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Synthesis of Some Novel 3,5-Diaryl-1,2,4-Triazole Derivatives and Investigation of Their Antimicrobial Activities

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A series of acylhydrazones (**2a-d)** was synthesized from the reactions of iminoester hydrochlorides (**1a-e)** with acyl hydrazines. 2,5-Dialkyl 1,3,4-oxadiazoles **(3a-d)** 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-(4-hydroxyphenyl)-5-phenyl-4*H*-1,2,4-triazole (**4a**) produced 4-amino-5-(4-acetoxyphenyl)-3-phenyl-4*H*-1,2,4-triazole **(9),** while the acetylation of 4-amino-3-(4-tolyl)-5-phenyl-4*H*-1,2,4-triazole **(4b)** gave 4-acetylamino-3-phenyl-5-(4-tolyl)-4*H*-1,2,4-triazole **(10)**. The treatment of compound **4b** with various aromatic aldehydes or acetophenone and 4-nitroacetophenone resulted in the formation of 4-arylidenamino-3,5-dialkyl-4*H*-1,2,4-triazoles (**5a-e** and **7a,b**). Sodium borohydride reduction of 4-arylidenamino derivatives of 1,2,4-triazoles afforded 4-alkylamino-3,5-dialkyl-4*H*-1,2,4-triazoles (**6a-e** and **8a,b**).

All newly synthesized compounds were screened for their antimicrobial and antifungal activities using agar-well diffusion. Compounds **5c** and **6d** showed marginal antimicrobial activities against *Staphylococcus aureus*, while compound **6b** displayed moderate antifungal activity towards *Candida tropicalis.*

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

Introduction

Triazole derivatives have been reported to have pharmacological, insecticidal, fungicidal, and herbicidal activities.[1−7] In one of our previous studies,[4] we reported that 4-(2-phenyl ethyliden or ethyl)amino-1,2,4-triazol-5-ones exhibit antitumoral activity, while 4-amino-1,2,4-triazol-5-ones possess no activity. Furthermore, arylidenhydrazides bearing amino-1,2,4-triazol-5-one ring were synthesized by us and found to possess

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antitumoral activity against only breast cancer.[8] In addition, it was reported that compounds having triazole moieties, such as Vorozole, Letrozole and Anastrozole, have been used as nonsteroidal aromatase inhibitors in medicine for treating breast cancer.[9−12] Moreover, 1,2,4-triazoles are a new class of antimicrobial agents. For instance, fluconazole and itraconazole are used as antimicrobial drugs in medicine.[13,14] Beside these, some biheterocyclic compounds incorporating 1,2,4-triazole ring have been reported as antimicrobial agents.[9,15−17] Among these, the commonly known systems are generally triazoles fused to pyridies, pyridazines, pyrimidines, pyrazines, and triazines. Although there are not many triazoles fused to thiadiazines or thiadiazoles, a number of them are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities.[16,18−21] However, in the last decades, the increasing drug resistance to the commonly used antibiotics has become a serious health problem. Therefore, for the effective treatment of infectious diseases, the synthesis of a new class of antibiotics having different structures from those commonly used is crucial.

In addition, diaryl heterocycles such as celecoxib, valdecoxib, rofecoxib and etoricoxib have been extensively used as anti-inflammatory drugs to treat acute or chronic inflammation by providing symptomatic pain relief. All these tricyclic molecules possess 1,2-diarylsubstitution on a central 5- or 6-membered ring system such as pyrazole, furanone isoxazole, and pyridine.[22]

Prompted by these observations, we aimed to obtain 1,2,4-triazole derivatives as possible biological active compounds.

Experimental

Chemistry

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 FTIR spectrometer. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compounds **1** were obtained as reported earlier.[23]

Microbiology

Antibacterial activity assay

All test microorganisms were obtained from the Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey) and were as follows: *Esherichia coli* ATCC 25922, *Pseudomonas auroginosa* ATCC 10145, *Yersinia pseudotuberculosis* ATCC 911, *Klepsiella pneumonia* ATCC 13883, *Enterococcus fecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* 709 ROMA.

A simple susceptibility screening test using agar-well diffusion[30] as adapted earlier[31] was used. Each bacterium was suspended in Mueller Hinton (Difco, Detroit, MI, USA) broth and diluted ca. 10^6 colony forming unit (cfu) per mL. They were "flood-inoculated" onto the surface of Mueller Hinton agar, which was then dried. The chemicals were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare extract stock solution of 10 mg/mL. Five millimeter diameter wells were cut from the agar using a sterile cork-borer, and 500 μg/50 $\mu\mu$L (10 mg/mL) of the chemical substances was 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 control antibiotics. DMSO served as solved control. The test results are given in the Table.

Table. Antibacterial and antifungal activities of the synthesized compounds (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
4a	5	5	5	5	5	5	5	5	5
4b	5	5	5	5	5	5	5	5	5
4d	5	5	5	5	5	5	5	5	5
5a	5	5	5	5	5	5	5	5	5
5b	5	5	5	5	5	5	5	5	5
5c	5	5	5	5	5	**8**	5	5	5
5d	5	5	5	5	5	5	5	5	5
5e	5	5	5	5	5	5	5	5	5
6a	5	5	5	5	5	5	5	5	5
6b	5	5	5	5	5	5	5	5	**11**
6c	5	5	5	5	5	5	5	5	5
6d	5	5	5	5	5	**9**	5	5	5
6e	5	5	5	5	5	5	5	5	5
7a	5	5	5	5	5	5	5	5	5
7b	5	5	5	5	5	5	5	5	5
8a	5	5	5	5	5	5	5	5	5
8b	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: Antimicrobial activity positive. 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.

