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Synthesis and *in vitro* Antimicrobial Screening of Benzofuran-3(2*H*)-one Linked *Geminal bis* 1,2,3-Triazole Hybrid Derivatives

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A series of novel hybrid derivatives, 2,2-di(1*H*-1,2,3-triazol-1-yl)benzofuran-3(2*H*)-ones, were synthesized from *o*-hydroxyacetophenones *via* a two-step process involving sodium azide, iodine and sodium bicarbonate, followed by Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) in aqueous conditions. These compounds were characterized using ^{1}H NMR, ^{13}C NMR and mass spectra and analyzed *in vitro* against bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*) and fungi (*Candida albicans*, *Aspergillus niger*). Compounds **3f**, **3h** and **3n** showed notable antibacterial activity (MIC = 50 µg/mL), comparable to streptomycin, while compounds **3b, 3f** and **3h** exhibited similar antifungal activity (MIC = 50 µg/mL), comparable to standard clotrimazole. Compound **3f** displayed superior antimicrobial efficacy among the tested compounds.

Keywords: Benzofuranone, 3-Coumaranone, *bis*-Triazole, Sodium azide, *gem*-Diazides.

INTRODUCTION

Benzofuranones and their derivatives are widely acknowledged as 'privileged' structures due to their prevalence in a diverse range of naturally occurring and biologically active compounds. Their versatile biological properties across multiple therapeutic domains have garnered significant interest from both synthetic and medicinal chemists. Several compounds with 2,2-disubstituted benzofuranone core have been identified in nature [1-5] and are being developed by researchers [6-8]. It has also been proved that these substances can be used for a wide range of medicinal purposes [9-11]. Geodin [12], rifamycin [13,14], griseofulvin [15], armeniaspiroles [16] and maesopsin [17] are a few examples of antibacterial compounds having 2,2-disubstituted benzofuranone core (Fig. 1).

On the other hand, 1,2,3-triazole and its analogues are attractive scaffolds in pharmaceutical chemistry [18-21] owing to their substantial biological efficacy, which includes antimicrobial [22,23], antituberculor [24,25], antimalarial [26], anticancer [27] and anti-HIV [28] properties. However, *geminal-*, *bis-* and *tris*-triazoles are a relatively rare and neglected group of triazole compounds that have received little attention from researchers [29-35]. Despite this, their biological functions have not been extensively studied and remain poorly understood.

In contemporary medicinal chemistry, chemists use the strategy of pharmacophore hybridization to create new chemical entities that are pertinent to biological systems [36,37]. Molecular hybridization is the method of amalgamating two or more established bioactive pharmacophoric fragments into a unified hybrid molecule that exhibits enhanced affinity and efficacy compared to the original medications. Hybrid molecules, in contrast to their parent drugs, possess the capacity to overcome drug resistance, expand their biological spectrum, reduce toxicity and enhance overall effectiveness [38,39].

Recently, several molecules possessing 1,2,3-triazole and benzofuranone fragments with promising biological activity have been reported. In particular, Lipeeva *et al.* [40,41], Liang *et al.* [42], Rama Kant *et al.* [43] synthesized 1,2,3-triazole tethered benzofuranone hybrids and evaluated their antibacterial and other biological activities. Inspired by the aforementioned findings and driven by our continuous investigation into the development of new heterocycles as potential bioactive compounds [44-48], we designed and synthesized a series of novel

