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ORIGINAL ARTICLE

Synthesis and antimicrobial evaluation of 1,4-disubstituted 1,2,3-triazoles with aromatic ester functionality

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Click chemistry;
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1,3-Dipolar cycloaddition;
Antibacterial activity;
Antifungal activity

Abstract A series of 1,4-disubstituted 1,2,3-triazoles having p-substituted aromatic ester functionality were synthesized *via* Cu(I) catalysed click reaction between p-substituted benzoic acid prop-2-ynyl esters and aralkyl azides. The synthesized triazoles were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral techniques. These compounds were evaluated for their antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* by two fold serial dilution method. Some of the synthesized 1,4-disubstituted 1,2,3-triazoles possess comparable or even better antibacterial, antitubercular and antifungal activities than reference drugs against tested bacterial, mycobacterial and fungal strains, respectively.

1. Introduction

The synthesis of substituted 1,2,3-triazoles is of key importance due to their large biological spectrum as antibiotic (Aufort et al., 2008), antimicrobial (Lal et al., 2012; Gaur et al., 2012; Demaray et al., 2008), antimalarial (D'hooghe et al., 2011), anticancer (Salmon et al., 2012), antihistaminic (Buckle et al., 1986), anti-HIV (Whiting et al., 2006) and antitubercular agents (Labadie et al., 2011). Good stability and high aqueous solubility of these compounds in biological system boost for appreciable biological activities. Further the 1,4-disubstituted 1,2,3-triazoles have also been used as ligation tool for the synthesis of neoglyco-conjugates (Perez-Balderas et al., 2003), multivalent dendrimeric peptides (Wu et al., 2004), ionic receptors (Kumar and Pandey, 2008), triazolophanes (Haridas et al., 2008), dendrimers (Haridas et al.,

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2007), cyclic peptides (Turner et al., 2007), peptide nanotubes (Horne et al., 2003), peptidomimetics (Angell and Burgess, 2007) etc. 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 (Huisgen, 1963). A practical solution to avoid the formation of isomeric mixture in products, was given by Sharpless (Rostovtsev et al., 2002) and Meldal (Tornøe et al., 2002) through the catchy term ''click chemistry'' which refers to facile, efficient, selective and versatile chemical transformation of reactant to a single isomeric product. These reactions are simple to perform, modular, high yielding and lead to excellent selectivity in the product. Among various reactions, Cu(I) catalysed variant of Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes to give only 1,4-disubstituted 1,2,3-triazoles has been generally pointed as the primary standard of click chemistry. Herein, we report the synthesis of a series of 1,4-disubstituted 1,2,3-triazoles (**3a–3p**) from various azides and alkynes containing p-substituted aromatic ester functionalities. All the synthesized 1,4-disubstituted 1,2,3-triazoles were characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and mass spectrometry and also screened for their antibacterial, antitubercular and antifungal activities.

2. Experimental

2.1. Measurements

Melting points of synthesized compounds were recorded in °C 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 a Waters Micromass Q-Tof Micro (ESI) spectrophotometer. The completion of reactions and the purity of the compounds were analysed by thin layer chromatography (TLC) using readymade silica gel plates (SIL G/UV254, ALUGRAM) and visualized under ultraviolet lamp.

2.2. General procedure for the synthesis of 1,4-disubstituted 1,2,3-triazoles

The starting reactants p-substituted benzoic acid prop-2-ynyl esters (**2**) were prepared by reacting p-substituted benzoyl chlorides (**1**) and propargyl alcohol in the presence of N,N-dimethylaminopyridine (DMAP) in dry dichloromethane at 0–10 °C. The 1,4-disubstituted 1,2,3-triazoles (**3a–3p**) were synthesized by stirring p-substituted benzoic acid prop-2-ynyl esters (1 mmol) with different aralkyl bromides (1 mmol) in the presence of sodium azide (3 mmol), copper sulphate pentahydrate (0.10 mmol) and sodium ascorbate (0.20 mmol) using N,N-dimethylformamide:water (8:2) mixture as solvent at room temperature for 6–12 h (Scheme 1).

The reaction workup was carried out with aqueous ammonia-ammonium chloride solution and the compound was extracted three times with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum to yield 1,4-disubstituted 1,2,3-triazoles.