Antifungal activity assay

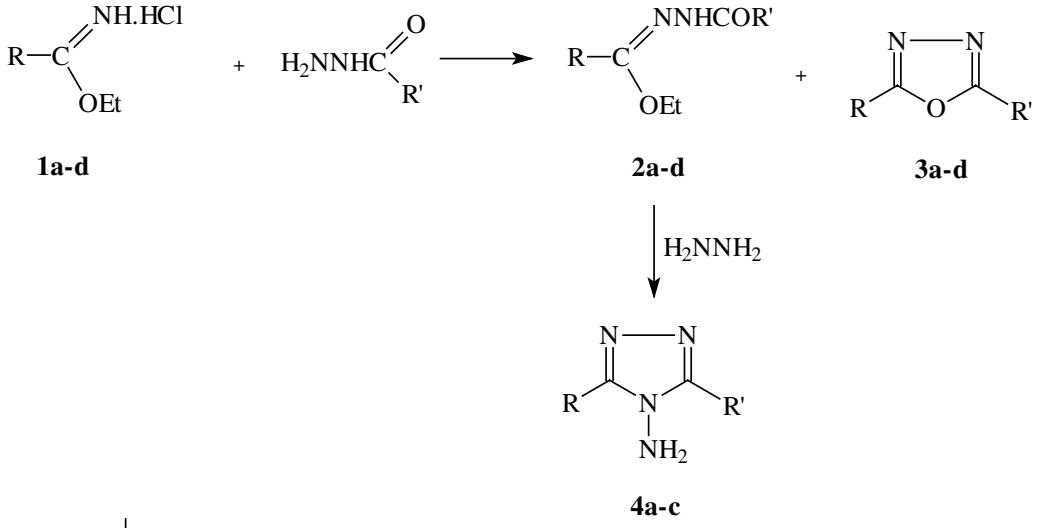
Candida albicans ATCC 60193 and *Candida tropicalis* ATCC 13803 were obtained from the Refik Saydam Hıfzıssıhha Institute. A simple susceptibility screening test using agar-well diffusion[31] as adapted earlier[32] was used. For *Candida*, Sabouraud Dextrose Agar (SDA) (Difco) was used. Triflucon (5 μg/50 μL) served as control antifungicide. DMSO served as solved control. The test results are given in the Table.

Results and Discussion

Chemistry

Compounds **2a-e** were obtained from the reaction of compounds **1a-e** with various acylhydrazines such as benzoyl and acetyl hydrazine (Scheme 1) and their structures were confirmed using IR, ^{1}H-NMR, and ^{13}C-NMR spectral data. Compound **2b** is known.[24] The IR spectrum of compounds **2a-d** displayed no absorption derived from –NH_2 stretching. In the NMR spectra of these compounds new signals originating

from the –OEt group were recorded. Compounds **3a-d** were obtained in the same reaction media with compounds **2a-d**, and they were separated from corresponding compound **2** using the differences in their solubility in benzene. Compounds **3a-e** are known.[25–27] In contrast to compounds **2a-d**, no absorption band corresponding to the –NH- group was observed in the IR spectra of compounds **3a-d**. Moreover, the signal belonging to carbonyl stretching was absent. The melting points of compounds **3a-d** were consistent with those reported in the literature.



1a-d **2a-d** **3a-d**

4a-c

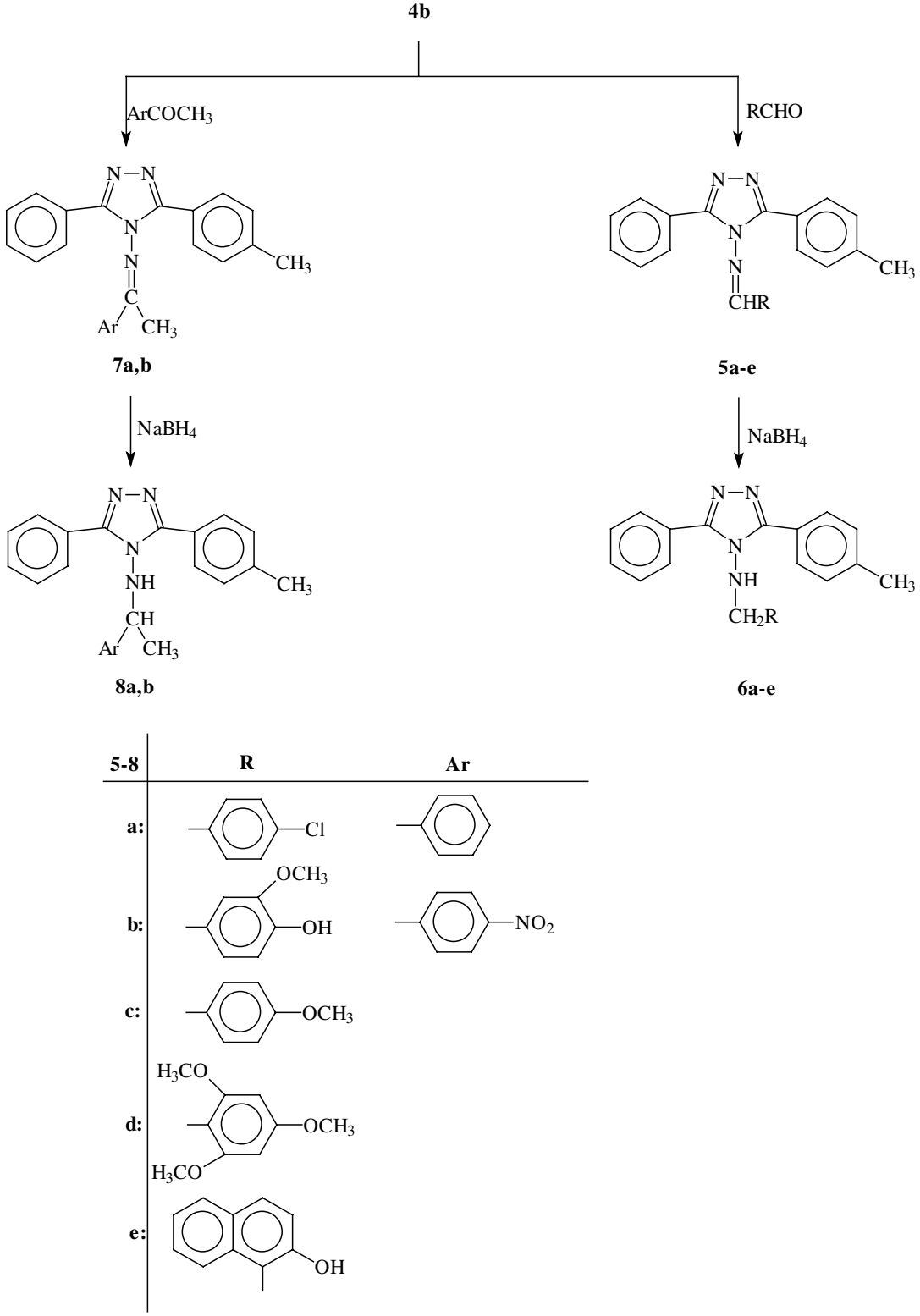
1-4	R	R'
a:	$-CH_3$	$-C_6H_5$
b:	$-C_6H_4OH(p-)$	$-C_6H_5$
c:	$-C_6H_4CH_3(p-)$	$-C_6H_5$
d:	$-C_6H_4N(p-)$	$-C_6H_5$
e:	$-C_6H_4N(p-)$	$-CH_3$
f:	$-C_6H_5$	$-CH_3$