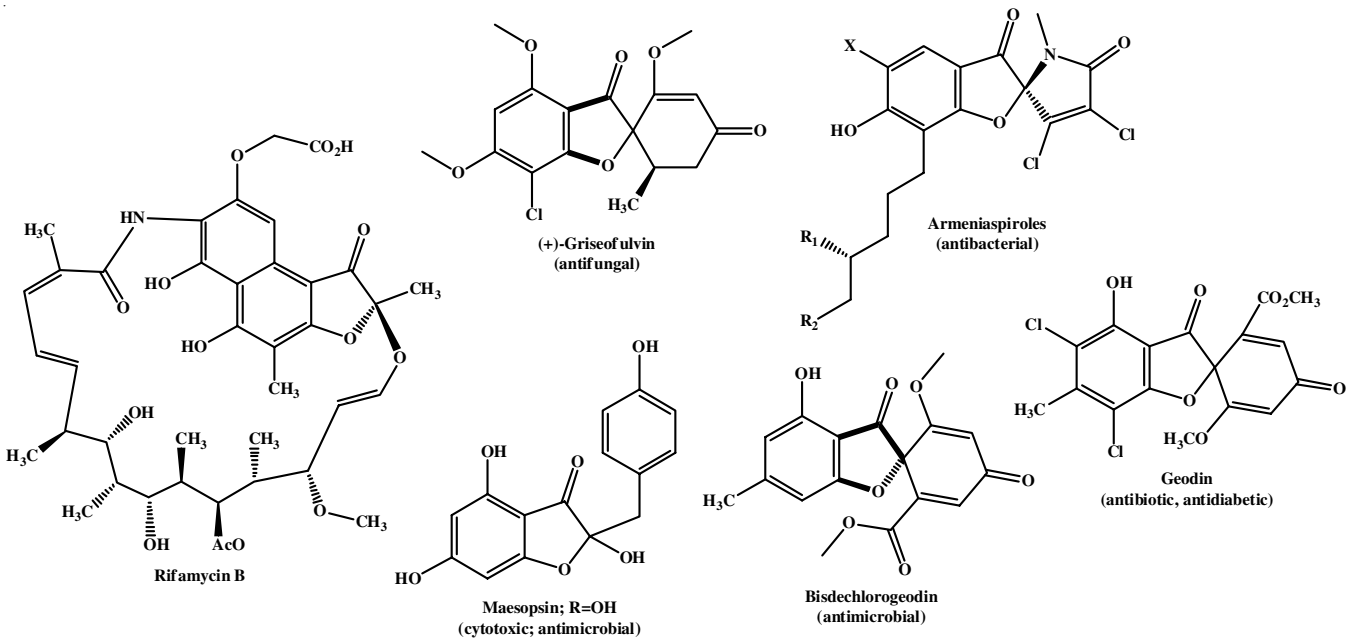


Fig. 1. Compounds with a 2,2-di-substituted benzofuranone core exhibiting antibacterial properties

compounds that incorporate benzofuranone, coupled with *bis* 1,2,3-triazole structural motifs, with the goal of creating potent antimicrobial agents.

EXPERIMENTAL

All chemicals were procured from the reputed commercial suppliers and employed them without additional purification. Thin-layer chromatography (TLC) was conducted using silica F_{254}-coated aluminum plates and visualization was achieved using UV light and iodine. The NMR spectrum was acquired on a Bruker NMR spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, with chemical shift (δ) values reported in parts per million (ppm). $CDCl_3$ served as the solvent and TMS was utilized as the internal standard.

Synthesis of 2,2-diazido-7-iodo-5-methylbenzofuran-3(2*H*)-one (2): Compound **2** was synthesized by following a previously reported procedure [49]. In a 100 mL round bottom flask, 5-methyl-2-hydroxy acetophenone (**1**) (1 equiv.), iodine (6 equiv.), sodium azide (7 equiv.), sodium bicarbonate (10 equiv.) and 5 mL of water were refluxed for 2 h. The reaction was then quenched with sodium thiosulfate solution. Subsequently, the compound was extracted with ethyl acetate and concentrated. Finally, the obtained crude product was purified through column chromatography using a hexane and ethyl acetate mixture.

2,2-Diazido-7-iodo-5-methylbenzofuran-3(2*H*)-one (2): White solid; yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.93 (t, 1H, *J* = 0.3 Hz), 7.47 (t, 1H, *J* = 0.6 Hz), 2.36 (s, 3h); 13C NMR (75 MHz, $CDCl_3$) δ ppm: 189.22, 167.25, 149.44, 136.13, 125.40, 117.94, 99.47, 77.48, 20.35; FT-IR (KBr, ν_{max}, cm^{-1}): 3371, 3220, 3021, 2925, 2402, 1765, 1620, 1490, 1461, 1307, 1261, 1215, 1172, 1105, 1040, 995, 930, 760, 669, 594.

