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Green aqueous synthesis and antimicrobial evaluation of 3,5-disubstituted 1,2,4-triazoles

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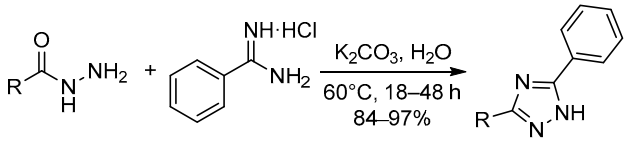


R = Ph, 4-HOC_6H_4, 4-*t*-BuC_6H_4, 4-$O_2NC_6H_4$, 3-$MeOC_6H_4$, 3-BrC_6H_4, 3-hydroxynaphthalen-2-yl, 4-pyridyl, 2-furyl, 5-chlorothiophen-2-yl, 4-methyl-1,2,3-thiadiazol-5-yl

An eco-friendly and simple procedure was proposed for the synthesis of 3,5-disubstituted 1,2,4-triazoles by optimized reaction of benzamidine hydrochloride and various aryl hydrazides. H_2O and K_2CO_3 were applied as green and available solvent and base, respectively. The products were generated in good to high yields in one step and sufficient purity after a simple workup. The designed process is applicable to other organic syntheses especially from poorly water-soluble reactants such as hydrazines or hydrazides. Inhibitory activity of all prepared derivatives was evaluated against 10 pathogenic bacteria strains including both Gram-positive and Gram-negative, as well as 2 mold and 1 yeast strains. The prepared derivatives showed good antimicrobial activities. 1,2,4-Triazoles containing 2-hydroxynaphthalen-3-yl and 5-chlorothiophen-2-yl substituents at position 3 showed the best antifungal and antibacterial properties, respectively.

Keywords: hydrazide, 1,2,4-triazole, antibacterial activity, antifungal activity, aqueous media, green synthesis.

1,2,4-Triazole ring forms an important structural motif of natural products, pharmaceuticals, and biologically active molecules (Fig. 1). Essramycin is a naturally occurring antibiotic extracted from marine *Streptomyces* sp., isolate Merv8102.[1] Sitagliptin decreases level of glucagon, and is prescribed to treat type 2 diabetes mellitus.[2] Rizatriptan is a selective serotonin receptor agonist having antimigrainous effects.[3] Maraviroc is used for the treatment of patients infected with CCR5-tropic HIV-1 virus.[4]

1,2,4-Triazole antifungals as an important class of medications affect a variety of pathogenic molds and yeasts.[5] Fluconazole, a popular antifungal drug, is a selective agonist of CYP51 (an essential enzyme in synthesizing fungal cell wall and lanosterol to ergosterol convertor).[6] Many publications have reported anti-inflammatory,[7,8] antinociceptive,[7] analgesic,[8] antioxidant,[9] antiurease,[9] antiacetylcholinesterase,[9] anticancer,[10,11] and antimicrobial[11] capacities of substituted or fused 1,2,4-triazole derivatives. Excellent inhibitory activities were observed with some 4-(benzylideneamino)-5-phenyl-4*H*-

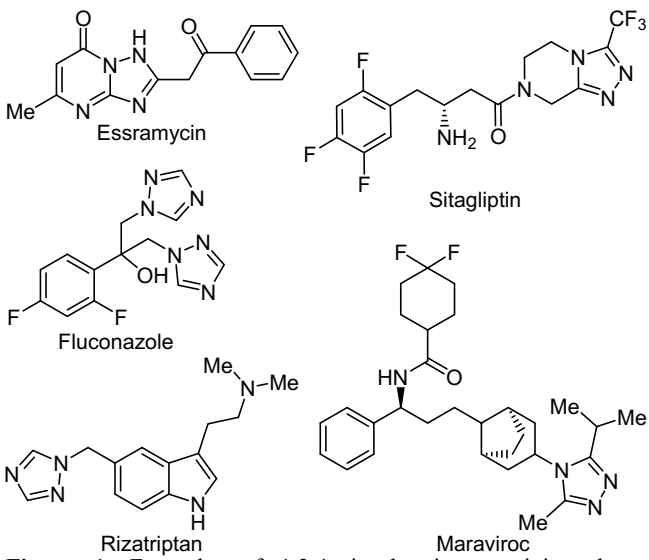


Figure 1. Examples of 1,2,4-triazole ring-containing drugs available on market.

