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

Mevlüt SERDAR¹, Nurhan GÜMRÜKÇÜOĞLU¹, Şengül ALPAY KARAOĞLU², Neslihan DEMİRBAŞ¹∗

¹Karadeniz Technical University, Department of Chemistry 61080 Trabzon-TURKEY
²Karadeniz Technical University, Department of Biology, 53100 Rize-TURKEY

e-mail: neslihan@ktu.edu.tr

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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-4H-1,2,4-triazole (4a) produced 4-amino-5-(4-acetoxyphenyl)-3-phenyl-4H-1,2,4-triazole (9), while the acetylation of 4-amino-3-(4-tolyl)-5-phenyl-4H-1,2,4-triazole (4b) gave 4-acetylamino-3-phenyl-5-(4-tolyl)-4H-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-4H-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-4H-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 ethylidene 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, aryldienehydrazides bearing amino-1,2,4-triazol-5-one ring were synthesized by us and found to possess

*Corresponding author
antitumoral activity against only breast cancer. 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. Moreover, 1,2,4-triazoles are a new class of antimicrobial agents. For instance, fluconazole and itraconazole are used as antimicrobial drugs in medicine. Beside these, some biheterocyclic compounds incorporating 1,2,4-triazole ring have been reported as antimicrobial agents. 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 thiazadiazines or thiazadiazoles, a number of them are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities. 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.

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.

Microbiology

Antibacterial activity assay

All test microorganisms were obtained from the Refik Saydam Hıfızıssıhha Institute (Ankara, Turkey) and were as follows: Escherichia 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 as adapted earlier 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 µµL (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).

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Microorganisms and inhibition zone (mm)</th>
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<td>DMSO</td>
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<td>Ampicillin</td>
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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 Hıfzüssıhha Institute. A simple susceptibility screening test using agar-well diffusion as adapted earlier 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, 1H-NMR, and 13C-NMR spectral data. Compound 2b is known. The IR spectrum of compounds 2a-d displayed no absorption derived from –NH2 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. 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.

In the second step of this study, 4-amino-3,5-dialkyl-4H-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 –NH₂ 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 ¹³C-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 –NH₂ group was recorded at 6.06-6.51 ppm in the ¹H-NMR spectra of these compounds (exchangeable with D₂O).

Among compounds 4a-c, 4a and 4b were treated with some aromatic aldehydes such as 4-chlorobenzaldehyde, 3-methoxy-4-hydroxybenzaldehyde, 3-methoxybenzaldehyde, 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.](image-url)
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 1H-NMR spectra of compounds 6a-e and 8a,b, and the signals due to –CH- (or –CH₂) 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 –NH₂ group, and gave compound 10. According to the literature, 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 nucleophile the acetic anhydride molecule than the 4-amino group. In the 1H-NMR spectrum of compound 9 there was a signal at 6.42 ppm representing a free –NH₂ group (exchangeable with D₂O), while the signal was absent in the spectra of compound 10. On the other hand, in the 1H-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 1H-NMR spectra and 2 new signals at 20.31-20.80 ppm and 168.50-169.07 ppm in the 13C-NMR spectra of compounds 9 and 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-hydroxybenzenecarboxyhydrzoneate (2a):** (Yield: 1.56 g, 55%). mp 164-165 °C (white crystals); IR (KBr) cm⁻¹: 3265 (νNH), 3200 (νOH), 1636 (νC=O), 1605 (νC=N); 1H-NMR (DMSO-d₆)δ ppm 1.10 (t, 3H, CH₃, J= 6.96 Hz), 4.20 (q, 2H, OCH₂, 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); 13C-NMR (DMSO-d₆)δ ppm 170.05 (C=O), 164.00 (C=N), ar-C: [158.65 (C), 129.02 (2CH), 129.60 (CH), 128.46 (2CH), 128.06 (2CH), 127.21 (C), 117.90 (C), 115.00 (2CH)], 62.05 (OCH₂), 14.06 (CH₃).
Ethyl N-benzoyl-4-methylbenzenecarboxyldrazonooate (2b): (Yield: 1.69 g, 60%). mp 77-78 °C (white crystals), ref.24 mp 78-79 °C; IR (KBr) cm⁻¹: 3162 (vNH), 1649 (vC=O), 1618 (vC=N).

