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Synthesis, characterization and microbicidal activity of some (1-substituted-1H-1,2,3-triazol-4-yl)methyl benzoates

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Abstract: Regioselective synthesis of some (1-substituted-1H-1,2,3-triazol-4-yl)methyl benzoates was carried out through Cu (I) catalyzed Huisgen [3+2] dipolar cycloaddition reaction between aralkyl azides and terminal alkynes. All the synthesized triazoles were characterized by analytical techniques like IR, ^{1}H NMR, ^{13}C NMR spectroscopy and mass spectrometry. To further confirm the structure of compound **3b**, X-ray crystallographic study was also carried out. The synthesized 1,4-disubstituted 1,2,3-triazoles i.e. (1-substituted-1H-1,2,3-triazol-4-yl)methyl benzoates in the present case were screened for their *in-vitro* bactericidal activity against two Gram negative bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa* and two Gram positive bacteria i.e. *Staphylococcus aureus*, *Bacillus subtilis* and one mycobacteria viz. *Mycobacterium tuberculosis*. These triazoles were also assessed for their *in-vitro* fungicidal activity against three fungal strains, namely *Candida albicans, Aspergillus niger* and *Aspergillus flavus*. It was found that increase in length of carbon chain at N-1 position of triazole ring leads to improved bactericidal potency of the compounds. However, fungicidal activity of triazole scaffolds with pyridine nucleus showed comparatively good results.

Keywords: Click reaction, Huisgen [3+2] dipolar cycloaddition, 1,4-Disubstituted 1,2,3-triazoles, Microbicidal activity.

Introduction

Ever increasing microbial resistance to antibiotics has come out as a major health concern to society from last few years [1-2]. Various drug resistant pathogenic bacterial and fungal strains cause serious diseases in the community. Owing to this, triazoles, a potential class of microbicidal compounds, can be used to replace ineffective antibiotics [3]. Among nitrogen heterocycles, triazole and their derivatives attracts special attention of researchers

due to their wild utility in medicines and agrochemicals [4-6]. 1,2,3-Triazoles are quite stable to acidic-basic hydrolysis and are highly polar in nature leading to effective hydrogen bonding and dipole-dipole interactions which enhance their aqueous solubility and binding efficiency with biological targets [7]. Huisgen 1,3-dipolar cycloaddition between terminal alkynes and azides is the common method for synthesis of disubstituted 1,2,3-triazoles, leads to the formation of 1,4- and 1,5-isomers in equal proportions. Modified copper (I) catalyzed Huisgen cycloaddition reported by Sharpless [8] and Meldal [9] resulted in to the regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles, can be termed as one of important click reaction in chemical science. The term "click reaction" refers to a facile, efficient, selective and versatile chemical transformation of reactant to single isomeric product. 1,4-Disubstituted 1,2,3-triazoles also posses broad spectrum of anti-infectious activities like antiviral [10], microbicidal [11-12], antiprotozoal [13], antimalarial [14], antitubercular [15-16], anti-HIV [17-18], anticancer[19-20] etc. Against this background, it has been planned to synthesize some 1,4-disubstituted 1,2,3-triazoles i.e. (1-substituted-1H-1,2,3-triazol-4-yl)methyl benzoates, containing pyridine nucleus [21]/ other moieties *via* click reaction and their screening for bactericidal, fungicidal and antitubercular activities.