1,2,4-triazole-3-thiol Schiff bases against *Microsporum gypseum* and *Staphylococcus aureus*.[12] Some of them exhibited acceptable antifungal effects on *Candida albicans* and *Aspergillus niger*, while were ineffective against *Escherichia coli*. Good antimicrobial properties of three fluconazol analogs, either alone or in binary mixtures, were demonstrated against *Trichoderma viride*, *Gloeophyllum trabeum*, *Coriolus versicolor*, and *Aspergillus niger*.[13] In addition, hybridization of 1,2,4-triazole derivatives with various antibiotics reduces the toxicity and improves the therapeutic effects, especially against drug-resistant bacteria.[14] To inhibit the growth of bacteria, 1,2,4-triazoles can disrupt nucleic acids, proteins, cell membrane and cell wall synthesis.[15]

The importance of molecules containing 1,2,4-triazole moiety, especially in the field of medicinal chemistry, has stimulated development of their synthesis methods. Pellizzari and Einhorn–Brunner reactions are usual procedures to prepare 1,2,4-triazole derivatives; they include interaction of hydrazides with amides and hydrazines with diacylamines, respectively.[16,17] These valuable heterocycles were also synthesized by cyclization of hydrazinecarboximidamide,[18] copper-catalyzed reaction of amidoximes with nitriles,[19] treatment of hydrazides with isothiocyanates or carbon disulfide and hydrazine,[20,21] interaction of carboxylic acids with hydrazinophthalazine,[22] electrochemical four-component reaction of aryl hydrazines, paraformaldehyde, ammonium acetate, and alcohols,[23] 1,3-dipolar cycloaddition of oximes with hydrazonoyl hydrochloride,[24] and nickel-catalyzed cyclocondensation of hydrazides with *N*-cyanobenzamide.[25]

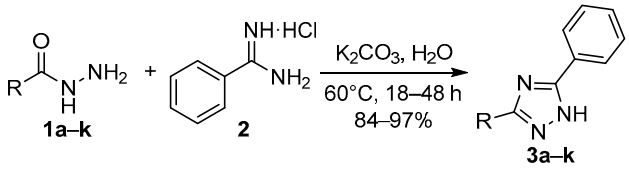
Arylamidines are highly efficient reagents to synthesize 1,2,4-triazole derivatives. They dimerize or react with imidates in basic solutions under catalytic oxidation to generate symmetric or asymmetric 1,2,4-triazoles, respectively.[26] Their interaction with nitriles in the presence of various oxidants affords 1,2,4-triazoles in good yields.[27] However, substituted 1,2,4-triazoles can be also produced *via* direct condensation of nitriles with hydrazides in *n*-BuOH under microwave irradiation at 150°C.[28] Amidrazones are useful intermediates or starting materials for the preparation of 1,2,4-triazoles. The reaction of neutralized acetamidine hydrochloride with hydrazides in EtOH at room temperature affords acetyl amidrazones; 1,2,4-triazoles are obtained thereof under subsequent thermal cyclization in xylene.[29] 1,2,4-Triazoles were also directly synthesized *via* heating of ethanolic solution containing benzamidine hydrochloride, hydrazides, and sodium methoxide.[29] In addition, amidrazones can be produced *via* reaction of arylamidines or corresponding imidic esters with hydrazides in ethanolic NaOEt or in PhH containing catalytic amounts of Et_3N under relatively mild conditions, and converted to 3,5-disubstituted 1,2,4-triazoles at higher temperatures.[30,31] In our new procedure described below, some new and known 1,2,4-triazole derivatives were eco-friendly prepared by reaction of aqueous solutions containing benzamidine hydrochloride, aryl hydrazides, and K_2CO_3.

