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RESEARCH ARTICLE

# Regio-selective synthesis of 1,4-disubstituted-1,2,3-triazoles and evaluation of their antimicrobial activity

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**ABSTRACT**
A series of 1,4-disubstituted 1,2,3-triazoles were synthesized via Cu(I) catalyzed reaction between terminal alkyne and substituted phenyl azides. The synthesized triazoles were characterized by 1H NMR and mass spectral techniques. The synthesized compounds were evaluated for their antimicrobial activity against Escherichia coli , Staphylococcus aureus, Aspergillus niger by well diffusion method.
**Keywords:** 1,2,3-triazoles, click chemistry, anti-fungal, antibacterial.

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**Introduction:**

Azoles are five membered heterocyclic compounds with two or more heteroatom in which at least one is nitrogen. Azoles are found widely in natural sources and there are several drugs available which contain azole ring importantly Isoxazole, Thiazole, pyrazole, triazole and tetrazole [1]. The triazole nucleus is one of the most important and well known azole which is a common and integral feature of a variety of natural products and medicinal agents [2]. The 1,2,3- triazole moiety does not occur in nature, although the synthetic molecules that contain 1,2,3-triazole units show diverse biological activities. Triazole derivatives are known to exhibit various pharmacological properties such as antimicrobial [3, 4], antitubercular [5], anticancer [6,7], anticonvulsant [8], antiinflamatory, analgesic [9] and antiviral [10]. Huisgen cycloaddition, the general method for the synthesis of 1,4-disubstituted 1,2,3-triazoles includes a 1,3-dipolar cycloaddition between azides and alkynes under thermal conditions to afford the equal mixture of 1,4- and 1,5- disubstituted isomers [11]. A practical solution to avoid the formation of isomeric mixture in products, was given by Sharpless [12] and Meldal [13] through the catchy term "click chemistry". The emerging field of click chemistry offers a unique approach to the synthesis of 1,2,3-triazole containing molecules. The Cu (I) catalyzed 1,2,3-triazole forming reaction between azides and terminal alkynes has become the gold standard of click chemistry due to its reliability, specificity and biocompatibility. Applications of click chemistry are increasingly found in all respects of drug discovery; they range from lead finding through combinatorial chemistry and target–templated *in vitro* chemistry [14]. Thus the development of the Cu (I) catalyzed triazole click chemistry has led to many intresting applications including the synthesis, medicinal chemistry [15], material science [16] and supramolecular chemistry [17].

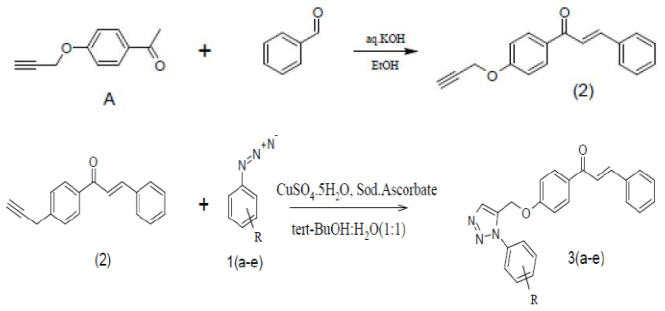
**Experimental**

**General:**

All the starting materials were commercially available research grade chemicals and used without further purification. The progress of all reactions was monitored by TLC on 2 x 5 cm precoated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV 254-366 nm and iodine. Melting points were determined on Buchi B-545 melting point apparatus and are uncorrected. $^1$H NMR spectra were recorded on Bruker AvIII HD-300MHz FT NMR spectrometer with TMS as internal Reference. Mass spectra were determined on JMS-T100LC, AccuTOF mass spectrometer (DART-MS).

**Synthesis:**

Herein**,** we report the synthesis of a series of 1,4-disubstituted 1,2,3-triazoles from terminal alkyne and various azides and their antibacterial and antifungal activities. The alkyne was prepared by treating propargylated p-hydroxy acetophenone with benzaldehyde in presence of aq.KOH in ethanol according to the literature procedure [18]. The click reaction between alkyne (**2)** and the azides **1(a-e)** was carried out in tert-BuOH:$H_2O$(1:1mixture) with Cu(I) as a catalyst and sodium ascorbate as the base [19]. It afforded the desired products **3(a-e)** in good yield, i.e., 69-90%.




*Where R= H, o-Nitro, p-Bromo, p-Methoxy, m-Bromo.

**Biology**

**Anti-bacterial activity:**

Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* in Dimethylsulphoxide(DMSO) by agar diffusion method on nutrient agar medium [20]. The sterile medium (Nutrient Agar Medium, 15 mL) in each petri-plates was uniformly smeared with cultures of Gram positive and Gram-negative bacteria. A well of 6mm diameter and 50µL volume was bored on the petriplates using a sterile cork borer. The solution of test compound 1000µg/ml was prepared in DMSO and the well bored on the medium was filled(50µg) with the test compound using a micropippete (20-200µL). The plates prepared were kept at room temperature for 10 minutes allowing the diffusion of test compound into the agar. Single well was bored on each plate and two plates were taken for each test compound and the experiment was repeated twice. Ciprofloxacin (10mg/ml) was used as a standard and was introduced into the well instead of the test compound. The plates were incubated at 37 ± 2°C for 24 h and the size of the resulting zone of inhibition was determined.

