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Design, Synthesis, Anticancer, Antimicrobial and Molecular Docking Studies of Substituted 2-[2,4-*Bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)phenyl]-1*H*-benzoimidazole Derivatives

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In this work, a novel series of substituted 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole derivatives (**9a-o**) has been designed and synthesized. These new compounds were subjected to analyze the cytotoxicity assays against MCF-7 (breast cancer), HeLa (cervical cancer) and PC-3 (prostrate cancer) cancer cell lines. Among them, compound **9m** was most prominent against all cancer cell lines and compounds **9b**, **9g**, **9j**, **9k**, **9n** and **9o** displayed effective inhibition of the cancer cell lines. Meanwhile, the synthesized 1,2,3-triazole derivatives (**9a-o**) were evaluated for *in vitro* antibacterial and antifungal activity against Gram-positive bacteria and Gram-negative bacteria as well as fungi, among them compound **9m** shows efficient activity. Furthermore, molecular docking investigations were conducted on the newly developed derivative compounds (**9a-o**), in conjunction with the standard drug doxorubicin, targeting the EGFR kinase protein (PDB ID: 3W33). Compounds **9a** and **9m** demonstrated exceptional binding energies of -12.1 kcal/mol and -12.2 kcal/mol, respectively, unveiling their superior binding affinity compared to doxorubicin.

Keywords: Substituted 1,2,3-triazoles, Click chemistry, Human cancer cell lines, Antimicrobial activity, Docking studies.

INTRODUCTION

Amongst different heterocycles, the five membered heterocyclic compounds- 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles exhibit different biological activities which makes them prominent motifs for the development of new drugs [1-3]. Among them, triazoles, with their unique configuration of three nitrogen atoms in a five-membered heterocyclic arrangement (Fig. 1), are renowned for their various biological functions [4,5]. They possess a broad spectrum of pharmacological effects, encompassing antibacterial [6], antifungal [7], antituberculosis [8], anticancer [9], antioxidant [10] and anti-inflammatory properties [11]. On the other hand, benzimidazole is a privileged structural motif in the development of wide range of pharmaceuticals (Fig. 2), with diverse biological activities which include antimicrobial [12], antiviral [13], anti-inflammatory [14], antihypertensive [15] and anticancer [16]. Cancer remains a leading cause of mortality in developed nations, underscoring the ongoing significance of discovering novel therapies in biomedical research [17-21]. In addition to these, conazoles such as itraconazole, fluconazole, voriconazole and ravuconazole which contains 1,2,4-triazole moiety constitute a major class of drugs being used for the treatment of fungal infections [3].

In developing and underdeveloped regions, prevalent cancers include lung, colon, breast and melanoma. Chemotherapy is the most common treatment for a variety of cancer forms worldwide [22-27]. Presently, combining chemotherapeutic agents with distinct mechanisms of action represents a strategy increasingly employed in cancer treatment [28,29]. Bacterial infections continue to pose a significant risk to human health due to the rising resistance of bacteria to current antibiotics, presenting a growing public health challenge [30-37]. As a result, there is an urgent requirement for the creation of novel antimicrobial agents that can effectively combat drug-resistant microorganisms [38-43]. This underscores the critical importance of ongoing research into both anticancer and antimicrobial agents, ensuring that investigations in these areas remain current and up-to-date [44-50]. On the other hand, 1,2,3-triazoles

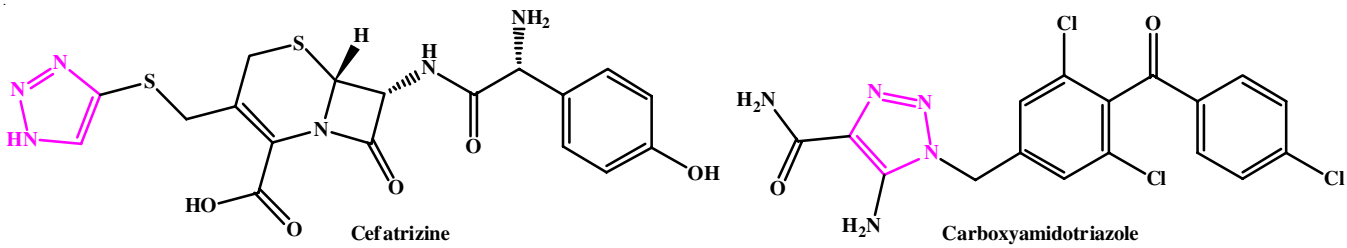


Fig. 1. Structure of some commerical anticancer drugs containing triazole scaffold

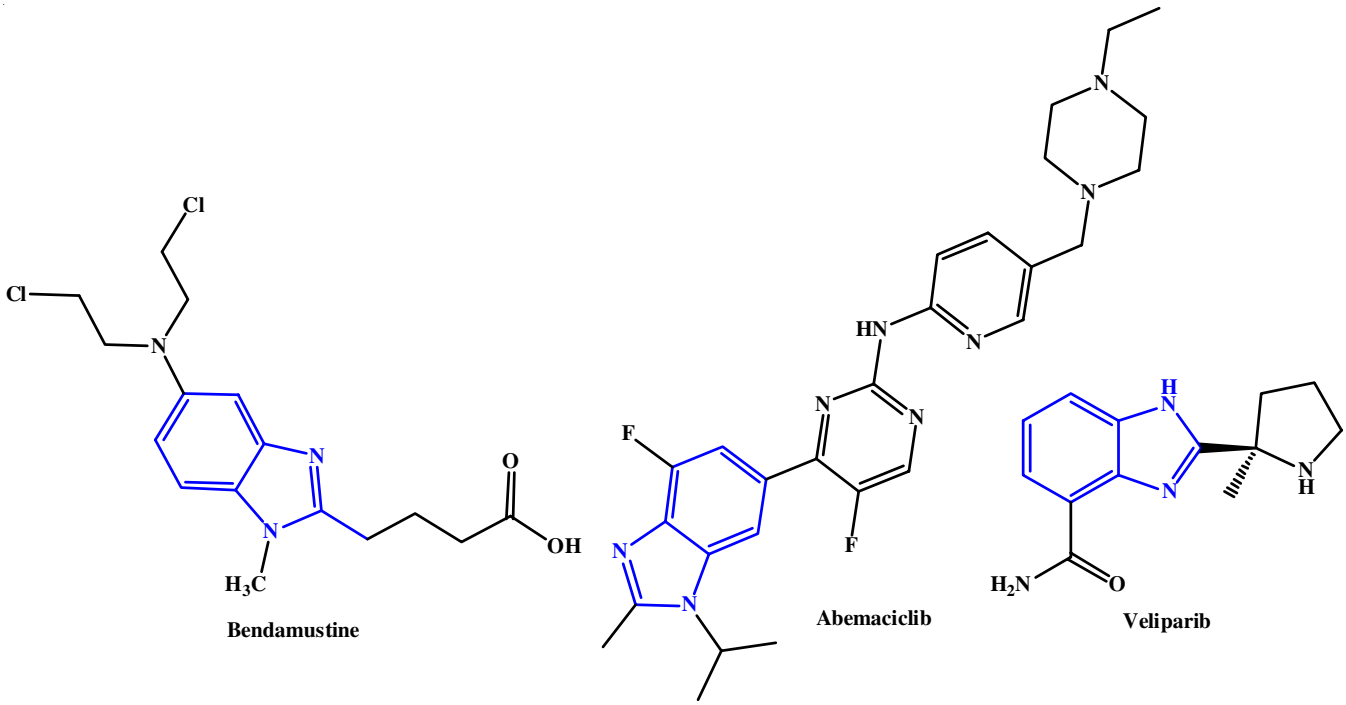


Fig. 2. Structure of some commerical anticancer drugs containing benzimidazole scaffold

were reported by 1,3-dipolar cycloaddition of azides with alkynes under thermal conditions, in the presence of copper or ruthenium catalysts [43-45]. Transition metal-free synthesis of 1,2,3-triazoles was reported by the cyclocondensation of organic azides with terminal alkynes in the presence of catalytic amount of tetramethylammonium hydroxide [46]. However, there are limitations to these methods, such as byproduct formation, handling of harsh and toxic reagents, use of anhydrous solvents, *etc*.