Environmental problems due to the use of most organic solvents make it necessary to find green alternatives. One of the main purposes of green chemistry is to design and develop reactions in aqueous media.[32] Although there have been many advances in the design of environmentally friendly organic solvents, water is still the best option. It is a safe, available, inexpensive, and eco-friendly liquid that can dissolve a variety of polar compounds and organic and inorganic salts. Chemical kinetics and reaction mechanisms can be affected by the presence of water as solvent. The insolubility of many nonpolar or less polar compounds and high-mass molecules in water leads to its limited application. The solubility of compounds in aqueous solutions can be improved *via* increasing the temperature, the use of multisolvent systems, addition of surfactants, and changing the pH.

To prove the utility of heterocyclic synthesis in aqueous media and search for potent antimicrobial agents, a series of 3-substituted 5-phenyl-1*H*-1,2,4-triazoles were synthesized in good to excellent yields *via* cyclocondensation of benzamidine hydrochloride and aryl hydrazides in basic aqueous medium. The reaction proceeded efficiently in the presence of K_2CO_3 as a green base. *In vitro* inhibitory activity of the prepared 1,2,4-triazoles was studied against some important fungal and bacterial pathogens.

3-Substituted 5-phenyl-1*H*-1,2,4-triazoles **3a**–**k** were prepared by reaction of aryl or heteroaryl hydrazides **1a**–**k** and benzamidine hydrochloride (**2**) under relatively mild conditions (Scheme 1). H_2O and K_2CO_3 were selected as green solvent and base, respectively, due to low cost, availability, and non-toxicity.[33,34]

Scheme 1. Green synthesis of 1,2,4-triazoles **3a**–**k** in aqueous media



a R = Ph, **b** R = 4-HOC_6H_4, **c** R = 4-*t*-BuC_6H_4, **d** R = 4-$O_2NC_6H_4$, **e** R = 3-$MeOC_6H_4$, **f** R = 3-BrC_6H_4, **g** R = 3-hydroxynaphthalen-2-yl, **h** R = 4-pyridyl, **i** R = 2-furyl, **j** R = 5-chlorothiophen-2-yl, **k** R = 4-methyl-1,2,3-thiadiazol-5-yl

The reaction conditions were optimized in terms of presence or absence of base, the type of base, and temperature. Interaction of 1.0 mmol of benzhydrazide (**1a**) with an equimolar amount of benzamidine hydrochloride (**2**) in 2 ml H_2O was studied under different conditions. The volume of H_2O was chosen as the minimum required to dissolve the reactants, including hydrazide **1a**. The reaction mixture must be heated to at least 50°C to improve the hydrazide solubility and the progress of reaction. The reaction did not occur in the absence of a base even at higher temperature. The reaction did not progress in the presence of different amounts (1.0, 1.5, and 2.0 mmol) of KOH at temperatures up to 100°C. No product was obtained in the presence of 1 mmol of K_2CO_3 at 50°C. The reaction progress was checked at 60°C when 1–2.5 mmol of K_2CO_3 was used as base (Table 1, entries 1–4). The lowest reaction time and the highest product yield were obtained in the presence of

Scheme 2. A possible mechanism for the formation of 1,2,4-triazoles **3a**–**k** in the presence of K_2CO_3

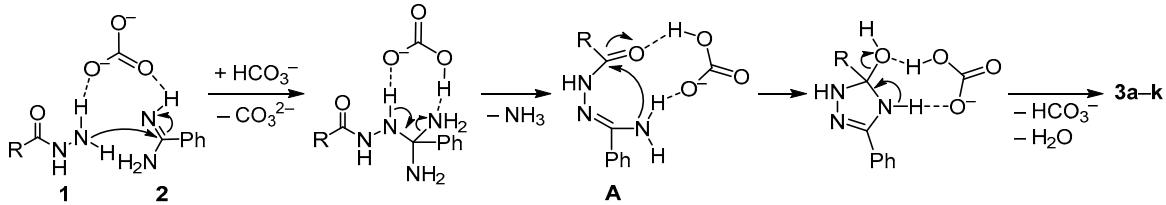


Table 1. Optimization of reaction conditions in the synthesis of 3,5-diphenyl-1*H*-1,2,4-triazole (**3a**)*

