Asian Journal of Chemistry; Vol. 36, No. 11 (2024), 2683-2688

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.32710

Synthesis and Characterization of Quinoxaline Anchored *Bis*(1,2,3-triazole) Derivatives as Potent Anticancer Agents

SHARADA ETNOORI[1], NAGENDRA BABU CHILAKALA[1], RAJU BAROTHU[1], A.M.S. KRISHNA[2], VISHNU THUMMA[3] and PREMALATHA KOKKU[1,4,*]

[1]Department of Chemistry, Osmania University, Hyderabad-500007, India
[2]Aragen Life Sciences Pvt. Ltd, Hyderabad-500076, India
[3]Department of Sciences and Humanities, Matrusri Engineering College, Hyderabad-500059, India
[4]Department of Chemistry, Telangana Mahila Viswavidyalayam, Hyderabad-500095, India

*Corresponding author: E-mail: premalathasheshu@gmail.com

Received: 13 September 2024; *Accepted*: 26 October 2024; *Published online*: 30 October 2024; AJC-21808

The synthesis of 1,4-*bis*((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione derivatives (**6a-l**) was accomplished through click chemistry protocol. The synthesized compounds were screened for their *in vitro* anticancer activity against MCF-7, PC-3 and HeLa cancer cell lines by MTT assay using doxorubicin as standard reference. Compounds **6g** (4-cyano), **6l** (4-bromo)**, 6c** (4-chloro) and **6i** (4-methyl) demonstrated promising percentage growth inhibition against all the three cell lines in comparison to doxorubicin. The determined IC_{50} value proved their efficacy against all cell lines, serve as effective inhibitors of MCF-7, PC-3 and HeLa cancer cells and should be considered in the development of chemotherapeutics during the drug discovery process.

Keywords: Breast cancer, Cervical cancer, Prostate cancer, Quinoxaline, *Bis*-1,2,3-triazole.

INTRODUCTION

Quinoxaline is commonly referred to as benzopyrazine and characterized as a bioisoster of quinoline, naphthalene and benzothiophene [1]. The pharmaceutical sector has garnered significant interest due to its extensive range of biological features. For instance, these compounds have demonstrated efficacy in combating various microorganisms such as bacteria [2], fungus [3], viruses [4], leishmania [5] and tuberculosis [6]. Moreover, they have shown potential in addressing conditions such as malaria [7], cancer [8], depression [9] and neurological disorders [10], among other applications. The presence of the quinoxaline structural nucleus enables the manifestation of these actions [11]. The quinoxaline structure serves as a precursor for the synthesis of numerous novel chemicals with a wide range of uses [12]. Since quinoxaline is already a component of several medications, including echinomycin, panadipion and riboflavin (vitamin B_2), (Fig. 1) which have shown significant efficacy against a wide range of transplantable tumors and demonstrated inhibitory effects on the proliferation of Gram positive bacteria, quinoxaline is an attractive core that enables medicinal chemists to develop a variety of biologically active compounds [13].

The molecular structure of 1,2,3-triazole exhibits distinctive physico-chemical properties, such as the presence of an amide bond, the mimicry of amide bioisosteres, low basicity, metabolic stability and the capacity to function as a hydrogen-bond acceptor [14]. These properties make it advantageous for binding biomolecular targets and enhancing solubility [15]. Mubritinib and carboxyamido-triazole demonstrate significant anticancer efficacy [16,17]. The establishment of innovative anticancer treatments with exceptional efficacy is crucial given the dire state of present anticancer medications. The structural properties of these 1,2,3-triazoles allow for a wide range of substituents to be placed around the core structures. This enables the development of many new bioactive compounds [18].

