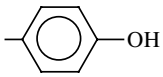
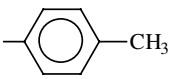
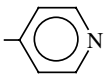
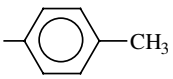
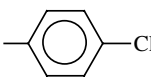
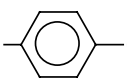
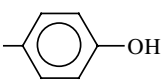
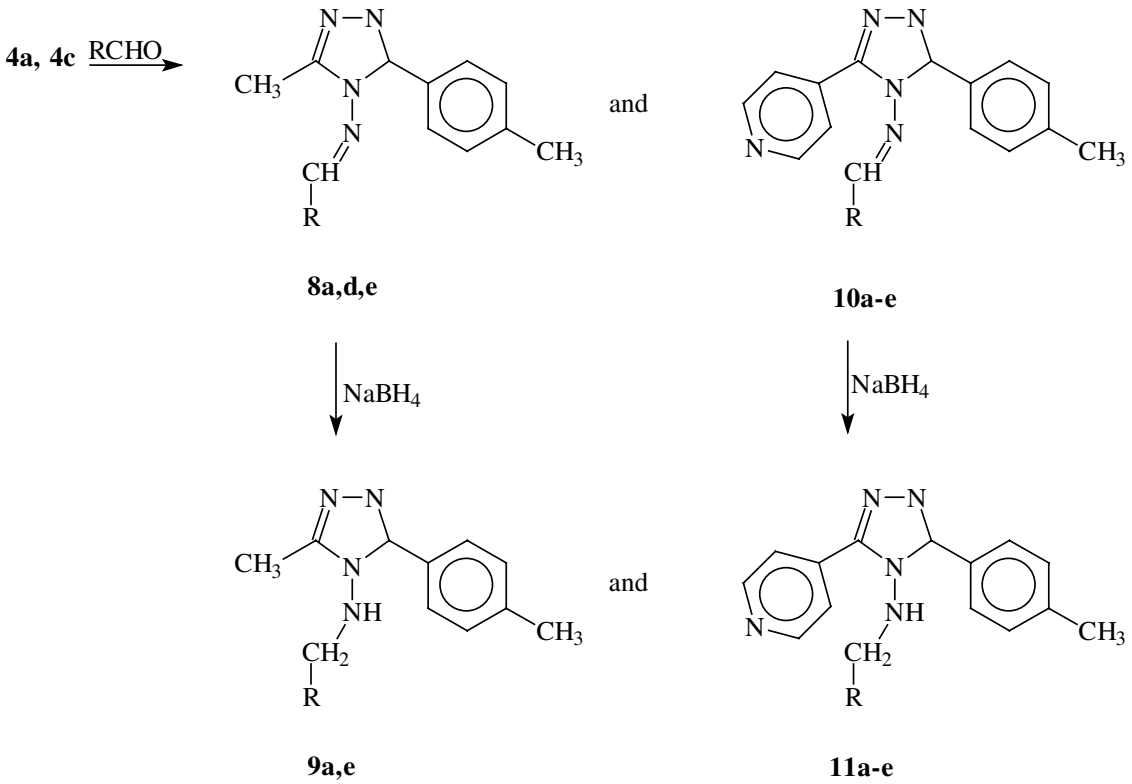
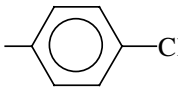
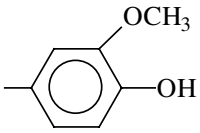
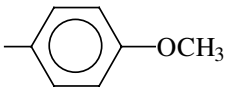
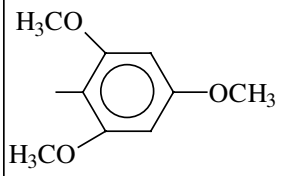
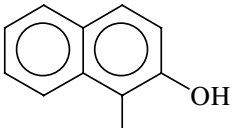
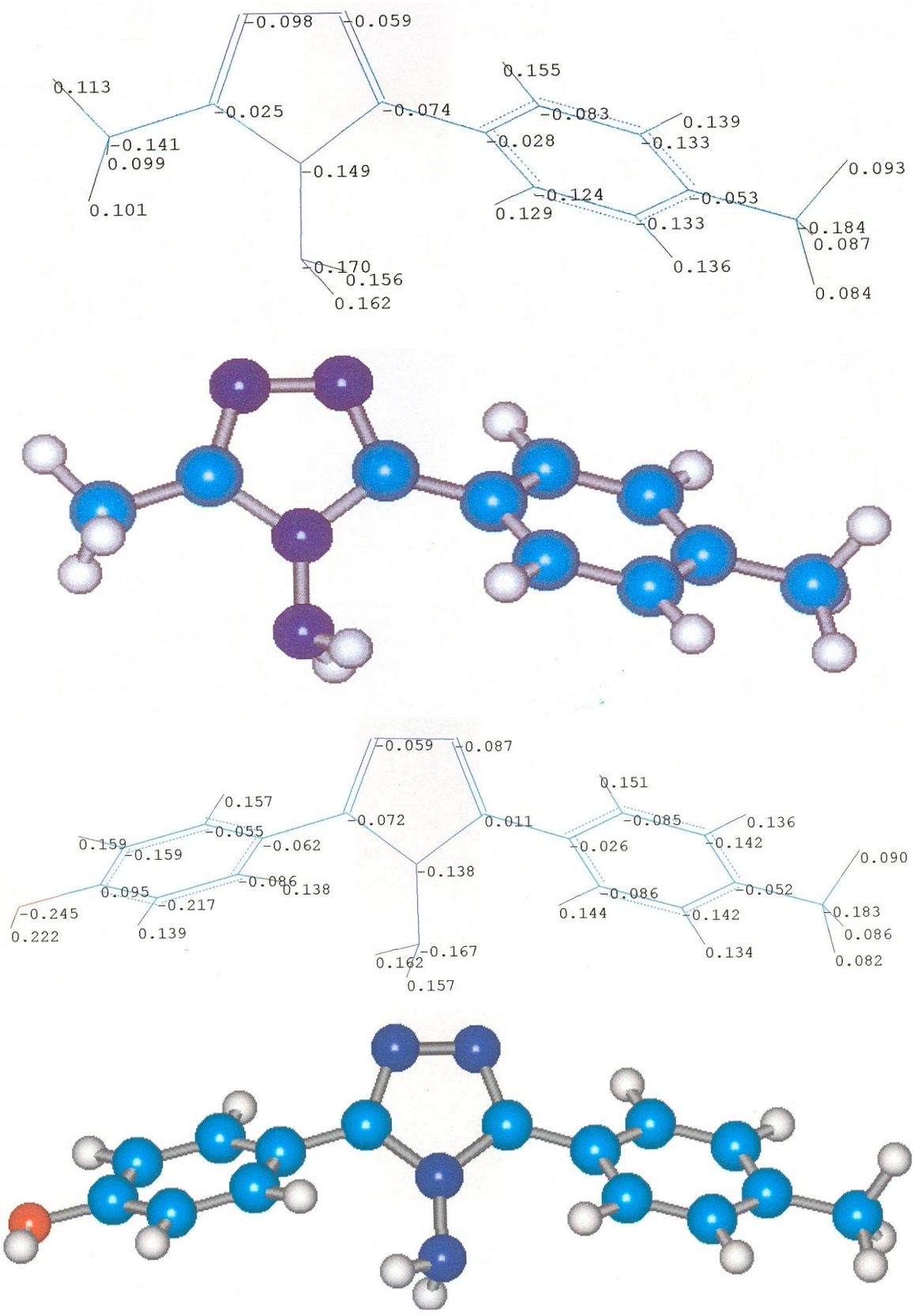
a		
b		
c		
d		
e		
f		