Scheme 1. Synthetic pathway for preparation of compounds **1-4**.

In the second step of this study, 4-amino-3,5-dialkyl-4*H*-1,2,4-triazoles (**4a-c**) were obtained by the reaction of compounds **2a-c** with hydrazine hydrate (Scheme 1). In the IR spectra of compounds **4a-c**, the stretching band derived from the $-NH_2$ group was present, while this signal was absent in the IR spectra of precursor compounds **2a-c**. In addition, no signal derived from the carbonyl group was observed in the IR or ^{13}C-NMR spectra of compounds **4a-c**. Beside this, the signals due to the ethoxy group were absent in the NMR spectra of compounds **4a-c**, while the peak corresponding to the $-NH_2$ group was recorded at 6.06-6.51 ppm in the ^{1}H-NMR spectra of these compounds (exchangeable with D_2O).

Among compounds **4a-c**, **4a** and **4b** were treated with some aromatic aldehydes such as 4-chlorobenzaldehyde, 3-methoxy-4-hydroxybenxaldehyde, 3-methoxybenxaldehyde, 2,4,6-trimethoxybenzaldehyde and 2-hydroxy-1-naphthaldehyde and some methylketones (acetophenone and 4-nitroacetophenone); thus, Schiff base derivatives (**5a-e** and **7a,b**) of compound **4b** and **4c** were obtained. Then compounds **5a-e** and **7a,b** were converted to their reduced derivatives (**6a-e** and **8a,b**) by treating with sodium borohydride

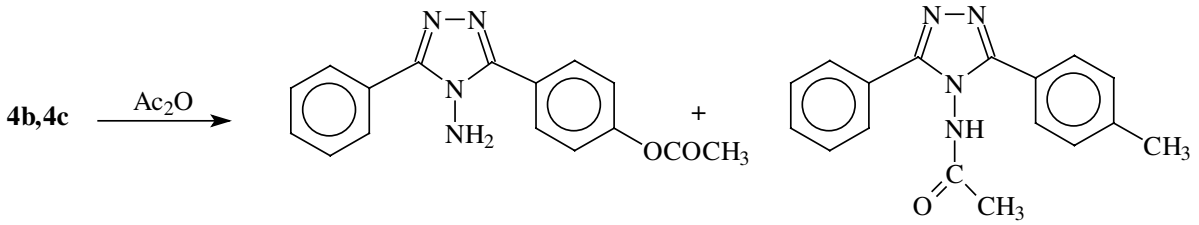
in methanol. Since sodium borohydride is a selective reducing agent, reduction took place only at the azomethyne bond and the 1,2,4-triazole ring remained unchanged (Scheme 2).



Scheme 2. Synthetic pathway for preparation of compounds **5-8**.

In contrast to those of compounds **4a-c**, the NMR spectra of compounds **5a-e** and **7a,b** displayed signals belonging to arylidenamino groups. When compounds **5a-e** and **7a,b** were converted to compounds **6a-e** and **8a,b** a new signal appeared at 6.71-7.35 ppm representing –NH- signals in the ^{1}H-NMR spectra of compounds **6a-e** and **8a,b**, and the signals due to –CH- (or –CH_2) group bearing 4-amino group were recorded at 3.67-3.80 ppm, while the =CH- group of compounds **5a-e** resonated at a lower region, 8.42-9.31 ppm.

In the last part of the synthesis reactions, compounds **4a** and **4b** were treated with acetic anhydride; thus, 2 different types of monoacetylated compounds (**9** and **10)** were obtained (Scheme 3). Compound **4a** was acetylated at phenolic –OH; thus compound **9** was obtained. On the other hand, the acetylation reaction of compound **4b** took place at the –NH_2 group, and gave compound **10**. According to the literature,[32] the 4-amino group on the 1,2,4-triazole ring could be partially hindered by adjacent bulky groups such as phenyl or 4-tolyl groups. Thus, the –OH group that is present at the tip of compound **4a** can attack easier as a nucleophyle the acetic anhydride molecule than the 4-amino group. In the ^{1}H-NMR spectrum of compound **9** there was a signal at 6.42 ppm representing a free –NH_2 group (exchangeable with D_2O), while the signal was absent in the spectra of compound **10.** On the other hand, in the ^{1}H-NMR spectrum of compound **9**, the –OH signal that appeared at 9.31 ppm of the parent compound (**4a**) is absent. In addition, new signals belonging to the acetyl group appeared at 2.03-2.42 ppm in the ^{1}H-NMR spectra and 2 new signals at 20.31-20.80 ppm and 168.50-169.07 ppm in the ^{13}C-NMR spectra of compounds **9** and **10**.