In contrast, we report herein a highly efficient protocol to develop a novel series of substituted 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole derivatives (**9a-o**), synthesize by using click chemistry and then evaluate the anticancer and animicrobial activities. The structural confirmation was established through spectral analyses employing FTIR, ^{1}H & ^{13}C NMR and Mass spectra. The synthesized compounds were subjected to screening for molecular docking, cytotoxicity and antimicrobial activities against Gram-positive bacteria (*B. subtilis*, *B. sphaericus* and *S. aureus*) and Gram-negative bacteria (*P. aeruginosa*, *K. aerogenes* and *C. violaceum*) as well as antifungal species *C. albicans*, *A. fumigatus, T. rubrum* and *T. mentagrophytes* and compared with the standard drugs.

EXPERIMENTAL

All the reactions were conducted in oven-dried apparatus. The reactions were monitored using thin layer chromatography (TLC) on silicon dioxide gel plates (60 F_{254}) and ultraviolet (UV) light as well as iodine vapour were used to view them. Column chromatography was performed on silica gel (60-120 mesh) with distilled hexane and ethyl acetate solutions. The ^{1}H and ^{13}C NMR spectra were acquired with 500 and 125 MHz spectrometers, mainly the Bruker Avance II 400 MHz devices, in $CDCl_3$ and DMSO solvents. Mass spectra were collected with the QSTAR XL GCMS mass spectrometer. The Shimadzu FT-IR-8400s device was used to record the infrared spectra. Melting points were determined in an open glass capillary tube using DbkProg melting point apparatus and are uncorrected.

Synthesis of 2,4-dihydroxy benzaldehyde (3): Initially, 2,4-dihydroxy benzaldehyde (**3**) was synthesized efficiently by reacting resorcinol (**1**, 1 mmol) and N,N-dimethylacetamide (**2**, 1.3 mmol) with $POCl_3$ (1.1 mmol) at room temperature for 2-3 h. After the reaction was completed, the starting material, along with the reaction mixture, was quenched by adding crushed ice. The resulting solid was then filtered to obtain the desired product 2,4-dihydroxy-benzaldehyde (**3**).

Synthesis of compound 5: To a solution of compound **3** (1 mmol) in anhydrous DMF was added anhydrous K_2CO_3 (2 mmol) stirred for 10 min and then added 3-bromo-propyne (**4**, 2.5 mmol)and stirred for 5-6 h at room temperature. After completion of the reaction, the reaction mixture was quenched by adding crushed ice. The resulting solid was then filtered to afford the product 2,4-*bis*-prop-2-ynyloxy-benzaldehyde (**5**).

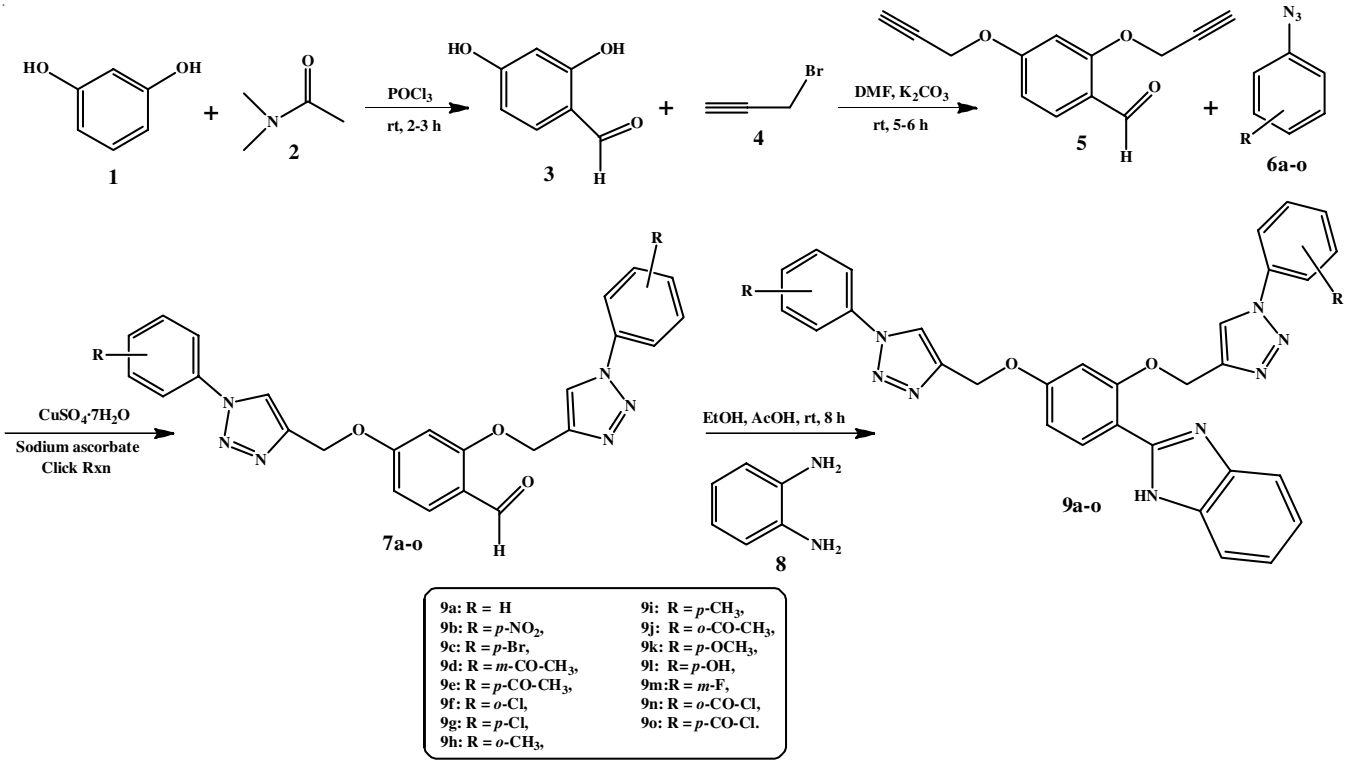
General procedure for the synthesis of compounds 7a-o: Substituted 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]benzaldehyde derivatives (**7a-o**) were synthesized using click chemistry. This involved reacting compound 5 (0.2 mmol) with various aromtaic azides (**6a-o**) (0.4 mmol) in a mixture of $CuSO_4{\cdot}5H_2O$, DMF and sodium ascorbate at room temperature for 16-17 h. The progress of the reaction was monitored using TLC. After completion, the reaction mixture was poured into a 100 mL beaker containing crushed ice. The resulting solid product was obtained by filtering through a Büchner funnel and drying under vacuum at reduced pressure. The crude compounds were further purified using column chromatography with a hexane/ethyl acetate solvent system (1:4 v/v), yielding excellent yields ranging from 68-80%.