2.3. Characterization of synthesized compounds

2.3.1. 4-Methylbenzoicacid-1-benzyl-1H-[1,2,3]triazol-4-ylmethylester (3a)

Appearance: white, crystalline solid; Yield: 82%; m.p. 118–120 °C; FT-IR (KBr): 3105 (C–H str., triazole ring), 3051, 2962, 1712, 1610, 1446, 1394 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.41 (s, 3H), 5.45 (s, 2H), 5.55 (s, 2H), 7.23 (d, 2H, J = 8 Hz), 7.28–7.31 (m, 3H), 7.38 (d, 2H, J = 12 Hz), 7.62 (s, 1H), 7.93 (d, 2H, J = 8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 21.7, 54.3, 57.9, 123.8, 127, 128.2, 128.9, 129.2, 129.8, 134.4, 143.4, 144, 166.5; MS m/z: 308.0 [M$^+$], 309.0 [M$^+$ + 1].

2.3.2. 4-Methylbenzoicacid-1-(phenylpropyl)-1H-[1,2,3]triazol-4-ylmethylester (3b)

Appearance: off-white solid; Yield: 65%; m.p. 90–92 °C; FT-IR (KBr): 3116 (C–H str., triazole ring), 3068, 2943, 1712, 1610, 1448, 1400 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 2.17–2.24 (m, 2H), 2.39 (s, 3H), 2.64 (t, 2H), 4.32 (t, 2H), 5.43 (s, 2H), 7.12–7.30 (m, 7H), 7.64 (s, 1H), 7.92 (d, 2H, J = 8 Hz); ^{13}C NMR (75 MHz, $CDCI_3$): 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: 336.0 [M$^+$], 337.0 [M$^+$ + 1].

2.3.3. 4-Methylbenzoicacid-1-(4-methylbenzyl)-1H-[1,2,3]triazol-4-ylmethylester (3c)

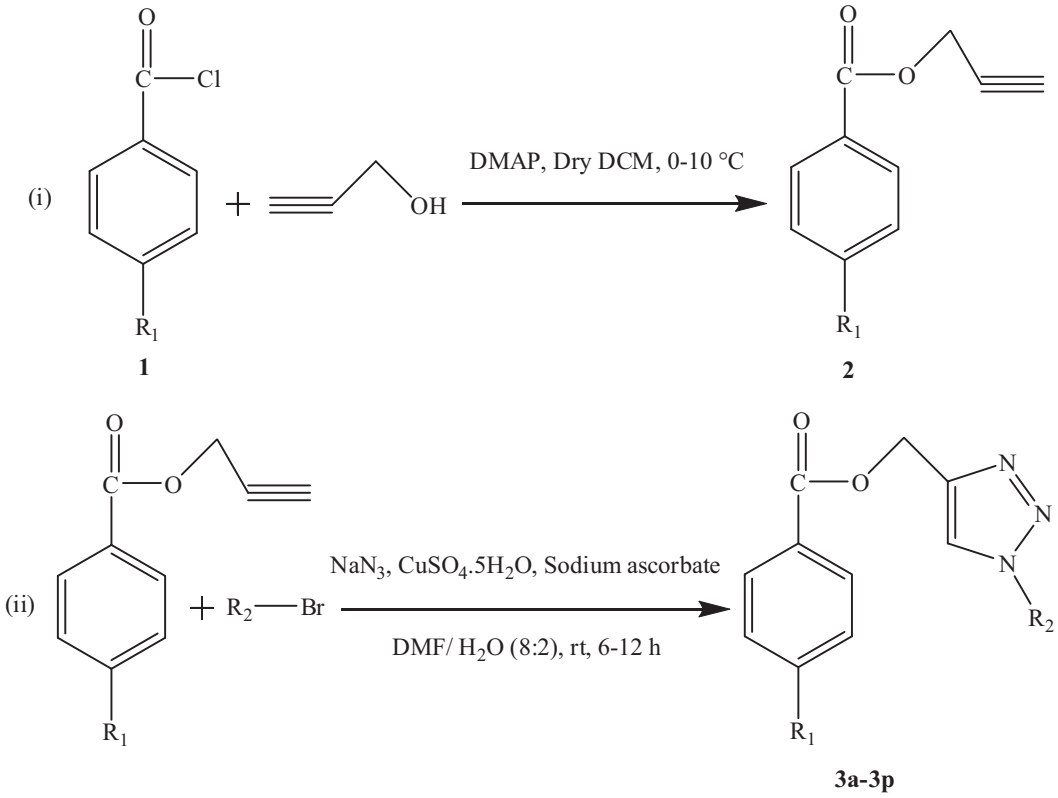
Appearance: creamy-white, crystalline solid; Yield: 68.5%. m.p. 104–106 °C; FT-IR (KBr): 3132 (C–H str., triazole ring), 3032, 2954, 2920, 1714, 1610, 1512, 1444 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 2.35 (s, 3H), 2.40 (s, 3H), 5.43 (s, 2H), 5.50 (s, 2H), 7.00–7.28 (m, 6H), 7.60 (s, 1H), 7.92 (d, 2H, J = 8 Hz); ^{13}C NMR (100 MHz, $CDCI_3$): 21.2, 21.7, 53.9, 54.1, 57.9, 123.7, 127, 127.2, 128.2, 129.1, 129.2, 129.8, 131.4, 138.8, 143.4, 143.9, 166.5; MS m/z: 322.0 [M$^+$], 323.0 [M$^+$ + 1].