Several *bis*-triazole containing compounds have been developed exhibiting enhanced characteristics in comparison to their original molecules [19]. These compounds have the ability to hinder the growth, spread and spread to other parts of tumors,

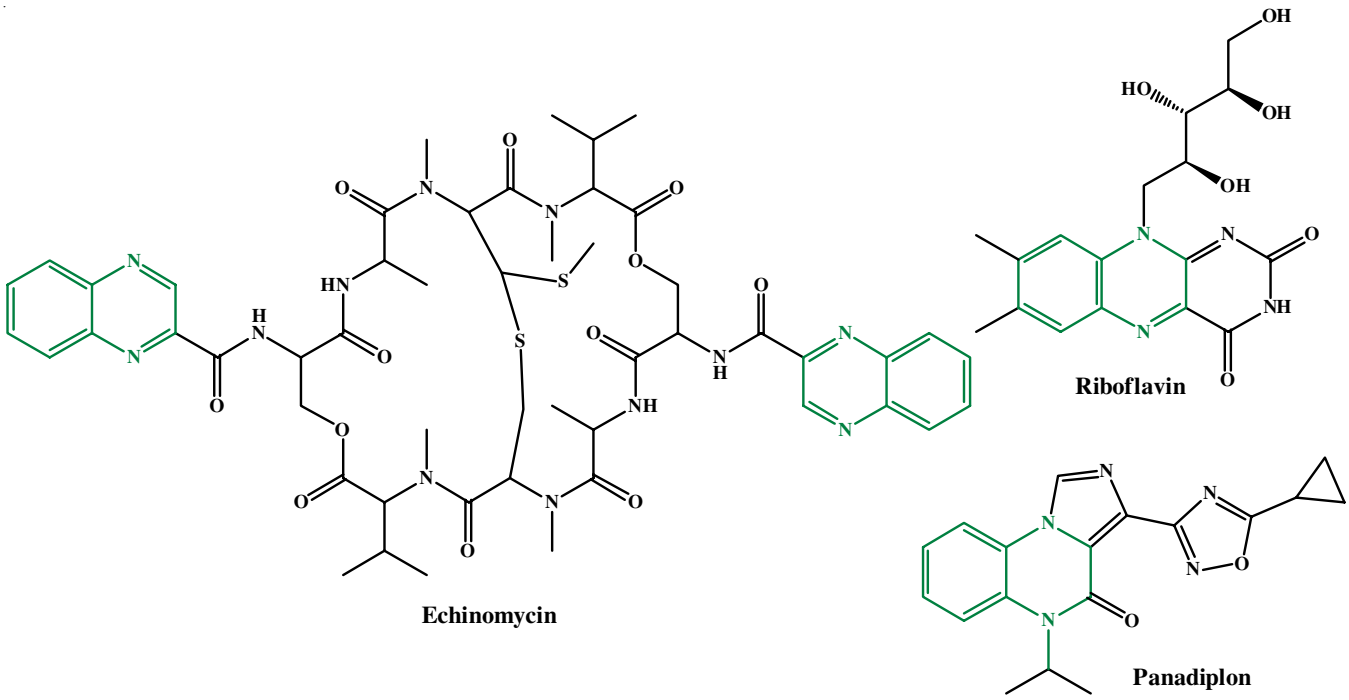


Fig. 1. Structure of some quinoxaline based commerical drugs

indicating their potential as possible candidates for anticancer treatment [20]. The discovery of innovative anticancer treatments with exceptional efficacy becomes essential given the dire state of present anticancer medications [21]. Dimerization is a valuable technique used to develop novel drug candidates that possess a wide range of biological effects, increased effectiveness and greater ability to combat drug resistance [22,23]. Some of the symmetric *bis*-1,2,3-traizoles scaffolds holds a significant inhibitory potency against α-glucosidase and acetylcholinesterase [24,25]. Based on the available literature on *bis*-1,2,3-triazoles and effective biological role of quinoxaline, aimed to synthesized *bis*-1,2,3-triazole linked quinoxaline derivatives, screened for their *in vitro* anticancer properties against MCF-7, PC-3 and HeLa cell lines, to understand binding interactions of most potent compounds further studied the molecular docking simulations on ligand-protein complex.