Materials and Methods

Experimental

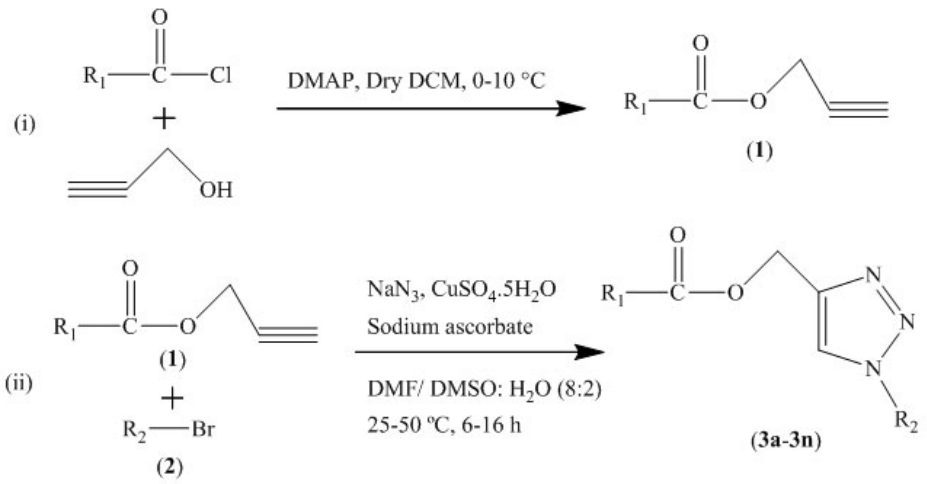
All chemicals used in present work were purchased from local suppliers and used without further purification. Nutrient broth and Sabouraud dextrose broth for microbicidal activity evaluation were procured from Hi-media, Mumbai. Melting points of synthesized compounds were recorded in ºC by applying open capillary method and are uncorrected. The IR spectra were recorded on Shimazdu IR Affinity-I FT-IR spectrophotometer using potassium bromide (KBr) powder and values are given in cm^{-1}. The ^{1}H NMR spectra were recorded on Bruker Avance II 400 MHz/ Bruker 300 MHz spectrophotometer and ^{13}C NMR on Bruker Avance II 400 at 100 MHz/ Bruker 300 at 75 MHz, in deuterated chloroform using tetramethylsilane (TMS) as an internal standard (chemical shift in δ, ppm). Coupling constant (J) values are given in Hertz (Hz). Mass spectra were recorded on Waters Micromass Q-Tof Micro (ESI) spectrophotometer. The completion of reactions and the purity of the compounds were determined by thin layer chromatography (TLC) using silica gel plates (SIL G/UV254, ALUGRAM) and visualized under ultraviolet lamp.

General procedure for the synthesis of (1-substituted-1H-1,2,3-triazol-4-yl)methyl benzoates (3a-3n)

To a solution of aralkyl bromide (**2**, 1.0 mmol) and sodium azide (3.0 mmol) in DMF: H_2O (8: 2) was added ester linked terminal alkyne (**1**, 1.0 mmol) [22], copper sulphate pentahydrate (10 mol %), sodium ascorbate (20 mol %) and the above reaction mixture was stirred at room temperature for 6-12 h. The products containing pyridine nucleus (**3f**, **3h**, **3j**, **3l** and **3n**) were synthesized at 45-50 ºC using DMSO: H_2O (8: 2) as a solvent by continuous stirring of 12-16 h. After completion of reaction, cold aqueous ammonia-ammonium chloride solution was added to the reaction mixture and product was extracted with ethyl acetate. Then organic layer was dried by adding anhydrous sodium sulphate, filtered and concentrated under vacuum to yield crude product. Crude product was purified by using column chromatography (**Scheme 1**).

Characterization of (1-substituted-1H-1,2,3-triazol-4-yl)methyl benzoates (3a-3n)

(1-benzyl-1H-1,2,3-triazol-4-yl)methyl benzoate (**3a**)



Scheme 1

Compound No.	R_1	R_2
3a	C_6H_5-	C_6H_5-CH_2-
3b	C_6H_5-	C_6H_5-CH_2-CH_2-
3c	C_6H_5-	C_6H_5-CH_2-CH_2-CH_2-
3d	C_6H_5-	4-CH_3-C_6H_5-CH_2-
3e	C_6H_5-	4-NO_2-C_6H_5-CH_2-
3f	C_6H_5-	4-C_5H_4N-CH_2-
3g	4- H_3C-C_6H_5-	C_6H_5-CH_2-CH_2-
3h	4- H_3C-C_6H_5-	4-C_5H_4N-CH_2-
3i	4-H_3CO-C_6H_5-	C_6H_5-CH_2-CH_2-
3j	4-H_3CO-C_6H_5-	4-C_5H_4N-CH_2-
3k	4-NO_2-C_6H_5-	C_6H_5-CH_2-CH_2-
3l	4-NO_2-C_6H_5-	4-C_5H_4N-CH_2-
3m	4-F-C_6H_5-	C_6H_5-CH_2-CH_2-
3n	4-F-C_6H_5-	4-C_5H_4N-CH_2-

Appearance: white, crystalline solid; Yield: 85%; m.p. 84-88 ºC; FT-IR (KBr): 3147 (C-H str., triazole ring), 3099 (C-H str., aromatic ring), 2956 (C-H str., aliphatic), 1712 (C=O str., ester), 1597, 1446 (C=C str., aromatic ring), 1261 (C-O str., asym., ester), 1099 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 5.43 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 7.23-7.31 (m, 5H, Ar-H), 7.36-7.45 (m, 3H, Ar-H), 7.60 (s, 1H, C-H triazole), 8.01 (d, 2H, Ar-H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 54.6, 58.3, 124.0, 127.1, 128.2, 129.3, 129.9, 133.4, 134.6, 143.4, 166.5; MS m/z for $C_{17}H_{15}N_3O_2Na$: 316.1224 $[M+Na]^+$.