General procedure for the synthesis of compounds 9a-o: The final target substituted 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]-triazol-4-ylmethoxy)phenyl]-1*H*-benzoimidazole (**9a-o**) derivatives were obtained by reacting compounds **7a-o** (1 mmol) with benzene-1,2-diamine (**8**, 1.2 mmol) in ethanol with acetic acid catalyst at room temperature for 8 h. After completion, ethanol was distilled off under reduced pressure. The obtained crude compound was quenched with crushed ice. The resulting pure solid product was obtained by filtering through a Büchner funnel and drying under vacuum at reduced pressure with good yields ranging from 60-75% (**Scheme-I**).

2-[2,4-*bis*-(1-Phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole (9a): Colour: pale yellow solid, yield: 78%, m.p.: 168-170 ºC. Elemental analysis of $C_{31}H_{24}N_8O_2$, calcd. (found) %: C, 68.88 (68.85); H, 4.47 (4.47); N, 20.73 (21.53); O, 5.92 (5.15); IR (KBr, ν_{max}, cm^{-1}): 3380.70, 3160.66, 3040.07, 2929.90, 1603.05, 1294.41, 878.12, 754.30, 661.02; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.09 (s, 2H), 7.98 (d, 4H, *J* = 7.21 Hz), 7.78 (d, 1H, *J* = 6.82 Hz), 7.62 (t, 4H, *J* = 7.62 Hz), 7.58 (d, 2H, *J* = 7.21 Hz), 7.18 (s, 1H), 6.87 (d, 1H, *J* = 6.96 Hz), 6.63 (d, 2H, *J* = 7.01 Hz), 6.51 (d, 2H, *J* = 7.02 Hz), 5.43 (s, 4H), 4.18 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 167.61, 164.65, 162.27, 142.54, 142.16, 134.46, 131.89, 130.63, 129.79, 128.61, 127.28, 126.99, 118.84, 108.28, 100.60, 46.97. ESI-MS: *m/z*: 540 $[M+1]^+$.

2-{2,4-*bis*-[1-(4-Nitro-phenyl)-1*H*-[1,2,3]triazol-4-ylmethoxy]phenyl}-1*H*-benzoimidazole (9b): Colour: yellow solid, yield: 79%, m.p.: 176-178 ºC. Elemental analysis of $C_{31}H_{22}N_{10}O_6$: calcd. (found) %: C, 59.05 (58.89); H, 3.52 (3.58); N, 22.21 (22.55); O, 15.22 (14.98); IR (KBr, ν_{max}, cm^{-1}): 3373.10, 3143.08, 2911.61, 2868.25, 1590.23, 1248.61, 758.91, 687.61; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.22 (s, 2H), 8.44 (m, 4H), 8.23 (m, 4H), 7.68 (d, 1H, *J* = 7.02 Hz), 7.18 (s, 1H), 6.84 (d, 1H, *J* = 7.02 Hz), 6.64 (d, 1H, *J* = 6.96 Hz), 6.63 (d, 2H, *J* = 7.01 Hz), 6.43 (d, 2H, *J* = 7.18 Hz), 5.46 (s, 2H), 5.42 (s, 2H), 4.15 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 164.54, 162.18, 153.85, 151.23, 148.23, 146.09, 143.89, 136.52, 129.90, 129.64, 128.80, 122.78, 120.23, 120.16, 118.66, 49.31. ESI-MS: *m/z*: 630 $[M+1]^+$.

2-{2,4-*bis*-[1-(4-Bromo-phenyl)-1*H*-[1,2,3]triazol-4-ylmethoxy]phenyl}-1*H*-benzoimidazole (9c): Colour: pale brown solid, yield: 81%, m.p.: 182-184 ºC. Elemental analysis



Scheme-I: Synthetic route of substituted 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole (**9a-o**) analogues

of $C_{31}H_{22}Br_2N_8O_2$: calcd. (found) %: C, 53.32 (53.45); H, 3.18 (3.18); N, 16.05 (16.02); O, 4.58 (4.38). IR (KBr, ν_{max}, cm^{-1}): 3378.65, 3157.72, 3038.49, 2992.59, 1592.45, 1278.64, 881.32, 756.48, 660.56. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.18 (s, 2H), 7.96 (m, 4H), 7.82 (m, 4H), 7.67 (d, 1H, *J* = 7.23 Hz), 7.18 (s, 1H), 6.86 (d, 1H, *J* = 7.18 Hz), 6.59 (d, 2H, *J* = 6.98 Hz), 6.46 (d, 2H, *J* = 7.19 Hz), 6.43 (d, 2H, *J* = 7.20 Hz), 5.48 (s, 2H), 5.43 (s, 2H), 4.10 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 165.77, 152.16, 147.19, 136.07, 132.94, 131.31, 129.83, 126.97, 126.51, 126.37, 125.95, 123.80, 122.34, 115.19, 113.42, 51.94. ESI-MS: *m/z*: 696 $[M+1]^+$.

1-(3-{4-[3-[1-(3-Acetyl-phenyl)-1*H*-[1,2,3]triazol-4-yl-methoxy]-4-(1*H*-benzoimidazol-2-yl)phenoxymethyl][1,2,3]-triazol-1-yl}phenyl)ethanone (9d): Colour: off-white solid, yield: 76%, m.p.: 186-188 ºC. Elemental analysis of $C_{35}H_{28}N_8O_4$: calcd. (found) %: C, 67.30 (67.81); H, 4.52 (4.85); N, 17.94 (17.56); O, 10.25 (9.78). IR (KBr, ν_{max}, cm^{-1}): 3363.61, 3148.52, 3012.29, 2996.41, 1695.86, 1565.32, 1272.43, 879.56, 745.48, 658.56; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.20 (s, 2H), 8.42 (s, 1H), 8.22 (d, 2H, *J* = 7.38 Hz), 8.08 (t, 2H, *J* = 7.52 Hz), 7.96 (s, 2H), 7.78 (d, 2H, *J* = 7.23 Hz), 7.18 (d, 2H, *J* = 6.72 Hz), 6.877 (d, 2H, *J* = 7.32 Hz), 6.57 (d, 2H, *J* = 7.08 Hz), 5.21 (s, 4H), 4.10 (s, 1H, NH), 2.76 (s, 3H), 2.70 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 187.62, 164.57, 162.21, 143.57, 143.15, 136.54, 129.97, 129.93, 128.89, 128.84, 123.27, 122.80, 121.50, 120.19, 118.68, 108.16, 100.32, 50.09, 16.09. ESI-MS: *m/z*: 624 $[M+1]^+$.