Scheme 1. Synthetic pathway for preparation of compounds **1-7**.



8-11	R
a:	
b:	
c:	
d:	
e:	

Scheme 2. Synthetic pathway for preparation of compounds **8-11**.



Scheme 3. Geometric optimizations and charge density of compounds **4a** and **4b**.

Experimental

Melting points were determined on a Gallenkamp 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. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compounds **1a-f** were synthesized using a published method.[30]

General method for the synthesis of ethyl carboxylate acylhydrazones (2a-f) and 2,5-dialkyl 1,3,4-oxadiazoles (3a,c,e)

To the solution of corresponding iminoester hydrochloride (**1**) in absolute ethanol (10 mmol) was added the solution of corresponding acyl hydrazine (10 mmol) in absolute ethanol and the mixture was stirred at 0-5 °C for 6 h. Then, the precipitated ammonium chloride was filtered off. After evaporating the solvent at 35-40 °C under reduced pressure, a white solid was obtained. This crude product was recrystallized from petroleum ether to afford compounds **2**. The part that did not dissolve in petroleum ether was recrystallized from ethanol; thus, compounds **3** were obtained.

Ethyl *N*-acetyl-4-methylbenzenecarbohydrazonoate (2a): (Yield: 1.10 g, 50%). mp 102-103 °C; IR (KBr) cm^{-1}: 3205 (ν_{NH}), 1669 ($\nu_{C=O}$), 1618 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$)δ ppm 1.30 (t, 3H, -CH$_3$, J = 6.96 Hz), 2.00 (s, 3H, -COCH$_3$), 2.08 (s, 3H, -CH$_3$), 4.15 (q, 2H, -O<u>CH$_2$</u>, J = 6.96 Hz), 7.54 (d, 2H, ar-H, J = 7.80 Hz), 7.88 (d, 2H, ar-H, J = 7.80 Hz), 10.60 (s, 1H, -NH); ^{13}C-NMR (DMSO-d$_6$)δ ppm 167.00 (C=O), 161.00 (C=N), ar-C: [138.00 (C), 129.00 (2CH), 128.05 (2CH), 125.00 (C)], 62.00 (OCH$_2$), 23.00 (ar-CH$_3$), 18.10 (O=C-<u>CH$_3$</u>), 13.00 (CH$_3$).

Ethyl *N*-(4-hydroxybenzoyl)-4-methylbenzencarbohydrazonoate (2b): (Yield: 1.79 g, 60%). mp 148-149 °C. IR (KBr) cm^{-1}: 3223 (ν_{NH}), 3200 (ν_{OH}), 1663 ($\nu_{C=O}$), 1609 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$)δ ppm 1.20 (t, 3H, -CH$_3$, J = 6.96 Hz), 2.38 (s, 3H, -CH$_3$), 4.30 (q, 2H, -OCH$_2$, J = 6.96 Hz), 6.90 (d, 2H, ar-H, J = 7.80 Hz), 7.36 (d, 2H, ar-H, J = 8.55 Hz), 7.82 (d, 2H, ar-H, J = 8.55 Hz), 7.95 (d, 2H, ar-H, J = 7.80 Hz), 9.95 (s, 1H, -NH), 10.15 (s, 1H, -OH); ^{13}C-NMR (DMSO-d$_6$)δ ppm 170.05 (C=O), 167.00 (C=N), ar-C: [158.36 (C), 139.00 (C), 129.91 (2CH), 128.86 (2CH), 127.99 (2CH), 124.33 (C) , 117.84 (C), 114.65 (2CH))], 63.00 (OCH$_2$), 24.72 (ar-CH$_3$), 20.94 (CH$_3$).

Ethyl *N*-isonicotinoyl-4-methylbenzencarbohydrazonoate (2c): (Yield: 1.50 g, 53%). mp 96-97 °C. IR (KBr) cm^{-1}: 3346 (ν_{NH}), 1665 ($\nu_{C=O}$), 1620 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$) δ ppm 1.30 (t, 3H, -CH$_3$, J = 6.96 Hz), 2.39 (s, 3H, -CH$_3$), 4.28 (q, 2H, -OCH$_2$, J = 6.96 Hz), 7.36 (d, 2H, ar-H, J = 7.80 Hz), 7.95 (d, 2H, ar-H, J = 7.80 Hz), 8.10 (bs, 2H, ar-H) 8.80 (bs, 2H, ar-H), 10.85 (s, 1H, -NH); ^{13}C-NMR (DMSO-d$_6$)δ ppm 169.96 (C=O), 163.00 (C=N), ar-C: [150.02 (2CH), 139.65 (C), 134.66 (C) , 129.18 (2CH)), 128.11 (2CH)), 124.00 (C), 122.04 (2CH)], 63.28 (OCH$_2$), 21.13 (ar-CH$_3$), 13.00 (CH$_3$).