Entry	Amount of K_2CO_3, mmol	Temperature, °C	Time, h	Yield, %
1	1.0	60	67	71
2	1.5	60	58	76
3	**2.0**	**60**	**48**	**86**
4	2.5	60	48	80
5	2.0	80	48	84
6	2.0	100	48	83

* For all entries, benzhydrazide (**1a**) (1.0 mmol), benzamidine hydrochloride (**2**) (1.0 mmol), H_2O (2 ml).

2 mmol of K_2CO_3 at 60°C (entry 3). Increasing the temperature did not improve the yields (entries 5, 6).

1,2,4-Triazole **3a** was not produced in the presence of only OH^- ions. Aqueous solutions of K_2CO_3 also contain other anions such as CO_3^- and HCO_3^-. It seems that they play a key role in the reaction as a buffering system, as shown in Scheme 2. An excess of K_2CO_3 is required for pH regulation along the progress of the reaction. K_2CO_3 as a base neutralizes benzamidine hydrochloride, improves the solubility of reactants, especially hydrazides, increases nucleophilicity of hydrazides through formation of strong hydrogen bonds, facilitates NH_3 removal process to form intermediate amidrazones **A**, as well as elimination of H_2O during the intramolecular cyclocondensation.

Finally, 1,2,4-triazole derivatives **3a**–**k** were prepared under the optimized conditions (twofold excess of K_2CO_3, 60°C) (Scheme 1). 1,2,4-Triazoles **3b**,**j**,**k** have not been described before. The chemical structure of all synthesized products was characterized and confirmed by FT-IR, ^{1}H and ^{13}C NMR spectroscopies and elemental analysis. In ^{1}H NMR spectra at ambient temperature, signals of NH groups of triazole rings appeared as singlets or broad singlets at 9.45–14.23 ppm. In ^{13}C NMR spectra, two signals were detected in the range 155.3–164.9 ppm attributed to the C-3 and C-5 carbon atoms of triazole rings **3b**–**k**. In IR spectra, the NH stretching vibrations were observed at ~3400 cm^{-1}.

In vitro antimicrobial properties of all synthesized 1,2,4-triazoles were studied against some important pathogenic fungi and Gram-positive and Gram-negative bacteria, as shown in Tables 2 and 3. The results were compared to the data for the antibiotic ampicillin and the antifungal drug clotrimazole.

Table 2. Antibacterial activity of 1,2,4-triazoles **3a**–**k** expressed as minimum inhibitory concentration (MIC), in μg·ml^{-1}, and minimum bactericidal concentration (MBC), in μg·ml^{-1}

Bacteria		Test compound											Reference
		3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	Ampicillin
Pseudomonas aeruginosa	MIC	256	1024	–*	2048	256	128	256	1024	2048	256	1024	0.063
	MBC	256	2048	–	2048	256	512	1024	2048	2048	1024	1024	0.063
Salmonella enterica subsp. *enterica*	MIC	256	1024	16	2048	1024	–	64	1024	1024	16	512	8
	MBC	1024	2048	64	2048	1024	–	64	2048	2048	64	512	8
Escherichia coli	MIC	2048	1024	–	256	2048	2048	256	2048	256	32	–	8
	MBC	2048	2048	–	1024	2048	2048	1024	2048	256	32	–	8
Klebsiella pneumoniae	MIC	1024	32	–	2048	–	2048	256	2048	2048	–	1024	4
	MBC	1024	64	–	2048	–	2048	1024	2048	2048	–	2048	4
Shigella dysenteriae	MIC	256	64	1024	1024	1024	1024	1024	256	64	256	1024	0.031
	MBC	1024	64	2048	2048	1024	1024	2048	1024	64	512	2048	0.063
Acinetobacter baumannii	MIC	256	256	–	512	512	–	32	1024	2048	–	–	16
	MBC	1024	256	–	1024	512	–	32	2048	2048	–	–	32
Bacillus cereus	MIC	1024	16	32	16	64	–	1024	2048	256	128	2048	0.25
	MBC	1024	64	64	32	64	–	2048	2048	1024	256	2048	4
Staphylococcus epidermidis	MIC	1024	512	64	128	2048	16	256	1024	256	512	512	1
	MBC	1024	1024	256	512	2048	64	1024	1024	256	1024	512	2
Listeria monocytogenes	MIC	1024	128	256	1024	2048	256	256	256	512	512	512	2
	MBC	1024	128	512	1024	2048	512	1024	1024	512	512	512	2
Streptococcus pyogenes	MIC	256	1024	1024	1024	32	1024	512	1024	256	64	–	2
	MBC	1024	1024	1024	2048	32	1024	512	1024	256	64	–	2