1-(4-{4-[3-[1-(4-Acetyl-phenyl)-1*H*-[1,2,3]triazol-4-yl-methoxy]-4-(1*H*-benzoimidazol-2-yl)phenoxymethyl]-[1,2,3]triazol-1-yl}phenyl)ethanone (9e): Colour: white solid, yield: 82%, m.p.: 192-194 ºC. Elemental analysis of $C_{35}H_{28}N_8O_4$: calcd. (found) %: C, 67.30 (67.49); H, 4.52 (4.87); N, 17.94 (17.85); O, 10.25 (9.79); IR (KBr, ν_{max}, cm^{-1}): 3348.53, 3151.58, 3018.78, 2998.64, 1698.46, 1568.43, 1276.43, 876.64, 732.48, 662.56; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.20 (s, 2H), 8.18 (m, 4H), 7.92 (m, 4H), 7.78 (d, 1H, *J* = 7.37 Hz), 7.18 (s, 1H), 6.82 (d, 1H, *J* = 7.31 Hz), 6.64 (d, 1H, *J* = 6.99 Hz), 6.51 (d, 2H, *J* = 6.56 Hz), 6.42 (d, 2H, *J* = 7.32 Hz), 5.43 (s, 4H), 4.10 (s, 1H, NH), 2.63 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 187.09, 169.78, 162.32, 159.35, 152.01, 147.31, 143.90, 136.93, 129.80, 123.79, 123.15, 121.82, 114.84, 113.34, 51.97, 20.75. ESI-MS: *m/z*: 624 $[M+1]^+$.

2-{2,4-*bis*-[1-(2-Chloro-phenyl)-1*H*-[1,2,3]triazol-4-ylmethoxy]phenyl}-1*H*-benzoimidazole (9f): Colour: brick red solid, yield: 80%, m.p.: 188-190 ºC, Elemental analysis of $C_{31}H_{22}Cl_2N_8O_2$: calcd. (found) %: C, 61.09 (61.12); H, 3.64 (3.64); N, 18.39 (18.35); O, 5.25 (5.23). IR (KBr, ν_{max}, cm^{-1}): 3349.45, 3150.51, 3017.65, 2999.45, 1569.56, 1277.49, 878.72, 727.83, 660.56; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.08 (s, 2H), 8.46 (d, 2H, *J* = 6.98 Hz), 8.18 (d, 2H, *J* = 7.01 Hz), 8.02 (d, 1H, *J* = 7.23 Hz), 7.78 (d, 1H, *J* = 6.91 Hz), 7.62 (d, 2H, *J* = 6.77 Hz), 7.18 (s, 1H), 6.84 (d, 1H, *J* = 6.67 Hz), 6.78 (t, 2H, *J* = 7.18 Hz), 6.52 (t, 2H, *J* = 6.87 Hz), 5.42 (s, 4H), 4.02 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 163.97, 162.21, 143.47, 143.15, 136.54, 129.97, 129.93, 129.68, 128.84, 124.27, 122.80, 121.50, 120.26, 120.19, 118.68, 108.68, 108.16, 101.32, 51.93. ESI-MS: *m/z*: 608 $[M+1]^+$.

2-{2,4-*bis*-[1-(4-Chloro-phenyl)-1*H*-[1,2,3]triazol-4-ylmethoxy]phenyl}-1*H*-benzoimidazole (9g): Colour: brick red solid, yield: 80%, m.p.: 188-190 ºC. Elemental analysis of $C_{31}H_{22}Cl_2N_8O_2$: calcd. (found) %: C, 61.09 (61.12); H, 3.64 (3.64); N, 18.39 (18.35); O, 5.25 (5.23). IR (KBr, ν_{max}, cm^{-1}): 3350.45, 3156.51, 3019.65, 1697.56, 1569.56, 1277.49, 878.72, 727.83, 660.56; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.08 (s, 2H), 8.0 (m, 4H), 7.78 (s, 4H), 7.42 (s, 1H), 7.10 (m, 4H), 6.94 (d, 1H, *J* = 7.21 Hz), 6.78 (d, 1H, *J* = 6.99 Hz), 6.58 (d, 2H, *J* = 6.82 Hz), 5.42 (s, 4H), 4.18 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 163.84, 161.45, 159.52, 153.90, 143.78, 142.55, 136.43, 134.59, 129.81, 128.81, 121.80, 121.51, 120.16, 118.58, 110.47, 108.68, 108.16, 101.32, 51.93. ESI-MS: *m/z*: 608 $[M+1]^+$.

2-[2,4-*bis*-(1-*o*-Tolyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole (9h): Colour: light yellow solid, yield: 78%, m.p.: 168-170 ºC. Elemental analysis of $C_{33}H_{28}N_8O_4$: calcd. (found) %: C, 69.70 (69.75); H, 4.96 (4.83); N, 19.71 (19.77); O, 5.63 (5.65). IR (KBr, ν_{max}, cm^{-1}): 3312.35, 3149.46, 3021.34, 2991.45, 1570.56, 1279.49, 881.72, 729.82, 658.74; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.12 (s, 2H), 8.77 (d, 2H, *J* = 6.99 Hz), 8.38 (s, 1H), 7.76 (d, 1H, *J* = 7.01 Hz), 7.70 (d, 2H, *J* = 6.95 Hz), 7.58 (d, 2H, *J* = 6.79 Hz), 7.38 (t, 2H, *J* = 7.42 Hz), 7.17 (t, 2H, *J* = 7.21 Hz), 6.87 (d, 1H, *J* = 7.32 Hz), 6.70 (s, 1H), 5.41 (s, 4H), 4.18 (s, 1H, NH), 3.82 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 163.47, 162.19, 161.03, 153.79, 143.85, 137.47, 131.78, 131.68, 121.87, 121.71, 121.33, 118.53, 115.94, 115.30, 110.49, 107.67, 107.41, 57.51, 35.68. ESI-MS: *m/z*: 600 $[M+1]^+$,

2-[2,4-*bis*-(1-*p*-Tolyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole (9i): Colour: yellow solid, yield: 76%, m.p.: 172-174 ºC. Elemental analysis of $C_{33}H_{28}N_8O_2$: calcd. (found) %: C, 69.70 (69.77); H, 4.96 (4.83); N, 19.71 (19.75); O, 5.63 (5.65). IR (KBr, ν_{max}, cm^{-1}): 3301.28, 3148.24, 3010.24, 2978.53, 1520.46, 1272.62, 878.14, 722.62, 660.24; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.01 (s, 2H), 8.18 (d, 1H, *J* = 6.78 Hz), 7.82 (d, 1H, *J* = 7.67 Hz), 7.78 (m, 4H), 7.40 (d, 2H, *J* = 6.68 Hz), 7.18 (s, 1H), 6.82 (d, 1H, *J* = 6.78 Hz), 6.51 (s, 1H), 5.42 (d, 4H), 4.12 (s, 1H, NH), 2.41 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 157.42, 153.90, 142.78, 142.22, 134.67, 134.32, 131.76, 130.52, 128.49, 125.91, 121.76, 121.50, 118.56, 110.53, 43.63, 28.11. ESI-MS: *m/z*: 568 $[M+1]^+$.

