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Microwave Assisted Synthesis of Benzoxazole-Triazole Hybrid Derivatives as Antimicrobial Agents

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A series of new 1,3-benzoxazole based 1,2,3-triazoles compounds were synthesized using click reaction under conventional and microwave irradiation methods. The microwave irradiation method gave higher yields, shorter reaction time as compared to conventional method using green solvents, eco-friendly reaction conditions. All the targeted compounds were characterized by IR, ^{1}H, ^{13}C NMR and mass spectral analysis. Furthermore, the titled compounds were screened for their *in vitro* antimicrobial activity against bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* as well as fungi such as *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*. Most of the compounds displayed good to excellent antimicrobial activity in comparison to standard drugs.

Keywords: Antimicrobial activity, 1,3-Benzoxazole, 1,2,3-Triazoles, Click chemistry, Microwave irradiation.

INTRODUCTION

Benzoxazole have active unique place in the arena of medicinal chemistry due to its wade wide range of activities. The benzoxazole derivatives have been testified to have various biological activities [1]. The substituted benzoxazoles have been showed to exhibit antimicrobial [2,3], antitubercular [4], antiviral [5], antitumor [6-9] and DNA-topoisomerase-I, II inhibitory activities [10]. Benzoxazoles are interesting fluorescent probes, which show high stokes shift and present thermal and photophysical stability due to an excited state intramolecular proton transfer mechanism [11]. The *bis*(benzoxazole) natural products are structurally unique class of streptomyces secondary metabolites as reported in the literature [12]. Similarly, triazoles compounds are the important class of *N*-heterocycles, are engaged in many pharmaceutical moieties. In particular, 1,2,3-triazole derivatives possess important biological activities, including antitumour [13,14], anti-HIV [15], anti-inflammatory [16] and antimicrobial [17] activities. In recent years, 1,2,3-triazoles have gained special attention and found wide applications in the drug discovery field especially as anticancer agents. Because of the growing use of copper catalyzed azide alkyne cycloaddition the click reaction [18,19], we used this reaction, for the synthesis of new triazoles derivatives.

In view of above literature and pharmacology importance of both benzoxazole and 1,2,3-triazole derivatives, we decided to design new hybrid molecules and synthesize the new 1,3-benzoxazole based 1,2,3-triazoles compounds *i.e.* 2-[4-(4-aryl-1*H*-1,2,3-triazol-1-yl)phenyl]-1,3-benzoxazole derivatives (**6a-j**) under conventional and microwave irradiation methods was achieved. All the synthesized compounds were screened for their *in vitro* antimicrobial activity against various micro-organisms.

EXPERIMENTAL

All the materials were of commercial purchased mostly from Sigma-Aldrich and used without further purification. The melting points were determined in open capillaries and are uncorrected. The purity of the newly synthesized compounds was checked by thin-layer chromatography (TLC) on silica gel 60 F_{254} (Merck). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 spectrometer using tetramethylsilane as internal standard. The IR spectra were recorded in KBr on a Shimadzu FTIR 8400S spectrometer. The mass spectra were obtained on a Shimadzu GCMS-QP 1000 instrument. Microwave-assisted reactions were carried out in a milestone multi-SYNTH microwave system. Elemental analysis was

performed by means of Perkin-Elmer 2400 CHN elemental analyzer.

Synthesis of 4-(1,3-benzoxazol-2-yl)aniline (3): Compound 2-amino-phenol (**1**) (1 mmol) and 4-aminobenzaldehyde (**2**) (1.1 mmol) were refluxed in ethanol (15 mL) for 3 h. After the reaction mixture was cooled, ethanol was removed in vacuum. The resulting Schiff base was dissolved in 20 mL of acetic acid and lead tetraacetate (1 mmol) was added and stirred at room temperature for 1 h. The reaction mixture was then diluted with 30 mL of H_2O, extracted with ethyl acetate and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum, and the crude product was purified by column chromatography with ethyl acetate-hexane (3:7) to afford pure compound **3** [20].

Synthesis of 2-(4-azidophenyl)-1,3-benzoxazole (4): Amine derivative (**3**) (1 mmol) was dissolved in 10% aq. HCl at room temperature. This reaction mixture upon cooling to 0 ºC and addition of a solution of $NaNO_2$ (1.1 mmol), allowed stirring for 10 min at 0-5 ºC. At this stage, sodium azide (1.1 mmol) was added and the mixture was stirred at room temperature for 2 h. When the reaction was completed, it was worked up by dilution with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation of solvent, the crude product was purified by column chromatography with ethyl acetate/hexane (1:9 *v*/*v*) to afford pure compound **4** [21].