General procedure for the synthesis of benzofuran-3(2*H*)-one linked *geminal-bis*-1,2,3-triazole hybrid derivatives (3a-n): A mixture containing 1-(2-hydroxy-5-methylphenyl)-ethan-1-one (1 equiv.), iodine (6 equiv.), sodium azide (7 equiv.), sodium bicarbonate (10 equiv.) and 5 mL of water was subjected to reflux for a duration of 2 h. Subsequently, the reaction mixture was allowed to cool to room temperature and the pH of the solution was adjusted to 7 by adding dilute HCl. Following this, two equivalents of 4-methyl phenyl acetylene were introduced, along with sodium ascorbate (15 mol%) and $CuSO_4$ (5 mol%) and the resulting mixture was stirred for 12-24 h at room temperature. The reaction was quenched using sodium thiosulphate ($Na_2S_2O_3$) solution. Ice-cold water was added in a small amount and the mixture was extracted with ethyl acetate. After evaporating ethyl acetate, the crude product was subjected to purification using column chromatography with a hexane and ethyl acetate mixture.

7-Iodo-5-methyl-2,2-*bis*(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)benzofuran-3(2*H*)-one (3a): Light yellow solid; yield: 61%; m.p.: 192-193 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.99 (s, 2H, triazole-H), 7.75 (d, 1H, Ar-H, *J* = 7.2 Hz), 7.41-7.43 (d, 4H, Ar-H), 7.29 (d, 1H, Ar-H, *J* = 6.8 Hz), 6.98-7.00 (d, 4H, Ar-H), 2.26 (s, 6H, 2-CH_3), 2.22 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 192.71, 168.99, 149.53, 146.10 (2C), 134.52 (4C), 129.53 (4C), 124.72 (2C), 124.38 (2C), 123.95 (2C), 117.52, 115.25, 112.69 (2C), 99.02, 21.60, 20.29 (2C); MS (ESI): *m/z* 588 $[M+H]^+$.

7-Iodo-2,2-*bis*(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-5-methylbenzofuran-3(2*H*)-one (3b): Light yellow solid; yield: 63%; m.p.: 202-203 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.33 (d, 1H, Ar-H), 8.23 (d, 1H, Ar-H), 7.89 (s, 2H, triazole-H), 7.53 (d, 4H, Ar-H), 7.45 (d, 4H, Ar-H), 3.72 (s, 6H, 2-OCH_3), 2.26 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 192.71, 168.99, 149.53, 146.10 (2C), 134.52 (4C), 129.53 (4C), 124.72 (2C), 124.38 (2C), 123.95 (2C), 117.52, 115.25, 112.69 (2C), 99.02, 21.60, 20.29 (2C); MS (ESI): *m/z* 588 $[M+H]^+$.

2,2-*Bis*(4-(4-bromophenyl)-1*H*-1,2,3-triazol-1-yl)-7-iodo-5-methylbenzofuran-3(2*H*)-one (3c): light yellow solid;

yield: 61%; m.p.: 198-199 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.92 s (2H, triazole-H), 7.34-7.4 m (9H, Ar-H), 7.33 (s, 1H, Ar-H), 2.26 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 193.69, 167.69, 140.98 (2C), 140.0 (2C),137.93 (4C), 132.82, 132.03 (4C), 126.93 (2C), 124.16, 122, 121.43, 116.33, 112.29 (2C), 98.92, 20.62.

5,7-Dimethyl-2,2-*bis*(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)benzofuran-3(2*H*)-one (3d): Light yellow solid; yield: 65%; m.p.: 208-209 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.81 (s, 2H, triazole-H), 7.40 (d, 4H, Ar-H), 7.80 (d, 1H, Ar-H), 6.99 (m, 5H, Ar-H), 2.25 (s, 6H, 2-CH_3), 2.18 (s, 3H, CH_3), 2.17 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 194.97, 165.57, 140.06 (2C), 136.43 (6C), 131.78, 129.4 (6C), 124.89 (2C), 122.17, 121.29 (2C), 119.17, 99.36, 21.27 (2C), 20.54, 14.03.

2,2-*Bis*(4-(4-chlorophenyl)-1*H*-1,2,3-triazol-1-yl)-5,7-dimethylbenzofuran-3(2*H*)-one (3e): Light yellow solid; yield: 60%; m.p.: 210-211 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.14 (s, 2H, triazole-H), 7.44 (d, 4H, Ar-H), 7.23 (d, 4H, Ar-H), 7.12 (d, 1H, Ar-H), 7.01 (d, 1H, Ar-H), 2.21 (s, 3H, CH_3), 2.17 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 194.26, 166.33, 140.67 (2C), 137.68 (6C), 136.54 (2C), 132.55, 128.97 (4C), 126.68 (2C), 122.17, 121.50 (2C), 118.85, 98.73, 20.57, 13.98.