1-(2-{4-[3-[1-(4-Acetyl-phenyl)-1*H*-[1,2,3]triazol-4-yl-methoxy]-4-(1*H*-benzoimidazol-2-yl)phenoxymethyl]-[1,2,3]triazol-1-yl}phenyl)ethanone (9j): Colour: pale yellow solid, yield: 80%, m.p.: 168-170 ºC. Elemental analysis of $C_{33}H_{28}N_8O_4$: calcd. (found) %: C, 67.30 (67.25); H, 4.52 (4.54); N, 17.94 (17.91); O, 10.24 (10.30). IR (KBr, ν_{max}, cm^{-1}): 3319.25, 3153.46, 3024.34, 2992.45, 1502.56, 1086.58, 886.72, 724.82, 655.74. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.03 (s, 2H), 8.48 (d, 2H, *J* = 7.52 Hz), 8.25 (d, 1H, *J* = 6.99 Hz), 7.78 (d, 1H, *J* = 7.32 Hz), 7.68 (d, 2H, *J* = 6.58 Hz), 7.58 (d, 2H, *J* = 6.79 Hz), 7.38 (t, 2H, *J* = 7.22 Hz), 7.19 (t, 2H, *J* = 7.35 Hz), 6.82 (d, 1H, *J* = 7.21 Hz), 6.59 (s, 1H), 5.42 (d, 2H), 3.82 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 187.44, 164.62, 162.22, 142.79, 141.85, 130.88, 129.77, 127.06, 126.82, 125.86, 125.56, 120.89, 121.33, 118.53, 115.94, 115.30, 110.49, 107.67, 107.41, 57.51, 35.68. ESI-MS: *m/z*: 624s $[M+1]^+$.

2-{2,4-*bis*-[1-(4-Methoxy-phenyl)-1*H*-[1,2,3]triazol-4-ylmethoxy]phenyl}-1*H*-benzoimidazole (9k): Colour: pale yellow solid, yield: 76%, m.p.: 178-180 ºC. Elemental analysis of $C_{33}H_{28}N_8O_4$: calcd. (found) %: 65.99 (65.95); H, 4.70 (4.71); N, 18.66 (18.63); O, 10.65 (10.71). IR (KBr, ν_{max}, cm^{-1}): 3349.92, 3150.92, 3019.73, 2992.90, 1522.56, 1275.34, 878.56, 730.45, 660.82; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.08 (s, 2H), 8.59 (d, 2H, *J* = 7.08 Hz), 7.84 (m, 4H), 7.76 (d, 1H, *J* = 7.41 Hz), 7.18 (m, 4H), 6.82 (d, 1H, *J* = 7.29 Hz), 6.68 (d, 2H, *J* = 6.88 Hz), 6.43 (s, 1H), 5.42 (s, 4H), 4.02 (s, 1H, NH), 3.82 (s, 6H); ^{13}C NMR 400 MHz (DMSO-d_6) δ ppm: 172.43, 168.54, 164.62, 162.22, 151.68, 144.63, 141.74, 130.90, 127.06, 126.82, 125.86, 125.58, 120.89, 113.02, 56.25, 35.67. ESI-MS: *m/z*: 600 $[M+1]^+$.

4,4′-(((4-(1*H*-Benzo[*d*]imidazol-2-yl)-1,3-phenylene)*bis*-(oxy))*bis*(1*H*-1,2,3-triazole-4,1-diyl)diphenol (9l): Colour: pale yellow solid, yield: 77%, m.p.: 178-180 ºC. Elemental analysis of $C_{31}H_{24}N_8O_4$: calcd. (found) %: C, 63.97 (63.81); H, 3.70 (3.75); N, 20.58 (20.49); O, 11.75 (11.95). IR (KBr, ν_{max}, cm^{-1}): 3640.67, 3349.92, 3150.92, 3019.73, 2992.90, 1522.56, 1275.34, 878.56, 730.45, 660.82. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.21 (s, 2H), 9.11 (s, 2H), 8.14 (s, 1H), 7.67 (m, 4H), 7.16 (s, 1H), 6.98 (m, 4H), 6.82 (d, 2H, *J* = 7.53 Hz), 6.52 (d, 1H, *J* = 6.56 Hz), 5.40 (s, 4H), 4.18 (s, 1H, NH); ^{13}C NMR 400 MHz (DMSO-d_6) δ ppm: 153.96, 151.65, 142.45, 142.21, 134.65, 130.92, 125.52, 121.77, 121.55, 120.92, 118.56, 113.01, 110.64, 56.13. ESI-MS: *m/z*: 572 $[M+1]^+$.

2-{2,4-*bis*-[1-(3-Fluoro-phenyl)-1*H*-[1,2,3]triazol-4-ylmethoxy]phenyl}-1*H*-benzoimidazole (9m): Colour: white solid, Yield: 79%, m.p.: 178-180 ºC, Elemental analysis of $C_{31}H_{22}F_2N_8O_2$: calcd. (found) %: C, 64.58 (63.81); H, 3.85 (3.47); N, 19.44 (19.56); O, 5.55 (6.18). IR (KBr, ν_{max}, cm^{-1}): 3369.61, 3151.52, 3016.29, 2998.41, 1698.86, 1568.32, 1276.43, 883.56, 749.48, 662.56; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.18 (s, 2H), 8.92 (d, 2H, *J* = 7.18 Hz), 8.45 (d, 2H, *J* = 7.27 Hz), 8.12 (s, 1H), 7.75 (d, 2H, *J* = 7.05 Hz), 7.56 (d, 2H, *J* = 7.28 Hz), 7.20 (d, 1H, *J* = 6.98 Hz), 6.84 (d, 1H, *J* = 7.14 Hz), 6.62 (t, 2H, *J* = 7.01 Hz), 6.51 (t, 2H, *J* = 7.56 Hz), 5.42 (s, 4H), 4.18 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 164.87, 161.34, 151.31, 149.05, 141.05, 141.95, 134.75, 131.75, 130.53, 128.49, 128.42, 127.32, 126.20, 119.16, 114.67, 113.52, 103.16, 55.84. ESI-MS: *m/z*: 576 $[M+1]^+$.

2,2′-(((4-(1*H*-Benzo[*d*]imidazol-2-yl)-1,3-phenylene)*bis*-(oxy))*bis*(methylene)*bis*(1*H*-1,2,3-triazole-4,1-diyl)dibenzoyl-chloride (9n): Colour: pale yellow solid, yield: 81%, m.p.: 168-170 ºC, Elemental analysis of $C_{33}H_{22}Cl_2N_8O_4$: C, 59.56 (59.51); H, 3.33 (3.38); N, 16.84 (16.56); O, 9.62 (9.72). IR (KBr, ν_{max}, cm^{-1}): 3324.25, 3159.46, 3029.34, 2995.45, 1720.56, 1510.56, 1089.58, 887.72, 726.82, 658.74; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.21 (s, 2H), 8.42 (d, 2H, *J* = 7.56 Hz), 8.14 (d, 2H, *J* = 7.65 Hz), 8.08 (d, 2H, *J* = 7.24 Hz), 7.98 (d, 2H, *J* = 6.72 Hz), 7.78 (d, 2H, *J* = 6.89 Hz), 7.08 (d, 1H, *J* = 7.34 Hz), 6.87 (t, 2H, *J* = 7.31 Hz), 6.78 (t, 2H, *J* = 7.01 Hz), 6.61 (s, 1H), 5.21 (s, 4H), 4.02 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 192.03, 163.07, 159.35, 151.33, 149.04, 142.74, 129.88, 126.23, 123.36, 121.87, 119.19, 114.88, 114.66, 113.42, 110.95, 103.11, 55.56. ESI-MS: *m/z*: 664 $[M+1]^+$.