General procedure for synthesis of 2-[4-(4-aryl-1*H*-1,2,3-triazol-1-yl)phenyl]-1,3-benzoxazole derivatives (6a-j)

Conventional method using $CuSO_4·5H_2O$ and sodium ascorbate: A mixture of 2-(4-azidophenyl)-1,3-benzoxazole (**4**) (1.1 mmol) and substituted phenyl acetylene (**5a-j**) (1 mmol) was dissolved in *t*-BuOH/H_2O 1:1 (20 mL) followed by sodium ascorbate (33 mg, 20 mol %), $CuSO_4$ (300 mg, 5 mol %) were added and stirred for 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, 40 mL of ice-cold water was added. The product was worked up by filtration, followed by extraction with dichloromethane (40 mL). The combined organic layers were washed with aqueous 5% NH_3 (30 mL), brine (10 mL) and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by column chromatography with ethyl acetate/hexane (3:7 v/v) to afford pure compound **6a-j**.

Microwave irradiation method using $CuSO_4·5H_2O$ and sodium ascorbate: A mixture of 2-(4-azidophenyl)-1,3-benzoxazole (**4**) (1.1 mmol) and substituted phenyl acetylene (**5a-j**) (1 mmol) was dissolved in *t*-BuOH/H_2O 1:1 (3.5mL, v/v), then sodium ascorbate (33 mg, 20 mol %), $CuSO_4$ (300 mg, 5 mol%) were taken into quartz tube and inserted into a Teflon vial with screw capped and then it was subjected to microwave irradiation at 100 W for 5 min. After completion of the reaction, 40 mL of ice cold water was added. The product was worked up by filtration, followed by extraction with dichloromethane (40 mL). The combined organic layers were washed with aqueous 5% NH_3 (30 mL), brine (10 mL) and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by column chromatography with ethyl acetate/hexane (3:7 v/v) to afford pure compound **6a-j**.

Conventional method using CuI: A mixture of 2-(4-azidophenyl)-1,3-benzoxazole (**4**) (1.1 mmol) and substituted phenyl acetylene (**5a-j**) (1 mmol), CuI (1.6 g, 8.47 mmol) and diisopropylethylamine (1.5 mL, 8.47 mmol) in DMF (10 mL) was stirred at room temperature for 2 h. Progress of the reaction was monitored by TLC. After completion of reaction, chilled water (40 mL) was added to it. The product was extracted with dichloromethane (100 mL). The organic layer was washed with aqueous 5% NH_3 (3 × 25 mL) followed by brine (25 mL) and then dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by column chromatography with ethyl acetate/hexane (3:7 v/v) to afford pure compound **6a-j**.

Microwave irradiation method using CuI: A mixture of 2-(4-azidophenyl)-1,3-benzoxazole (**4**) (1.1 mmol) and substituted phenyl acetylene (**5a-j**) (1 mmol), CuI (1.6 g, 8.47 mmol) and diisopropylethylamine (1.5 mL, 8.47 mmol) in DMF (2 mL) was taken into quartz tube and inserted into a Teflon vial with screw capped and then it was subjected to microwave irradiation at 320 watts for 3 min. Progress of the reaction was monitored by TLC. After completion of reaction, chilled water (40 mL) was added to it and then the product was extracted with dichloromethane (100 mL). The organic layer was washed with aqueous 5% NH_3 (3 × 25 mL) followed by brine (25 mL) and then dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by column chromatography with ethyl acetate/hexane (3:7 v/v) to afford pure compound **6a-j**.