EXPERIMENTAL

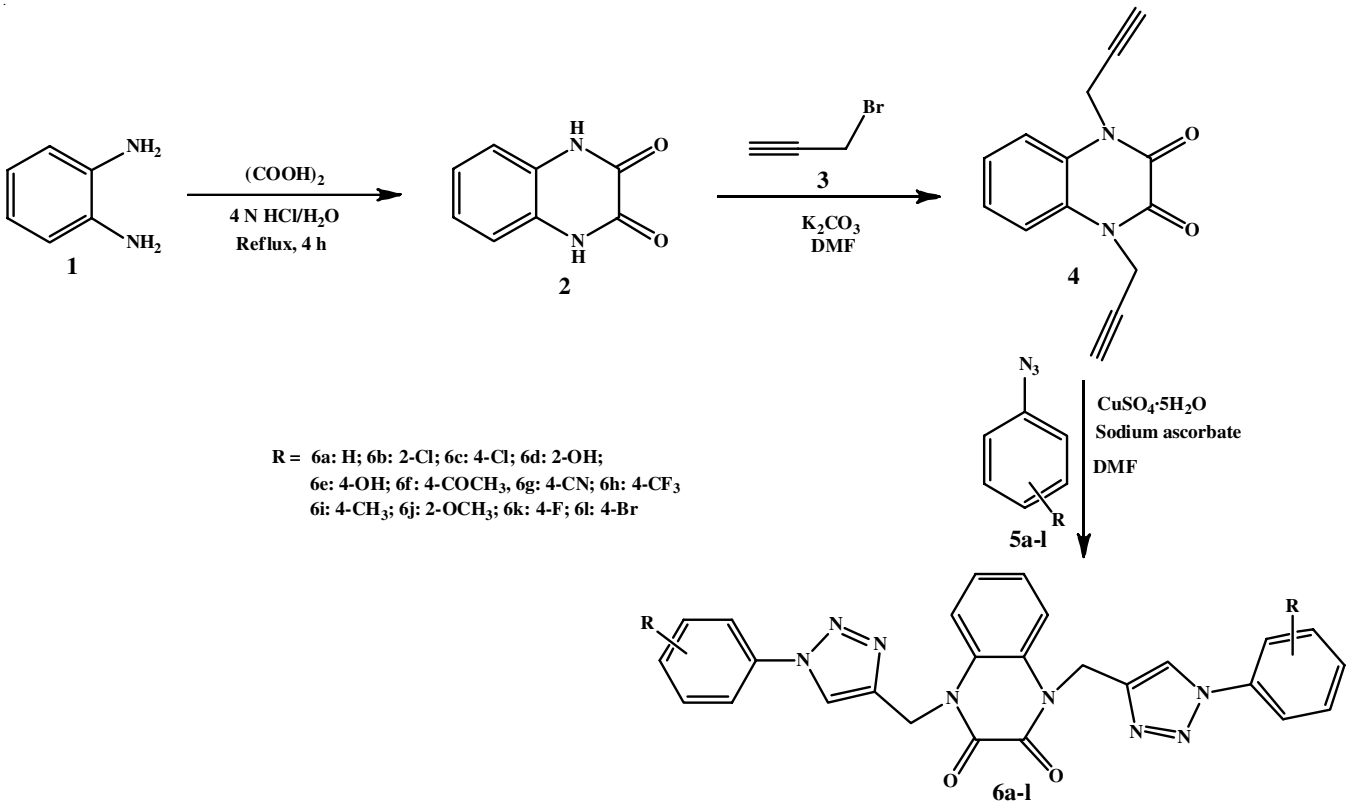
Unless otherwise specified, all the reagents and solvents were purchased commercially and utilized without additional purification. A Varian 400 MHz spectrometer was used to record NMR spectra. TMS was used as the internal standard and the solvents used were DMSO-d_6 or $CDCl_3$. A Jeol JMSD-300 spectrometer was utilized to obtain mass spectra (ESI). Using KBr pellets, IR spectra were acquired using a Perkin-Elmer 100S spectrometer. Melting points are provided uncorrected, having been obtained in open capillaries with a Buchi melting point equipment. Using hexane/ethyl acetate as the eluent, thin-layer chromatography was used to monitor the purity and progress of the reaction on aluminum sheets coated with F_{254} silica gel. A UV-visible spectrometer was used to perform UV-vis spectral studies. An automatic elemental analyzer, the Carlo Erba EA 1108, was used to do elemental analyses.