4,4′-(((4-(1*H*-Benzo[*d*]imidazol-2-yl)-1,3-phenylene)*bis*-(oxy))*bis*(methylene)*bis*(1*H*-1,2,3-triazole-4,1-diyl)dibenzoyl-chloride (9o): Colour: white solid, yield: 80%, m.p.: 172-174 ºC. Elemental analysis of $C_{33}H_{22}Cl_2N_8O_4$: C, 59.56 (59.51); H, 3.33 (3.38); N, 16.84 (16.56); O, 9.62 (9.72). IR (KBr, ν_{max}, cm^{-1}): 3350.53, 3153.58, 3020.78, 2997.64, 1699.46, 1556.43, 1281.43, 871.64, 731.48, 661.56; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.18 (s, 2H), 8.17 (m, 4H), 7.98 (m, 4H), 7.75 (d, 1H, *J* = 7.41 Hz), 7.21 (d, 1H, *J* = 6.98 Hz), 6.85 (d, 1H, *J* = 7.28 Hz), 6.62 (d, 2H, *J* = 6.98 Hz), 6.48 (d, 2H, *J* = 6.86 Hz), 5.42 (d, 4H), 4.08 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 187.57, 164.53, 162.18, 138.50, 138.43, 134.27, 130.25, 129.62, 123.11, 122.65, 120.09, 120.02, 118.64, 108.12, 100.25, 52.06. ESI-MS: *m/z*: 664 $[M+1]^+$.

Docking studies: Newly synthesized compounds **9a-o** were subjected to molecular docking analysis alongside the standard drug doxorubicin, focusing on their interactions with the EGFR kinase protein (PDB ID: 3W33) to assess their binding strengths and interaction patterns. EGFR is frequently found in excessive amounts or with mutations in different cancer types like lung, colorectal and head and neck cancers. This abnormal EGFR activity promotes unregulated cell growth, survival and spread, which are key factors in cancer advancement. Therefore, targeting EGFR kinase represents a hopeful avenue in crafting anticancer medications.

Cytotoxicity activity: The effectiveness of newly synthesized derivatives containing 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]-triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole (**9a-o**) core was assessed for anticancer properties against three specific tumor cell lines (MCF-7, PC-3 and HeLa) using the MTT assay. The evaluation of anticancer activity occurred in two stages. Initially, all the compounds were tested at two concentrations (5 and 10 μM) across the mentioned cell lines. Subsequently, the compounds chosen for their promising anticancer activity underwent further testing with a broader range of concentrations. This step aimed to determine the IC_{50} value of each compound, which serves as a crucial parameter for evaluating and comparing the potency of these compounds against the standard drug utilized in this study.

MTT assay: The cytotoxic effects of substituted 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)phenyl]-1*H*-benzoimidazole (**9a-o**) derivatives were assessed using the MTT assay. Initially, stock solutions of the synthesized derivatives were diluted with culture medium. At a density of 5×10^4 cells per well, cells were seeded into 96-well plates and incubated until 90–95% confluence was achieved. Subsequently, each well was treated with 100 μL of medium containing the desired concentrations of synthesized derivatives and incubated for 48 h. Afterward, 20 μL of MTT working solution (5 mg/mL) was added to each well and incubated for an additional 4 h. Following incubation, the medium was carefully aspirated and 200 μL of DMSO was added. The optical density was then measured at 490 nm and 630 nm using a microplate reader.

The formula for calculating the percentage of cell growth inhibition is:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Sample group OD490} - \text{Sample group OD630}}{\text{Control group OD490} - \text{Control group OD630}}\right) \times 100$$

Antimicrobial activity

Antibacterial activity: The synthesized compounds **9a-o** were evaluated for their antibacterial activity against three Gram-negative strains [*P. Aeruginosa* (MTCC 741), *K. aerogenes* (MTCC 39) and *C. violaceum* (MTCC 2656)] and three Gram-positive strains [*B. subtilis* (MTCC 441), *B. sphaericus* (MTCC 11) and *S. aureus* (MTCC 96)]. The antibacterial assay was conducted using the disc diffusion method. For the assay, standard inoculums with a concentration of 1-2 × 10^7 cfu/mL using 0.5 McFarland standards were spread evenly onto the surface of sterile agar plates using a sterile glass spreader. Discs with a diameter of 6.26 mm were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 ºC for 1 h. These sterile discs were then soaked in a known concentration of the test compounds and placed on nutrient agar medium in the plates. The plates were inverted and incubated for 24 h at 37 ºC to allow the bacterial growth and assess the inhibition zones around the discs.

Antifungal activity: Compounds **9a-o** were also evaluated for the antifungal activity against the four strains *viz. C. albicans* (ATCC 10231), *A. fumigatus* (HIC 6094), *T. rubrum* (IFO 9185) and *T. mentagrophytes* (IFO 40996) using the disc diffusion method in DMSO. Amphotericin B serves as the standard drug for comparison. The mean inhibition zone (MZI) values were measured and recorded along the control values to assess the efficacy of the screened compounds.

RESULTS AND DISCUSSION

In **Scheme-I**, the process for synthesizing substituted [1,2,3]triazole conjugated with benzoimidazole derivatives (**9a-o**) was outlined. Initially, 2,4-dihydroxy-benzaldehyde (**3**) was synthesized efficiently by reacting resorcinol (**1**) and N,N-dimethylacetamide (**2**) with $POCl_3$ at room temperature for 2-3 h. This resulted in the formation of compound **3**, which subsequently, reacted with propargyl bromide (**4**) and K_2CO_3 in DMF at room temperature for 5-6 h, yielding compound 2,4-*bis*(prop-2-ynyloxy)benzaldehyde (**5**). Substituted 2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)benzaldehyde (**7a-o**) were synthesized by reacting compound **5** with different substituted aromatic azides (**6a-o**) under click reaction conditions. Finally, the target substituted 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)phenyl]-1*H*-benzoimidazole (**9a-o**) derivatives were obtained by reacting compounds **7a-o** with benzene-1,2-diamine (**8**) in an ethanol solvent with acetic acid at room temperature for 8 h. All the newly synthesized compounds were confirmed using ^1H & ^{13}C NMR, Mass and IR spectroscopic techniques. The ^1H NMR spectra of all the synthesized compounds was characterized by the disappearance of CHO signal, formerly appearing at ~ δ 10.23 ppm in the spectrum of compounds **7a-o**. The proton signals of triazole protons appeared as singlet in between δ 8.45 to 9.30 ppm, the methylene protons planked between the phenyl ring and triazole appeared as singlet or doublet signals in between at δ 5.40- 5.80 ppm and the remaining all protons signals appeared at their corresponding integral values. The carbon signals of triazole ring appeared at 121-130 ppm and O-CH_2 signals appeared at 45-60 ppm. All other spectral data were consistent with the assumed structures. The mass spectra of the target compounds showed the (M+1) peaks and are in agreement with their molecular formulae.

Biological assays

MTT assays: Target substituted 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole derivatives were evaluated for their cytotoxicity against MCF-7 (breast cancer), HeLa (cervical cancer) and PC-3 (prostrate cancer) cancer cell lines at different concentrations by the well known method, MTT assay method using doxorubicin as standard drug. According to the preliminary screening studies, compound **9m** exhibited significant activity, while compounds **9b**, **9g**, **9j**, **9k**, **9n** and **9o** effectively inhibited the growth of cancer cells compared to other compounds. The % growth inhibition for these compounds was above 70% at tested concentrations of 5 μM and 10 μM (Table-1).