(1-phenethyl-1H-1,2,3-triazol-4-yl)methyl benzoate (3b)

Appearance: white, highly crystalline solid;

Yield: 81%; m.p. 90-94 ºC; FT-IR (KBr): 3122 (C-H str., triazole ring), 3051 (C-H str., aromatic ring), 2947 (C-H str., aliphatic), 1716 (C=O str., ester), 1597, 1456 (C=C str., aromatic ring), 1267 (C-O str., asym., ester), 1099 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 3.12 (t, 2H, CH_2, J=7.2 Hz) 4.50 (t, 2H, CH_2, J=7.2 Hz), 5.34 (s, 2H, CH_2), 6.98-7.00 (m, 2H, Ar-H), 7.11-7.18 (m, 3H, Ar-H), 7.32-7.37 (m, 3H, Ar-H), 7.48 (s, 1H, C-H triazole), 7.93 (d, 2H, Ar-H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 37.2, 52.2, 58.5, 124.8, 127.5, 128.8, 129.2, 130.2, 133.6, 137.2, 143.3, 166.8; MS m/z for $C_{18}H_{17}N_3O_2Na$: 330.1218 $[M+Na]^+$.

(1-(3-phenylpropyl)-1H-1,2,3-triazol-4-yl) methyl benzoate (3c)

Appearance: off-white solid; Yield: 76%; m.p. 70-74 ºC; FT-IR (KBr): 3155 (C-H str., triazole ring), 3053 (C-H str., aromatic ring), 2935 (C-H str., aliphatic), 1707 (C=O str., ester), 1595, 1454 (C=C str., aromatic ring), 1269 (C-O str., asym., ester), 1101 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 2.18 (p, 2H, CH_2, J=7.2 Hz), 2.58 (t, 2H, CH_2, J=7.2 Hz), 4.27 (t, 2H, CH_2, J=7.2 Hz), 5.39 (s, 2H, CH_2) 7.07-7.14 (m, 3H, Ar-H), 7.18-7.23 (m, 2H, Ar-H), 7.32-7.36 (m, 2H, Ar-H), 7.48 (t, 1H, Ar-H, J=6.8 Hz) 7.57 (s, 1H, C-H triazole), 7.97 (d, 2H, Ar-H, J=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 32.0, 32.9, 50.0, 58.5, 124.4, 126.8, 128.8, 129.0, 130.2, 133.6, 140.4, 143.3, 166.9; MS m/z for $C_{19}H_{19}N_3O_2Na$: 344.1327 $[M+Na]^+$.

(1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl) methyl benzoate (3d)

Appearance: creamy-white, crystalline solid; Yield: 78.5%. m.p. 78-82 ºC; FT-IR (KBr): 3167 (C-H str., triazole ring), 3045 (C-H str., aromatic ring), 2958 (C-H str., aliphatic), 1712 (C=O str., ester), 1608, 1444 (C=C str., aromatic ring), 1255 (C-O str., asym., ester), 1095 (C-O str., sym., ester) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): 2.35 (s, 3H, CH_3), 5.43 (s, 2H, CH_2), 5.50 (s, 2H, CH_2), 7.00-7.50 (m, 7H, Ar-H), 7.59 (s, 1H, C-H triazole), 8.02 (d, 2H, Ar-H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 21.2, 54.1, 57.0, 123.7, 127.2, 128.2, 129.2, 129.8, 131.4, 138.8, 143.4, 166.6; MS m/z for $C_{18}H_{17}N_3O_2Na$: 330.1216 $[M+Na]^+$.

(1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl) methyl benzoate (3e)

Appearance: white solid; Yield: 75.5%; m.p. 140-144 ºC; FT-IR (KBr): 3122 (C-H str., triazole ring), 3072 (C-H str., aromatic ring), 2964 (C-H str., aliphatic), 1712 (C=O str., ester), 1598, 1448 (C=C str., aromatic ring), 1525 (N-O str., asym., NO_2), 1340 (N-O str., sym., NO_2), 1263 (C-O str., asym., ester), 1107 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ): 5.41 (s, 2H, CH_2), 5.58 (s, 2H, CH_2), 7.33-7.51 (m, 5H, Ar-H), 7.64 (s, 1H, C-H triazole), 7.94-7.99 (m, 2H, Ar-H), 8.16 (d, 2H, Ar-H, J=8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 53.7, 57.6, 58.4, 124.7, 127.1, 128.8, 129.1, 129.9, 133.7, 141.8, 143.3, 148.6, 166.9; MS m/z for $C_{17}H_{14}N_4O_4Na$: 361.1145 $[M+Na]^+$.