Ethyl *N*-(4-chlorobenzoyl)-4-methylbenzencarbohydrazonoate (2d): (Yield: 1.81 g, 57%). mp 125-126 °C. IR (KBr) cm^{-1}: 3225 (ν_{NH}), 1687 ($\nu_{C=O}$), 1598 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$) δ ppm 1.25 (t, 3H, -CH$_3$, J = 6.96 Hz), 1.96 (s, 3H, -CH$_3$), 4.18 (q, 2H, -OCH$_2$, J = 6.96 Hz), 7.20 (d, 2H, ar-H, J = 7.80 Hz), 7.50 (bs, 2H, ar-H), 7.70 (d, 2H, ar-H, J = 7.20 Hz), 7.85 (d, 2H, ar-H, J = 7.20 Hz), 10.58 (s, 1H, -NH); ^{13}C-NMR (DMSO-d$_6$)δ ppm 167.02 (C=O), 161.00 (C=N), ar-C: [139.86(C), 135.64(C), 132.18(C),

131.06 (2CH), 130.44 (2CH), 128.18 (2CH), 128.00 (2CH), 126.94 (C)], 61.96 (OCH_2), 16.00 (ar-CH_3), 14.05 (CH_3).

Ethyl *N*-(4-chlorobenzoyl)ethanhydrazonoate (2e): (Yield: 1.06 g, 44%). mp 129-130 °C. IR (KBr) cm^{-1}: 3279 (ν_{NH}), 1662 ($\nu_{C=O}$), 1625 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 1.30 (t, 3H, CH_3, -J = 6.96 Hz), 1.95 (s, 3H, N=C-CH_3), 4.14 (q, 2H, O-CH_2, J = 6.96 Hz), 7.55 (d, 2H, ar-H, J = 7.20 Hz), 7.85 (d, 2H, ar-H, J = 7.20 Hz), 10.59 (s, 1H, -NH); ^{13}C-NMR (DMSO-d_6)δ ppm 166.84 (C=O), 161.00 (C=N), ar-C: [135.39 (C), 132.27 (C), 128.75 (2CH), 127.92 (2CH)], 61.56 (OCH_2), 15.22 (N=C-$\underline{CH}_3$), 13.69 (CH_3).

Ethyl *N*-(4-hydroxybenzoyl)ethanhydrazonoate (2f): (Yield: 1.11 g, 50%). mp 153-154 °C. IR (KBr) cm^{-1}: 3261 (ν_{NH}), 3200 (ν_{OH}), 1636 ($\nu_{C=O}$), 1606 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6) δ ppm 1.25 (t, 3H, -CH_3, J = 6.96 Hz), 1.96 (s, 3H, N=C-CH_3), 4.22 (q, 2H, O-CH_2, J = 6.96 Hz), 6.80 (d, 2H, ar-H, J = 8.55 Hz), 7.70 (d, 2H, ar-H, J = 8.55 Hz), 10.02 (s, 1H, -NH) 10.18 (s, 1H, -OH); ^{13}C-NMR (DMSO-d_6)δ ppm 170.02 (C=O), 162.94 (C=N), ar-C: [160.02 (C), 129.56 (2CH), 124.00 (C), 115.11 (2CH)], 61.93 (OCH_2), 24.18 (N=C-$\underline{CH}_3$), 17.38 (CH_3).

2-Methyl-5-(4-tolyl)-1,3,4-oxadiazole (3a): (Yield: 0.61 g, 35%). mp 134-135 °C (white crystals); IR (KBr) cm^{-1}: 1594 and 1577($\nu_{2C=N}$); ^{1}H-NMR ($CDCl_3$)δ ppm 2.40 (s, 3H, -CH_3), 2.60 (s, 3H, N=C-CH_3), 7.30 (d, 2H, ar-H,J = 7.80 Hz), 7.90 (d, 2H, ar-H, J = 7.80 Hz); ^{13}C-NMR ($CDCl_3$)δ ppm 164.00 (triazole C_3), 163.00 (triazole C_5), ar-C: [139.00 (C), 129.71 (2CH), 128.00 (2CH), 125.00 (C)], 21.61 (ar-CH_3), 10.01 (CH_3).

2-Pyridin-4-yl-5-(4-tolyl)-1,3,4-oxadiazole (3c): (Yield: 0.83 g, 35%). mp 151-152 °C (white crystals), (ref.[29]mp 153 °C); IR (KBr) cm^{-1} 1606 and 1536 ($\nu_{2C=N}$).

2-Methyl-5-(4-chlorophenyl)-1,3,4-oxadiazole (3e): (Yield: 0.82 g, 42%). mp 143-144 °C (white crystals); IR (KBr) cm^{-1}: 1601 and 1577 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.38 (s, 3H, N=C-CH_3), 7.55 (d, 2H, ar-H, J = 7.20 Hz), 7.85 (d, 2H, ar-H, J = 7.20 Hz); ^{13}C-NMR (DMSO-d_6)δ ppm 163.86 (triazole C_3), 161.12 (triazole C_5), ar-C: [135.92 (C), 132.08 (C), 129.46 (2CH), 128.14 (2CH)], 10.00 (CH_3).

General method for the synthesis of 4-amino-3,5-dialkyl-1,2,4-triazoles (4a-c)

The corresponding compound **2** (10 mmol) was refluxed with hydrazine hydrate (25 mmol) in propanol for 24 h. Then, the reaction mixture was cooled to room temperature and a solid was obtained. This crude product was filtered off, washed with benzene 4 times, and recrystallized from an appropriate solvent to afford the desired compound.