TABLE-1
CYTOTOXIC ASSAY DATA OF SYNTHESIZED COMPOUNDS (**9a-o**)

Compd.	MCF-7		PC-3		HeLa	
	5 μM	10 μM	5 μM	10 μM	5 μM	10 μM
9a	70	75	58	67	66	73
9b	86	94	73	80	79	84
9c	76	82	62	69	71	75
9d	75	85	67	72	73	78
9e	66	73	56	64	63	70
9f	73	80	60	64	69	71
9g	75	83	61	67	69	74
9h	65	71	54	60	62	70
9i	67	73	57	62	65	71
9j	91	97	72	80	88	94
9k	89	94	79	86	81	90
9l	72	79	58	67	68	72
9m	92	96	82	91	86	95
9n	88	89	77	78	80	87
9o	86	88	76	77	81	89
Doxorubicin	93	99	84	93	87	97
Control	10	10	10	10	10	10

Therefore, the compounds displaying the most promising % growth inhibitory activity were chosen for further evaluation. They were subjected to additional testing under the same conditions to determine the IC_{50} value at concentrations of 2, 4, 8, 16 and 32 μM (Table-2). Among the target compounds tested, **9m** exhibited excellent IC_{50} values 2.62 ± 0.03 μM (MCF-7), 3.87 ± 0.05 μM (PC-3) and 3.23 ± 0.05 μM (HeLa). The other targets **9b**, **9g**, **9j**, **9k**, **9n** and **9o** also showed good to moderate IC_{50} values against all the cancer cell lines in comparison to doxorubicin. These results clearly implies that the most potent target compound, is the one having 3-fluoro-phenyl substituent on the triazole rings.

Antimicrobial studies

Antibacterial assays: The antibacterial activity of all the newly synthesized compounds **9a-o** was assayed against three Gram-positive bacteria *B. subtilis*, *B. sphaericus*, *S. aureus* and three Gram-negative bacteria *P. aeruginosa*, *K. aerogenes*,

TABLE-2
ANTICANCER IC_{50} VALUES OF SYNTHESIZED COMPOUNDS (**9a-o**) WITH STANDARD DOXORUBICIN

Compd.	IC_{50} (μM ± SEM)		
	MCF-7	PC-3	HeLa
9b	5.62 ± 0.03	9.30 ± 0.09	9.41 ± 0.06
9c	7.34 ± 0.05	11.27 ± 0.07	7.24 ± 0.08
9d	6.92 ± 0.04	8.43 ± 0.06	7.16 ± 0.09
9f	8.39 ± 0.05	11.62 ± 0.14	8.07 ± 0.11
9g	5.73 ± 0.08	11.51 ± 0.12	7.86 ± 0.14
9j	2.77 ± 0.03	4.13 ± 0.06	3.68 ± 0.05
9k	3.28 ± 0.05	4.08 ± 0.03	3.51 ± 0.02
9l	5.48 ± 0.03	10.79 ± 0.11	7.60 ± 0.12
9m	2.62 ± 0.03	3.87 ± 0.05	3.23 ± 0.05
9n	2.78 ± 0.05	3.92 ± 0.03	3.32 ± 0.02
9o	2.98 ± 0.06	3.91 ± 0.03	3.45 ± 0.06
Doxorubicin	2.70 ± 0.04	3.81 ± 0.03	3.13 ± 0.08

C. violaceum by disc-diffusion method. Among these compounds, **9m** showed high activity with mean zone of inhibition (MZI in mm) almost equal to the standard drug streptomycin against all the strains of Gram-positive and Gram-negative bacteria.The highest acitivity of compound **9m** can be attributed to the presence 3-fluorophenyl group on the triazole ring. Besides, compounds **9b**, **9c**, **9e**, **9h**, **9i**, **9n** and **9o** demonstarted potent antibacterial activity compared to the standard drug.The significant inhibitory activity of the compunds **9b**, **9c**, **9e**, **9h**, **9i**, **9n** and **9o** is likely due to the presence of specific substituents on the triazole moiety of the target compounds, **9b** (4-nitrophenyl), **9c** (4-bromophenyl), **9e** (4-phenylacetyl), **9h** (*o*-tolyl), **9i** (*m*-tolyl), **9n** (2-benzenecarbonylchloride) and **9o** (4-benzenecarbonylchloride) (Table-3).

Antifungal activity: The synthesized compounds **9a-o** were evaluated for the antifungal activity against four strains of fungi, *C. albicans*, *A. fumigatus, T. mentagrophytes* and *T. rubrum.* Substituted 1,2,3-triazole derivatives (**9a-o**) effectively inhibited the fungal growth of all the four strains (Table-4). Compound **9m** with 3-fluorophenyl as substituent on triazole rings is showing activity almost equal to the standard drug amphotericin B. Meanwhile, other compounds **9b**, **9c**, **9g**, **9n** and **9o** exhibited potent and good antifungal activity compared to the standard drug at the tested concentrations. Compound **9b** with 4-nitrophenyl substituent and compound **9c** with 4-bromophenyl substituent and **9g** with 4-chlorophenyl as substituent on trizole rings displayed efficient antifungal activity. Compounds **9n** and **9o** with 2-phenylcarbonylchloride and 4-phenylcarbonyl chloride substituents on triazole ring showed the good antifungal activity.

TABLE-4
ANTIFUNGAL ASSAY DATA OF SYNTHESIZED COMPOUNDS (**9a-o**)

Compound	Mean zone inhibition (MZI)[a] in 100 μg/mL			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. mentagrophytes*
9a	17	19	21	22
9b	25	28	26	25
9c	25	28	25	26
9d	23	25	24	25
9e	26	27	26	26
9f	17	19	23	21
9g	24	27	25	24
9h	20	23	21	22
9i	22	20	23	22
9j	21	19	20	21
9k	20	21	17	19
9l	21	19	18	21
9m	26	29	26	25
9n	25	27	26	25
9o	25	26	27	26
Amphotericin B	26	29	27	27

Amphotericin B (100 μg/disc) was used as positive reference and compounds **9a-o** derivatives (100 μg/disc). [a]Values are mean (n = 3)

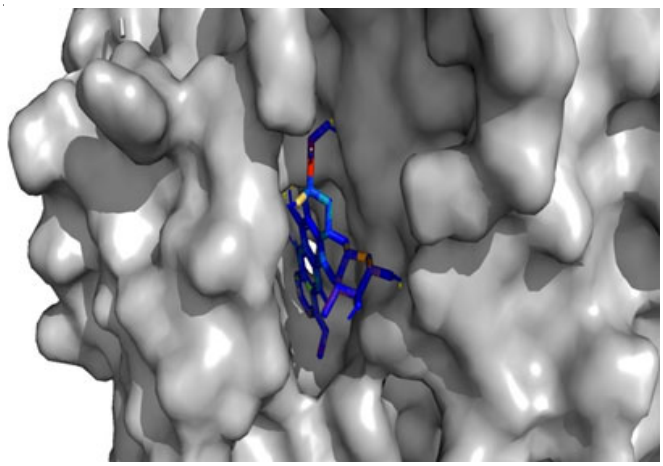
Molecular modeling assays: In this study, the molecular docking investigations were conducted on the newly developed derivatives **9a-o**, in conjunction with the standard drug doxorubicin, targeting the EGFR kinase protein (PDB ID: 3W33).