(1-(pyridin-4-ylmethyl)-1H-1,2,3-triazol-4-yl)methyl benzoate (3f)

Appearance: dull-white solid; Yield: 60.5%; m.p. 132-136 ºC; FT-IR (KBr): 3142 (C-H str., triazole ring), 3043 (C-H str., aromatic ring), 2964 (C-H str., aliphatic), 1708 (C=O str., ester), 1598, 1516, 1444 (C=C & C=N str., aromatic & pyridine ring), 1273 (C-O str., asym., ester), 1101 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 5.41 (s, 2H, CH_2), 5.49 (s, 2H, CH_2), 7.06 (d, 2H, Ar-H, J=4.8 Hz), 7.36 (t, 2H, Ar-H, J=7.6 Hz), 7.50 (t, 1H, Ar-H, J=7.6 Hz), 7.64 (s, 1H, C-H triazole), 7.96 (d, 2H, Ar-H, J=7.6 Hz), 8.55 (d, 2H, Ar-H, J=4.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 53.2, 58.4, 122.6, 124.7, 128.9, 130.0, 130.1, 133.7, 143.8, 144.3, 151.0, 166.9; MS m/z for $C_{16}H_{14}N_4O_2Na$: 317.1196 $[M+Na]^+$.

(1-phenethyl-1H-1,2,3-triazol-4-yl)methyl 4-methylbenzoate (3g)

Appearance: white, crystalline solid; Yield: 78%; m.p. 106-108 ºC; FT-IR (KBr): 3126 (C-H str., triazole ring), 3043 (C-H str., aromatic ring), 2956 (C-H str., aliphatic), 1710 (C=O str., ester), 1610, 1450 (C=C str., aromatic ring), 1267 (C-O str., asym., ester), 1103 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 2.42 (s, 3H, CH_3), 3.22 (t, 2H, CH_2, J=7.2 Hz), 4.60 (t, 2H, CH_2, J=7.2 Hz), 5.42 (s, 2H, CH_2), 7.09 (d, 2H, Ar-H, J=6.4), 7.10-7.29 (m, 5H, Ar-H), 7.42 (s, 1H, C-H triazole), 7.93 (d, 2H, Ar-H, J=8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 21.7, 36.8, 51.7, 57.9, 124.3, 127, 127.1, 128.7, 128.8, 129.1, 129.8, 136.9, 142.8, 143.9, 166.5; MS m/z: 322.0 [M^+], 323.0 [M^++1].

(1-(pyridin-4-ylmethyl)-1H-1,2,3-triazol-4-yl)methyl 4-methylbenzoate (3h)

Appearance: light yellow, crystalline solid; Yield: 51%; m.p. 114-116 ºC; FT-IR (KBr): 3142 (C-H str., triazole ring), 3041 (C-H str., aromatic ring), 2950 (C-H str., aliphatic), 1710 (C=O str., ester), 1600, 1508, 1417 (C=C & C=N str., aromatic & pyridine ring), 1272 (C-O str., asym., ester), 1097 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 2.38 (s, 3H, CH_3), 5.45 (s, 2H, CH_2), 5.57 (s, 2H, CH_2), 7.04-7.14 (m, 4H, Ar-H), 7.75 (s, 1H, C-H triazole), 8.03 (d, 2H, Ar-H, J=7.2 Hz), 8.60 (d, 2H, Ar-H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 22.1, 52.6, 57.9, 115.7, 122.2, 124.4, 125.8, 132.2, 143.5, 150.4, 163.3, 165.2, 168.3; MS m/z: 309.0 [M^+], 310.0 [M^++1].