4-Amino-3-methyl-5-(4-tolyl)-4*H*-1,2,4-triazole (4a): White crystals from ethyl acetate (yield: 1.64 g, 87%). mp 215-216 °C. IR (KBr) cm^{-1}: 3245-3142 (ν_{NH2}), 1652 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.38 ($2CH_3$), 6.05 (s, 2H, -NH_2), 7.30 (d, 2H, ar-H, J = 7.80 Hz), 7.92 (d, 2H, ar-H, J = 7.80 Hz); ^{13}C-NMR (DMSO-d_6)δ ppm 153.06 (triazole C_3), 152.10 (triazole C_5), ar-C: [138.67 (C), 128.81 (2CH), 127.57 (2CH), 124.71 (C)], 20.83 (ar-CH_3), 9.80 (CH_3).

4-Amino-3-(4-hydroxyphenyl)-5-(4-tolyl)-4*H*-1,2,4-triazole (4b): White crystals from ethyl acetate (yield: 2.42 g, 91%). mp 283-284 °C; IR (KBr) cm^{-1}: 3318-3275 (ν_{NH2}), 3200 (ν_{OH}), 1619 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.38 (s, 3H, ar-$\underline{CH}_3$), 6.16 (s, 2H, NH_2), 6.94 (d, 2H, ar-H, J = 7.80 Hz), 7.40 (d, 2H, ar-H, J = 8.55 Hz), 7.85 (d, 2H, ar-H, J = 7.80 Hz), 8.00 (d, 2H, ar-H, J = 8.55 Hz), 9.91 (s,

1H,-OH); ^{13}C-NMR (DMSO-d_6)δ ppm, 154.00 (triazole C_3), 153.45 (triazole C_5), ar-C: [158.50 (C), 138.86 (C), 129.68 (2CH), 128.85 (2CH), 127.95 (2CH), 124.47 (C), 117.86 (C), 115.07 (2CH)], 20.83 (ar-CH_3).

4-Amino-3-(pyridin-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (4c): White crystals from 1-propanol (yield: 1.56 g, 62%). mp 265-266 °C; IR (KBr) cm^{-1}: 3321-3192 (ν_{NH2}), 1602 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.39 (s, 3H, ar-CH_3), 6.37 (s, 2H, -NH_2), 7.40 (d, 2H, ar-H, J = 7.80 Hz), 7.92 (d, 2H, ar-H, J = 7.80 Hz), 8.06 (d, 2H, ar-H, J = 6.10 Hz), 8.75 (bs, 2H, ar-H); ^{13}C-NMR (DMSO-d_6)δ ppm 155.00 (triazole C_3), 152.00 (triazole C_5), ar-C: [149.93 (2CH), 138.94 (C), 134.28 (C), 129.02 (2CH), 128.16 (2CH), 124.10 (C), 121.81 (2CH)], 20.96 (ar-CH_3).

General method for the synthesis of compounds 5-7

The corresponding compound **4** (10 mmol) was refluxed with acetic anhydride (10 mL) for 1 h, protected from moisture. After cooling the reaction mixture to room temperature, 40 mL of ethanol was added, followed by refluxing for an additional 30 min. Then, the excess of acetic anhydride was removed under reduced pressure at 55-60 °C and the obtained solid was recrystallized from acetone-petroleum ether (v/v: 1:2) to afford the desired compound.

4-Acetylamino-3-methyl-5-(4-tolyl)-4*H*-1,2,4-triazole (5): (Yield: 1.36 g, 59%). mp 235-236 °C (white crystals); IR (KBr) cm^{-1}: 3104 (ν_{NH}), 1710 ($\nu_{C=O}$), 1617($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.18 (s, 3H, -CH_3), 2.37 (s, 3H, -N=C-CH_3), 2.49 (s, 3H, -CH_3), 7.50 (d, 2H, ar-H, J = 7.80 Hz), 7.80 (d, 2H, ar-H, J = 7.80 Hz), 11.70 (s, 1H, -NH)]; ^{13}C-NMR (DMSO-d_6) δ ppm 168.57 (C=O), 152.05 (triazole C_3), 152.00 (triazole C_5), ar-C: [139.69 (C), 129.35 (2CH), 126.87 (2CH), 123.24 (C)], 20.83 (ar-CH_3), 20.31 (O=C-$\underline{CH_3}$), 9.24 (N=C-$\underline{CH_3}$).

4-Acetylamino-3-pyridin-4-yl-5-(4-tolyl)-4*H*-1,2,4-triazole (6): (Yield: 2.14 g, 73%). mp 230-231 °C (white crystals); IR (KBr) cm^{-1}: 3290 (ν_{NH}), 1719 ($\nu_{C=O}$), 1604 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.02 (s, 3H, CH_3), 2.40 (s, 3H, -CH_3), 7.40 (d, 2H, ar-H, J = 7.80 Hz), 7.73 (d, 2H, ar-H, J = 7.80 Hz), 7.84 (d, 2H, ar-H, J = 6.10 Hz), 8.83 (bs, 2H, ar-H), 12.10 (bs, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ ppm 168.68 (C=O), 154.59 (triazole C_3), 151.56 (triazole C_5), ar-C: [150.52 (2CH), 140.54 (C), 133.05 (C), 129.57 (2CH), 127.44 (2CH), 122.45 (C), 121.12 (2CH)], 20.92 (ar-CH_3), 20.38 (O=C-$\underline{CH_3}$).

4-Amino-3-(4-acetoxyphenyl)-5-(4-tolyl)-4*H*-1,2,4-triazole (7): (Yield: 2.09 g, 68%). mp 208-209 °C (white crystals); IR (KBr) cm^{-1}: 3225-3108 (ν_{NH2}), 1716 ($\nu_{C=O}$), 1611($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.04 (s, 3H, -CH_3), 2.45 (s, 3H, -CH_3), 6.40 (s, 2H, N-NH_2), 7.02 (d, 2H, ar-H, J = 7.80 Hz), 7.48 (d, 2H, ar-H, J = 7.80 Hz), 7.90-8.00 (m, 2H, ar-H), 8.00-8.10 (m, 2H, ar-H); ^{13}C-NMR (DMSO-d_6)δ ppm 170.02 (C=O), 154.10 (triazole C_3), 154.00 (triazole C_5), ar-C: [158.21 (C), 139.06 (C), 129.87 (2CH), 128.73 (2CH), 128.00 (2CH), 124.32 (C), 117.98 (C), 114.93 (2CH)], 20.86 (O=C-$\underline{CH_3}$), 17.32 (ar-CH_3).