* – No noticeable antibacterial effect at concentration 2048 μg·ml^{-1}.

Table 3. Antifungal activity of 1,2,4-triazoles **3a**–**k** expressed as minimum inhibitory concentration (MIC), in μg·ml^{-1}, and minimum fungicidal concentration (MFC), in μg·ml^{-1}

Fungi		Test compound											Reference
		3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	Clotrimazole
Aspergillus fumigatus	MIC	256	512	1024	–	–	–	16	512	1024	–	1024	32
	MFC	1024	512	2048	–	–	–	32	512	1024	–	2048	32
Fusarium oxysporum	MIC	2048	64	16	256	256	16	128	2048	512	–	32	256
	MFC	2048	64	64	1024	1024	32	128	2048	512	–	64	512
Candida albicans	MIC	16	512	–*	1024	1024	32	16	128	32	–	512	256
	MFC	64	512	–	2048	2048	32	32	512	64	–	512	512

* – No noticeable antibacterial effect at concentration of 2048 μg·ml^{-1}.

According to the results presented in Table 2, products **3a**,**b**,**d**,**g**,**h**,**i** were effective against all tested bacteria. All synthesized triazoles could inhibit the growth of Gram-negative *Shigella dysenteriae* and Gram-positive *Staphylococcus epidermidis* and *Listeria monocytogenes*. The best antibacterial activities were observed for triazole **3j** containing 3-(5-chlorothiophen-2-yl) and 5-phenyl substituents, however, it was completely ineffective against the studied fungi. 1,2,4-Triazole **3j** was the only chlorine-containing compound among the prepared derivatives.

All fungal strains were inhibited by compounds **3a**,**b**,**g**,**h**,**i**,**k** (Table 3). 3-(5-Phenyl-1*H*-1,2,4-triazol-3-yl)-naphthalen-2-ol (**3g**) was recorded as possessing the highest antifungal potency. As expected from literature data,[5] compounds **3a**–**k** were more successful in blocking fungal than bacterial strains.

In this study, a new modified procedure was proposed for the preparation of 3,5-disubstituted 1,2,4-triazoles. The use of green, easy available and inexpensive solvent and base, simple workup and purification, and acceptable yields are the advantages of the designed method. The efficiency of this eco-friendly system can be evaluated in other heterocyclic synthesis from starting materials, such as hydrazines or hydrazides. Inhibitory potentials of synthesized products were proved against a broad spectrum of bacterial and fungal pathogens. Chlorinated aryls or sulfurated heterocycles at positions 3 and 5 of the triazole ring may improve antimicrobial effects.

Experimental

FT-IR spectra were recorded on a Bruker Tensor-27 FT-IR spectrometer in thin layer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker FT-NMR Ultra Shield-400 spectrometer (400 and 100 MHz, respectively) using DMSO-d_6 residual solvent peak as internal standard (for ^{1}H nuclei δ 2.48 ppm, for ^{13}C nuclei δ 39.9 ppm). Elemental analyses for C, H, N, and S atoms were performed on a Termo Finnigan Flash EA microanalyzer. Melting points are uncorrected and were determined using a Kruss-type KSP1N melting point apparatus. The progress of the reactions was monitored by aluminum TLC plates precoated with silica gel with fluorescent indicator F254, different mixtures of MeOH and CH_2Cl_2 were used as the mobile phase.