9 **10**

Scheme 3. Synthetic pathway for preparation of compounds **9** and **10**.

General procedure for the preparation of compounds 2a-d and 3a-d

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 the solvent was evaporated at 35-40 °C under reduced pressure, a white solid appeared. This crude product was recrystallized from benzene-petroleum ether (1:2) to afford compounds **2**. The part of the reaction mixture that did not dissolve in benzene was recrystallized from an appropriate solvent; thus compounds **3** were obtained.

Ethyl *N*-benzoyl-4-hydroxybenzencarbohydrazonoate (2a): (Yield: 1.56 g, 55%). mp 164-165 °C (white crystals); IR (KBr) cm^{-1}: 3265 (ν_{NH}), 3200 (ν_{OH}), 1636 ($\nu_{C=O}$), 1605 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 1.10 (t, 3H, CH_3, J= 6.96 Hz), 4.20 (q, 2H, OCH_2, J= 6.96 Hz), [ar-H: 6.90 (d, 2H, J= 8.55 Hz), 7.40-7.65 (m, 3H)], 7.90 (d, 2H, J=8.55 Hz), 8.00-8.15 (m, 2H)], 9.45 (s, 1H, NH), 10.02 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6)δ ppm 170.05 (C=O), 164.00 (C=N), ar-C: [158.65 (C), 129.92 (2CH), 129.60 (CH), 128.46 (2CH), 128.06 (2CH), 127.21 (C), 117.90 (C), 115.00 (2CH)], 62.05 (OCH_2), 14.06 (CH_3).

Ethyl N-benzoyl-4-methylbenzenecarbohydrazonoate (2b): (Yield: 1.69 g, 60%). mp 77-78 °C (white crystals), ref.[24] mp 78-79 °C; IR (KBr) cm^{-1}: 3162 (ν_{NH}), 1649 ($\nu_{C=O}$), 1618 ($\nu_{C=N}$).

Ethyl N-benzoyl-pyridine-4-yl-carbohydrazonoate (2c): (Yield: 1.35 g, 50%). mp 90-91 °C (white crystals); IR (KBr) cm^{-1}: 3133 (ν_{NH}), 1676 ($\nu_{C=O}$), 1610 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 1.22 (t, 3H, CH_3, J= 6.96 Hz), 4.25 (q, 2H, OCH_2, J= 6.96 Hz), [ar-H: 7.50-7.70 (m, 3H), 7.80-8.00 (d, 2H, J= 7.60 Hz), 8.10 (d, 2H, J= 6.10 Hz), 8.85 (bs, 2H)], 9.60 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6)δ ppm 172.10 (C=O), 163.00 (C=N), ar-C: [150.36 (2CH) 135.00 (C), 130.86 (2CH), 129.18 (2CH), 128.08 (C), 127.84 (2CH), 121.98 (2CH)], 67.00 (OCH_2), 15.92 (CH_3).

Ethyl N-acetyl-pyridine-4-yl-carbohydrazonoate (2d): (Yield: 0.85 g, 41%). mp 109-110 °C (white crystals); IR (KBr) cm^{-1}: 3186 (ν_{NH}), 1668 ($\nu_{C=O}$), 1640 and 1621 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 1.30 (t, 3H, CH_3, J= 6.96 Hz), 2.34 (s, 3H, N=C-CH_3), 4.30 (q, 2H, OCH_2, J= 6.96 Hz), [ar-H: 8.06 (bs, 2H), 8.76 (bs, 2H)], 10.28 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm 170.00 (C=O), 160.05 (C=N), ar-C: [150.00 (2CH), 134.05 (C), 122.00 (2CH)], 63.15 (OCH_2), 20.05 (N=C-CH_3), 13.86 (CH_3).

2-Phenyl-5-(4-hydroxyphenyl)-1,3,4-oxadiazole (3a): Recrystallization from acetone-petroleum ether (1:2) (yield: 30%). mp 253-254 °C, ref.[25] mp 253 °C; IR (KBr) cm^{-1}: 3370 (ν_{OH}), 1608 and 1559 ($\nu_{2C=N}$).

2-Phenyl-5-(4-tolyl)-1,3,4-oxadiazole (3b): Recrystallization from ethanol (yield: 31%). mp 121-122 °C, ref.[25] mp 122-123 °C, IR (KBr) cm^{-1}: 1611 and 1549 ($\nu_{2C=N}$).

2-Phenyl-5-(pyridine-4-yl)-1,3,4-oxadiazole (3c): Recrystallization from ethanol (yield: 39%). mp 146-147 °C, ref.[26] mp 145-147 °C, IR (KBr) cm^{-1}: 1608 and 1547 ($\nu_{2C=N}$).

2-Methyl-5-(pyridine-4-yl)-1,3,4-oxadiazole (3d): Recrystallization from ethanol (yield: 43%). mp 148-149 °C, ref.[27] mp 149-151 °C, IR (KBr) cm^{-1}: 1578 and 1567 ($\nu_{2C=N}$).

General procedure for the preparation of compounds 4a-d

A solution of the corresponding compound **2** (10 mmol) in n-propanol was refluxed with hydrazine hydrate (25 mmol) for 24 h. After it was cooled to room temperature, a white solid appeared. This crude product was filtered off, washed with benzene 3 times, and recrystallized from an appropriate solvent to afford the desired compound.