Ethyl N-benzoyl-pyridine-4-yl-carboxyldrazonooate (2e): (Yield: 1.35 g, 50%). mp 90-91 °C (white crystals); IR (KBr) cm⁻¹: 3133 (vNH), 1676 (vC=O), 1610 (vC=N); 1H-NMR (DMSO-d₆) δ ppm 1.22 (t, 3H, CH₃, J= 6.96 Hz), 4.25 (q, 2H, OCH₂, 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); 13C-NMR (DMSO-d₆) 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 (OCH₂), 15.92 (CH₃).

Ethyl N-acetyl-pyridine-4-yl-carboxyldrazonooate (2d): (Yield: 0.85 g, 41%). mp 109-110 °C (white crystals); IR (KBr) cm⁻¹: 3186 (vNH), 1660 (vC=O), 1636 (vC=N); 1H-NMR (DMSO-d₆) δ ppm 1.30 (t, 3H, CH₃, J= 6.96 Hz), 2.34 (s, 3H, N=C-CH₃), 4.30 (q, 2H, OCH₂, J= 6.96 Hz), [ar-H: 8.06 (bs, 2H), 8.76 (bs, 2H)], 10.28 (s, 1H, NH); 13C-NMR (DMSO-d₆) δ ppm 170.00 (C=O), 160.05 (C=N), ar-C: [150.00 (2CH), 134.05 (C), 122.00 (2CH)], 63.15 (OCH₂), 20.05 (N=C-CH₃), 13.86 (CH₃).

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 (νOH), 1608 and 1559 (νC=O).

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 (νC=O).

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 (νC=O).

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 (νC=O).

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-1,2,4-triazole (4a): Recrystallization from ethyl acetate. mp 229-230 °C (white crystals); IR (KBr) cm⁻¹: 3358-3289 (νNH₂), 3200 (νOH), 1613 (νC=O); 1H-NMR (DMSO-d₆) δ ppm 6.21 (s, 2H, NH₂), [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); 13C-NMR (DMSO-d₆) δ ppm 154.21 (triazole C₃), 153.65 (triazole C₅), 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-1,2,4-triazole (4b): Recrystallization from 1-propanol (yield: 85%). mp 282-283 °C, ref.28,29 mp 282-284 °C. IR (KBr) cm⁻¹: 3345-3285 (νNH₂), 1636 (νC=O).

4-Amino-3-(pyridine-4-yl)-5-phenyl-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 (νNH₂), 1607 (νC=O); 1H-NMR (DMSO-d₆) δ ppm 6.51 (s, 2H, NH₂), [ar-H: 7.60-7.80 (m, 3H), 8.05-8.30 (m, 4H), 8.82

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(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 ºC (white crystals); IR (KBr) cm$^{-1}$: 1609 and 1595 ($\nu_2$C$=$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 ºC (white crystals); IR (KBr) cm$^{-1}$: 3204 ($\nu$OH), 1589 and 1514 ($\nu_2$C$=$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$_3$), 149.88 (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 ºC (white crystals); IR (KBr) cm$^{-1}$: 1595 and 1569 ($\nu_2$C$=$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), 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$_3$).

3-Phenyl-4-[(2,4,6-trimethoxyhydroxyphenyl)methylenamino]-5-(4-tolyl)-4H-1,2,4-triazole (5d): (Yield: 4.02 g, 94%). mp 200-201 ºC (white crystals); IR (KBr) cm$^{-1}$: 1595 and 1569 ($\nu_2$C$=$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), 152.31 (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$_3$).
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 (νOH), 1602 and 1578 (νC=N); ¹H-NMR (DMSO-d₆) δ ppm 2.42 (s, 3H, ar-CH₃), [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); ¹³C-NMR (DMSO-d₆) δ ppm 168.00 (N=CH), 150.16 (triazole C₅), 150.00 (triazole C₅), 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 (2CH), 126.53 (C), 124.00 (CH), 123.61 (CH), 118.07 (CH), 107.93 (C)], 20.79 (ar-CH₃).