7-Iodo-5-methoxy-2,2-*bis*(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)benzofuran-3(2*H*)-one (3f): Light yellow solid; yield: 62%; m.p.: 199-201 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.06 (d, 1H, Ar-H), 7.92 (s, 2H, triazole-H), 7.73 (d, 1H, Ar-H), 7.43 (d, 4H, Ar-H), 7.00 (d, 4H, Ar-H), 3.81 (s, 3H, CH_3), 2.26 (s, 6H, 2-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 191.96, 171.95, 168.52, 140.09 (2C), 136.49 (4C), 129.59 (4C), 125.9 (2C), 124.73 (2C), 123.49, 112.98, 111.72 (2C), 100.89, 95.63 (2C), 55.92, 21.92 (2C).

5-Chloro-7-iodo-2,2-*bis*(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)benzofuran-3(2*H*)-one (3g): Light Yellow solid; yield: 65%; m.p.: 203-204 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.09 (s, 2H, triazole-H), 7.28 (d, 4H, Ar-H), 7.27 (d, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.00 (d, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 193.09, 169.58, 144.46, 140.48 (2C), 136.62 (6C), 129.69 (4C), 125.41 (2C), 124.17 (2C), 123.30 (2C), 118.56, 113.08, 100.79, 21.30 (2C).

5-Bromo-7-iodo-2,2-*bis*(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)benzofuran-3(2*H*)-one (3h): Light yellow solid; yield: 62%; m.p.: 212-213 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.89 (s, 2H, triazole-H), 7.43 (d, 4H, Ar-H), 7.29 (d, 1H, Ar-H), 7.00 (d, 4H, Ar-H), 6.74 (d, 1H, Ar-H), 2.26 (s, 6H, 2-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 193.29, 169.45, 140.49 (2C), 136.62 (6C), 133.09, 129.67 (4C), 126.10 (2C), 125.46 (2C), 124.17, 118.95, 116.13 (2C), 100.6, 21.31 (2C).

7-Iodo-5-nitro-2,2-*bis*(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)-benzofuran-3(2*H*)-one (3i): Yellow solid; yield: 60%; m.p.: 206-207 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.26 d (1H, Ar-H), 8.22 d (1H, Ar-H), 8.09 s (2H, triazole-H), 7.44 d (4H, Ar-H), 2.25 s (6H, 2-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 192.76, 172.15, 142.70, 140.96 (2C), 136.74 (2C), 132.98 (2C), 129.86 (6C), 123.45 (2C), 121.18 (2C), 120.26, 113.23, 102.49, 21.32 (2C).

5-Acetyl-7-iodo-2,2-*bis*(4-(*p*-tolyl)-1*H*-1,2,3-triazol-1-yl)benzofuran-3(2*H*)-one (3j): Light yellow solid; yield: 64%; m.p.: 201-202 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.33 (d, 1H, Ar-H), 8.24 (d, 1H, Ar-H), 7.89 (s, 2H, triazole-H), 7.39 (d, 4H, Ar-H), 7.02 (d, 4H, Ar-H), 2.89 (s, 3H, $COCH_3$), 2.25 (6H, 2-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 194.69, 168.80, 166.16, 140.37 (2C), 136.49 (6C), 133.23, 131.57, 129.66 (4C), 124.43 (2C), 119.65, 114.91 (2C), 113.0 (2C), 100.12, 24.36, 21.31 (2C).

5-Acetyl-2,2-*bis*(4-(4-chlorophenyl)-1*H*-1,2,3-triazol-1-yl)-7-iodobenzofuran-3(2*H*)-one (3k): Light yellow solid; yield: 63%; m.p.: 221-222 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.09 (d, 1H, Ar-H), 8.05 (d, 4H, Ar-H), 7.86 (s, 2H, triazole-H), 7.63 (d, 1H, Ar-H), 7.51 (d, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 191.40, 182.52, 158.25, 153.39, 139.89 (2C), 135.07 (2C), 131.01 (6C), 129.10 (4C), 128.98 (2C), 126.83 (2C), 124.99, 124.16, 116.79, 15.22.