2-[4-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)phenyl]-1,3-benzoxazole (6a): Pale yellow coloured solid; m.p.: 262-264 ºC; R_f : 0.60; IR (KBr, ν_{max}, cm^{-1}): 3036, 1557, 1429, 1331; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 6.82-6.99 (m, 2H, Ar-H), 7.12-7.19 (m, 2H, Ar-H), 7.24-7.33 (m, 2H, Ar-H), 7.48 (dd, 2H, Ar-H), 7.57 (d, 1H, *J* = 8.23 Hz, Ar-H), 7.57 (d, 2H, *J* = 8.23 Hz, Ar-H), 7.82 (s, 1H, Triazole-H), 7.97 (d, 2H, *J* = 8.23 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 108.1, 114.5, 118.8, 121.3, 122.1, 123.4, 124.6, 125.0, 125.2, 127.1, 127.3, 130.3, 133.1, 133.7, 141.4, 141.8, 147.4, 148.9, 158.2, 159.9. Elemental analysis calcd. (found) % of $C_{21}H_{14}N_4O$: C, 74.48 (74.54); H, 4.19 (4.17); N, 16.59 (16.56). Mass spectrum: *m/z* 339 $[M+H]^+$ (100%).

2,4-[4-(3,4,5-Trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenyl-1,3-benzoxazole (**6b**): Pale yellow coloured solid; m.p.: 250-252 ºC; R_f: 0.75; IR (KBr, ν_{max}, cm^{-1}): 3075, 1673, 1557, 1216, 757. ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 3.62 (s, 3H, OCH_3), 3.73 (s, 6H, 2 × OCH_3), 7.37 (s, 2H, Ar-H), 7.46 (m, 1H, Ar-H), 7.51 (d, 2H, *J* = 8.01 Hz, Ar-H), 7.53-7.61 (m, 3H, Ar-H), 7.97 (d, 2H, *J* = 8.01 Hz, Ar-H), 8.16 (s, 1H, Triazole-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 54.9, 55.2, 110.0, 110.8, 116.1, 118.6, 122.8, 123.2, 124.9, 125.2, 128.6, 140.1, 140.7, 143.2, 146.8, 148.1, 155.2, 160.1. Elemental analysis calcd. (found) % of $C_{24}H_{20}N_4O_4$: C, 67.28 (67.34); H, 4.71 (4.66); N, 13.08 (13.11). Mass spectrum: *m/z* 429 $[M+H]^+$ (100%).

2,4-[4-(4-Trifluoromethyl) phenyl-1*H*-1,2,3-triazol-1-yl]phenyl-1,3-benzoxazole (**6c**): Pale yellow coloured solid; m.p.: 240-242 ºC; R_f: 0.65; IR (KBr, ν_{max}, cm^{-1}): 3054, 1661,

1552, 1229, 760. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.27-7.36 (m, 2H, Ar-H), 7.52-7.61 (m, 2H, Ar-H), 7.70 (d, 2H, *J* = 8.60 Hz, Ar-H), 7.83 (d, 2H, *J* = 8.03 Hz, Ar-H), 7.97 (d, 2H, *J* = 8.03, Ar-H), 8.07 (d, 2H, *J* = 8.63 Hz, Ar-H), 8.13 (s, 1H, Triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 109.2, 112.8, 114.5, 117.8, 123.1, 123.9, 124.6, 125.8, 127.9, 130.7, 131.8, 134.1, 141.8, 142.0, 146.3, 148.7, 159.0, 160.9. Elemental analysis calcd. (found) % of $C_{22}H_{133}N_4OF$: C, 65.03 (65.11); H, 3.22 (3.19); N, 13.79 (13.81). Mass spectrum: *m/z* 407 $[M+H]^+$ (100%).

2,4-[4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-phenyl-1,3-benzoxazole (6d): Pale yellow coloured solid; m.p.: 270-272 ºC; R_f : 0.72; IR (KBr, ν_{max}, cm^{-1}): 3058, 1642, 1570, 1225, 763. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.91 (s, 3H, OCH_3), 6.86 (d, 2H, *J* = 9.02 Hz, Ar-H), 7.35-7.46 (m, 4H, Ar-H), 7.54 (d, 2H, *J* = 8.02 Hz, Ar-H), 7.76 (d, 2H, *J* = 8.02 Hz, Ar-H), 7.88 (d, 2H, *J* = 8.02 Hz, Ar-H), 8.09 (s, 1H, Triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 54.9, 110.1, 114.2, 114.9, 117.7, 122.7, 123.6, 124.0, 125.1, 125.9, 126.6, 133.9, 141.7, 141.9, 146.0, 147.7, 148.8, 158.9, 160.9. Elemental analysis calcd. (found) % of $C_{22}H_{16}N_4O_2$: C, 71.68 (71.72); H, 4.41 (4.38); N, 15.23 (15.21). Mass spectrum: *m/z* 369 $[M+H]^+$ (100%).