4-Amino-3-(4-hydroxyphenyl)-5-phenyl-4H-1,2,4-triazole (4a): Recrystallization from ethyl acetate. mp 229-230 °C (white crystals); IR (KBr) cm^{-1}: 3358-3289 (ν_{NH2}), 3200 (ν_{OH}), 1613 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 6.21 (s, 2H, NH_2), [ar-H: 6.95 (d, 2H, J= 8.55 Hz), 7.50-7.70 (m, 3H), 7.86 (d, 2H, J= 7.60 Hz), 8.10 (d, 2H, J= 8.55 Hz)], 9.94 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6)δ ppm 154.21 (triazole C_3), 153.65 (triazole C_5), ar-C: [158.57 (C), 129.74 (2CH), 129.30 (CH), 128.33 (2CH), 128.08 (2CH), 127.30 (C), 117.80 (C), 115.13 (2CH)].

4-Amino-3-(4-tolyl)-5-phenyl-*4H*-1,2,4-triazole (4b): Recrystallization from 1-propanol (yield: 85%). mp 282-283 °C, ref.[28,29] mp 283-284 °C. IR (KBr) cm^{-1}: 3345-3285 (ν_{NH2}), 1636 ($\nu_{C=N}$).

4-Amino-3-(pyridine-4-yl)-5-phenyl-4H-1,2,4-triazole (4c): Recrystallization from ethyl acetate (Yield: 1.30 g, 55%). mp 269-270 °C (white crystals); IR (KBr) cm^{-1}: 3359-3260 (ν_{NH2}), 1607 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 6.51 (s, 2H, NH_2), [ar-H: 7.60-7.80 (m, 3H), 8.05-8.30 (m, 4H), 8.82

(bs, 2H)]; ^{13}C-NMR (DMSO-d_6)δ ppm 155.80 (triazole C_3), 152.77 (triazole C_5), ar-C: [150.65 (2CH), 135.03 (C), 130.55 (CH), 129.21 (2CH), 129.03 (2CH), 127.30 (C), 122.60 (2CH)].

General procedure for the preparation of compounds 5a-e and 7a,b

The solution of corresponding compound **4b** (10 mmol) in acetic acid was refluxed with an aromatic aldehyde (for compounds **5a-e**) or acetophenone and 4-nitroacetophenone (for compounds **7a** and **7b**, respectively) 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 (**5a,c,d** and **7b**) or ethyl acetate (**5b,e** and **7a**) to afford pure compounds.

4-[(4-Chlorophenyl)methylenamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (5a): (Yield: 3.06 g, 82%). mp 191-192 °C (white crystals); IR (KBr) cm^{-1}: 1609 and 1595 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6) δ ppm 2.47 (s, 3H, ar-CH_3), [ar-H: 7.48 (d, 2H, J= 7.80 Hz), 7.67 (d, 2H, J= 7.80 Hz), 7.75 (d, 2H, J= 8.60 Hz), 7.84 (d, 2H, J= 8.60 Hz), 7.90-8.02 (m, 5H)], 8.77 (s, 1H, N=CH); ^{13}C-NMR (DMSO-d_6) δ ppm 169.79 (N=CH), 150.06 (triazole C_3), 149.88 (triazole C_5), ar-C: [139.52 (C), 138.13 (C), 130.56 (2CH), 130.05 (C), 129.74 CH), 129.46 (2CH), 129.37 (2CH), 128.80 (2CH), 128.11 (2CH), 128.05 (2CH), 126.21 (C), 123.30 (C)], 21.00 (ar-CH_3).

4-[(3-Methoxy-4-hydroxyphenyl)methylenamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (5b): (Yield: 2.88 g, 75%). mp 234-235 °C (white crystals); IR (KBr) cm^{-1}: 3204 (ν_{OH}), 1589 and 1514 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.24 (s, 3H, ar-CH_3), 3.73 (s, 3H, OCH_3), [ar-H: 6.80 (d, 1H, J= 8.09 Hz), 7.14 (d, 1H, J= 8.09 Hz), 7.24 (d, 2H, J= 7.80 Hz), 7.30-7.40 (m, 1H), 7.40-7.54 (m, 3H), 7.70 (d, 2H, J= 7.80 Hz), 7.80 (d, 2H, J= 7.60 Hz)], 8.42 (s, 1H, N=CH), 10.15 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6)δ ppm 171.11(N=CH), 150.30 (triazole C_3), 150.13 (triazole C_5), ar-C: [152.31 (C), 148.30 (C), 139.52 (C), 129.57 (CH), 129.31 (2CH), 128.74 (2CH), 127.97 (2CH), 127.92 (2CH), 126.65 (C), 125.14(CH), 123.75 (C), 122.79 (C), 115.79 (CH), 110.76 (CH)], 55.58 (OCH_3), 20.87 (ar-CH_3).

4-[(4-Methoxyphenyl)methylenamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (5c): (Yield: 3.20 g, 87%). mp 159-160 °C (white crystals); IR (KBr) cm^{-1}: 1595 and 1569 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6) δ ppm 2.35 (s, 3H, ar-CH_3), 3.85 (s, 3H, OCH_3), [ar-H: 7.10 (d, 2H, J= 7.00 Hz), 7.35 (d, 2H, J= 7.00 Hz), 7.40-7.60 (bs, 3H), 7.75 (d, 2H, J= 7.80 Hz), 7.80-8.00 (m, 4H)], 8.71 (s, 1H, N=CH); ^{13}C-NMR (DMSO-d_6)δ ppm 170.54 (N=CH), 150.00 (triazole C_3), 149.86 (triazole C_5), ar-C: [164.02 (C), 139.36 (C), 131.70 (2CH), 130.54 (2CH), 130.26 (CH), 129.98 (2CH), 129.41 (2CH), 128.62 (2CH), 127.07 (C), 124.42 (C), 124.15 (C), 115.43 (2CH)], 56.18 (OCH_3), 21.47 (ar-CH_3).