2.3.4. 4-Methylbenzoicacid-1-(4-nitrobenzyl)-1H-[1,2,3]triazol–4-ylmethylester (3d)

Appearance: white, crystalline solid; Yield: 62.4%; m.p. 170–172 °C; FT-IR (KBr): 3130 (C–H str., triazole ring), 3082, 2962, 1699, 1606, 1525, 1435, 1348 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 2.40 (s, 3H), 5.46 (s, 2H), 5.65 (s, 2H), 7.23 (d, 2H), 7.43 (d, 2H, J = 8.4 Hz), 7.73 (s, 1H), 7.91 (d, 2H, J = 8.0 Hz), 8.23 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCI_3$): 21.7, 53.2, 57.8, 124.1, 126.8, 129.2, 129.5,129.8, 141.4, 142.7, 144.1, 145.4, 148.1, 166.5; MS m/z: 353.0 [M$^+$], 354.0 [M$^+$ + 1].

2.3.5. 4-Methoxybenzoicacid-1-benzyl-1H-[1,2,3]triazol-4-ylmethylester (3e)

Appearance: off-white solid; Yield: 79%; m.p. 118–120 °C; FT-IR (KBr): 3136 (C–H str., triazole ring), 3064, 2951, 1712, 1604, 1508, 1452, 1323 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 3.82 (s, 3H), 5.39 (s, 2H), 5.50 (s, 2H), 6.86 (d, 2H, J = 8 Hz), 7.28–7.33 (m, 5H), 7.60 (s, 1H), 7.95 (d, 2H,



Compound No.	R_1	R_2
3a	CH_3	C_6H_5-CH_2-
3b	CH_3	C_6H_5-CH_2-CH_2-CH_2-
3c	CH_3	4-CH_3-C_6H_5-CH_2-
3d	CH_3	4-NO_2-C_6H_5-CH_2-
3e	OCH_3	C_6H_5-CH_2-
3f	OCH_3	C_6H_5-CH_2-CH_2-CH_2-
3g	OCH_3	4-CH_3-C_6H_5-CH_2-
3h	OCH_3	4-NO_2-C_6H_5-CH_2-
3i	NO_2	C_6H_5-CH_2-
3j	NO_2	C_6H_5-CH_2-CH_2-CH_2-
3k	NO_2	4-CH_3-C_6H_5-CH_2-
3l	NO_2	4-NO_2-C_6H_5-CH_2-
3m	F	C_6H_5-CH_2-
3n	F	C_6H_5-CH_2-CH_2-CH_2-
3o	F	4-CH_3-C_6H_5-CH_2-
3p	F	4-NO_2- C_6H_5-CH_2-

Scheme 1 Synthesis of 1,4-disubstituted 1,2,3-triazoles(**3a**–**3p**).

J = 8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 54.10, 55.28, 57.71, 113.54, 122.07, 130.73, 128.07, 129.09, 131.81, 134.44, 143.48, 163.45, 165.96; MS m/z: 324.0 [M^+], 325.0 [M^+ + 1].