2,4-[4-(3,5-Di(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl]phenyl-1,3-benzoxazole (6e): Pale yellow coloured solid; m.p.: 282-284 ºC; R_f: 0.75; IR (KBr, ν_{max}, cm^{-1}): 3059, 1697, 1557, 1229, 759. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.17-7.28 (m, 2H, Ar-H), 7.42-7.54 (m, 2H, Ar-H), 7.59 (d, 2H, *J* = 8.18 Hz, Ar-H), 7.82 (s, 1H, Ar-H), 7.88 (s, 2H, Ar-H), 7.99 (d, 2H, *J* = 8.23 Hz, Ar-H), 8.09 (s, 1H, Triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 110.8, 111.3, 114.2, 116.9, 121.3, 122.4, 123.8, 124.7, 125.0, 128.4, 130.0, 132.7, 133.9, 141.2, 142.0, 157.8, 158.1, 159.9. Elemental analysis calcd. (found) % of $C_{23}H_{12}F_6N_4O$: C, 58.24 (58.27); H, 2.55 (2.58); N, 11.81 (11.65). Mass spectrum: *m/z* 475 $[M+H]^+$ (100%).

2,4-[4-(3,5-Difluorophenyl)-1*H*-1,2,3-triazol-1-yl]-phenyl-1,3-benzoxazole (6f): Pale yellow coloured solid; m.p.: 255-257 ºC; R_f: 0.80; IR (KBr, ν_{max}, cm^{-1}): 3058, 2966, 1689, 1552, 1233, 758. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.04-7.19 (m, 3H, Ar-H), 7.40-7.47 (m, 2H, Ar-H), 7.54 (s, 2H, Ar-H), 7.62 (d, 2H, *J* = 8.93 Hz, Ar-H), 8.03 (d, 2H, *J* = 9.03 Hz, Ar-H), 8.13 (s, 1H, Triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 100.3, 109.7, 112.3, 114.9, 122.7, 126.0, 126.6, 132.4, 133.8, 134.8, 141.6, 141.9, 148.8, 159.1, 160.6. Elemental analysis calcd. (found) % of $C_{21}H_{12}N_4OF_2$: C, 67.38 (67.43); H, 3.22 (3.18); N, 14.97 (14.98). Mass spectrum: *m/z* 375 $[M+H]^+$ (100%).

2-[4-(4-*p*-Tolyl-1*H*-1,2,3-triazol-1-yl)phenyl]-1,3-benzoxazole (6g): Pale yellow coloured solid; m.p.: 260-262 ºC; R_f: 0.70; IR (KBr, ν_{max}, cm^{-1}): 3105, 2964, 1684, 1547, 1254, 752. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.02 (s, 3H, CH_3), 7.09 (s, 2H, Ar-H), 7.14-7.20 (m, 3H, Ar-H), 7.40-7.49 (m, 3H, Ar-H), 7.66 (d, 2H, *J* = 8.23 Hz, Ar-H), 8.05 (d, 2H, *J* = 8.21 Hz, Ar-H), 8.18 (s, 1H, Triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 20.1, 108.0, 114.6, 117.7, 122.8, 123.2, 124.9, 125.1, 126.9, 128.8, 132.7, 134.8, 136.7, 141.2, 141.6, 145.4, 148.6, 160.7. Elemental analysis calcd. (found) % of $C_{22}H_{16}N_4O$: C, 74.77 (74.98); H, 4.30 (4.58); N, 15.73 (15.90). Mass spectrum: *m/z* 353 $[M+H]^+$ (100%).

2,4-[4-(3,5-Dimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl]phenyl-1,3-benzoxazole (6h): Pale yellow coloured solid; m.p.: 280-282 ºC; R_f: 0.80; IR (KBr, ν_{max}, cm^{-1}): 3062, 1693, 1558, 1263, 761. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.68 (s, 6H, OCH_3), 6.61 (s, 1H, Ar-H), 7.11 (m, 1H, Ar-H), 7.30-7.38 (m, 3H, Ar-H, 7.51 (s, 2H, Ar-H), 7.61 (d, 2H, *J* = 8.09 Hz, Ar-H), 8.09 (d, 2H, *J* = 8.11, Ar-H), 8.24 (s, 1H, Triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 56.5, 96.7, 109.4, 111.6, 114.9, 123.3, 124.7, 125.1, 131.8, 133.8, 141.2, 141.7, 147.8, 148.6, 158.9, 160.4. Elemental analysis calcd. (found) % of $C_{23}H_{18}N_4O_3$: C, 69.34 (69.23); H, 4.55 (4.59); N, 14.06 (14.09). Mass spectrum: *m/z* 399 $[M+H]^+$ (100%).