3-Phenyl-4-[(2,4,6-trimethoxhydroxyphenyl)methylenamino]-5-(4-tolyl)-4H-1,2,4-triazole (5d): (Yield: 4.02 g, 94%). mp 200-201 °C (white crystals); IR (KBr) cm^{-1}: 1598 and 1572 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6) δ ppm 2.42 (s, 3H, ar-CH_3), 3.78 (s, 6H, $2OCH_3$), 3.89 (s, 3H, OCH_3), [ar-H: 6.30 (s, 2H), 7.38 (d, 2H, J= 7.80 Hz), 7.40-7.60 (m, 3H), 7.90 (d, 2H, J= 7.80 Hz), 8.00 (d, 2H, J= 7.60 Hz)], 8.45 (s, 1H, N=CH); ^{13}C-NMR (DMSO-d_6)δ ppm, 163.87 (N=CH), 150.00 (triazole C_3), 149.37 (triazole C_5), ar-C: [165.14 (C), 161.42 (2C), 139.14 (C), 129.42 (CH), 129.18 (2CH), 128.61 (2CH), 128.06 (2CH), 127.98 (2CH), 127.00 (C), 124.00 (C), 101.98 (C), 90.95 (2CH)], 56.02 (OCH_3), 55.66 (OCH_3), 20.83 (Aryl-CH_3).

4-(2-Hydroxy-1-naphthylidenamino)-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (5e): (1:2) (Yield: 3.11 g, 77%). mp 173-174 °C (white crystals); IR (KBr) cm^{-1}: 3204 (ν_{OH}), 1602 and 1578 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.42 (s, 3H, ar-CH_3), [ar-H: 7.26 (d, 1H, J= 8.80 Hz), 7.40 (d, 2H, J= 7.80 Hz), 7.50-7.70 (m, 6H), 7.90 (d, 2H, J= 7.80 Hz), 7.90-8.05 (m, 2H), 8.15 (d, 1H, J= 8.85 Hz), 8.92 (d, 1H, J= 8.85 Hz)], 9.31 (s, 1H, N=CH), 11.45 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6)δ ppm 168.00 (N=CH), 150.16, (triazole C_3), 150.00 (triazole C_5), ar-C: [160.62 (C), 139.41 (C), 136.66 (CH), 131.37 (C), 129.65 (CH), 129.29 (2CH), 128.96 (2CH), 128.72 (2CH), 128.24 (2CH), 128.14 (2CH), 127.79 (C), 126.53 (C), 124.00 (CH), 123.61 (CH), 118.07 (CH), 107.93 (C)], 20.79 (ar-CH_3).

4-(1-Phenylethyliden)amino)-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (7a): (Yield: 2.46 g, 70%). mp 150-151 °C (white crystals); IR (KBr) cm^{-1}: 1598 and 1569 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6) δ ppm 1.86 (s, 3H, CH_3), 2.32 (s, 3H, ar-CH_3), [ar-H: 7.30 (d, 2H, J= 7.80 Hz), 7.40-7.50 (m, 3H), 7.50-7.70 (m, 3H), 7.75 (d, 2H, J= 7.80 Hz), 7.80-7.90 (m, 2H), 8.05 (d, 2H, J= 7.60 Hz)]; ^{13}C-NMR (DMSO-d_6)δ ppm 178.43 (C=N), 149.77 (triazole C_3), 149.58 (triazole C_5), ar-C: [139.83 (C), 134.80 (C), 132.75 (CH), 130.02 (CH), 129.63 (2CH), 129.13 (2CH), 129.06 (2CH), 127.89 (2CH), 127.41 (2CH), 127.34 (2CH), 126.59 (C), 123.71 (C)], 21.00 (ar-CH_3), 16.86 (CH_3).

4-[1-(4-Nitrophenyl)ethylidenamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (7b): (Yield: 2.94 g, 74%). mp 169-170 °C (white crystals); IR (KBr) cm^{-1}: 1600-1586 ($\nu_{2C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 1.95 (s, 3H, CH_3), 2.34 (s, 3H, ar-CH_3), [ar-H: 7.30 (d, 2H, J= 7.80 Hz), 7.40-7.60 (m, 3H), 7.70 (d, 2H, J= 7.80 Hz), 7.80 (d, 2H, J= 7.60 Hz), 8.23 (d, 2H, J= 9.16 Hz), 8.39 (d, 2H, J= 9.16 Hz)]; ^{13}C-NMR (DMSO-d_6)δ ppm 177.63 (C=N), 149.94 (triazole C_3), 149.81 (triazole C_5), ar-C: [150.00 (C), 140.33 (C), 139.97 (C), 130.15 (CH), 129.69 (2CH), 129.37 (2CH), 129.12 (2CH), 127.49 (2CH), 127.42 (2CH), 126.39 (C), 124.08 (2CH), 123.52 (C)], 21.02 (ar-CH_3), 17.41 (CH_3).

General procedure for the preparation of compounds 6a-e and 8a,b

A solution of corresponding compound **5** or **7** (10 mmol) in absolute methanol was treated with a solution of $NaBH_4$ (10 mmol) in absolute methanol. Then the mixture was refluxed for 1 h. After the solvent was evaporated at 35-40 °C under reduced pressure a solid was obtained. This crude product was treated with water, filtered off, and washed with water 3 times. The obtained solid was recrystallized from ethyl acetate to afford the desired compound.