5-Acetyl-7-iodo-2,2-*bis*(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzofuran-3(2*H*)-one (3l): Light yellow solid; yield: 67%; m.p.: 215-216 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.05-8.10 (m, 4H, Ar-H), 7.86 (s, 2H, triazole-H), 7.54-7.69 (m, 8H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 191.47, 183.98, 158.32, 153.63, 136.97, 133.40 (2C), 133.34 (2C), 129.59 (6C), 128.91 (4C), 128.77, 126.98, 124.96, 124.22, 116.84, 100.03, 15.25.

5-Bromo-2,2-*bis*(4-(4-bromophenyl)-1*H*-1,2,3-triazol-1-yl)-7-iodobenzofuran-3(2*H*)-one (3m): Light yellow solid; yield: 61%; m.p.: 212-213 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.09 (s, 2H, triazole-H), 7.75 (d, 1H, Ar-H), 7.43 (d, 4H, Ar-H), 7.30 (d, 1H, Ar-H), 7.00 (d, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 192.64, 169.46, 164.91, 163.24, 145.01, 138.95 (4C), 125.49 (2C), 123.81 (2C), 122.80, 118.23, 116.26 (4C), 116.12 (2C), 113.00 (2C), 100.34.

5-Bromo-2,2-*bis*(4-(4-chlorophenyl)-1*H*-1,2,3-triazol-1-yl)-7-iodobenzofuran-3(2*H*)-one (3n): Light yellow solid; yield: 63%; m.p.: 214-215 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 8.10-8.13 (m, 4H, Ar-H), 7.80 (d, 1H, Ar-H), 7.41-7.45 (m, 5H, Ar-H), 7.75 (s, 2H, triazole-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ, ppm: 192.64, 169.46, 164.91, 163.24, 145.01, 138.95 (4C), 125.49 (2C), 123.81 (2C), 122.80, 122.78, 118.23 (4C), 116.12 (2C), 113.00 (2C), 100.34.

Antimicrobial activity: All the newly synthesized compounds **3a-n** were screened for their *in vitro* antimicrobial activity against a panel of microorganisms, including four bacterial strains *viz*. *Staphylococcus aureus* (MTCC 121), *Bacillus subtilis* (MTCC 96), *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453), as well as two fungal strains, *Candida albicans* and *Aspergillus niger*. Positive control drugs, namely streptomycin and clotrimazole, were used as references, respectively. The agar well diffusion method [49,50] was employed for *in vitro* screening in triplicates for accuracy. To prepare wells for sample loading, sterile cork borers with a diameter of 6 mm were used. The test compounds were prepared at a concentration of 100 µg/mL, while the positive control consisted of streptomycin and clotrimazole at 30 µg/mL, with DMSO serving as the negative control. Incubation was carried out at 37 ºC for 24 h for bacteria and at 28 ºC for 48 h for fungi.

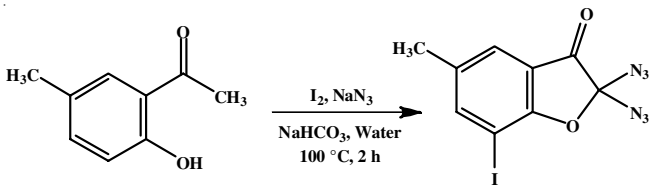
Following the appropriate incubation periods, the diameter of the zone of inhibition (ZOI) around each well was measured in millimeters.

The minimum inhibitory concentration (MIC) for both the tested compounds and standard substances was determined in µg/mL using the Broth dilution test [51]. To conduct this test, the bacterial strains *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* as well as the fungi *C. albicans* and *A. niger* were diluted 100-fold in nutrient broth (with 100 µL of bacterial cultures in 10 mL of nutrient broth). Various concentrations of the test samples (1.25, 2.5, 5, 10, 20 and 40 µL of the stock solution, equivalent to 6.25, 12.5, 25, 50, 100 and 200 µg/well of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All tubes were then incubated for 24 h at 37 ºC for bacteria and 48 h at 28 ºC for fungi. The tubes were examined for visible turbidity, with nutrient broth used as a control. Additionally, simultaneous testing was performed using controls with and without test samples. Compounds exhi-biting notable antibacterial and antifungal activities underwent further assessment for minimum bactericidal concentration (MBC) [52] and minimum fungicidal concentration (MFC) [53].