*2.3.6. 4-Methoxybenzoicacid-1-(phenylpropyl)-1H-[1,2,3]triazol-4-ylmethylester (**3f**)*

Appearance: creamy-white solid; Yield: 64%; m.p. 78–80 °C; FT-IR (KBr): 3109 (C–H str., triazole ring), 3070, 2947, 1712, 1600, 1502, 1448, 1377 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 2.13–2.29 (m, 2H, J = 7.2 Hz), 2.65 (t, 2H, J = 7.2 Hz), 3.83 (s, 3H), 4.34 (t, 2H J = 7.2 Hz), 5.43 (s, 2H), 6.89 (d, 2H, J = 8.7 Hz), 7.14–7.30 (m, 5H), 7.65 (s, 1H), 7.99 (d, 2H, J = 8.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 31.6, 32.5, 49.6, 55.5, 57.9, 113.7, 122.2, 126.4, 128.3, 128.4, 128.6, 131.8, 140.0, 143.2, 163.6, 166.3; MS m/z: 352.0 [M^+], 353.0 [M^+ + 1].

*2.3.7. 4-Methoxybenzoicacid-1-(4-methylbenzyl)-1H-[1,2,3]triazol-4-ylmethylester (**3g**)*

Appearance: dull-white solid; Yield: 69.4%; m.p. 108–110 °C; FT-IR (KBr): 3188 (C–H str., triazole ring), 3022, 2947, 2920, 1705, 1606, 1512, 1448 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 2.34 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 5.40 (s, 2H, CH_2), 5.47 (s, 2H, CH_2), 6.88 (d, 2H, J = 8.7 Hz), 7.17–7.26 (m, 4H), 7.64 (s, 1H), 7.97 (d, 2H, J = 8.7 Hz); ^{13}C NMR (75 MHz, $CDCI_3$): 21.2, 54.1, 55.4, 57.8, 113.6, 122.1, 128.2, 129.8, 131.4, 131.8, 138.8, 143.5, 163.5, 166.2; MS m/z: 338.0 [M^+], 339.0 [M^+ + 1].

*2.3.8. 4-Methoxybenzoicacid-1-(4-nitrobenzyl)-1H-[1,2,3]triazol-4-ylmethylester (**3h**)*

Appearance: shiny creamy-white solid; Yield: 72.8%; m.p. 172–174 °C; FT-IR (KBr): 3124 (C–H str., triazole ring), 3080, 2848, 1701, 1606, 1523, 1431, 1340 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 3.84 (s, 3H), 5.43 (s, 2H), 5.63 (s, 2H), 6.88 (d, 2H, J = 8.7 Hz), 7.41 (d, 2H, J = 8.4 Hz), 7.70 (s, 1H), 7.96 (d, 2H, J = 8.7 Hz), 8.21 (d, 2H, J = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCI_3$): 51.52, 53.17, 55.46, 56.96, 57.66, 113.67, 121.89, 124.33, 128.69, 131.79, 141.41, 144.15, 148.10, 163.63, 166.19; MS m/z: 369.0 [M^+], 370.0 [M^+ + 1].

*2.3.9. 4-Nitrobenzoicacid-1-benzyl-1H-[1,2,3]triazol-4-ylmethylester (**3i**)*

Appearance: creamy-white solid; Yield: 81%; m.p. 134–136 °C; FT-IR (KBr): 3124 (C–H str., triazole ring), 3076, 1726, 1604, 1521, 1435, 1350 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 5.50 (s, 2H), 5.56 (s, 2H), 7.28–7.33 (m, 2H), 7.40–7.41 (m, 3H),7.63 (s,1H), 8.21 (d, 2H J = 6.8 Hz), 8.29 (d, 2H J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCI_3$): 54.3, 58.8, 123.5, 124, 128.2, 129, 129.2, 130.9, 134.2, 135.1, 142.5, 164.6; MS m/z: 339.0 [M^+], 340.0 [M^+ + 1].

*2.3.10. 4-Nitrobenzoicacid-1-(phenylpropyl)-1H-[1,2,3]triazol-4-ylmethylester (**3j**)*

Appearance: off-white solid; Yield: 70.15%; m.p. 106–108 °C; FT-IR (KBr): 3124 (C–H str., triazole ring), 3076, 1722, 1606, 1516, 1454, 1348 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 2.27–2.31 (m, 2H), 2.67 (t, 2H J = 6.9 Hz), 4.40 (t, 2H J = 6.9 Hz), 5.52 (s, 2H), 7.15–7.32 (m, 5H), 7.9 (s, 1H), 8.26 (m, 4H); ^{13}C NMR (75 MHz, $CDCI_3$): 31.6, 32.5, 49.7, 58.8, 123.6, 126.5, 128.4, 128.7, 130.9, 135.1, 139.9, 142.1, 150.7, 164.6; MS m/z: 367.0 [M^+], 368.0 [M^+ + 1].

