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Synthesis and Antimicrobial Activity of 2-(4-((4-substituted phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-arylacetamides

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ABSTRACT

2-(4-((4-substituted phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-arylacetamides (7a-7j) were synthesized through copper(I)-catalyzed click reaction of 1-methyl-4-(prop-2-ynyloxy)benzene (3a) and 1-nitro-4-(prop-2-ynyloxy)benzene (3b) with 2-azido-N-arylacetamides. The structural confirmation of the synthesized compounds was carried out by using various spectral techniques (FT-IR, ^{1}H-NMR, ^{13}C-NMR, HRMS). The synthesized compounds which reflects in general the various 1,4-disubstituted 1,2,3-triazoles were screened for in vitro antibacterial activity against Escherichia coli, Pseudomonas aeruginosa (Gram negative bacteria), Bacillus subtilis, Staphylococcus aureus (Gram positive bacteria) and antifungal activity against Candida albicans, Aspergillus niger. Compound 7i displayed good antimicrobial activity against tested bacterial and fungal strains.

Keywords: Click reaction, 1,4-disubstituted-1,2,3-triazoles, Antibacterial activity, Antifungal activity

INTRODUCTION

The development of drug resistance in microbial strains to common antibiotics is the major issue for the public health in the world [1]. This problem has thrown a challenge to the researchers for development of newer potent antimicrobials with diverse biological spectrum to combat the microbial diseases effectively. The N-heterocycles especially 1,2,3-triazoles [2] has attracted the attention of organic chemists in the design of potential drug candidates owing to their good biocompatibility and significant pharmacological actions in the form of antiviral [3], antibacterial [4], antifungal [5], anti-HIV [6], antitubercular [7], antimalarial [8], antitripanosomal [9], anticancer [10], analgesic [11], anti-allergic [12], antihypertensive [13], anticonvulsant [14] and CNS depressant [15] agents. In addition, 1,2,3-triazole is a stable scaffold, forms hydrogen bonding in biological systems to have good aqueous solubility and binding efficiency towards targeted microbial enzymes [16]. Further, 1,2,3-triazoles are polar in nature, which leads to good dipole-dipole and pi-stacking interactions with microbial proteins to suppress their growth, hence, provides a platform to serve as potent antimicrobials.

Huisgen 1,3-dipolar cycloaddition between terminal alkynes and organic azides is the classical approach towards the synthesis of disubstituted 1,2,3-triazoles results into formation of both 1,4- and 1,5-isomers [17]. This reaction is slow, requires high temperature and gives poor yield of products without any selectivity. The issue of selectivity in products was amicably resolved by Sharpless [18] and Meldal [19] in 2001, by introducing the copper(I) catalyst in the classical Huisgen's method which leads to regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles as sole product. This Sharpless-Meldal's pioneered 1,3-dipolar cycloaddition with fragrance of copper(I) as catalyst has been acknowledged as one of the premier click reaction in heterocyclic chemistry. Generally, click reaction refers to facile, selective, robust, insensitive and versatile chemical transformation of reactants to single regio-isomeric product. The libraries of medicinally important 1,4-disubstituted 1,2,3-triazoles can be synthesized by using different azides and terminal alkynes *via* this click reaction [20]. Moreover, 1,4-disubstituted 1,2,3-triazoles also find applications in the form of cyclic peptides [21], dendrimers [22], peptidomimetics [23], ionic receptors [24], triazolophanes [25], nanotubes [26] and liquid crystals [27].

So, keeping above aspects of 1,2,3-triazoles in mind, we focused our research on synthesis, characterization and antimicrobial evaluation of 1,4-disubstituted 1,2,3-triazoles. Earlier also, we reported the synthesis of various 1,4-disubstituted 1,2,3-triazoles [28-33] having appreciable antimicrobial efficacy. In continuation of our above work, here, 2-(4-((4-substituted phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-arylacetamides (7a-7j) have been synthesized *via* click reaction of 1-methyl-4-(prop-2-ynyloxy)benzene (3a) and 1-nitro-4-(prop-2-ynyloxy)benzene (3b) with 2-azido-*N*-arylacetamides (prepared *in-situ* by the reaction of sodium azide with 2-bromo-*N*-arylacetamides). To the best of our knowledge, all the ten synthesized amide-ether linked 1,4-disubstituted 1,2,3-triazoles are new. The synthesized triazoles were characterized by spectroscopic techniques like FT-IR, ^{1}H-NMR, ^{13}C-NMR, HRMS and evaluated for antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*.