RESULTS AND DISCUSSION

A synthetic approach was employed to synthesize novel 1,2,3-triazole derivatives linked with benzofuranone as illustrated in **Schemes I** and **II**. The initial step involved the synthesis of 2,2-diazido-7-iodo-5-methylbenzofuran-3(2*H*)-one by refluxing 1-(2-hydroxy-5-methylphenyl)ethan-1-one with iodine, sodium azide and $Na(HCO_3)_2$ in water, following the method [54]. Subsequently, compound 2,2-diazido-7-iodo-5-methylbenzofuran-3(2*H*)-one was subjected to a reaction with two equivalents of 4-methyl-phenyl acetylene in the presence of sodium ascorbate (15 mol%) and $CuSO_4$ (5 mol%) in an aqueous medium, resulting in the production of compound **3a** with a high yield of 87%.

Despite their inherent dangers and explosive characteristics, the production and isolation of organic *gem*-diazides have not been widely promoted [32]. Consequently, it is strongly recommended to generate organic azides *in situ*. Various researchers [55-57] developed methods for producing 1,4-disubstituted 1,2,3-triazoles in a single reaction vessel without isolating the organic azide intermediate. In reference to these approaches, an attempt was made to synthesize benzofuranone-triazole hybrid **3a** in a one-pot process without the need for intermediate separation as depicted in **Scheme-II**. As anticipated, compound **3a** was successfully obtained in a yield of 52% within 14 h. The structural confirmation of **3a** was achieved through Mass spectrometry, which revealed a molecular ion peak at *m/z* 588. Additionally, ^1H and ^{13}C NMR spectral studies further validated its structure, with a distinctive signal at 7.99 ppm (representing triazole-H) and 146.10 ppm. Encouraged by these results, we proceeded to synthesize derivatives **3b-n** using the same procedure.

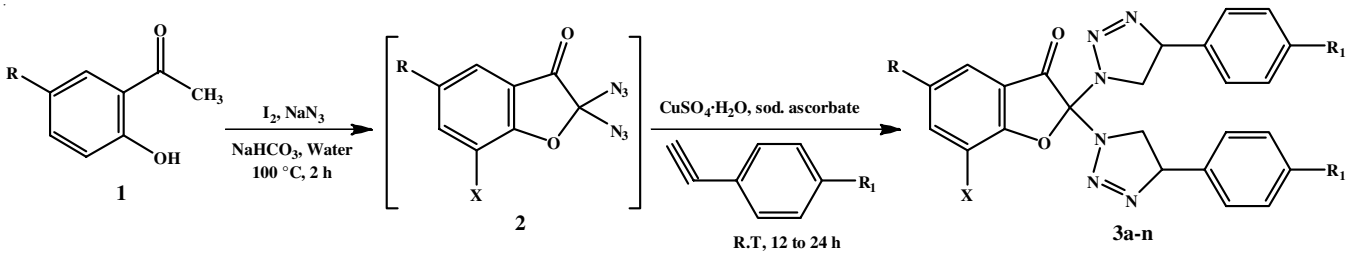


1-(2-Hydroxy-5-methylphenyl) ethan-1-one

2,2-Diazido-7-iodo-5-methylbenzofuran-3(2*H*)-one

Scheme-I

Antimicrobial activity: All the synthesized derivatives (**3a-n**) were evaluated for their *in vitro* antimicrobial activity against a panel of four bacterial strains, namely *Staphylococcus aureus* (MTCC 121), *Bacillus subtilis* (MTCC 96), *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453), along with two fungal strains, *Candida albicans* and *Aspergillus niger*. Positive control drugs streptomycin and clotrimazole were employed as references. The assessment was conducted utilizing the Agar well diffusion method and the results are depicted in Table-1. It was observed that compounds **3b**, **3f**, **3g**, **3h** and **3n**, exhibited comparable zones of inhibition against *S. aureus*, measuring 21, 21, 20, 20 and 20 mm, respectively, which were on par with the standard drug streptomycin (zone of inhibition: 22 mm). Similarly, compounds **3b**, **3m**, **3c**, **3e** and **3j** demonstrated comparable efficacy against *Bacillus subtilis*, exhibited zones of inhibition at 19, 18 and 17 mm, respectively, which were comparable to streptomycin's zone