*2.3.11. 4-Nitrobenzoicacid-1-(4-methyl-benzyl)-1H-[1,2,3]triazol-4-ylmethylester (**3k**)*

Appearance: off-white solid; Yield: 77.3%; m.p. 136–138 °C; FT-IR (KBr): 3116 (C–H str., triazole ring), 3066, 2951, 1722, 1606, 1521, 1442, 1350 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 2.37 (s, 3H), 5.49 (s, 2H), 5.51 (s, 2H), 7.21–7.28 (m, 4H), 7.61 (s, 1H), 8.21 (d, 2H, J = 8.8 Hz), 8.28 (d, 2H, J = 8.8 Hz); ^{13}C NMR (100 MHz, $CDCI_3$): 21.2, 54.2, 58.8, 123.5, 123.8, 128.3, 129.9, 130.9, 142.4, 167.0; MS m/z: 353.0 [M^+], 354.0 [M^+ + 1].

*2.3.12. 4-Nitrobenzoicacid-1-(4-nitrobenzyl)-1H-[1,2,3]triazol-4-ylmethylester (**3l**)*

Appearance: very light yellow coloured solid; Yield: 79.9%; m.p. 142–144 °C; FT-IR (KBr): 3151 (C–H str., triazole ring), 3078, 1724, 1606, 1541, 1436, 1352 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 5.0 (s, 2H), 5.17 (s, 2H), 6.94 (d, 2H, J = 10), 7.66–7.78 (m, 6H), 7.7 (s, 1H). ^{13}C NMR (75 MHz, $CDCI_3$): 53.14, 58.54, 123.51, 124.31, 128.69, 130.84, 134.90, 141.27, 142.98, 148.12, 150.65, 164.40; MS m/z: 384.0 [M^+], 385.0 [M^+ + 1].

*2.3.13. 4-Flourobenzoicacid-1-benzyl-1H-[1,2,3]triazol-4-ylmethylester (**3m**)*

Appearance: white puffy solid; Yield: 85.3%; m.p. 98–100 °C; FT-IR (KBr): 3120 (C–H str., triazole ring), 3068, 1720, 1602, 1508, 1450, 1242 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 5.45 (s, 2H), 5.51 (s, 2H), 7.10 (t, 2H, J = 8.8 Hz), 7.28–7.32 (m, 3H), 7.38–7.40 (d, 2H), 7.62 (s, 1H), 8.04–8.07 (d, 2H); ^{13}C NMR (100 MHz, $CDCI_3$): 54.3, 58.8. 115.6, 123.8, 126, 128.2, 128.9, 129.2, 132.3, 134.3, 143.2, 164.6, 165.5; MS m/z: 312.0 [M^+], 313.0 [M^+ + 1].

*2.3.14. 4-Flourobenzoicacid-1-(phenyl-propyl)-1H-[1,2,3]triazol-4-ylmethylester (**3n**)*

Appearance: white solid; Yield: 72.1%; m.p. 80–82 °C; FT-IR (KBr): 3111 (C–H, str., triazole ring), 3066, 1716, 1598, 1502, 1450, 1236 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 2.24–2.32 (m, 2H), 2.68 (t, 2H J = 7.2 Hz), 4.37 (t, 2H, J = 7.2 Hz), 5.50 (s, 2H), 7.12 (d, 2H, J = 8.4 Hz), 7.17–7.33 (m, 5H) 7.67 (s, 1H), 8.07 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCI_3$): 31.6, 32.5, 49.6, 58.2, 115.5, 115.7, 124.0, 126.0, 126.4, 128.4, 128.6, 132.3, 132.4, 140.0, 142.8, 164.6, 165.5, 167.2; MS m/z: 340.0 [M^+], 341.0 [M^+ + 1].