All solvents and reagents were commercially purchased from Sigma-Aldrich and Merck and used without further purification.

Synthesis of 1,2,4-triazoles 3a–k (General method). A suspension of aryl hydrazide **1a**–**k** (1.0 mmol), benzamidine hydrochloride (**2**) (0.157 g, 1.0 mmol), and K_2CO_3 (0.276 g, 2.0 mmol) in double-distilled H_2O (2 ml, conductivity 1.76 μS·cm^{-1}) was vigorously stirred at 60°C for 18–48 h. The progress of the reaction was monitored by TLC. The reaction mixture was cooled in an ice bath and subsequently neutralized with 1.0 M HCl solution. The precipitate was filtered off, washed with cold H_2O (5 ml) and EtOH (5 ml), and dried in an oven to afford 1,2,4-triazoles **3a**–**k** without the need for additional purification.

3,5-Diphenyl-1*H*-1,2,4-triazole (3a). Reaction time 48 h. Yield 190 mg (86%), mp 188–190°C (mp 190–192°C[35]). IR spectrum, ν, cm^{-1}: 3414 (NH), 1617 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.41–7.50 (6H, m, H-3,4,5 Ph); 8.07 (4H, d, *J* = 6.8, H-2,6 Ph); 14.23 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 126.3; 129.2; 129.5; 130.5; 159.1. Found, %: C 76.03; H 4.99; N 18.98. $C_{14}H_{11}N_3$. Calculated, %: C 76.00; H 5.01; N 18.99.

4-(5-Phenyl-1*H*-1,2,4-triazol-3-yl)phenol (3b). Reaction time 45 h. Yield 216 mg (91%), mp 265–267°C. IR spectrum, ν, cm^{-1}: 3418 (NH), 3342 (OH), 1620 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 6.87 (2H, d, *J* = 8.1, H-3,5 Ar); 7.44–7.48 (3H, m, H-3,4,5 Ph); 7.87 (2H, d, *J* = 8.1, H-2,6 Ar); 8.04 (2H, d, *J* = 6.2, H-2,6 Ph); 10.16 (1H, br. s, OH); 14.13 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 116.0; 126.3; 128.1; 128.6; 129.2; 129.6; 130.0; 159.3; 164.9; 160.8. Found, %: C 70.85; H 4.66; N 17.75. $C_{14}H_{11}N_3O$. Calculated, %: C 70.87; H 4.67; N 17.71.

3-(4-*tert*-Butylphenyl)-5-phenyl-1*H*-1,2,4-triazole (3c).[36] Reaction time 41 h. Yield 269 mg (97%), mp 177–179°C. IR spectrum, ν, cm^{-1}: 3414 (NH), 1644 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.28 (9H, s, $3CH_3$); 7.40–7.50 (5H, m, H-3,5 Ar, H-3,4,5 Ph); 8.06 (2H, d, *J* = 8.1, H-2,6 Ar); 8.14 (2H, d, *J* = 7.0, H-2,6 Ph); 14.13 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 31.4; 34.8; 125.9; 126.3; 126.4; 129.1; 129.5; 130.6; 131.6; 152.3; 158.5; 159.2. Found, %: C 77.90; H 6.93; N 15.17. $C_{18}H_{19}N_3$. Calculated, %: C 77.95; H 6.90; N 15.15.

3-(4-Nitrophenyl)-5-phenyl-1*H*-1,2,4-triazole (3d). Reaction time 18 h. Yield 253 mg (95%), mp 248–250°C (mp 246–247°C[27]). IR spectrum, ν, cm^{-1}: 3415 (NH), 1621 (C=N), 1551 (N–O). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.43–7.46 (3H, m, H-3,4,5 Ph); 7.85 (2H, d, *J* = 7.7, H-2,6 Ph); 8.30–8.09 (4H, m, H Ar); 14.06 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 124.5; 126.4; 127.2; 129.3; 130.6; 133.7;

138.5; 147.2; 159.3; 162.2. Found, %: C 63.19; H 3.79; N 21.02. $C_{14}H_{10}N_4O_2$. Calculated, %: C 63.15; H 3.79; N 21.04.