	R	R^1	X
3a	$-CH_3$	$-CH_3$	I
3b	$-CH_3$	$-OCH_3$	I
3c	$-CH_3$	-Br	I
3d	$-CH_3$	$-CH_3$	$-CH_3$
3e	$-CH_3$	-Cl	$-CH_3$
3f	$-OCH_3$	$-CH_3$	I
3g	-Cl	$-CH_3$	I

	R	R^1	X
3h	-Br	$-CH_3$	I
3i	$-NO_2$	$-CH_3$	I
3j	$-COCH_3$	$-CH_3$	I
3k	$-COCH_3$	-Cl	I
3l	$-COCH_3$	-H	I
3m	-Br	-Br	I
3n	-Br	-Cl	I

Scheme-II: Synthetic strategy of compounds **3a-n**

TABLE-1
In vitro ANTIMICROBIAL ACTIVITY OF COMPOUNDS **3a-n**

Compound	Antibacterial activity								Antifungal activity	
	Gram-positive				Gram-negative				*C. albicans*	*A. niger*
	S. aurus		*B. subtilis*		*E. coli*		*P. aeruginosa*			
	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
3a	12	200	11	200	12	100	10	200	9	10
3b	21	50	19	50	18	100	19	50	20	18
3c	19	100	17	50	14	100	18	100	17	14
3d	13	200	14	200	14	100	16	100	11	19
3e	19	100	17	50	12	200	14	100	18	14
3f	21	50	19	100	18	50	19	50	19	16
3g	20	50	18	100	17	100	18	50	17	14
3h	20	50	19	100	18	50	16	100	18	17
3i	18	200	15	200	15	100	15	100	11	8
3j	19	100	17	50	15	100	11	200	15	11
3k	18	200	15	200	16	100	17	100	10	8
3l	14	200	12	100	13	100	9	200	9	8
3m	19	100	18	50	14	200	11	100	16	14
3n	20	50	18	100	17	100	18	50	18	16
Streptomycin	22	25	21	12.5	20	12.5	20	12.5	–	–
Clotrimazole	–	–	–	–	–	–	–	–	24	20

ZOI = Zone of inhibition in millimetre for analogs (**3a-n**) at 100 µg/mL; positive control drugs streptomycin and clotrimazole at 30 µg/mL; MIC = Minimum inhibitory concentration values in µg/mL.

TABLE-2
MBC AND MFC VALUES (µg/mL) OF COMPOUNDS **3b**, **3f**, **3h** AND **3n**

Compound	Antibacterial activity				Antifungal activity	
	Gram-positive		Gram-negative		*C. albicans*	*A. niger*
	S. aurus	*B. subtilis*	*E. coli*	*P. aeruginosa*		
	MBC	MBC	MBC	MBC	MFC	MFC
3b	12.5	6.25	12.5	6.25	12.5	12.5
3f	6.25	12.5	12.5	6.25	6.25	6.25
3h	12.5	25.0	25.0	25.0	12.5	12.5
3n	12.5	12.5	12.5	12.5	6.25	12.5

MBC = Minimum bactericidal concentration; MFC = Minimum fungicidal concentration

of inhibition at 21 mm. Additionally, compounds **3f** and **3h** exhibited 18 mm zone of inhibition against *E. coli*, closely resembling streptomycin's zone of inhibition at 20 mm.

To delve into the structure-activity relationship (SAR), we explored the impact of various substituents attached to the benzofuranone ring and the benzene ring linked to the triazole on *in vitro* antibacterial activities. Present findings revealed that the compounds **3b**, **3f**, **3h** and **3n**, which contained groups such as -OCH_3 and Br on the benzofuranone ring, exhibited greater activity compared to the other compounds. Further investigations involved assaying the compounds with higher antibacterial and antifungal activities for minimum bactericidal concentration (MBC) [52] and minimum fungicidal concentration (MFC) [53], with the corresponding values are listed in Table-2.

Conclusion

In summary, novel *geminal bis*-triazoles linked to benzofuran-3(2*H*)-one compounds were synthesized successfully in one pot reaction. This synthesis was achieved using Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) starting from various *o*-hydroxyacetophenones and terminal alkynes in an aqueous environment, without the need to isolate the *gem*-diazides. Notably, four of the synthesized compounds demonstrated *in vitro* antimicrobial activity comparable to that of standard drugs. These results suggest that further modifications to these molecules could yield promising lead compounds with enhanced antimicrobial properties.

CONFLICT OF INTEREST

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

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