MATERIALS AND METHODS

General

The chemicals used in the present work were purchased from Sigma-Aldrich, Hi-Media, Qualigens, CDH and Alfa-Aesar. The solvents were purified as per literature procedures. Melting points of the synthesized compounds were recorded in °C by applying open capillary method using an electrothermal apparatus and are uncorrected. The IR spectra were recorded on Shimazdu IR Affinity-I FT-IR spectrophotometer using potassium bromide (KBr) powder and absorption frequencies are stated in cm^{-1}. The NMR spectra were recorded at 400 (^{1}H-NMR), and 100 (^{13}C-NMR) MHz, respectively, on a commercial Bruker Avance II instrument, in deuterated dimethylsulphoxide-d_6 using Tetramethylsilane (TMS) as an internal standard. The chemical shift value are noted in parts per million (δ ppm). Coupling constant (J) values are given in Hertz (Hz). High Resolution Mass spectra were recorded on Waters Micromass Q-Tof Micro (ESI) spectrophotometer. The completion of reactions and the purity of the compounds were analyzed by Thin Layer Chromatography (TLC) using readymade silica gel plates (SIL G/UV$_{254}$, ALUGRAM) and visualized under ultraviolet lamp.

Synthesis

General procedure for the synthesis of 1-methyl / 1-nitro-4-(prop-2-ynyloxy)benzenes (3a-3b)

To a stirred solution of p-cresol 1a (1.0 mmol)/ p-nitrophenol 1b (1.0 mmol) in dry dimethylformamide, propargyl bromide 2 (1.5 mmol), potassium carbonate (3.0 mmol) was added and the stirring was continued at 35-45°C for 5-8 h (Scheme 1) [34]. The progress of reaction was monitored by TLC. After completion of reaction, 2 N hydrochloric acid solution was added into the reaction mixture, and the contents were stirred further for 10 min. The product was extracted from the reaction contents with ethyl acetate (50 ml ×3). Washed the organic layer with saturated brine solution and dried using anhydrous sodium sulphate. Finally, filtered and evaporated the organic layer to get the desired 1-methyl/1-nitro-4-(prop-2-ynyloxy)benzenes (3a-3b).

General procedure for the synthesis of 2-bromo-N-arylacetamides (6a-6e)

To a solution of aromatic amines 4a-4e (1.0 mmol) and triethylamine (3.0 mmol) in dry dichloromethane (15 ml) under stirring, bromoacetyl bromide 5 (1.2 mmol) was added dropwise and the stirring of reaction mixture was continued at 0-15°C for 4-6 h (Scheme 2) [35]. The progress of reaction was monitored by TLC. After completion of reaction, added 50 ml of dichloromethane to the reaction mixture, then, the content was stirred and transferred into a separating funnel. Separated organic layer was washed with 2 N hydrochloric acid, followed by saturated sodium bicarbonate solution and finally with brine solution. The organic layer was dried using anhydrous sodium sulphate, filtered and evaporated to get 2-bromo-N-arylacetamides (6a-6e).

General procedure for the synthesis of 2-(4-((4-substituted phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-arylacetamides (7a-7j)

For the synthesis of 2-(4-((4-substituted phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-arylacetamides, to a stirred solution of 2-bromo-*N*-arylacetamides (1.0 mmol) (6a-6e) in dimethylformamide, aqueous solution of sodium azide (3.0 mmol) was added. Then, after 1 h, 1-methyl/1-nitro-4-(prop-2-ynyloxy)benzenes 3a-3b (1.0 mmol), copper sulphate pentahydrate (0.1 mmol) and sodium ascorbate (0.2 mmol) were added successively [36]. Stirring of the reaction content was continued at 55-60°C for 9-12 h (Scheme 3). The progress of reaction was monitored by TLC. After completion of the reaction, cold water was added to the reaction mixture, filtered the precipitated solid and product was washed with aqueous ammonia solution and finally with water. The crude products were purified by washing with ethyl acetate and dried by applying vacuum to furnish the desired 1,4-disubstituted 1,2,3-triazoles (7a-7j) in good yields.