*2.3.15. 4-Flourobenzoicacid-1-(4-methyl-benzyl)-1H-[1,2,3]triazol-4-ylmethylester (**3o**)*

Appearance: white solid; Yield: 76.0%; m.p. 108–110 °C; FT-IR (KBr): 3118 (C–H str., triazole ring), 3066, 2951, 1716, 1598, 1506, 1444, 1224 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 2.37 (s, 3H), 5.44 (s, 2H), 5.50 (s, 2H), 7.10 (dd, 2H, J = 8.0), 7.22 (m, 4H), 7.59 (s,1H), 8.05 (dd, 2H, J = 8.0); ^{13}C NMR (100 MHz, $CDCI_3$): 21.2, 54.1, 58.1, 115.4, 115.7, 123.7, 126.0, 128.2, 129.8, 131.3, 132.3, 138.9, 143.1, 164.6, 165.5, 167.2; MS m/z: 326.0 [M^+], 327.0 [M^+ + 1].

*2.3.16. 4-Flourobenzoicacid-1-(4-nitro-benzyl)-1H-[1,2,3]triazol-4-ylmethylester (**3p**)*

Appearance: light yellow solid; Yield: 79.3%; m.p. 122–124 °C; FT-IR (KBr): 3130 (C–H str., triazole ring), 3068, 1707, 1602, 1510, 1462, 1226 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 5.40 (s, 2H), 5.66 (s, 2H), 7.08 (d, 2H, J = 8.0 Hz), 7.43 (d, 2H, J = 10.0 Hz), 7.74 (s, 1H), 8.03 (d, 2H, J = 8.0 Hz), 8.20 (d, 2H, J = 10.0 Hz); ^{13}C NMR (75 MHz, $CDCI_3$): 53.07, 57.90, 115.78, 124.26, 128.66, 132.39, 141.40, 143.61, 148.06, 163.33, 165.29, 168.40; MS m/z: 357.0 [M^+], 358.0 [M^+ + 1].

2.4. Determination of antimicrobial activities

2.4.1. Antibacterial/antitubercular activity evaluation

All the synthesized compounds were assessed for their *in vitro* antibacterial activity against two gram negative bacteria, *Escherichia coli* (MTCC 1231) and *Pseudomonas aeruginosa* (MTCC 1036), two gram positive bacteria, *Staphylococcus*

Table 1 MIC (μg/ml) values of compounds **3a**–**3p** for Antibacterial activity.

Compound no.	*E. coli* (MTCC 1231)	*S. aureus* (MTCC 7443)	*B. subtilis* (MTCC 9023)	*P. aeruginosa* (MTCC 1036)	*M. tuberculosis* (ATCC 27294)
3a	100	500	100	250	50
3b	25	12.5	12.5	12.5	12.5
3c	100	100	200	50	50
3d	100	250	200	250	12.5
3e	100	250	500	500	50
3f	50	200	100	200	25
3g	200	1000	500	500	25
3h	100	250	200	200	50
3i	100	200	250	100	25
3j	50	100	50	100	6.25
3k	100	500	50	200	50
3l	200	500	250	500	50
3m	500	500	100	250	12.5
3n	100	50	50	50	3.75
3o	100	100	100	100	25
3p	100	500	500	500	50
Ciprofloxacin	25	50	12.5	12.5	–
Isoniazid	–	–	–	–	3.75

Table 2 MIC (μg/ml) values of compounds **3a**–**3p** for antifungal activity.

Compound no.	*C. albicans* (MTCC 854)	*A. niger* (MTCC 282)	*A. flavus* (MTCC 873)
3a	500	500	1000
3b	25	100	100
3c	50	25	50
3d	500	100	100
3e	500	250	125
3f	100	50	100
3g	200	100	100
3h	100	200	500
3i	500	200	250
3j	500	500	500
3k	250	200	100
3l	500	1000	500
3m	50	100	50
3n	12.5	12.5	25
3o	25	25	50
3p	100	100	200
Amphotericin B	25	25	50

aureus (MTCC 7443) and *Bacillus subtilis* (MTCC 9023). The *in vitro* activities of newly synthesized triazoles were tested in nutrient broth (NB, Hi-media, Mumbai) by two fold serial dilution method using a stock solution of 1000 μg/ml concentration. Dimethylsulphoxide was used as solvent control. The stock solutions of test compounds and reference drug were serially diluted to get concentrations of 500, 250, 200, 100, 50, 25 and 12.5 μg/ml. These dilutions were inoculated with 100 μL suspension of respective microorganisms in sterile saline and incubated at 37 °C for 24 h. To check the effect of solvent on bacterial growth, a control test was performed with the test medium supplemented with dimethylsulphoxide at same dilution as used in the experiment. The antibacterial potency of the compounds was compared with a broad spectrum antibiotic ciprofloxacin.