General method for the synthesis of compounds (8a,d,e and 10a,e)

The solution of corresponding compound **4** (10 mmol) in acetic acid was refluxed with an aromatic aldehyde for 4 h. Then, the reaction mixture was poured into ice-water under stirring. The precipitated product was filtered off and washed with water. The obtained white solid was recrystallized from ethanol (for compounds

8) or ethyl acetate (for compounds **10**).

4-(4-Chlorophenylmethylenamino)-3-methyl-5-(4-tolyl)-4*H*-1,2,4-triazole (8a): (Yield: 2.49 g, 80%); mp 118-119 °C (white crystals); IR (KBr) cm^{-1}: 1612 and 1595 ($\nu_{2C=N}$); ^{1}H-NMR ($CDCl_3$)δ ppm 2.38 (s, 3H, CH_3), 2.56 (s, 3H, ar-CH_3), 7.20 (d, 2H, ar-H, J = 7.80 Hz), 7.45 (d, 2H, ar-H, J = 7.80 Hz), 7.64 (d, 2H, ar-H, J = 8.60 Hz) 7.80 (d, 2H, ar-H, J =8.60 Hz), 8.30 (s, 1H, N=CH); ^{13}C-NMR ($CDCl_3$)δ ppm 164.19 (N=CH), 150.00 (triazole C_3), 149.10 (triazole C_5), ar-C: [140.00 (C), 139.14 (C), 131.00 (2CH), 130.07 (4CH), 129.58 (2CH), 124.00 (C), 123.98 (C)], 21.43 (ar-CH_3), 11.20 (CH_3).

3-Methyl-4-(2,4,6-trimethoxyphenylmethylenamino)-5-(4-tolyl)-4*H*-1,2,4-triazole (8d): (Yield: 3.44 g, 94%); mp 140-141 °C (white crystals); IR (KBr) cm^{-1}: 1615 and 1561 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.05 (s, 3H, CH_3), 2.12 (s, 3H, ar-CH_3), 3.56 (s, 9H, O-CH_3), 5.98 (bs, 2H, ar-H), 6.99 (bs, 2H, ar-H), 7.60 (bs, 2H, ar-H), 8.32 (s, 1H, N=CH); ^{13}C-NMR (DMSO-d_6)δ ppm, 162.23 (N=CH) 149.00 (triazole C_3), 147.00 (triazole C_5), ar-C: [164.95 (C), 161.33 (2C), 139.00 (C), 129.00 (2CH), 127.32 (2CH), 124.00 (C), 101.98 (C), 90.92 (2CH)], 56.12 ($2OCH_3$), 55.61 (OCH_3), 21.00 (ar-CH_3), 11.00(CH_3).

4-(2-Hydroxy-1-naphthylmethylenamino)-3-methyl-5-(4-tolyl)-4*H*-1,2,4-triazole (8e): (Yield: 2.70 g, 79%); mp 207-208 °C (white crystals); IR (KBr) cm^{-1}: 3370 (ν_{OH}), 1624 and 1601 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.32 (s, 3H, CH_3), 2.52 (s, 3H, ar-CH_3), 7.25-7.40 (m, 1H, ar-H), 7.30 (d, 2H, ar-H, J = 7.80 Hz), 7.45 (t, 1H, ar-H, J = 8.55 Hz), 7.62 (t, 1H, ar-H, J = 8.80 Hz), 7.75 (d, 2H, ar-H, J =7.80 Hz), 7.92 (d, 1H, ar-H, J = 8.85 Hz), 8.10 (d, 1H, ar-H, J = 8.55 Hz), 8.92 (d, 1H, ar-H, J = 8.85 Hz), 9.47 (s, 1H, N=CH), 11.60 (bs, 1H, OH); ^{13}C-NMR (DMSO-d_6)δ ppm 176.54 (N=CH), 149.25 (triazole $C_3$and triazole C_5), ar-C: [170.49 (C), 159.41 (C), 157.85 (C), 146.39 (2CH), 141.47 (C), 139.24 (2CH), 138.92 (2CH), 137.73 (2CH), 133.99 (CH), 133.64 (CH), 128.18 (CH), 118.30 (C)], 30.80 (ar-CH_3), 20.73 (CH_3).

4-(4-Chlorophenylmethylenamino)-3-(pyridin-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (10a): (Yield: 2.80 g, 75%); mp 191-192 °C (white crystals); IR (KBr) cm^{-1}: 1596 and 1564 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6) δ ppm 2.34 (s, 3H, CH_3), 7.20-7.40 (m, 2H, ar-H), 7.60-7.80 (m, 4H, ar-H), 7.80-8.10 (m, 4H, ar-H), 8.60-8.90 (m, 2H, ar-H), 8.68 (m, 1H, N=CH); ^{13}C-NMR (DMSO-d_6)δ ppm 170.48 (N=CH), 150.74 (triazole C_3), 148.09 (triazole C_5), ar-C: [150.31 (2CH), 139.88 (C), 138.42 (C), 133.55 (C), 130.79 (2CH), 129.88 (C), 129.48 (2CH) 129.37 (2CH), 128.20 (2CH), 122.83 (C), 121.70 (2CH)], 20.84 (ar-CH_3).