Characterization of synthesized compounds

N-phenyl-2-(4-(p-tolyloxymethyl)-1*H*-1,2,3-triazol-1-yl)acetamide (7a)

Appearance: white solid; Yield: 77%; m.p. 182-184°C; FTIR (KBr): 3269 (N-H str., amide), 3143 (C-H str., triazole ring), 3072, 2943, 1674 (C=O str., amide), 1606, 1550, 1510, 1249, 1010 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=2.24 (s, 3H, CH_3), 5.13 (s, 2H, OCH_2), 5.35 (s, 2H, NCH_2), 6.94 (d, 2H, Ar-H, J=8.0 Hz), 7.09-7.11 (m, 3H, Ar-H), 7.34 (t, 2H, Ar-H, J=8.0 Hz), 7.59 (d, 2H, Ar-H, J=8.0 Hz), 8.24 (s, 1H, C-H triazole), 10.49 (s, 1H, N-H amide); ^{13}C-NMR (100 MHz, DMSO-d_6): δ=20.6, 52.7, 61.4, 114.9, 119.7, 124.2, 126.7 (C-5 triazole), 129.4, 129.9, 130.3, 138.9, 143.1 (C-4 triazole), 156.4, 164.7 (C=O amide); HRMS m/z for $C_{18}H_{18}N_4O_2$: 322.9697 $[M+H]^+$.

N-(4-methoxyphenyl)-2-(4-(p-tolyloxymethyl)-1*H*-1,2,3-triazol-1-yl)acetamide (7b)

Appearance: white solid; Yield: 79%; m.p. 196-198°C; FTIR (KBr): 3275 (N-H str., amide), 3140 (C-H str., triazole ring), 3093, 2947, 1674 (C=O str., amide), 1610, 1550, 1512, 1246, 1037 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=2.24 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 5.12 (s, 2H, OCH_2), 5.31 (s, 2H, NCH_2), 6.91 (d, 2H, Ar-H, J=8.0 Hz), 6.94 (d, 2H, Ar-H, J=8.0 Hz), 7.10 (d, 2H, Ar-H, J=8.0 Hz), 7.50 (d, 2H, Ar-H, J=8.0 Hz), 8.23 (s, 1H, C-H triazole), 10.35 (s, 1H, N-H amide); ^{13}C-NMR (100 MHz, DMSO-d_6): δ20.6, 52.6, 55.6, 61.4, 114.5, 114.9, 121.2, 126.6 (C-5 triazole), 129.9, 130.3, 132.0, 143.1 (C-4 triazole), 156.0, 156.4, 164.1 (C=O amide); HRMS m/z for $C_{19}H_{20}N_4O_3$: 352.9937 $[M+H]^+$.

N-(4-bromophenyl)-2-(4-(p-tolyloxymethyl)-1*H*-1,2,3-triazol-1-yl)acetamide (7c)

Appearance: white solid; Yield: 84%; m.p. 222-224°C; FTIR (KBr): 3261 (N-H str., amide), 3142 (C-H str., triazole ring), 3057, 2945, 1672 (C=O str., amide), 1612, 1541, 1512, 1249, 1109, 1012 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=2.24 (s, 3H, CH_3), 5.13 (s, 2H, OCH_2), 5.36 (s, 2H, NCH_2), 6.94 (d, 2H, Ar-H, J=8.0 Hz), 7.10 (d, 2H, Ar-H, J=8.0 Hz), 7.54 (d, 4H, Ar-H, J=8.0 Hz), 8.24 (s, 1H, C-H triazole), 10.63 (s, 1H, N-H amide); ^{13}C-NMR (100 MHz, DMSO-d_6): δ=20.6, 52.7, 61.4, 114.9, 115.9, 121.6, 126.6 (C-5 triazole), 129.9, 130.3, 132.2, 138.2, 143.2 (C-4 triazole), 156.4, 164.9 (C=O amide); HRMS m/z for $C_{18}H_{17}BrN_4O_2$: 402.9213 $[M+2]^+$.