4-[(4-Chlorophenyl)methylamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole 6a: Yield: 3.15 g, 84%; mp 187-188 °C (white crystals); IR (KBr) cm^{-1}: 3294 (ν_{NH}), 1620 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.45 (s, 3H, ar-CH_3), 3.73 (d, 2H, CH_2, J= 4.58 Hz), 7.28 (t, 1H, NH, J= 4.58 Hz), [ar-H: 6.80 (d, 2H, J= 7.80 Hz), 7.16 (d, 2H, J= 8.60 Hz), 7.40 (d, 2H, J= 7.80 Hz), 7.50-7.68 (m, 3H), 7.76-8.00 (m, 4H)]; ^{13}C-NMR (DMSO-d_6)δ ppm 153.80 (triazole C_3), 153.74 (triazole C_5), ar-C: [139.57 (C), 134.29 (C), 132.11 (C), 130.37 (2CH), 129.74 (CH), 129.12 (2CH), 128.42 (2CH), 127.88 (2CH), 127.73 (2CH), 126.93 (C), 124.08 (C)], 53.06 (CH_2), 20.94 (ar-CH_3).

4-[(3-Methoxy-4-hydroxyphenyl)methylamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (6b): Yield: 3.36 g, 87%; mp 191-192 °C (white crystals); IR (KBr) cm^{-1}: 3320 (ν_{NH}), 3206 (ν_{OH}), 1598 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.42 (s, 3H, ar-CH_3), 3.80 (bs, OCH_3 + CH_2) 7.12 (bs, 1H, NH), [ar-H: 6.13 (d, 1H, J= 8.00 Hz), 6.30 (s, 1H), 6.50 (d, 2H, J= 8.00 Hz), 7.42 (d, 2H, J= 7.80 Hz), 7.54-7.70 (m, 3H), 7.86 (d,

2H, J= 7.80 Hz), 7.90-8.10 (m, 2H)], 10.25 (s, 1H, OH); ^{13}C-NMR (DMSO-d$_6$)δ ppm 154.05 (triazole C$_3$), 154.00 (triazole C$_5$), ar-C: [147.95 (C), 146.10 (C), 139.90 (C), 129.80 (CH), 129.30 (2CH), 128.62 (2CH), 127.87 (2CH), 127.76 (2CH), 127.10 (C), 125.74 (C), 124.20 (C), 120.95 (CH), 114.53 (CH), 112.13 (CH)], 55.00 (OCH$_3$), 53.80 (CH$_2$), 21.08 (ar-CH$_3$).

4-[(4-Methoxyphenyl)methylamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (6c): Yield: 3.50 g, 95%; mp 151-152 °C (white crystals); IR (KBr) cm^{-1}: 3278 (ν_{NH}), 1604 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$)δ ppm 2.42 (s, 3H, ar-CH$_3$), 3.67 (bs, OCH$_3$ + CH$_2$), 7.14 (bs, 1H, NH), [ar-H: 6.64 (d, 2H, J= 7.00 Hz), 6.70 (d, 2H, J= 7.00 Hz), 7.40 (d, 2H, J= 7.80 Hz), 7.50-7.70 (m, 3H), 7.70-8.07 (m, 4H)]; ^{13}C-NMR (DMSO-d$_6$)δ ppm, 153.98 (triazole C$_3$), 153.91 (triazole C$_5$), ar-C: [158.82 (C), 139.30 (C), 129.86 (2CH), 129.65 (CH), 129.10 (2CH), 128.42 (2CH), 127.89 (2CH), 127.72 (2CH), 127.40 (C), 127.15 (C), 124.92 (C), 113.40 (2CH)], 54.87 (OCH$_3$), 53.30 (CH$_2$), 21.00 (ar-CH$_3$).

3-Phenyl-4-[(2,4,6-trimethoxhydroxyphenyl)methylamino]-5-(4-tolyl)-*4H*-1,2,4-triazole (6d): Yield: 4.09 g, 95%; mp 131-132 °C (white crystals); IR (KBr) cm^{-1}: 3318 (ν_{NH}), 1609 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$) δ ppm 2.39 (s, 3H, ar-CH$_3$), 3.48 (s, 3H, OCH$_3$), 3.78 (s, 6H, 2OCH$_3$), 3.85 (s, 2H, CH$_2$), 6.71(t, 1H, NH, *J*= 4.58 Hz), [ar-H: 6.00 (s, 2H), 7.30 (d, 2H, *J*= 7.80 Hz), 7.40-7.60 (m, 3H), 7.80-8.10 (m, 4H); ^{13}C-NMR (DMSO-d$_6$)δ ppm, 153.92 (triazole C$_3$), 153.89 (triazole C$_5$), ar-C: [160.94 (C), 158.91 (2C), 139.00 (C), 129.20 (CH), 128.79 (2CH), 128.12 (2CH), 127.72 (2CH), 127.63 (2CH), 127.00 (C), 124.04 (C), 103.11 (C), 90.00 (2CH)], 55.09 (C) (OCH$_3$), 55.02 (2C) (OCH$_3$), 42.10 (CH$_2$), 21.00 (ar-CH$_3$).

4-(2-Hydroxy-1-naphthyl)methylamino)-3-phenyl-5-(4-tolyl)-*4H*-1,2,4-triazole (6e): Yield: 3.74 g, 92%; mp 209-210 °C (white crystals); IR (KBr) cm^{-1}: 3339 (ν_{NH}), 3206 (ν_{OH}), 1631 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$)δ ppm 2.45 (s, 3H, ar-CH$_3$), 4.30 (d, 2H, CH$_2$, *J*= 4.58 Hz), 7.10 (bs, 1H, NH), [ar-H: 7.25 (d, 1H, *J*= 8.80 Hz), 7.40-7.60 (m, 6H), 7.60-7.85 (m, 5H), 8.00-8.30 (m, 3H)], 9.82 (s, 1H, OH); ^{13}C-NMR (DMSO-d$_6$)δ ppm, 153.72 (triazole C$_3$), 153.64 (triazole C$_5$), ar-C: [154.01 (C), 139.00 (C), 133.29 (C), 132.43 (C), 131.80 (CH), 129.38 (CH), 128.82 (2CH), 128.45 (2CH), 128.20 (CH), 127.97 (2CH), 127.56 (2CH), 127.03 (C), 125.87 (CH), 124.13 (C), 122.32 (CH), 121.99 (CH), 117.41 (CH), 112.09 (C)], 44.59 (CH$_2$), 20.94 (ar-CH$_3$).