2,4-[4-(4-Nitrophenyl)-1*H*-1,2,3-triazol-1-yl]phenyl-1,3-benzoxazole (6i): Pale yellow coloured solid; m.p.: 270-272 ºC; R_f: 0.65; IR (KBr, ν_{max}, cm^{-1}): 3058, 1654, 1538, 1275, 760. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.16-7.22 (m, 2H, Ar-H), 7.44-7.50 (m, 3H, Ar-H), 7.60 (d, 2H, *J* = 8.01 Hz, Ar-H), 7.71 (d, 2H, *J* = 8.36 Hz, Ar-H), 8.01 (d, 1H, *J* = 8.03 Hz, Ar-H), 8.15 (d, 2H, *J* = 8.36 Hz, Ar-H), 8.34 (s, 1H, Triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 109.6, 115.0, 117.9, 122.9, 123.2, 123.7, 124.6, 125.3, 131.0, 134.2, 135.4, 141.3, 141.9, 145.6, 145.9, 148.0, 158.2. Elemental analysis calcd. (found) % of $C_{21}H_{13}N_5O_3$: C, 65.79 (65.83); H, 3.42 (3.46); N, 18.27 (18.31). Mass spectrum: *m/z* 384 $[M+H]^+$ (100%).

2,4-[4-(4-Chlorophenyl)-1*H*-1,2,3-triazol-1-yl]phenyl-1,3-benzoxazole (6j): Pale yellow coloured solid; m.p.: 292-294 ºC; R_f: 0.75; IR (KBr, ν_{max}, cm^{-1}): 3061, 1673, 1557, 1230, 757. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.75 (s, 1H, Ar-H), 6.86 (d, 1H, *J* = 8.01 Hz, Ar-H), 7.11-7.21 (m, 2H, Ar-H), 7.30-7.39 (m, 3H, Ar-H), 7.54 (d, 2H, *J* = 9.02 Hz, Ar-H), 7.73 (d, 1H, *J* = 8.07 Hz, Ar-H) 7.93 (d, 2H, *J* = 9.02 Hz, Ar-H), 8.17 (s, 1H, Triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 109.7, 112.1, 114.8, 115.9, 121.8, 122.4, 123.2, 125.0, 125.8, 129.9, 131.8, 133.7, 142.2, 142.7, 146.6, 147.8, 157.7, 160.8. Elemental analysis calcd. (found) % of $C_{21}H_{13}N_4OCl$: C, 67.73 (7.66); H, 3.38 (3.51); N, 15.21 (15.23). Mass spectrum: *m/z* 373 $[M+H]^+$ (100%).

Antibacterial activity: All the synthesized compounds were screened for their antibacterial activity against different bacterial strains, such as Gram-positive bacterial strains (*B. subtilis* and *S. aureus*) and Gram-negative bacterial strains (*K. pneumoniae* and *E. coli*) at a concentration of 20 µg/mL and 40 µg/mL. The cultures were diluted with 5% saline autoclaved and the final volume was done with concentration approximately 10^5-10^6 CFU/mL. The synthesized compounds were diluted in DMSO for antibacterial biological assays. For disc diffusion method, the liquid form of test compound was soaked onto the disc and then allowed to air dry, such that the disc gets completely saturated with the test compound. The saturated chemical discs were introduced onto the upper layer of the medium evenly flooded with the bacteria. The discs were dipped in different chemical samples, were placed over the evenly spread bacterial nutrient media, and incubated at 37 ºC for 2-3 days for better inhibition of bacteria. The zones of inhibition were measured after 24-48 h. All the experiments were carried

out in triplicates and the results were expressed as zone of inhibition in mm. The zones of inhibition of synthesized compounds **6a-j** were compared with the zone of inhibition of standard antibiotic concentrations of gatifloxacin (20 µg/mL and 40 µg/mL).