N-(4-nitrophenyl)-2-(4-(p-tolyloxymethyl)-1*H*-1,2,3-triazol-1-yl)acetamide (7d)

Appearance: brown solid; Yield: 87%; m.p. 198-200 ºC; FTIR (KBr): 3259 (N-H str., amide), 3155 (C-H str., triazole ring), 3095, 2926, 1714 (C=O str., amide), 1616, 1562, 1506 (N-O str., asym., NO_2), 1486, 1338 (N-O str., sym., NO_2), 1234, 1111 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=2.24 (s, 3H, CH_3), 5.13 (s, 2H, OCH_2), 5.45 (s, 2H, NCH_2), 6.94 (d, 2H, Ar-H, J=8.0 Hz), 7.10 (d, 2H, Ar-H, J=8.0 Hz), 7.83 (d, 2H, Ar-H, J=12.0 Hz), 8.26 (d, 2H, Ar-H, J=12.0 Hz), 8.27 (s, 1H, C-H triazole), 11.11 (s, 1H, N-H amide); ^{13}C NMR (100 MHz, DMSO-d_6): δ=20.5, 52.8, 61.4, 115.0, 119.5, 125.6, 126.7 (C-5 triazole), 129.9, 130.3, 143.1 (C-4 triazole), 145.0, 156.4, 165.8 (C=O amide); HRMS m/z for $C_{18}H_{17}N_5O_4$: 367.9635 $[M+H]^+$.

N-(naphthalen-1-yl)-2-(4-(p-tolyloxymethyl)-1*H*-1,2,3-triazol-1-yl)acetamide (7e)

Appearance: white solid; Yield: 79%; m.p. 208-210°C; FTIR (KBr): 3257 (N-H str., amide), 3132 (C-H str., triazole ring), 3049, 2920, 1678 (C=O str., amide), 1598, 1546, 1514, 1238, 1049 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=2.24 (s, 3H, CH_3), 5.14 (s, 2H, OCH_2), 5.57 (s, 2H, NCH_2), 6.95 (d, 2H, Ar-H, *J*=8.0 Hz), 7.10 (d, 2H, Ar-H, *J*=8.0 Hz), 7.50-7.61 (m, 3H, Ar-H), 7.73 (d, 1H, Ar-H, *J*=8.0 Hz), 7.81 (d, 1H, Ar-H, *J*=8.0 Hz), 7.97 (d, 1H, Ar-H, *J*=8.0 Hz), 8.18 (d, 1H, Ar-H, *J*=8.0 Hz), 8.30 (s, 1H, C-H triazole), 10.46 (s, 1H, N-H amide); ^{13}C NMR (100 MHz, DMSO-d_6): δ=20.6, 52.5, 61.5, 115.0, 122.0, 123.1, 126.0, 126.2, 126.5, 126.7 (C-5 triazole), 128.0, 128.7, 129.9, 130.3, 133.2, 134.2, 143.2 (C-4 triazole), 156.4, 165.6 (C=O amide); HRMS m/z for $C_{22}H_{20}N_4O_2$: 373.0114 $[M+H]^+$.

2-(4-((4-nitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-phenylacetamide (7f)

Appearance: white solid; Yield: 92%; m.p. 198-200°C; FTIR (KBr): 3255 (N-H str., amide), 3145 (C-H str., triazole ring), 3082, 2947, 1685 (C=O str., amide), 1598, 1550, 1506 (N-O str., asym., NO_2), 1446, 1348 (N-O str., sym., NO_2), 1255, 1111 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=5.37 (s, 2H, OCH_2), 5.38 (s, 2H, NCH_2), 7.09 (t, 1H, Ar-H, *J*=8.0 Hz), 7.28-7.36 (m, 4H, Ar-H), 7.59 (d, 2H, Ar-H, *J*=8.0 Hz), 8.23 (d, 2H, Ar-H, *J*=8.0 Hz), 8.33 (s, 1H, C-H triazole), 10.52 (s, 1H, N-H amide); ^{13}C-NMR (100 MHz, DMSO-d_6): δ=52.7, 62.3, 115.8, 119.7, 124.3, 126.3, 127.2 (C-5 triazole), 129.4, 138.9, 141.5, 142.0 (C-4 triazole), 163.8, 164.6 (C=O amide); HRMS m/z for $C_{17}H_{15}N_5O_4$: 353.9750 $[M+H]^+$.