**Anti-fungal activity:**

The synthesized compounds were screened for their antifungal activity against *Aspergillus niger* in DMSO by agar diffusion method. Potato Dextrose Agar (PDA)

media was prepared and about 15 mL of PDA was poured into each petriplate and allowed to solidify and 10µL of fungal broth was spread on the surface of PDA plates. A well of 6mm diameter and 50µL volume was bored on the petriplates using a sterile cork borer. The well was filled with test solution(1000µg/mL) prepared in DMSO using a micropippete(20-200µL) Single well was bored on each plate and two plates were taken for each test compound and the experiment was repeated twice. Fluconazole (10mg/ml) was used as a standard and was introduced into the well instead of the test compound. The plates containing the test organism and the test material in contact were incubated at 27 ± 0.2$^0$C for 48hrs. After incubation the size of the resulting zone of inhibition was measured in mm.

## Results and Discussion

### Chemistry:

Structures of compounds **3(a-e)** was confirmed by $^1$HNMR and mass spectroscopic techniques. The formation of triazoles was apparent due to appearance of characteristic singlet in $^1$HNMR due to triazolyl protons in the region of δ =7.26 ppm.

| Compound | Appearance | m.p(ºC) | Yield(%) | Recrystallization solvent | TLC | Mol. Formula | Mol. weight |
|---|---|---|---|---|---|---|---|
| **3a** | Shiny straw color solid | 120-122 | 69 | Diethylether | Hexane:Ethylacetate(7:3) | $C_{24}H_{19}O_2N_3$ | 381.44 |
| **3b** | Light yellow solid | 160-162 | 90 | Diethylether | Hexane:Ethylacetate(7:3) | $C_{24}H_{18}O_4N_4$ | 426.34 |
| **3c** | Copper color puffy solid | - | 75 | Diethylether | Hexane:Ethylacetate(7:3) | $C_{24}H_{18}O_2N_3Br$ | 460.33 |
| **3d** | Off white solid | 152-154 | 80 | Diethylether | Hexane:Ethylacetate(7:3) | $C_{25}H_{21}O_3N_3$ | 411.46 |
| **3e** | Creamy white solid | 140-142 | 73 | Diethylether | Hexane:Ethylacetate(7:3) | $C_{24}H_{18}O_2N_3Br$ | 460.33 |

**Table 1: Physical data of synthesized compounds.**

| S.No | Compounds | Zone of inhibition (mm) Antibacterial activity | | Zone of inhibition(mm) Antifungal activity |
|---|---|---|---|---|
| | | *S.aureus* | *E.coli* | *A. niger* |
| 1 | 3a | 19.00 | 19.00 | 16.00 |
| 2 | 3b | 20.00 | 18.00 | 17.00 |
| 3 | 3c | 22.00 | 18.00 | 23.00 |
| 4 | 3d | 20.00 | 17.00 | 20.00 |
| 5 | 3e | 18.00 | 17.00 | 19.00 |
| 6 | Ciprofloxacin | 40.00 | 35.00 | - |
| 7 | Fluconazole | - | - | 20.00 |

**Table 2**: ***In vitro*** **antibacterial and antifungal screening of the title compounds-3a-3e.**

### Antifungal and antibacterial activity

Well diameter/vol. -6mm/50µl for antibacterial activity and 5mm/50µl for antifungal activity; Ciprofloxacin, standard for antibacterial activity. Fuconazole, standard for antifungal activity.

The results obtained from their antibacterial studies revealed that compounds **3(a-e)** showed inhibitory activity against both the test pathogenic bacteria. Among test compounds**3c** showed highest activity against *S.aureus* with zone of inhibition of 22mm followed by **3d** and **3b** with zone of inhibition of 20mm each. Among test compounds **3a** showed maximum activity against *E.coli* with zone of inhibition of 19mm.

The antifungal activity results indicated that all the synthesized compounds exhibited good activity against the tested fungal strain with zone of inhibition values ranging from 16-23mm. According to **(Table2)** compound **3c** showed the maximum zone of inhibition of 23mm followed by the standard fluconazole and compound **3d** with zone of inhibition of 20mm each. Compounds **3e, 3b** and **3a** showed good to moderate activity.

### Discussion:

By comparing the antibacterial activity of the synthesized compounds it was found that the tested compounds were more effective against the gram positive bacteria(*S.aureus*) than gram negative bacteria(*E.coli*). The difference in the antibacterial activity of the compounds is related to cell wall structure of the bacteria. This is possible because the cell wall is essential for the survival of many bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan. Gram positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids, but in contrast, Gram negative bacteria have a relatively thin

cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysachharides and lipoproteins. These differences in cell wall structure can produce differences in the antibacterial susceptibility and some antibiotics that can kill only Gram-positive bacteria are ineffective against Gram negative bacteria [21]. A comparison within each series shows that a substituent at position 3 of the benzyl ring has negative effect on antimicrobial activity [22].

The results obtained from their antifungal studies also revealed that compounds **3(a-e)** showed inhibitory activity against *Aspergillus niger.*

It is evident from the data that even the position of substituent on the aromatic ring influences the relative activity which can be attributed to their differences in either the bioavailability or the protein-binding properties.

## Conclusion

A series of 1,4-disubstituted 1,2,3-triazole compounds were synthesized through an easy and convenient Cu(I) catalyzed click reaction and evaluated for their *invitro* antimicrobial activity against *Staphylococcus aureus, Escherchia coli and Aspergillus niger.* The antimicrobial activity studies revealed that all the compounds screened showed moderate to good activities .The significant antimicrobial activity of some of the synthesized compounds highlights them as promising molecules for further synthetic and biological exploration.

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