(1-phenethyl-1H-1,2,3-triazol-4-yl)methyl 4-methoxybenzoate (3i)

Appearance: white, crystalline solid; Yield: 74%; m.p. 76-78 ºC; FT-IR (KBr): 3126 (C-H str., triazole ring), 3068 (C-H str., aromatic ring), 2954 (C-H str., aliphatic), 1708 (C=O str., ester), 1610, 1454 (C=C str., aromatic ring), 1267 (C-O str., asym., ester), 1097 (C-O str., sym., ester) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 3.19 (t, 2H, CH_2, J=7.2 Hz), 3.85 (s, 3H, CH_3), 4.57 (t, 2H, CH_2, J=7.2 Hz), 5.38 (s, 2H, CH_2), 6.90 (d, 2H, Ar-H, J=8.4 Hz), 7.07 (d, 2H, Ar-H, J=7.2 Hz), 7.22-7.26 (m, 3H, Ar-H), 7.40 (s, 1H, C-H triazole), 7.97 (d, 2H, Ar-H, J=8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 36.8, 51.7, 55.5, 57.8, 113.6, 122.2, 124.3, 127.1, 128.7, 131.8, 136.8, 142.9, 163.6, 166.2; MS m/z: 338.0 [M^+], 339.0 [M^++1].

(1-(pyridin-4-ylmethyl)-1H-1,2,3-triazol-4-yl)methyl 4-methoxybenzoate (3j)

Appearance: slightly yellow solid; Yield: 45%; m.p. 148-150 ºC; FT-IR (KBr): 3126 (C-H str., triazole ring), 3032 (C-H str., aromatic ring), 2962 (C-H str., aliphatic), 1701 (C=O str., ester), 1604, 1519, 1438 (C=C & C=N str., aromatic & pyridine ring), 1269 (C-O str., asym., ester), 1097 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 3.85 (s, 3H, CH_3), 5.45 (s, 2H, CH_2), 5.55 (s, 2H, CH_2), 6.90 (d, 2H, Ar-H, J=7.2 Hz), 7.13 (d, 2H, Ar-H, J=7.2 Hz), 7.70 (s, 1H, C-H triazole), 7.98 (d, 2H, Ar-H, J=7.2 Hz), 8.62 (d, 2H, Ar-H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 36.8, 55.4, 57.6, 113.6, 121.9, 124.4, 126.1, 131.8, 144.2, 150.4, 163.6, 166.2; MS m/z: 325.0 [M^+], 326.0 [M^++1].

(1-phenethyl-1H-1,2,3-triazol-4-yl)methyl 4-nitrobenzoate (3k)

Appearance: creamy white solid; Yield: 76%; m.p. 124-126 ºC; FT-IR (KBr): 3138 (C-H str., triazole ring), 3057 (C-H str., aromatic ring), 2947 (C-H str., aliphatic), 1718 (C=O str., ester), 1604, 1444 (C=C str., aromatic ring), 1521 (N-O str., asym., NO_2), 1350 (N-O str., sym., NO_2), 1280 (C-O str., asym., ester), 1103 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 3.23 (t, 2H, CH_2, J=7.2 Hz), 4.63 (t, 2H, CH_2, J=7.2 Hz), 5.50 (s, 2H, CH_2), 7.08 (d, 2H, Ar-H, J=6.8 Hz), 7.19 -7.25 (m, 3H, Ar-H), 7.52 (s, 1H, C-H triazole), 8.20 (d, 2H, Ar-H, J=8.4 Hz), 8.28 (d, 2H, Ar-H, J=8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 36.7, 51.8, 58.8, 123.6, 124.5, 127.2, 128.7, 130.9, 135.2, 136.8, 141.8, 150.7, 164.5; MS m/z: 353.0 [M^+], 354.0 [M^++1].

(1-(pyridin-4-ylmethyl)-1H-1,2,3-triazol-4-yl)methyl 4-nitrobenzoate (3l)

Appearance: white solid; Yield: 42%; m.p. 138-140 ºC; FT-IR (KBr): 3132 (C-H str., triazole ring), 3056 (C-H str., aromatic ring), 2958 (C-H str., aliphatic), 1705 (C=O str., ester), 1598, 1508, 1424 (C=C & C=N str., aromatic & pyridine ring), 1523 (N-O str., asym., NO_2), 1354 (N-O str., sym., NO_2), 1271 (C-O str., asym., ester), 1105 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 5.53 (s, 2H, CH_2), 5.65 (s, 2H, CH_2), 7.10-7.14 (m, 2H, Ar-H), 7.78 (s, 1H, C-H triazole), 8.22-8.27 (m, 4H, Ar-H), 8.69 (d, 2H, Ar-H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 53.7, 57.8, 123.6, 124.0, 125.8, 131.4, 135.1, 142.1, 145.1, 150.8, 152.1, 164.3; MS m/z: 340.0 [M^+], 341.0 [M^++1].