N-(4-methoxyphenyl)-2-(4-((4-nitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide(7g)

Appearance: white solid; Yield: 93%; m.p. 218-220°C; FTIR (KBr): 3277 (N-H str., amide), 3134 (C-H str., triazole ring), 3086, 2945, 1670 (C=O str., amide), 1600, 1552, 1506 (N-O str., asym., NO_2), 1456, 1340 (N-O str., sym., NO_2), 1253, 1114 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=3.72 (s, 3H, OCH_3), 5.32 (s, 2H, OCH_2), 5.36 (s, 2H, NCH_2), 6.91 (d, 2H, Ar-H, *J*=8.0 Hz), 7.29 (d, 2H, Ar-H, *J*=8.0 Hz), 7.50 (d, 2H, Ar-H, *J*=8.0 Hz), 8.23 (d, 2H, Ar-H, *J*=8.0 Hz), 8.32 (s, 1H, C-H triazole), 10.36 (s, 1H, N-H amide); ^{13}C-NMR (100 MHz, DMSO-d_6): δ=52.6, 55.6, 62.3, 114.5, 115.8, 121.2, 126.3, 127.2 (C-5 triazole), 131.9, 141.5, 142.0 (C-4 triazole), 156.0, 163.8, 164.1 (C=O amide); HRMS m/z for $C_{18}H_{17}N_5O_5$: 384.0025 $[M+H]^+$.

N-(4-bromophenyl)-2-(4-((4-nitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (7h)

Appearance: light yellow solid; Yield: 89%; m.p. 210-212°C; FTIR (KBr): 3259 (N-H str., amide), 3122 (C-H str., triazole ring), 3042, 2943, 1664 (C=O str., amide), 1598, 1548, 1502 (N-O str., asym., NO_2), 1458, 1344 (N-O str., sym., NO_2), 1257, 1112 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=5.37 (s, 2H, OCH_2), 5.38 (s, 2H, NCH_2), 7.29 (d, 2H, Ar-H, *J*=12.0 Hz), 7.52 (d, 2H, Ar-H, *J*=8.0 Hz), 7.56 (d, 2H, Ar-H, *J*=8.0 Hz), 8.23 (d, 2H, Ar-H, *J*=12.0 Hz), 8.32 (s, 1H, C-H triazole), 10.64 (s, 1H, N-H amide); ^{13}C-NMR (100 MHz, DMSO-d_6): δ=52.7, 62.3, 115.8, 121.6, 126.3, 127.2 (C-5 triazole), 132.2, 138.2, 141.5, 142.0 (C-4 triazole), 163.8, 164.8 (C=O amide); HRMS m/z for $C_{17}H_{14}BrN_5O_4$: 433.8008 $[M+2]^+$.

2-(4-((4-nitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-(4-nitrophenyl)acetamide (7i)

Appearance: brown solid; Yield: 72%; m.p. 222-224°C; FTIR (KBr): 3286 (N-H str., amide), 3151 (C-H str., triazole ring), 3089, 2937, 1708 (C=O str., amide), 1595, 1552, 1506 (N-O str., asym., NO_2), 1460, 1338 (N-O str., sym., NO_2), 1253, 1114 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=5.38 (s, 2H, OCH_2), 5.47 (s, 2H, NCH_2), 7.29 (d, 2H, Ar-H, *J*=8.0 Hz), 7.83 (d, 2H, Ar-H, *J*=8.0 Hz), 8.23 (d, 2H, Ar-H, *J*=8.0 Hz), 8.25 (d, 2H, Ar-H, *J*=8.0 Hz), 8.35 (s, 1H, C-H triazole), 11.10 (s, 1H, N-H amide); ^{13}C-NMR (100 MHz, DMSO-d_6): δ=52.8, 62.3, 115.8, 119.5, 125.6, 126.3, 127.2 (C-5 triazole), 141.5, 142.0 (C-4 triazole), 143.1, 144.9, 163.8, 165.8 (C=O amide); HRMS m/z for $C_{17}H_{14}N_6O_6$: 399.0476 $[M+H]^+$.

N-(naphthalen-1-yl)-2-(4-((4-nitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetamide (7j)