3-(3-Methoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole (3e). Reaction time 18 h. Yield 231 mg (92%), mp 184–186°C (mp 178–180°C[35]). IR spectrum, ν, cm^{-1}: 3419 (NH), 1623 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.81 (3H, s, CH_3); 7.01 (1H, d, *J* = 7.7, H-4 Ar); 7.38–7.49 (4H, m, H-2 Ar, H-3,4,5 Ph); 7.66–7.71 (2H, m, H-5,6 Ar); 8.11 (2H, d, *J* = 6.8, H-2,6 Ph); 9.85 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 55.5; 111.5; 115.7; 118.8; 126.4; 129.2; 129.8; 130.0; 130.4; 131.2; 158.6; 158.9; 160.0. Found, %: C 71.73; H 5.19; N 16.68. $C_{15}H_{13}N_3O$. Calculated, %: C 71.70; H 5.21; N 16.72.

3-(3-Bromophenyl)-5-phenyl-1*H*-1,2,4-triazole (3f).[37] Reaction time 40 h. Yield 255 mg (85%), mp 210–212°C. IR spectrum, ν, cm^{-1}: 3417 (NH), 1643 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.45–7.50 (5H, m, H-2,5 Ar, 3,4,5 Ph); 7.62 (1H, d, *J* = 7.3, H-4 Ar); 8.06 (2H, d, *J* = 6.7, H-2,6 Ph); 8.22 (1H, s, H-6 Ar); 10.64 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 122.5 125.3; 126.5; 128.8; 129.4; 130.3; 131.5; 132.0; 132.5; 133.1; 158.3; 158.7. Found, %: C 56.04; H 3.35; N 13.97. $C_{14}H_{10}BrN_3$. Calculated, %: C 56.02; H 3.36; N 14.00.

3-(5-Phenyl-1*H*-1,2,4-triazol-3-yl)naphthalen-2-ol (3g). Reaction time 38 h. Yield 270 mg (94%), mp 247–249°C (mp 244–246°C[38]). IR spectrum, ν, cm^{-1}: 3414 (NH), 3339 (OH), 1622 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.03–7.10 (2H, m, H-6,7 Ar); 7.24–7.30 (1H, m, H-1 Ar); 7.44–7.48 (3H, m, H-3,4,5 Ph); 7.50–7.54 (2H, m, H-5,8 Ar); 7.71 (1H, d, *J* = 8.0, H-4 Ar); 7.88–7.90 (2H, m, H-2,6 Ph); 8.51 (1H, s, OH); 14.10 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 110.9; 121.3; 123.1; 125.2; 125.4; 127.0; 127.1; 128.7; 128.9; 129.7; 130.4; 133.7; 136.8; 151.4; 161.6; 165.1. Found, %: C 75.24; H 4.60; N 14.61. $C_{18}H_{13}N_3O$. Calculated, %: C 75.25; H 4.56; N 14.63.

4-(5-Phenyl-1*H*-1,2,4-triazol-3-yl)pyridine (3h). Reaction time 28 h. Yield 189 mg (85%), mp 242–243°C (mp 241–242°C[32]). IR spectrum, ν, cm^{-1}: 3418 (NH), 1619 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.48–7.54 (3H, m, H-3,4,5 Ph); 7.70 (2H, d, *J* = 4.7, H-3,5 Py); 7.99 (2H, d, *J* = 4.7, H-2,6 Py); 8.07 (2H, d, *J* = 7.3, H-2,6 Ph); 14.08 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 120.5; 126.6; 128.2; 129.4; 130.6; 137.6; 150.8; 157.6; 158.5. Found, %: C 70.21; H 4.53; N 25.26. $C_{13}H_{10}N_4$. Calculated, %: C 70.25; H 4.54; N 25.21.