TABLE-3
ANTIBACTERIAL ASSAY DATA OF SYNTHESIZED COMPOUNDS (**9a-o**)

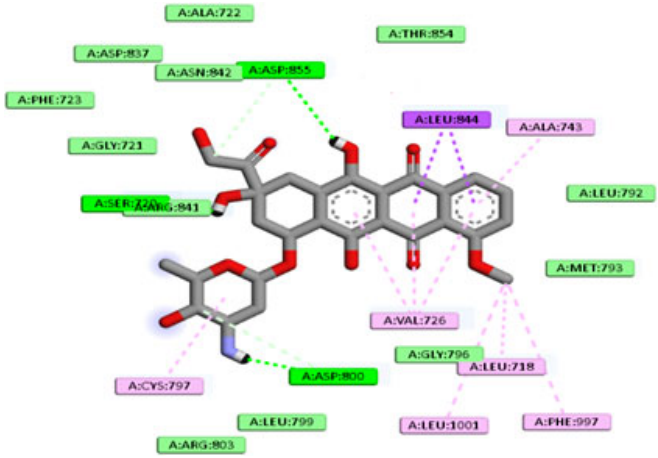
Compound	Mean zone inhibition (MZI)[a] in 100 μg/mL					
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
9a	26	26	23	25	23	21
9b	31	29	31	26	26	27
9c	30	28	31	26	26	28
9d	21	19	17	19	21	17
9e	30	27	31	26	26	27
9f	15	19	23	21	19	21
9g	17	21	19	21	23	19
9h	31	28	31	26	26	28
9i	31	30	32	29	29	31
9j	23	21	19	21	19	21
9k	21	19	23	22	21	25
9l	17	19	20	18	20	21
9m	31	30	32	27	27	28
9n	30	28	30	25	26	29
9o	28	29	28	24	26	26
Streptomycin	31	30	32	27	27	29

Streptomycin (100 μg/disc) was used as positive reference andcompounds **9a-o**(100 μg/disc) ([a]Values are mean (n = 3).

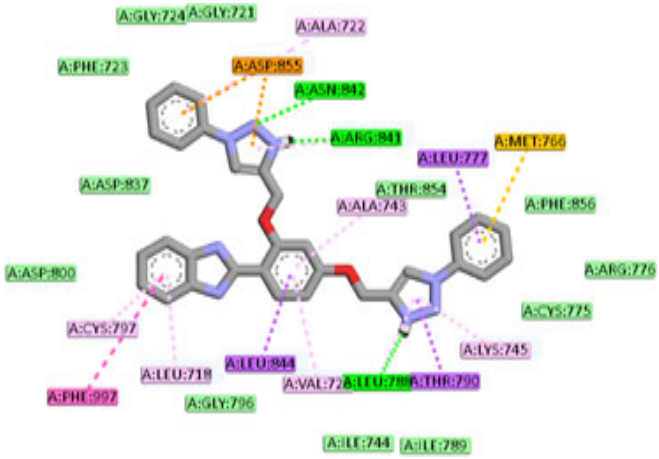
Following the successful docking of compounds **9a-o** in the conducted experiments, the results revealed significant interactions between the ligands and all receptor proteins. The newly synthesized compounds **9a-o** exhibited binding energies ranging from -10 to -12.2 kcal/mol, surpassing the binding energy of doxorubicin (-10.2 kcal/mol). Particularly, compounds **9a** and **9m** demonstrated exceptional binding energies of -12.1 kcal/mol and -12.2 kcal/mol, respectively, showcasing their superior binding affinity compared to doxorubicin. These favourable binding energies can be attributed to a range of interactions, including conventional π-alkyl, π-σ, π-cation, π-anion, C-H & H bonding's and van der Waals interactions between the triazole derivatives **9a-o** and the EGFR kinase protein (Fig. 3).



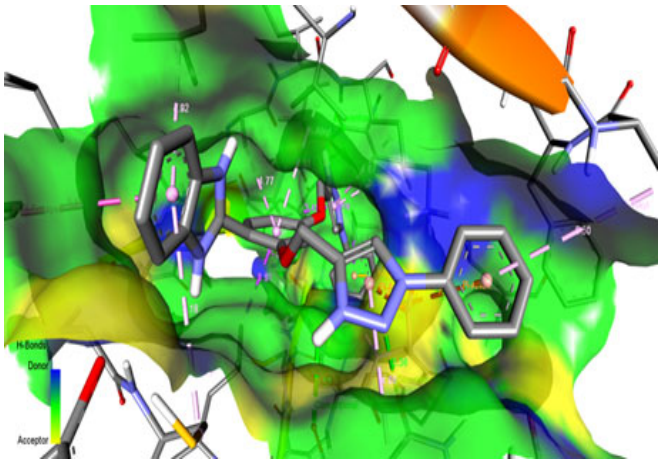
Doxo in the binding pocket of the EGFR kinase



2D Interaction image of Doxo with the EGFR kinase

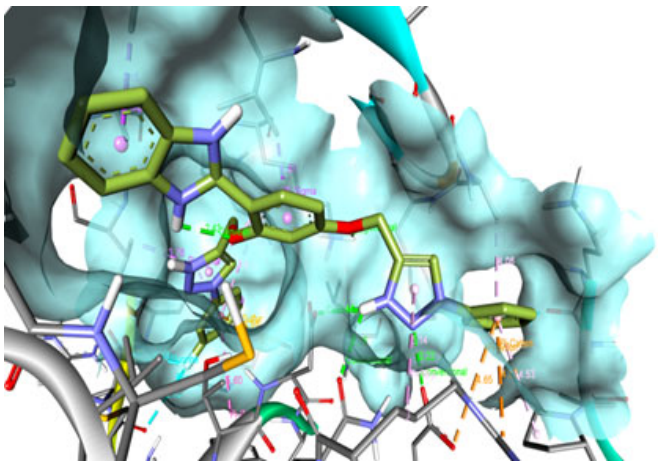


2D Interaction image of **9a** with the EGFR kinase



3D Interaction image of **9a** with the EGFR kinase

2D Interaction image of **9m** with the EGFR kinase



3D Interaction image of **9m** with EGFR kinase

Fig. 3. The 2D and 3D images of the molecular docking interaction of compounds **9a** and **9m** and doxorubicin with EGFR kinase

Conclusion

In summary, we have successfully synthesized novel derivatives of 2-[2,4-*bis*-(1-phenyl-1*H*-[1,2,3]triazol-4-ylmethoxy)-phenyl]-1*H*-benzoimidazole (**9a-o**). Their structures were confirmed using spectral analyses such as ^{1}H & ^{13}C NMR, FTIR and Mass spectral methods. The biological studies conducted on compounds **9a-o** included, cytotoxicity testing, evaluation of antimicrobial activity (both antibacterial and antifungal) and molecular modelling. Several compounds demonstrated equal or superior activity compared to standard drugs. Significantly, the presence of specific substituents on the triazole moiety, appeared to contribute significantly to their 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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