(1-phenethyl-1H-1,2,3-triazol-4-yl)methyl 4-fluorobenzoate (3m)

Appearance: white, crystalline solid; Yield: 80%; m.p. 72-74 ºC; FT-IR (KBr): 3122 (C-H str., triazole ring), 3059 (C-H str., aromatic ring), 2945 (C-H str., aliphatic), 1716 (C=O str., ester), 1602, 1454 (C=C str., aromatic ring), 1278 (C-O str., asym., ester), 1109 (C-O str., sym., ester) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 3.19 (t, 2H, CH_2, J=7.0 Hz), 4.58 (t, 2H, CH_2, J=7.0 Hz), 5.39 (s, 2H, CH_2), 7.03-7.22 (m, 7H, Ar-H), 7.41 (s, 1H, C-H triazole), 8.02 (d, 2H, Ar-H, J=7.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): 36.7, 51.6, 58.0, 115.7, 124.3, 127.0, 128.7, 132.3, 136.7, 142.3, 163.2, 165.2; MS m/z: 326.0 [M^+], 327.0 [M^++1].

(1-(pyridin-4-ylmethyl)-1H-1,2,3-triazol-4-yl)methyl 4-fluorobenzoate (3n)

Appearance: Shiny white, crystalline solid; Yield: 56%; m.p. 118-120 ºC; FT-IR (KBr): 3143 (C-H str., triazole ring), 3043 (C-H str., aromatic ring), 2962 (C-H str., aliphatic), 1708 (C=O str., ester), 1600, 1508, 1436 (C=C & C=N str., aromatic & pyridine ring), 1274 (C-O str., asym., ester), 1099 (C-O str., sym., ester) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 5.49 (s, 2H, CH_2), 5.58 (s, 2H, CH_2), 7.09-7.15 (m, 4H, Ar-H), 7.71 (s, 1H, C-H triazole), 8.07 (d, 2H, Ar-H, J=7.2 Hz), 8.64 (d, 2H, Ar-H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 52.8, 58.0, 115.7, 122.2, 124.3, 125.8, 132.3, 143.3, 143.7, 150.6, 165.5, 167.2; MS m/z: 313.0 [M^+], 314.0 [M^++1].

Microbicidal activity assays

The Minimal inhibitory concentrations (MICs) of the ester linked 1,4-disubstituted 1,2,3-triazoles were obtained through standard serial dilution method [28]. Ciprofloxacin, Isoniazid and Amphotericin B were used as standard drugs for bactericidal, antitubercular and fungicidal activity respectively.

Bactericidal activity

The synthesized (1-substituted-1H-1,2,3-triazol-4-yl)methyl benzoates were tested for their *in vitro* bactericidal activity against two Gram negative bacteria i.e. *Escherichia coli* (MTCC 1231), *Pseudomonas aeruginosa* (MTCC 1036) and two Gram positive bacteria i.e. *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 9023) using double strength nutrient broth by serial dilution method. Dimethylsulphoxide was used as solvent control. The stock solutions of 1000 µg/ml concentration of test compounds and reference drug were serially diluted to get concentrations of 500 to 12.5 µg/ ml and then inoculated with 100 µL suspension of respective microorganisms in sterile saline and incubated at 37 ± 1 ºC for 24 h. A blank test was also carried out with nutrient media containing dimethylsulphoxide at experimental condition to observe the effect of solvent on bacterial growth. The ciprofloxacin, a broad spectrum antibiotic was used as a standard drug.

Antitubercular activity of the ester linked triazoles was assessed against *Mycobacterium*

tuberculosis (ATCC 27294) in microtiter plates by adding 10 ml aliquots of a culture suspension [whose turbidity was equal to that of a no. 0.5 McFarland standard containing $1.5X10^8$ colony forming units (CFU)/ml] to 80 ml of Middlebrook 7H9 medium containing 0.5% glycerol and 10% albumin-dextrose-catalase (ADC) and various concentrations of test compounds. Plates were then incubated for 9 days at 37 ± 1 °C. At the end of incubation, the numbers of viable mycobacterium were determined by the MTT method.

Fungicidal activity

The synthesized molecules were screened for their *in vitro* fungicidal activity against *Candida albicans* (MTCC 854), *Aspergillus niger* (MTCC 282) and *Aspergillus flavus* (MTCC 873). Sabouraud dextrose broth was used as culture media while dimethylsulphoxide as solvent control and amphotericin B as reference drug. A spore suspension in sterile saline was prepared from three days old culture of fungus growing on sabourauds dextrose broth. The final spore concentration was 100 µL/ ml. The stock solutions of 1000 µg/ml of test compounds and standard drug were serially diluted to get concentrations of 500, 250, 200, 100, 50, 25 and 12.5 µg/ ml. These dilutions were inoculated with suspension of respective microorganism in their culture media and were incubated at 25 ± 1 °C for 48 h in case of *Candida albicans* and at 25 ± 1 °C for 120 h in case of *Aspergillus niger* and *Aspergillus flavus*.