Appearance: white solid; Yield: 94%; m.p. 208-210°C; FTIR (KBr): 3257 (N-H str., amide), 3147 (C-H str., triazole ring), 3072, 2953, 1670 (C=O str., amide), 1591, 1556, 1504 (N-O str., asym., NO_2), 1463, 1342 (N-O str., sym., NO_2), 1251, 1114 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ=5.38 (s, 2H, OCH_2), 5.60 (s, 2H, NCH_2), 7.30 (d, 2H, Ar-H, *J*=8.0 Hz), 7.51-7.60 (m, 3H, Ar-H), 7.73 (d, 1H, Ar-H, *J*=8.0 Hz), 7.80 (d, 1H, Ar-H, *J*=8.0 Hz), 7.96 (d, 1H, Ar-H, *J*=8.0 Hz), 8.19 (d, 1H, Ar-H, *J*=8.0 Hz), 8.23 (d, 2H, Ar-H, *J*=8.0 Hz), 8.39 (s, 1H, C-H triazole), 10.51 (s, 1H, N-H amide); ^{13}C-NMR (100 MHz, DMSO-d_6): δ=52.6, 62.4, 115.8, 122.0, 123.1, 126.0, 126.2, 126.3, 126.5, 126.7, 127.3 (C-5 triazole), 128.0, 128.7, 133.2, 134.2, 141.5, 142.0 (C-4 triazole), 163.8, 165.6 (C=O amide); HRMS m/z for $C_{21}H_{17}N_5O_4$: 403.9587 $[M+H]^+$.

Antibacterial activity

The synthesized amide-ether linked 1,4-disubstituted 1,2,3-triazoles (7a-7j) were screened for *in vitro* antibacterial potential against two Gram-negative bacteria i.e., *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 1652) and 2 Gram-positive bacteria i.e., *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 7443) by two fold serial dilution method [37] using stock solutions of 2000 and 400 µg/ml. Dimethyl Sulphoxide (DMSO) was employed as a solvent control. Double strength nutrient broth was used as a culture media in the preparation of dilutions of test compounds. One ml nutrient broth was added in each of 9 test tubes. To the first test-tube 1.0 ml solution of test compound (2000 µg/ml) was added aseptically to get the concentration of 1000 µg/ml. From this concentration, other dilutions were prepared by serial dilution method to get final solutions of 500 and 250 µg/ml concentrations. From second stock solution, 1.0 ml solution of test compound (400 µg/ml) was taken in fourth test tube, to get the concentration of 200 µg/ml. From above (200 µg/ml) solution, other solutions were prepared by serial dilution technique to get final concentrations of 100, 50, 25, 12.5 and 6.25 µg/ml in test-tube number 5-9, respectively. All the test tubes were inoculated under aseptic condition by 0.1 ml (100 µl) of desired bacterial strain taken in sterile saline solution. The microbial loaded test samples were then incubated at 37 ± 1°C for 24 h (*B. subtilis*, *S. aureus*, *P. aeruginosa*) and 48 h (*E. coli*) in incubator and the results were recorded in terms of minimum inhibitory concentrations (MIC) expressed in µmol/ml as given in Table 1. Ciprofloxacin was used as reference drug and also tested under similar experimental conditions for comparison with test compounds. A control test was also performed with test medium supplemented with dimethylsulphoxide at same dilution as used in experiment to check the effect of solvent on bacterial growth.

Antifungal activity

The *in vitro* antifungal activity screening of synthesized triazoles (7a-7j) was performed against two fungal strains *i.e. Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 1344) by serial dilution method [37] using stock solutions of 2000 and 400 µg/ml of compounds. Sabouraud Dextrose Broth was used as a fungal culture media. DMSO was used as a solvent control. One ml of freshly prepared sterile culture media was added under aseptic conditions in each test tube followed by serial dilution with test compounds to get solutions of 1000, 500, 250, 200, 100, 50, 25, 12.5 and 6.25 µg/ml concentrations. Further these dilutions were inoculated with 0.1 ml of suspension of respective microorganism contained in sterilized saline solution.

Then samples of compounds loaded with microorganisms incubated at 25 ± 1°C for 2 days in case of *C. albicans* and for seven days in case of *A. niger*. Fluconazole was used as a reference drug. The results of antifungal activity are recorded in terms of Minimum Inhibitory Concentration (MIC) expressed in µmol/ml as given in Table 2.