4-(1-Phenylethyl)amino-3-phenyl-5-(4-tolyl)-*4H*-1,2,4-triazole (8a): Yield: 2.90 g, 82%; mp 181-182 °C (white crystals); IR (KBr) cm^{-1}: 3345 (ν_{NH}), 1617 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$) δ ppm 1.05 (d, 3H, CH$_3$,*J*= 6.41 Hz), 2.40 (s, 3H, ar-CH$_3$), 3.85 (d, 1H, CH, *J*= 6.41 Hz), 7.35 (bs, 1H, NH), [ar-H: 6.85 (d, 2H, *J*= 7.80 Hz), 7.05-7.30 (m, 3H), 7.40-7.60 (m, 2H), 7.60-7.80 (m, 4H), 7.80-8.20 (m, 3H)]; ^{13}C-NMR (DMSO-d$_6$)δ ppm 150.18 (triazole C$_3$), 150.05 (triazole C$_5$), ar-C: [140.94 (C), 136.00 (C), 134.00 (CH), 131.48 (CH), 130.83 (2CH), 130.46 (2CH), 130.39 (2CH), 129.17 (2CH), 128.87 (2CH), 128.05 (2CH), 127.98 (C), 124.66 (C)], 58.01 (NH-<u>CH</u>), 21.00 (ar-CH$_3$), 20.00 (CH$_2$).

4-[1-(4-Nitrophenyl)ethyl]amino-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole (8b): Yield: 2.83 g, 71%; mp 199-200 °C (white crystals); IR (KBr) cm^{-1}: 3346 (ν_{NH}), 1608 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d$_6$)δ ppm 1.00 (d, 3H, CH$_3$,J= 6.41 Hz), 2.35 (s, 3H, ar-CH$_3$), 3.78 (d, 1H, CH, J= 6.41 Hz), 7.30 (s, 1H, NH), [ar-H: 6.78 (d, 2H, J= 7.80 Hz), 7.00-7.20 (m, 3H), 7.45-7.65 (m, 4H), 7.80 (d, 2H, J= 9.16 Hz), 8.00-8. (Yield: 0.93 g, 20 (m, 2H)]; ^{13}C-NMR (DMSO-d$_6$)δ ppm 150.16 (triazole C$_3$), 150.08 (triazole C$_5$), ar-C: [147.14 (C), 145.83 (C), 140.86 (C), 132.18 (CH), 131.09 (2CH), 130.98 (2CH), 130.03 (2CH), 129.36 (2CH), 129.04 (2CH), 128.02 (C), 125.47 (C), 123.01 (2CH)], 58.00 (NH-CH), 21.06 (ar-CH$_3$), 17.05 (CH$_2$).

General procedure for the preparation of compounds 9 and 10

The corresponding compound **4a (**for **9)** or **4b (**for **10)** (10 mmol) was refluxed with acetic anhydride (10 mL) for 1 h. Then the mixture was cooled to room temperature and 40 mL of ethanol was added, followed by refluxing for an additional 30 min. After the excess of acetic anhydride was removed under reduced pressure at 55-60 °C, a white solid was obtained. This was recrystallized from acetone-petroleum ether (1:2) to afford the desired compound.

4-Amino-5-(4-acetoxyphenyl)-3-phenyl-4*H*-1,2,4-triazole (9): Yield: 2.00 g, 65%; mp 239-240 °C (white crystals); IR (KBr) cm^{-1}: 3321-3192 (ν_{NH2}), 1758 ($\nu_{C=O}$), 1608 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.42 (s, 3H, O=C-CH_3), 6.42 (s, 2H, NH_2), [ar-H: 7.42 (d, 2H, J= 7.60 Hz), 7.60-7.78 (m, 3H), 8.14 (d, 2H, J= 8.55 Hz), 8.20 (d, 2H, J= 8.55 Hz)]; ^{13}C-NMR (DMSO-d_6)δ ppm 169.07 (C=O), 154.18 (triazole C_3), 153.60 (triazole C_5), ar-C: [151.33 (C), 129.46 (2CH), 129.45 (CH), 128.41 (2CH), 128.20 (2CH), 127.02 (C), 124.68 (C), 121.98 (2CH)], 20.80 (ar-CH_3).

4-Acetylamino-3-phenyl-5-(4-tolyl)-4*H*-1,2,4-triazole (10): Yield: 1.81 g, 62%; mp 213-214 °C (white crystals); IR (KBr) cm^{-1}: 3297 (ν_{NH}), 1711 ($\nu_{C=O}$), 1615 ($\nu_{C=N}$); ^{1}H-NMR (DMSO-d_6)δ ppm 2.03 (s, 3H, ar-CH_3), 2.48 (s, 3H, O=C-CH_3), [ar-H: 7.40-7.60 (bs, 2H), 7.60-7.80 (bs, 3H), 7.80-7.90 (bs, 2H), 7.90-8.10 (bs, 2H)], 11.72 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ ppm 168.50 (C=O), 153.84 (triazole C_3), 153.72 (triazole C_5), ar-C: [140.12 (C), 130.26 (CH), 129.45 (2CH), 128.89 (2CH), 127.34 (2CH), 127.26 (2CH), 125.78 (C), 123.00 (C)], 20.88 (ar-CH_3), 20.31(O=C-$\underline{CH_3}$).

Microbiology

Compounds **5c** and **6d** showed marginal antimicrobial activities against *Staphylococcus aureus*, while compound **6b** displayed moderate antifungal activity towards *Candida tropicalis*. This result does not allow an evaluation of the structure-activity relationship. However, this stimulated us to investigate structural modifications in the 1,2,4-triazole ring to obtain possible antimicrobial activity.

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