Antifungal activity: The antifungal activity of synthesized compounds **6a-j** was tested against three pathogenic fungi such as *A. niger*, *Fusarium oxysporum* and *A. flavus* by the poison plate technique at a concentration of 50 µg/mL. Three kinds of fungi were incubated in potato dextrose agar (PDA) at 25 ± 1 ºC for 5 days to get new mycelium for antifungal assay, then mycelia as discs of approximately 0.45 cm diameter cut from the culture medium were picked up with a sterilized inoculation needle and inoculated in the center of PDA plate. Test compounds were dissolved in DMSO (10 mL) then added to PDA medium (PDA, 40 mL). The final concentration of compounds in the medium was adjusted to 50 µg/mL. The inoculated plates were incubated at 25 ± 1 ºC for 5 days. DMSO in sterilized distilled water was used as control while clotrimazole was used as standard drug for all the treatments; three replicates were performed. The radial growth of fungal colonies was measured on the 4th day and the data were statistically analyzed. The *in vitro* inhibitory effects of the test compounds on the fungi were calculated by the given formula:

$$CV = \frac{A - B}{A}$$

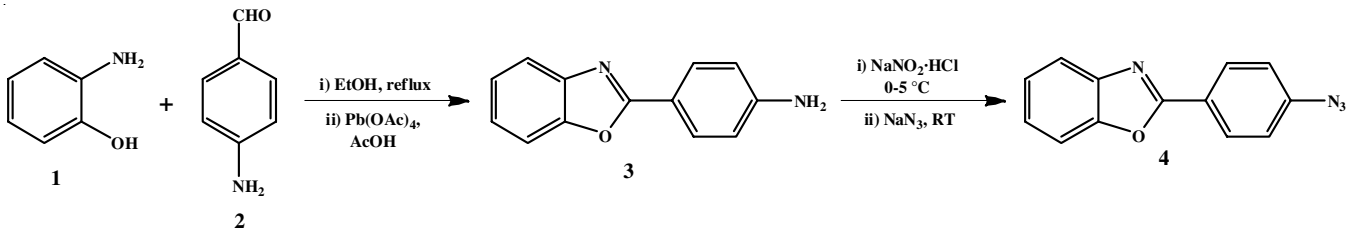
where, A represents the diameter of fungi growth on untreated PDA, B represents the diameter of fungi on treated PDA and CV represents the rate of inhibition.

RESULTS AND DISCUSSION

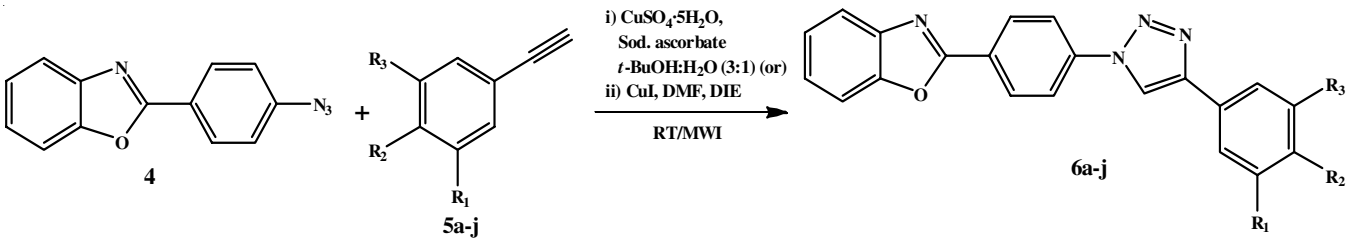
The synthetic method, in **Scheme-I** planned for all final compounds under of this study comprised of the initial preparation of the required 4-(1,3-benzoxazol-2-yl)aniline (**3**) from 2-aminophenol (**1**) and 4-aminobenzaldehyde (**2**) were refluxed in ethanol for 3 h [20]. After the reaction mixture was cooled, ethanol was removed in vacuum. The resulting Schiff base was dissolved in acetic acid and lead tetraacetate was added and stirred at room temperature for 1 h to afford 4-(1,3-benzoxazol-2-yl)aniline (**3**). Later the obtained compound **3** was dissolved in 10% aq. HCl at room temperature, then reaction mixture upon cooling to 0 ºC and addition of a solution of $NaNO_2$, allowed to stir for 10 min at 0-5 ºC. Sodium azide was added dropwise drop and the mixture was stirred at room temperature for 2 h to yield 2-(4-azidophenyl)-1,3-benzoxazole (**4**).