4-(4-Hydroxy-3-methoxyphenylmethylenamino)-3-(pyridin-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (10b): (Yield: 2.35 g, 73%); mp 129-130 °C (white crystals); IR (KBr) cm^{-1}: 3188 (ν_{OH}), 1605 and 1577 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.34 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.95 (bs, 1H, ar-H), 7.19-7.40 (m, 2H, ar-H), 7.48 (bs, 2H, ar-H), 7.78 (d, 2H, ar-H,J = 7.80 Hz), 7.87 (bs, 2H, ar-H), 8.88 (bs, 2H, ar-H), 8.52 (s, 1H, N=CH), 10.30 (bs, 1H, OH); ^{13}C-NMR (DMSO-d_6)δ ppm 171.50 (N=CH), 152.39 (triazole C_3), 150.85 (triazole C_5), ar-C: [150.30 (2CH), 148.17 (C), 148.08 (C), 139.73 (C), 133.73 (C), 129.44 (2CH), 128.08 (2CH) 125.29 (CH), 123.05 (C), 122.40 (C), 121.54 (2CH), 115.65 (CH), 110.63 (CH)], 55.58 (OCH_3), 20.83 (ar-CH_3).

4-(4-Methoxyphenylmethylenamino)-3-(pyridin-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (10c): (Yield: 2.77 g, 75%); mp 185-186 °C (white crystals); IR (KBr) cm^{-1}: 1605 and 1567 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6) δ ppm 2.34 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 7.16 (d, 2H, ar-H, J = 7.00 Hz), 7.34 (d, 2H, ar-

H,J = 7.00 Hz), 7.78 (d, 2H, ar-H,J = 7.80 Hz), 7.80-8.10 (m, 4H), 8.78 (bs, 2H, ar-H), 8.60 (s, 1H, N=CH); ^{13}C-NMR (DMSO-d_6)δ ppm 170.15 (N=CH), 150.15 (triazole C_3), 149.25 (triazole C_5), ar-C: [162.54 (C), 149.78 (2CH), 138.71 (C), 132.90 (C), 130.26 (2CH), 128.39 (2CH), 127.08 (2CH), 123.10 (C), 122.54 (C), 121.98 (2CH), 113.82 (2CH)], 54.54 (OCH_3), 19.79 (ar-CH_3).

3-(Pyridin-4-yl)-4-(2,4,6-trimethoxyphenylmethylenamino)-5-(4-tolyl)-4*H*-1,2,4-triazole (10d): (Yield: 3.39 g, 79%); mp 208-209 °C (white crystals); IR (KBr) cm^{-1}: 1599 and 1572 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6) δ ppm 2.09 (s, 3H, CH_3), 3.18 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 6.00 (s, 2H, ar-H), 7.10 (d, 2H, ar-H, J = 7.80 Hz), 7.55 (d, 2H, ar-H,J = 7.80 Hz), 7.78 (d, 2H, ar-H,J = 6.10 Hz), 8.50 (d, 2H, ar-H,J = 6.10 Hz), 8.13 (s, 1H, N=CH); ^{13}C-NMR (DMSO-d_6)δ ppm, 164.37 (N=CH), 150.53 (triazole C_3), 148.00 (triazole C_5), ar-C: [165.45 (C), 161.62 (2C), 150.27 (2CH), 139.63 (C), 134.18 (C), 129.43 (2CH), 128.34 (2CH), 123.58 (C), 121.74 (2CH), 101.22 (C), 91.10 (2CH)], 56.20 ($2OCH_3$), 55.85 (OCH_3), 21.02 (ar-CH_3).

4-(2-Hydroxy-1-naphthylmethylenamino)-3-(pyridin-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (10e): (Yield: 3.04 g, 75%); mp 207-208 °C (white crystals); IR (KBr) cm^{-1}: 3188 (ν_{OH}), 1623 and 1608 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.33 (s, 3H, CH_3), 7.20 (d, 1H, ar-H,J = 8.85 Hz), 7.40 (d, 2H, ar-H,J = 7.80 Hz), 7.50 (d, 1H, ar-H,J = 8.60 Hz), 7.60 (d, 1H, ar-H,J = 8.60 Hz), 7.80 (d, 2H, ar-H,J = 7.80 Hz), 7.80-8.10 (m, 2H, ar-H), 8.05 (bs, 2H, ar-H), 8.10 (d, 1H, ar-H,J = 8.85 Hz), 8.90 (d, 2H, ar-H,J = 6.10 Hz), 9.23 (s, 1H, N=CH), 9.75 (bs, 1H, OH); ^{13}C-NMR (DMSO-d_6)δ ppm 168.49 (N=CH), 150.84 (triazole C_3), 148.06 (triazole C_5), ar-C: [160.94 (C), 150.20 (2CH), 139.69 (C), 136.90 (2CH), 133.90 (C), 131.37 (C), 129.34 (2CH), 129.08 (CH), 129.00 (CH), 128.25 (2CH), 127.79 (C), 124.02 (CH), 123.74 (CH), 123.20 (C), 118.09 (2CH), 108.00 (C)], 21.00 (ar-CH_3).

General method for the synthesis of compounds 9a,e and 11a-e

The mixture of corresponding compounds **8** or **10** (10 mmol) was refluxed with $NaBH_4$ (10 mmol) in absolute methanol for 1 h, protected from moisture. After the solvent was refluxed at 35-40°C under reduced pressure, a solid was obtained. This product was treated with water, filtered off, and washed with water twice. The obtained white solid was recrystallized from an appropriate solvent to afford the desired compound.

4-(4-Chlorobenzyl)amino-3-methyl-5-(4-tolyl)-4*H*-1,2,4-triazole (9a): White crystals from ethanol (yield: 2.41 g, 77%); mp 132-133 °C; IR (KBr) cm^{-1}: 3202 (ν_{NH}), 1528 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.31 (s, 3H, -CH_3), 2.37 (s, 3H, -CH_3), 3.90 (d, 2H, -CH_2, J = 4.58 Hz), 7.11 (bs, 1H, -NH), 7.12 (d, 2H, ar-H, J = 7.80 Hz), 7.26 (d, 2H, ar-H, J = 7.80 Hz), 7.30 (d, 2H, ar-H, J = 8.60 Hz), 7.82 (d, 2H, ar-H, J =8.60 Hz); ^{13}C-NMR (DMSO-d_6)δ ppm 152.42 (triazole C_3), 151.82 (triazole C_5), ar-C: [139.11 (C), 135.02 (C), 132.35 (C), 130.90 (2CH), 129.02 (2CH), 128.14 (2CH), 127.39 (2CH), 124.53 (C)], 53.58 (CH_2), 20.91 (ar-CH_3), 9.90 (CH_3).