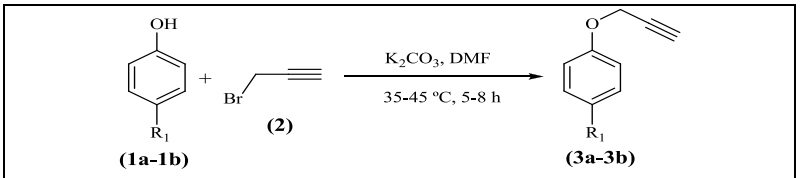
RESULTS AND DISCUSSION

Chemistry

Various 1,4-disubstituted 1,2,3-triazoles i.e. 2-(4-((4-substituted phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-arylacetamides (7a-7j), in the present case, were synthesized [36] *via* copper(I)-catalyzed click reaction of 1-methyl-4-(prop-2-ynyloxy)benzene (3a) and 1-nitro-4-(prop-2-ynyloxy)benzene (3b) with 2-azido-*N*-arylacetamides (Scheme 3). 2-Azido-*N*-arylacetamides were prepared *in situ* by reacting corresponding 2-bromo-*N*-arylacetamides (6a-6e) with aqueous sodium azide.

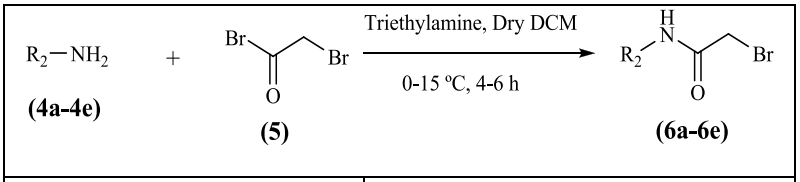
1-methyl-4-(prop-2-ynyloxy)benzene (3a) and 1-nitro-4-(prop-2-ynyloxy)benzene (3b) were synthesized [34] by reacting propargyl bromide (2) with p-cresol (1a) and p-nitro-phenol (1b), respectively, using potassium carbonate as a base in dimethylformamide (Scheme 1), whereas, 2-bromo-*N*-arylacetamides (6a-6e) were synthesized [35] by reacting bromoacetyl bromide (5) with various aromatic amines (4a-4e) using triethylamine as base in dry dichloromethane (Scheme 2).

The ether linked terminal alkynes i.e., 1-methyl-4-(prop-2-ynyloxy)benzene (3a) and 1-nitro-4-(prop-2-ynyloxy)benzene (3b) were reacted with 2-bromo-*N*-arylacetamides (6a-6e) in the presence of sodium azide, copper sulphate pentahydrate and sodium ascorbate in dimethylformamide-water to furnish desired 2-(4-((4-substituted phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-arylacetamides 7a-7j (Scheme 3).



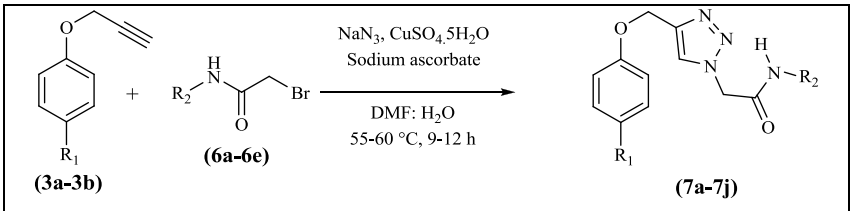
Compound	R_1
1a; 3a	CH_3
1b; 3b	NO_2

Scheme 1: Synthesis of 1-methyl/1-nitro-4-(prop-2-ynyloxy)benzene (3a-3b)



Compound	R_2
4a; 6a	C_6H_5
4b; 6b	4-$CH_3OC_6H_4$
4c; 6c	4-BrC_6H_4
4d; 6d	4-$NO_2C_6H_4$
4e; 6e	α-$C_{10}H_7$ (α-Naphthyl)

Scheme 2: Synthesis of 2-bromo-*N*-arylacetamides (6a-6e)



Compound	R_1	R_2
7a	CH_3	C_6H_5
7b	CH_3	4-$CH_3OC_6H_4$
7c	CH_3	4-BrC_6H_4
7d	CH_3	4-$NO_2C_6H_4$
7e	CH_3	α-$C_{10}H_7$ (α-Naphthyl)
7f	NO_2	C_6H_5
7g	NO_2	4-$CH_3OC_6H_4$
7h	NO_2	4-BrC_6H_4
7i	NO_2	4-$NO_2C_6H_4$
7j	NO_2	α-$C_{10}H_7$ (α-Naphthyl)

Scheme 3: Synthesis of 1,4-disubstituted 1,2,3-triazoles (7a-7j)