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 (νC=N); ¹H-NMR (DMSO-d₆) δ ppm 1.86 (s, 3H, CH₃), 2.32 (s, 3H, ar-CH₃), [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)]; ¹³C-NMR (DMSO-d₆) δ ppm 178.43 (C=N), 149.77 (triazole C₃), 149.58 (triazole C₅), 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-CH₃), 16.86 (CH₃).

4-(1-Phenylethyliden)amino)-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 (νC=N); ¹H-NMR (DMSO-d₆) δ ppm 1.95 (s, 3H, CH₃), 2.34 (s, 3H, ar-CH₃), [ar-H: 7.30 (d, 2H, J = 7.80 Hz), 7.40-7.60 (m, 3H), 7.70 (d, 2H, J = 7.80 Hz), 8.23 (d, 2H, J = 9.16 Hz), 8.39 (d, 2H, J = 9.16 Hz)]; ¹³C-NMR (DMSO-d₆) δ ppm 177.63 (C=N), 149.94 (triazole C₃), 149.81 (triazole C₅), 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-CH₃), 17.41 (CH₃).

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 NaBH₄ (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 (νNH), 1620 (νC=N); ¹H-NMR (DMSO-d₆) δ ppm 2.45 (s, 3H, ar-CH₃), 3.73 (d, 2H, CH₂, 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)]; ¹³C-NMR (DMSO-d₆) δ ppm 153.80 (triazole C₃), 153.74 (triazole C₅), 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 (CH₂), 20.94 (ar-CH₃).

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 (νNH), 3206 (νOH), 1598 (νC=N); ¹H-NMR (DMSO-d₆) δ ppm 2.42 (s, 3H, ar-CH₃), 3.80 (bs, OCH₃ + CH₂), 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 = 8.00 Hz)].
2H, J = 7.80 Hz), 7.90-8.10 (m, 2H), 10.25 (s, 1H, OH); 13C-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$_3$), 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 ($\nu_{NH}$), 1604 ($\nu_{C=O}$); 1H-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)]; 13C-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-trimethoxyphenyl]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 ($\nu_{NH}$), 1609 ($\nu_{C=O}$); 1H-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)]; 13C-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)amino)-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 ($\nu_{NH}$), 3206 ($\nu_{OH}$), 1631 ($\nu_{C=O}$); 1H-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); 13C-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 (2CH), 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 ($\nu_{NH}$), 1617 ($\nu_{C=O}$); 1H-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)]; 13C-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-CH$_2$), 21.00 (ar-CH$_3$), 20.00 (CH$_2$).

4-[1-(4-Nitrophenethyl)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 ($\nu_{NH}$), 1608 ($\nu_{C=O}$); 1H-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)]; 13C-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$_2$), 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 (\(\nu\)NH\(_2\)), 1758 (\(\nu\)C=O), 1608 (\(\nu\)C=N); \(^1\)H-NMR (DMSO-d\(_6\)) \(\delta\) 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)]; \(^1\)C-NMR (DMSO-d\(_6\)) \(\delta\) 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 (\(\nu\)NH), 1711 (\(\nu\)C=O), 1615 (\(\nu\)C=N); \(^1\)H-NMR (DMSO-d\(_6\)) \(\delta\) 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); \(^1\)C-NMR (DMSO-d\(_6\)) \(\delta\) 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), 129.45 (CH), 128.41 (2CH), 128.20 (2CH), 127.34 (2CH), 127.26 (2CH), 125.78 (C), 123.00 (C)], 20.88 (ar-CH\(_3\)), 20.31(O=C-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.

References

Synthesis of Some Novel 3,5-Diaryl-1,2,4-Triazole..., M. SERDAR, et al.,