Results and discussion

Chemistry

The 1,4-disubstituted 1,2,3-triazoles (**3a-3n**) were synthesized by click reaction between terminal alkynes containing benzoate esters (**1**) and aralkyl bromides (**2**) in the presence of NaN_3, $CuSO_4.5H_2O$ and sodium ascorbate using DMF/ DMSO: H_2O (8: 2) with continuous stirring of 6-16 h at 25-50 °C with good yields. The characterization of synthesized compounds was carried out by using various analytical techniques like IR, ^{1}H NMR, ^{13}C NMR spectroscopy and Mass spectrometry. The formation of triazoles was confirmed by the presence of absorption bands in the region at 3167-3122 (C-H, str., triazole ring), 1718-1701 (C=O str., ester), 1280-1255 (C-O asym. str., ester), 1107-1097 (C-O sym. str., ester) cm^{-1} in IR spectra. The presence of characteristic singlets in the region (δ values in ppm) at 5.34-5.65 (OC**H**$_2$), 7.40-7.78 (C**H** triazole) in ^{1}H NMR spectra and 57.0-58.8 (O**C**H$_2$), 128.7-132.3 (**C**-5 triazole ring), 141.8-144.2 (**C**-4 triazole ring), 164.3-166.9 (**C**=O, ester) in ^{13}C NMR spectra also confirmed the formation of target compounds. The results obtained from mass spectral analysis were found in accordance to their theoretically predicted molecular masses. To further confirm the structure of synthesized triazoles, an X-ray crystallographic study of the compound **3b** was also carried out (**Fig. 1**).

Microbicidal activity

The (1-substituted-1H-1,2,3-triazol-4-yl)methyl benzoates (**3a-3n**) were screened for their *in vitro* bactericidal activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*, moreover, fungicidal activity was evaluated against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* by using standard serial dilution technique.

Bactericidal activity

All the synthesized 1,4-disubstituted 1,2,3-triazoles (**3a-3n**) were assayed for their bactericidal activity against two Gram negative bacteria i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, two Gram positive bacteria i.e. *Staphylococcus aureus*, *Bacillus subtilis* and one

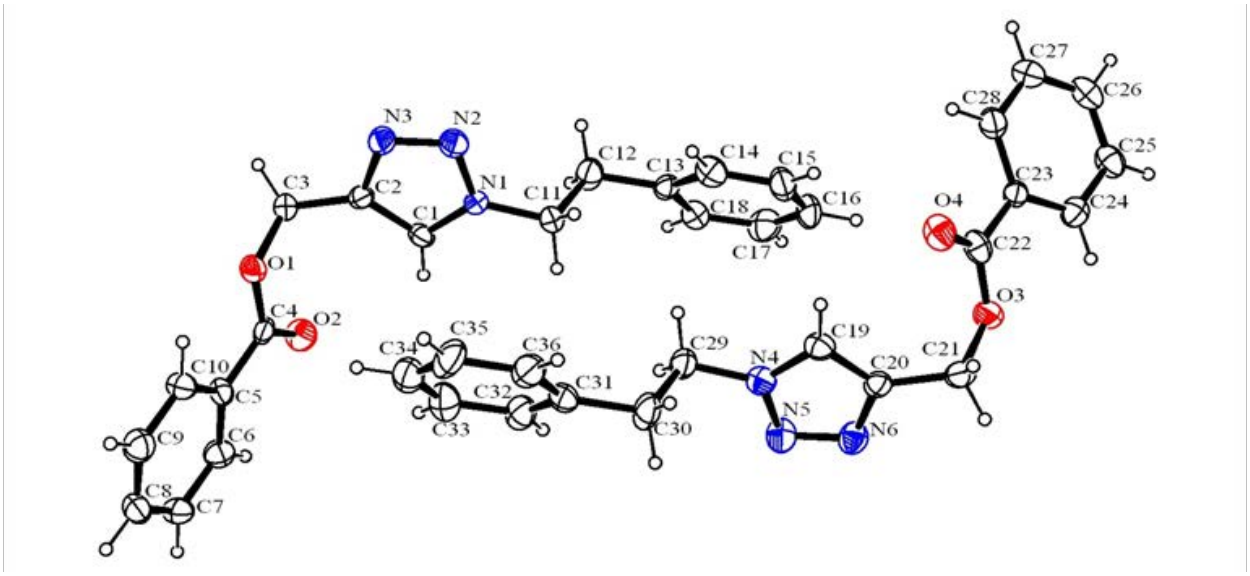


Figure 1. X-ray structure of compound 3b.