In **Scheme-2**, a mixture of 2-(4-azidophenyl)-1,3-benzoxazole (**4**) and substituted 1-ethynylbenzenes (**5a-j**) was dissolved in *t*-BuOH/H_2O (1:1) To this mixture, sodium ascorbate, $CuSO_4$ were added and stirred for 4-6 h at room temperature to get 2-[4-(4-aryl-1*H*-1,2,3-triazol-1-yl)phenyl]-1,3-benzoxazole derivatives (**6a-j**). Other methods used with CuI, diisopropylethyl amine was dissolved in DMF and stirred at room temperature for 2-4 h to get 2-[4-(4-aryl-1*H*-1,2,3-triazol-1-yl)phenyl]-1,3-benzoxazole derivatives (**6a-j**). The synthesis of final compound **6a-j** under both conventional and microwave irradiation methods.

It was determined that microwave irradiation induced faster conversion of the compounds leading to higher yield (reaction time 5-7 min, yield 79-83% in $CuSO_4 \cdot 5H_2O$ and



Scheme-I: Synthesis of 2-(4-azidophenyl)-1,3-benzoxazole (**4**)



Compound	a	b	c	d	e	f	g	h	i	j
R_1	H	OCH_3	H	H	CF_3	F	H	OCH_3	H	H
R_2	H	OCH_3	CF_3	OCH_3	H	H	CH_3	H	NO_2	Cl
R_3	H	OCH_3	H	H	CF_3	F	H	OCH_3	H	H

Scheme-II: Synthesis of 2-[4-(4-aryl-1*H*-1,2,3-triazol-1-yl)phenyl]-1,3-benzoxazole derivatives (**6a-j**)

reaction time 3-4 min, yield 89-93% in CuI as catalyst) than conventional heating (reaction time 4-6 h, yield 61-68% in $CuSO_4·5H_2O$ and reaction time 3-4 min, yield 73-79% in CuI as catalyst) (Table-1).

Structures of the synthesized compounds **6a-j** were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. As a representative case, spectral data of 2-(4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl)benzo[*d*]oxazole (**6a**) has been discussed. In the IR spectrum of compound **6a**, peaks at 1557 cm^{-1} which indicated the presence of N=N group in aromatic 1,2,3-triazole ring and characteristic bands at 1429 cm^{-1} confirmed the presence of C-N groups, respectively. The characteristic bands at 3036 and 1429 cm^{-1} indicated the presence of C–H and C=C groups, respectively. The ^{1}H NMR spectrum of the representative compound (dissolved in $CDCl_3$) showed a sharp singlet at 7.82 δ ppm indicates 1,2,3-triazole ring. The compound showed two doublets at 7.97 δ ppm (J = 8.23 Hz) and 7.57 δ ppm (J = 8.23 Hz) indicating that the A_2B_2 pattern of meddle phenyl ring. The remaining aromatic peaks appeared in the range 6.82-7.97 δ ppm with corresponding multiplicity. In case of ^{13}C NMR spectrum, in triazole ring, the carbon attached to phenyl ring appeared at 148.9 δ ppm and the carbon bearing hydrogen appeared at 141.4 δ ppm. The peak in benzo-[*d*]oxazole ring, the C=N carbon appeared as singlet at 159.9 δ ppm, C-O linked carbon appeared at 158.2 δ ppm and C-N attached carbon peak at 148.9 δ ppm. The entire peaks in ^{13}C NMR spectrum support the formation of product. Mass spectrum exhibited the base peak as $[M+H]^+$ peak at *m/z* 339. Thus, on the basis of above studies has been assigned structure 2-(4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl)benzo[*d*]oxazole (**6a**)

Antibacterial activity: All the synthesized compounds **6a-j** were screened *in vitro* for their antibacterial activity against Gram-positive bacterial strains [*Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633)] and Gram-negative bacterial strains [*Escherichia coli* (ATCC25922), *Klebsiella pneumonia* (ATCC 13883)] using ciprofloxacin as the standard drug. The activity was determined using filter paper disc method by measuring the zone of inhibition in mm. The compounds were screened at the concentration of 20 µg/mL and 40 µg/mL in DMSO. From the screening studies (Table-2), most of the compounds showed relatively excellent activity against Gram-positive bacterial strains and Gram-negative bacterial strains. Among all, compounds **6b**, **6d, 6e** and **6h** were showed good to excellent activity against bacterial strains and remaining compounds **6a**, **6c**, **6f**, **6g**, **6i** and **6j** were showed better activity than standard drug.