3-(Furan-2-yl)-5-phenyl-1*H*-1,2,4-triazole (3i). Reaction time 43 h. Yield 177 mg (84%), mp 187–188°C (mp 190–193°C[39]). IR spectrum, ν, cm^{-1}: 3415 (NH), 1622 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 6.64 (1H, s, H-4 Fur); 7.02 (1H, s, H-3 Fur); 7.45–7.50 (3H, m, H-3,4,5 Ph); 7.84 (1H, s, H-5 Fur); 8.03 (2H, d, *J* = 6.8, H-2,6 Ph); 14.21 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 110.0; 112.2; 126.4; 129.3; 130.1; 144.4; 145.5; 152.3; 158.1; 160.8. Found, %: C 68.21; H 4.32; N 19.88. $C_{12}H_9N_3O$. Calculated, %: C 68.24; H 4.29; N 19.89.

3-(5-Chlorothiophen-2-yl)-5-phenyl-1*H*-1,2,4-triazole (3j). Reaction time 42 h. Yield 238 mg (91%), mp 260–262°C. IR spectrum, ν, cm^{-1}: 3416 (NH), 1635 (C=N), 823 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 7.16 (1H, s, H-4 Het); 7.47–7.50 (4H, m, H-3 Het, H-3,4,5 Ph); 8.02 (2H, d, *J* = 6.2, H-2,6 Ph); 14.15 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm (*J*, Hz): 125.8; 126.5; 127.1; 128.3; 128.5; 129.4; 130.3; 132.5; 155.3; 157.5. Found, %: C 55.08; H 3.10; N 16.03; S 12.21. $C_{12}H_8ClN_3S$. Calculated, %: C 55.07; H 3.08; N 16.06; S 12.25.

4-Methyl-5-(5-phenyl-1*H*-1,2,4-triazol-3-yl)-1,2,3-thiadiazole (3k). Reaction time 46 h. Yield 231 mg (95%), mp 258–259°C. IR spectrum, ν, cm^{-1}: 3418 (NH), 1621 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.96 (3H, s, CH_3); 7.50–7.52 (3H, m, H-3,4,5 Ph); 7.97–8.00 (2H, m, H-2,6 Ph); 14.17 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 14.1; 126.7; 129.5; 131.1; 141.1; 153.9; 156.4; 156.6; 162.1. Found, %: C 54.35; H 3.70; N 28.80; S 13.15. $C_{11}H_9N_5S$. Calculated, %: C 54.31; H 3.73; N 28.79; S 13.17.

Biological evaluation. Mueller–Hinton broth, Mueller–Hinton agar, RPMI 1640 medium (Roswell Park Memorial Institute 1640) buffered to pH 7.0 with morpholine propane sulfonic acid, ampicillin, and clotrimazole were obtained from HiMedia and Sigma-Aldrich companies. Appropriate concentration of microbial suspensions was prepared with a Jenway 6405 UV/Vis spectrophotometer. Gram-positive bacterial strains including *Streptococcus pyogenes* (PTCC 1447), *Staphylococcus epidermidis* (PTCC 1435), *Listeria monocytogenes* (PTCC 1297), *Bacillus cereus* (PTCC 1665), Gram-negative bacterial strains including *Pseudomonas aeruginosa* (PTCC 1310), *Salmonella enterica* subsp. *enterica* (PTCC 1709), *Shigella dysenteriae* (PTCC 1188), *Klebsiella pneumoniae* (PTCC 1290), *Acinetobacter baumannii* (PTCC 1855), *Escherichia coli* (PTCC 1399), molds including *Aspergillus fumigatus* (PTCC 5009) and *Fusarium oxysporum* (PTCC 5115) and yeast *Candida albicans* (PTCC 5027) were obtained from the Persian Type Culture Collection (PTCC), Karaj, Iran. Antimicrobial susceptibility tests were performed according to published procedures.[40] The reported results are taken as the mean of three independent experiments.

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