4-[(2-Hydroxy-1-naphthyl)methyl]amino-3-methyl-5-(4-tolyl)-4*H*-1,2,4-triazole (9e):White crystals from ethanol (yield: 2.86 g, 83%); mp 222-223 °C; IR (KBr) cm^{-1}: 3368 (ν_{OH}), 3305 (ν_{NH}), 1628 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.30 (-2CH_3), 4.30 (bs, 2H, CH_2), 6.70 (bs, 1H, NH), 7.00-7.40 (m, 5H, ar-H), 7.70 (d, 2H, ar-H, J = 7.80 Hz), 7.80-8.00 (m, 3H, ar-H), 9.94 (s, 1H, -OH); ^{13}C-NMR (DMSO-d_6)δ ppm 152.34 (triazole C_3), 151.62 (triazole C_5), ar-C: [153.73 (2C), 138.44 (C), 133.07 (C), 129.14 (CH),

128.38 (2CH), 127.72 (CH), 127.12 (2CH), 125.80 (CH), 123.99 (C), 122.15 (CH), 122.08 (C), 121.88 (CH), 117.22 (CH)], 44.19 (CH_2), 20.48 (ar-CH_3), 9.24 (CH_3).

4-(4-Chlorophenyl)-amino-3-(pyridyn-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (11a): White crystals from ethyl acetate (yield: 3.01 g, 80%); mp 237-238 °C; IR (KBr) cm^{-1}: 3271 (ν_{NH}), 1601 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.33 (s, 3H, -CH_3), 3.67 (bs, 2H, -CH_2), 7.30 (bs, 1H, -NH), 6.70 (d, 2H, ar-H, J = 7.80 Hz), 7.04 (d, 2H, ar-H, J = 7.80 Hz), 7.30 (d, 2H, ar-H, J = 8.60 Hz), 7.80 (d, 2H, ar-H, J = 6.10 Hz), 7.88 (d, 2H, ar-H, J = 8.60 Hz), 8.65 (bs, 2H^{13}C NMR (DMSO-d_6) δ ppm 154.05 (triazole C_3), 151.49 (triazole C_5), ar-C: [149.48 (2CH), 139.62 (C), 139.03 (C), 133.73 (C), 131.91 (C), 130.22 (2CH), 128.97 (2CH), 127.56 (2CH), 127.39 (2CH), 123.92 (C), 121.00 (2CH)], 52.86 (CH_2), 20.57 (ar-CH_3).

4-[(4-Hydroxy-3-methoxyphenyl)methyl]amino-3-(pyridyn-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (11b): White crystals from ethyl acetate (yield: 3.17 g, 82%); mp 181-182 °C; IR (KBr) cm^{-1}: 3318 (ν_{OH}), 3225 (ν_{NH}), 1608 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.33 (s, 3H, CH_3), 3.54 (s, 3H, OCH_3), 3.60 (bs, 2H, CH_2), 7.20 (bs, 1H, NH), 6.02 (d, 1H, ar-H, J = 8.00 Hz), 6.20 (s, 1H, ar-H), 6.40 (d, 1H, ar-H, J = 8.00 Hz), 7.35 (d, 2H, ar-H, J = 7.80 Hz), 7.78-8.00 (m, 4H, ar-H), 8.80 (bs, 2H, ar-H), 10.28 (bs, 1H, OH); ^{13}C-NMR (DMSO-d_6)δ ppm 154.61 (triazole C_3), 151.88 (triazole C_5), ar-C: [149.76 (2CH), 147.04 (C), 145.99 (C), 139.90 (C), 134.31 (C), 129.33 (2CH), 127.70 (2CH), 125.46 (C), 123.98 (C), 121.15 (2CH), 120.00 (CH), 114.59 (CH), 112.34 (CH)], 54.69 (OCH_3), 53.64 (CH_2), 20.92 (ar-CH_3).

4-[(4-Methoxyphenyl)methyl]amino-3-(pyridyn-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (11c): White crystals from ethyl acetate (yield: 3.00 g, 81%); mp 181-182 °C; IR (KBr) cm^{-1}: 3244 (ν_{NH}), 1613 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.43 (s, 3H, CH_3), 3.66 (bs, 5H, OCH_3 + CH_2), 7.36 (bs, 1H, NH), 6.57 (d, 2H, ar-H, J = 7.00 Hz), 6.66 (d, 2H, ar-H, J = 7.00 Hz), 7.45 (d, 2H, ar-H, J = 7.80 Hz), 7.80 (d, 2H, ar-H, J = 7.80 Hz), 7.88 (d, 2H, ar-H, J = 6.10 Hz), 8.70 (bs, 2H, ar-H); ^{13}C-NMR (DMSO-d_6)δ ppm, 154.49 (triazole C_3), 151.80 (triazole C_5), ar-C: [158.27 (C), 149.05 (2CH), 140.16 (C), 133.72 (C), 129.73 (2CH), 129.19 (2CH), 127.37 (2CH), 126.09 (C), 122.85 (C), 121.77 (2CH), 112.94 (2CH)], 54.50 (OCH_3), 52.74 (CH_2), 20.52 (ar-$\underline{CH}_3$).