The synthesized amide-ether linked 1,4-disubstituted 1,2,3-triazoles i.e. 7a-7j were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and high resolution mass spectrometry. The formation of compounds was confirmed by the presence of absorption bands in the region 3286-3255 cm^{-1} (N-H str., amide), 3155-3122 cm^{-1} (C-H str., triazole ring) and 1714-1664 cm^{-1} (C=O str., amide) in FTIR spectra. The presence of characteristic singlet in the region δ=5.12-5.38 (OCH_2), δ=5.31-5.60 (NCH_2), δ=8.23-8.39 (C-H triazole), δ=10.35-11.51 (N-H amide) in ^{1}H-NMR spectra, while, the peaks in ^{13}C-NMR spectra at δ=52.5-52.8 (NCH_2), δ=61.4-62.4 (OCH_2), δ=126.6-127.3 (C-5 triazole ring), δ=142.0-143.2 (C-4 triazole ring), δ=164.1-165.8 (C=O amide) also confirmed the formation of target compounds. The results obtained from high resolution mass spectral analysis were found in accordance to calculated values.

Antibacterial activity

The synthesized triazoles 7a-7j were tested for antibacterial activity against four bacterial strains *B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa* by using serial dilution method [37]. The antibacterial efficacy of the compounds was compared with reference drug, ciprofloxacin and minimum inhibitory concentration (MIC) values were recorded in µmol/ml as shown in Table 1.

Table 1: Antibacterial activity of 1,4-disubstituted 1,2,3-triazoles 7a-7j in terms of MIC in µmol/ml

	Gram-positive bacteria		Gram-negative bacteria	
Compound	***Bacillus subtilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***
7a	0.3102	0.7755	0.6204	0.7755
7b	0.7094	0.5676	0.7094	0.7094
7c	0.4984	0.6230	0.6230	0.4984
7d	0.1361	0.5444	0.2722	0.2722
7e	0.5370	0.6713	1.3426	1.3426
7f	0.2830	0.2830	0.1415	0.1415
7g	0.2609	0.5217	0.6521	0.6521
7h	0.4627	0.5784	0.4627	0.4627
7i	0.0628	0.1255	0.0628	0.0628
7j	0.1239	0.4958	0.2479	1.2395
Ciprofloxacin	0.0377	0.1509	0.0377	0.0754

The perusal of antibacterial activity data revealed that the synthesized triazoles displayed moderate to good activity against tested bacterial strains. Compound 7i exhibited good antibacterial activity against all tested bacterial strains, while, compound 7f displayed good bactericidal potential against *S. aureus* and *E. coli*.

Antifungal activity

The 1,4-disubstituted 1,2,3-triazoles 7a-7j were tested for antifungal activity against two fungal strains *C. albicans*, *A. niger* by using serial dilution method [37]. Fluconazole was used as standard drug. The results were recorded in terms of MIC in µmol/ml as given in Table 2.

Table 2: Antifungal activity of 1,4-disubstituted 1,2,3-triazoles 7a-7j in terms of MIC in µmol/ml

Compound	***Candida albicans***	***Aspergillus niger***
7a	0.6204	0.3102
7b	0.5676	0.5676
7c	0.6230	0.6230
7d	0.0681	0.1361
7e	0.5370	1.3426
7f	0.1415	0.0354
7g	0.5217	0.5217
7h	1.1568	0.5784
7i	0.0628	0.0628
7j	0.2479	0.2479
Fluconazole	0.0408	0.0816

The antifungal activity data showed that the synthesized compounds exhibited moderate to excellent activity against tested fungal strains. Compound 7i appeared as appreciable antifungal agent against both tested fungal strains, while, compounds 7d and 7f displayed remarkable appreciable inhibition effect against *C. albicans* and *A. niger*, respectively. From the above results, it was observed that the presence of nitro group at p-position of both phenoxy and anilide ring improved the antimicrobial potency against tested microbial strains.

CONCLUSION

Here, 1,3-dipolar cycloaddition between 1-methyl-4-(prop-2-ynyloxy)benzene/1-nitro-4-(prop-2-ynyloxy)benzene and 2-azido-*N*-arylacetamides using copper(I) as catalyst, has been carried out to synthesize 2-(4-((4-substituted phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-arylacetamides. The synthesized triazoles were examined *in vitro* for antimicrobial activity against *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans* and *A. niger*. The compound 7i displayed good to excellent antimicrobial activity against the tested bacterial and fungal strains. Further, among the synthesized molecules, 7d and 7f exhibited remarkable fungicidal potential against *C. albicans* and *A. niger*, respectively.

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