Antifungal activity: The antifungal activity of synthesized compounds **6a-j** was tested against three pathogenic fungi, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* using amphotericin-B as the standard drug. The activity was

TABLE-1
TIME AND YIELD OF SYNTHESIZED COMPOUNDS (**6a-j**)

Compound	Conventional				MWI			
	Time (h)		Yield (%)		Time (min)		Yield (%)	
	$CuSO_4·5H_2O$	CuI	$CuSO_4·5H_2O$	CuI	$CuSO_4·5H_2O$	CuI	$CuSO_4·5H_2O$	CuI
6a	4.0	2.0	62	73	5	3	81	90
6b	4.0	3.0	63	75	6	4	80	93
6c	5.5	2.5	65	76	6	4	79	89
6d	6.0	3.0	67	74	7	5	82	91
6e	5.0	4.0	68	77	5	4	83	90
6f	6.0	3.5	64	78	7	3	79	92
6g	5.0	3.0	66	76	5	3	80	93
6h	5.0	3.0	63	74	5	4	83	89
6i	4.5	4.0	61	77	6	4	81	93
6j	6.0	3.5	65	76	5	3	79	89

TABLE-2
ANTIBACTERIAL ACTIVITY OF COMPOUNDS (**6a-j**)

Compound	Zone of inhibition (mm)							
	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		*B. subtilis*		*K. pneumoniae*		*E. coli*	
	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL
6a	13	20	14	15	06	14	14	18
6b	16	29	17	31	24	36	19	36
6c	14	20	13	15	08	14	15	15
6d	17	30	19	33	25	38	20	37
6e	19	31	18	32	26	37	22	38
6f	12	17	15	16	04	05	16	18
6g	11	19	13	12	05	07	12	19
6h	18	24	20	34	29	39	23	39
6i	10	16	14	12	04	16	14	20
6j	12	20	12	16	05	21	16	22
Ciprofloxacin	15	28	16	30	23	35	18	35

determined using the cup-plate agar diffusion method by measuring the zone of inhibition in mm. The compounds were screened at a concentration of 50 µg/mL in DMSO. Among all the synthesized compounds, compounds **6c** (R_2= CF_3), **6e** (R_1 & R_3= CF_3), **6f** (R_1 & R_3 = F) and **6i** (R_2 = NO_2) with substtution on ring showed maximum activity against *A. niger*, *A. flavus* and *F. oxysporum*. Investigations of antifungal activity revealed that presence of electron withdrawing groups (-CF_3, -F & -NO_2) showed higher activity (Table-3).

TABLE-3
ANTIFUNGAL ACTIVITY OF COMPOUNDS (**6a-j**)

Compounds	Zone of inhibition (mm)		
	A. niger	*A. flavus*	*F. oxysporum*
	50 µg/mL	50 µg/mL	50 µg/mL
6a	6.6 ± 0.2	10.6 ± 0.4	5.5 ± 0.4
6b	9.6 ± 0.8	9.0 ± 0.2	8.2 ± 0.4
6c	15.5 ± 0.4	15.9 ± 0.4	17.5 ± 0.3
6d	8.6 ± 0.6	9.4 ± 0.6	14.5 ± 0.7
6e	16.5 ± 0.3	14.7 ± 0.8	17.9 ± 0.1
6f	16.0 ± 0.4	14.9 ± 0.4	17.4 ± 0.6
6g	10.2 ± 0.2	7.4 ± 0.6	10.0 ± 0.4
6h	9.7 ± 0.6	7.7 ± 0.4	9.7 ± 0.2
6i	17.5 ± 0.4	13.9 ± 0.4	18.0 ± 0.4
6j	10.2 ± 0.6	7.4 ± 0.6	10.2 ± 0.6
Amphotericin-B	15 ± 0.4	13.5 ± 0.2	17.2 ± 0.5

Conclusion

A convenient, simple, high yielding route for the synthesis of new substituted 2-[4-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)-phenyl]-1,3-benzoxazoles (**6a-j**) under conventional and microwave irradiation methods is reported. The microwave irradiation method was showed to be high yielding with higher rate of acceleration and ecofriendly. The compounds were evaluated for their *in vitro* antimicrobial activity. The compounds **6a**, **6d**, **6e** and **6h** showed excellent antibacterial activity and compounds **6c**, **6e**, **6f** and **6i** showed good to excellent antifungal activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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