5-(4-Methylphenyl)-3-(pyridyn-4-yl)-4-[(2,4,6-tolyl)methyl]amino-4*H*-1,2,4-triazole (11d): White crystals from ethyl acetate (yield: 4.05 g, 94%); mp 224-225 °C; IR (KBr) cm^{-1}: 3292 (ν_{NH}), 1608 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.31 (s, 3H, -CH_3), 3.60 (bs, 11H, 3OCH_3+ CH_2), 6.80 (bs, 1H, -NH), 5.82 (s, 2H, ar-H), 7.30 (d, 2H, ar-H, J = 7.80 Hz), 7.60-7.80 (m, 4H, ar-H), 8.55 (bs, 2H, ar-H,); ^{13}C-NMR (DMSO-d_6)δ ppm, 154.44 (triazole C_3), 151.35 triazole C_5), ar-C: [160.59 (C), 158.55 (2C), 149.12 (2CH), 139.04 (C), 133.86 (C), 128.66 (2CH), 127.31 (2CH), 123.55 (C), 121.18 (2CH), 102.48 (C), 90.00 (2CH)], 54.70 (3OCH_3), 41.70 (CH_2), 20.54 (ar-CH_3).

4-[(2-Hydroxy-1-naphthyl)methyl]amino-3-(pyridyn-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (11e): White crystals from ethyl acetate (yield: 3.58 g, 88%); mp 245-246 °C; IR (KBr) cm^{-1}: 3318 (ν_{OH}), 3264 (ν_{NH}), 1610 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.32 (s, 3H, -CH_3), 3.60 (bs, 5H, -CH_3 + -CH_2), 7.14 (t, 1H, -NH, J = 4.58 Hz), 6.85 (d, 1H, ar-H, J = 8.80 Hz), 7.20-7.30 (m, 2H, ar-H), 7.40 (d, 2H, ar-H, J = 7.80 Hz), 7.44 (d, 1H, ar-H, J = 8.55 Hz), 7.60 (d, 1H, ar-H, J = 8.80 Hz), 7.64 (d, 1H, ar-H, J = 8.85 Hz), 7.86 (d, 2H, ar-H, J = 6.10 Hz), 7.94 (d, 2H, ar-H, J = 7.80 Hz), 8.52 (bs, 2H, ar-H), 9.58 (s, 1H, -OH); ^{13}C-NMR (DMSO-d_6)δ ppm 154.98 (triazole C_3), 151.70 (triazole C_5), ar-C: [154.16 (2C), 149.32 (2CH),

139.55 (C), 133.86 (CH), 133.25 (CH), 129.49 (CH), 129.08 (2CH), 128.07 (2CH), 127.92 (CH), 127.64 (C), 125.90 (C), 123.86 (2CH), 122.26 (C), 122.07 (C), 117.30 (CH), 111.95 (CH)], 44.36 (CH_2), 20.98 (ar-CH_3).

Antimicrobial activity

All test microorganisms were obtained from the Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey), which included Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 10145, Yersinia pseudotuberculosis ATCC 911, Klepsiella pneumonia ATCC 13883, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, Bacillus cereus 709 ROMA, Candida albicans ATCC 60193, and Candida tropicalis ATCC 13803. The chemicals were weighed and dissolved in dimethylsulfoxide (DMSO) to prepare extract stock solutions of 10 mg/mL.

Table. Screening for antimicrobial activity of the selected compounds in DMSO (dimethylsulfoxide) solvent (10 mg/mL).

Compound no.	Microorganisms and inhibition zone (mm)								
	Ec	Pa	Yp	Kp	Ef	Sa	Bc	Ca	Ct
3a	5	5	5	5	5	5	5	5	5
3c	5	5	5	5	5	5	5	5	5
3e	5	**25**	5	**11**	5	5	5	**15**	**10**
4a	5	5	5	5	5	5	5	5	5
4b	5	5	5	5	5	5	5	5	**9**
4c	5	5	5	5	5	5	5	5	**9**
5	5	5	5	5	5	5	5	5	5
6	5	**10**	5	5	5	5	5	5	**10**
7	5	5	5	5	5	5	5	5	**7**
8a	5	5	5	5	5	**11**	5	5	5
8d	5	5	5	5	5	5	5	5	**8**
8e	5	5	5	5	5	**8**	5	**20**	**16**
9a	5	**10**	5	5	5	**10**	5	5	5
9e	5	5	5	5	5	**10**	5	5	**9**
10a	5	5	5	5	5	5	5	5	5
10b	5	5	5	5	5	5	5	5	5
10c	5	5	5	5	5	5	5	5	5
10d	5	5	5	5	5	**10**	5	5	5
10e	5	5	5	5	5	5	5	5	5
11a	5	5	5	5	5	5	5	5	5
11b	5	5	5	5	5	5	5	5	5
11c	5	5	5	5	5	5	5	**9**	**10**
11d	5	5	5	5	5	**10**	5	5	5
11e	5	5	5	5	5	5	5	5	5
DMSO	5	5	5	5	5	5	5	5	5
Ampicillin	8	5	5	5	11	15	14		
Triflucan								25	25

Results were interpreted in terms of the diameter of the inhibition zone (5 mm: no antimicrobial activity; > 5 mm: positive antimicrobial activity). Ec: *Escherichia coli* ATCC 25922; Pa: *Pseudomonas aeruginosa* ATCC 10145; Yp: *Yersinia pseudotuberculosis* ATCC 911; Kp: *Klebsiella pneumonia* ATCC 13883; Ef: *Enterococcus faecalis* ATCC 29212; Sa: *Staphylococcus aureus* ATCC 25923; Bc: *Bacillus cereus* 709 ROMA; Ca: *Candida albicans* ATCC 60193; Ct: *Candida tropicalis* ATCC 13803.

Agar well diffusion method

A simple susceptibility screening test using agar-well diffusion[31] (**1**) as adapted earlier[32] was used. Each microorganism was suspended in Mueller Hinton (Difco, Detroit, MI, USA) broth and diluted to ca. 10^6 colony forming units (cfu) per mL. They were flood-inoculated onto the surface of Mueller Hinton agar and Sabouraud dextrose agar (SDA) (Difco), which were then dried. For *C. albicans* and *C. tropicalis*, SDA was used. From the agar, 5-mm diameter wells were cut using a sterile cork-borer and 500 μg/50 μL (10 mg/mL) of the chemical 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/50 μL) served as the control antibiotic. Triflucan (5 μg/50 μL) served as the control fungicide. DMSO served as the solvent control. The results are shown in the Table.

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