Mycobacterium tuberculosis (ATCC 27294) were assessed 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 1.5×10^8 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 °C. At the end of incubation, the number of viable mycobacterium was determined by the MTT method.

2.4.2. Antifungal activity evaluation

All synthesized triazole compounds were evaluated for their *in vitro* antifungal activity against three fungal strains viz. *Candida albicans* (MTCC 854), *Aspergillus niger* (MTCC 282), *Aspergillus flavus* (MTCC 873) and amphotericin B was used

as the standard drug. Sabouraud dextrose broth was employed as culture media and dimethylsulphoxide as solvent control. A spore suspension in sterile saline was prepared from one day old culture of fungus growing on sabourauds dextrose broth (SDB, Hi-Media, Mumbai). The final spore concentration was 100 μL/ml. The stock solutions of 1000 μg/ml of test compounds and standard drug were diluted to get concentrations of 500, 250, 200, 100, 50, 25 and 12.5 μg/ml. These dilutions were inoculated with the suspension of respective microorganism in their culture media and were incubated at 25 °C for 48 h in case of *C. albicans* and at 25 °C for 120 h in case of *A. niger* and *A. flavus*.

3. Results and discussion

3.1. Chemistry

The triazole compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral techniques. The formation of triazole was confirmed by the presence of an absorption band in the region 3188–3105 cm^{-1} in IR spectra due to =C–H stretching of triazole ring. The presence of characteristic singlet in ^{1}H NMR due to triazolyl protons in the region of δ 7.59–7.9 and δ 129.8–132.4 in ^{13}C NMR due to C-5 of the triazole ring confirmed the formation of the triazole ring. The results obtained from mass spectral analysis were found to be in accordance to their molecular weights.

3.2. Antibacterial/antitubercular activity

All compounds were assessed for their *in vitro* antibacterial/antitubercular activity against *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa* and *M. tuberculosis*. The antibacterial/ antitubercular potency of the compounds was compared with standard drugs ciprofloxacin and isoniazid. The minimum inhibitory concentration (MIC) values were calculated as summarized in Table 1.

The results obtained from their antibacterial studies revealed that compounds **3a** to **3p** showed moderate to excellent activities against tested bacterial strains. Compound **3b** showed an excellent activity against both gram positive and gram negative bacterial strains with MIC values ranging from 12.5–25 μg/ml. Compound **3j** exhibited good to moderate activity against *E. coli, B. subtilis, M. tuberculosis* and compound **3n** has better inhibition effect against *S. aureus, B. subtilis, P. aeruginosa* and *M. tuberculosis*. Compounds **3c** and **3f** were found to possess apparent activity against *P. aeruginosa* and *E. coli*, respectively.

3.3. Antifungal activity

The *in vitro* antifungal activity of the synthesized compounds was examined against three fungal strains viz. *C. albicans, A. niger, A. flavus* and Amphotericin B was used as the standard drug. The MIC values of the test compounds and the standard are furnished in Table 2.

Antifungal activity results indicated that most of the synthesized compounds exhibited good to moderate activity against the tested fungal strains. The compounds **3c**, **3n** and **3o** showed excellent antifungal activity against all tested fungal strains with MIC values ranging from 12.5–50 μg/ml. Compounds **3b**, **3f** and **3m** exhibited good antifungal activity against *C. albicans, A. niger* and *A. flavus*, respectively.

4. Conclusion

A series of 1,4-disubstituted 1,2,3-triazole compounds were synthesized through an easy, convenient, Cu(I) catalysed click reaction and evaluated for their *in vitro* antimicrobial activity against *E. coli, P. aeruginosa, S. aureus, B. subtilis, M. tuberculosis, C. albicans, A. niger* and *A. flavus*. Compound **3b** exhibited significant antibacterial activity against all tested bacterial strains, whereas, compounds **3n**, **3o** possess excellent antifungal activity among the used fungal strains. Rest of synthesized molecules have moderate to good antimicrobial activity. 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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