Table No. 1. Bactericidal activity of compounds 3a-3n in terms of MIC's (µg/ml)

Compound No.	*E. coli*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*M. tuberculosis*
3a	200	250	200	500	50
3b	25	50	50	100	25
3c	50	25	12.5	12.5	25
3d	200	100	250	100	50
3e	200	200	100	250	12.5
3f	100	100	200	100	50
3g	50	50	25	100	25
3h	250	250	500	100	25
3i	100	200	250	250	50
3j	200	200	500	250	25
3k	100	100	200	50	12.5
3l	100	200	100	250	25
3m	250	100	100	200	6.25
3n	100	50	50	50	12.5
Ciprofloxacin	25	50	12.5	12.5	-----
Isoniazid	-----	-----	-----	-----	3.75

mycobacterium, *Mycobacterium* tuberculosis. Ciprofloxacin, a broad spectrum antibiotic and isoniazid were used as a standard drug. The minimum inhibitory concentrations (MIC's) values in µg/ml were determined as given in **Table No. 1.**

Most of the synthesized triazoles exhibited moderate to good activity against tested bacterial strains. Compounds **3b, 3c, 3g** and **3n** displayed potent bactericidal activity against all tested bacterial strains with 12.5-100 µg/ml of minimum inhibitory concentrations. Whereas, the compound **3m** showed better antitubercular

Table No. 2. Fungicidal activity of compounds 3a-3n in terms of MIC's (µg/ml)

Compound No.	*C. albicans*	*A. niger*	*A. flavus*
3a	500	1000	1000
3b	1000	500	500
3c	50	50	100
3d	25	50	50
3e	1000	200	100
3f	200	100	50
3g	1000	1000	500
3h	25	25	12.5
3i	100	200	500
3j	50	250	100
3k	500	250	500
3l	100	50	50
3m	200	100	500
3n	25	12.5	12.5
Amphotericin B	25	25	50

activity with MIC value 6.25 µg/ml against *Mycobacterium tuberculosis* and the compounds **3e, 3k, 3n** displayed intermediate antitubercular activity. From the results obtained, it has been observed that increase in length of carbon chain at N-1 position of triazole ring leads to increase in bactericidal potency of the compounds.

Fungicidal activity

The ester linked 1,4-disubstituted 1,2,3-triazoles (**3a-3n**) were examined for their *in-vitro* fungicidal activity against three fungal strains i.e. *Candida albicans, Aspergillus niger, Aspergillus flavus* and Amphotericin B was used as the standard drug. The MIC's values of the test compounds and the standard drug are given in **Table No. 2.**

Results obtained from the fungicidal activity screening revealed that the synthesized compounds exhibited intermediate to significant activity against all tested fungal strains. The compounds **3d**, **3h, 3l** and **3n** were found to exhibit promising fungicidal activity against *Candida albicans, Aspergillus niger* and *Aspergillus flavus* with MIC values ranging from 12.5-50 µg/ml. It was also observed that triazoles scaffolded with pyridine nuclei exhibited better fungicidal activity than remaining compounds.

Conclusion

In the present work, we have synthesized some (1-substituted-1H-1,2,3-triazol-4-yl) methyl benzoates (**3a-3n**) through Cu(I) catalyzed Huisgen [3+2] dipolar cycloaddition reaction between aralkyl azides and ester linked terminal alkynes. The synthesized ester linked 1,4-disubstituted 1,2,3-triazoles were screened for their *in vitro* microbicidal activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Mycobacterium tuberculosis, Candida albicans, Aspergillus niger* and *Aspergillus flavus*. The compounds **3b, 3c, 3g** and **3n** exhibited potent bactericidal activity against all tested bacterial strains and the compound **3m** appeared as better antitubercular triazole, whereas the compounds

3d, **3h, 3l** and **3n** found to possess remarkable fungicidal activity among the tested fungal strains. The above results clearly indicates that the (1-substituted-1H-1,2,3-triazol-4-yl) methyl benzoates displayed wide range of microbicidal activity against tested microbial strains.

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