Synthesis of 1,4-dihydroquinoxaline-2,3-dione (2): Charged *o*-phenylenediamine (**1**, 1 equiv.), oxalic acid dihydrate (1.5 equiv.) and 4 N HCl (5.3 mL) into a clean, dry 250 mL round-bottom flask at room temperature. Stirred the mixture at reflux temperature for 4-5 h. Checked completion of reaction with TLC. Cooled the reaction mixture to 30 ºC, then filtered and washed the white solid product with ethyl acetate to obtain 1,4-dihydroquinoxaline-2,3-dione (**2**) [26].

Synthesis of 1,4-di(prop-2-yn-1-yl)-1,4-dihydroquinoxaline-2,3-dione (4): To a solution of 1,4-dihydroquinoxaline-2,3-dione (**2**, 1 equiv.) in DMF (20 mL), added K_2CO_3 and propargyl bromide (**3**, 2.5 equiv.) at room temperature under nitrogen gas atmosphere and stirred the reaction mixture for 3-4 h. After TLC checked, quenched the reaction with crushed ice, stirred for 5 min and then collected the obtained solid by filtration.

General procedure for the synthesis of 1,4-*bis*((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione derivatives (6a-l): To a individual solution of 1,4-di-(prop-2-yn-1-yl)-1,4-dihydroquinoxaline-2,3-dione (**4**, 1 equiv.) in DMF (20 mL), added $CuSO_4{\cdot}5H_2O$, sodium ascorbate and substituted aromatic azides (**5a-l**, 2.2 equiv.) at 30 ºC under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 4-5 h and quenched with crushed ice. The obtained solid was filtered and purified by column chromatography using ethyl acetate:pet. ether (1:3) as eluents to obtain title compounds **6a-l** in good yields (**Scheme-I**).

1,4-*Bis*((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione (6a): Off-white solid; yield: 80%; m.p.: 221-223 ºC; IR (KBr, ν_{max}, cm^{-1}): 3165 (C=C-H), 2930 (C-C-H), 1718 (CO), 1499 (N-CH_2), 810 (C-H, Ar), 706 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 9.21 (s, CH-triazole, 2H), 8.48-8.46 (m, 2H), 8.27-8.25 (m, 2H), 8.02



Scheme-I: Synthesis of 1,4-*bis*((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione derivatives (**6a-l**)

(d, *J* = 8.0 Hz, 4H), 7.86-7.84 (m, 4H), 7.45-7.21 (m, 2H), 5.45 (s, 4H, $2CH_2$); ^{13}C NMR (DMSO, 101 MHz) δ ppm: 192.4 (CO), 159.7 (=C= triazole), 157.5, 145.7, 142.3, 131.6, 128.6, 121.5, 119.7, 116.2 (CH-triazole), 60.9 (CH_2). ESI-MS: *m/z* 546.15 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{26}H_{20}N_8O_2$: C, 65.54 (65.49); H, 4.23 (4.19); N, 23.52 (23.47).

1,4-*Bis*((1-(2-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1,4-dihydroquinoxaline-2,3-dione (6b): Pale yellow solid; yield: 76%; m.p.: 230-232 ºC; IR (KBr, ν_{max}, cm^{-1}): 3155 (C=C-H), 2950 (C-C-H), 1727 (CO), 1433 (N-CH_2), 854 (C-H, Ar), 721 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 9.03 (s, CH-triazole, 2H), 8.76 (d, *J* = 8.0 Hz, 2H), 8.76-7.98 (m, 2H), 7.96-7.84 (m, 2H), 7.80-7.60 (m, 4H), 7.45 (d, *J* = 8.0 Hz, 2H), 5.41-5.43 (m, 4H, $2CH_2$); ^{13}C NMR (DMSO, 101 Hz) δ ppm: 185.3 (CO), 159.9 (=C= triazole), 145.8, 142.9, 142.4, 136.4, 131.7, 129.9, 128.8, 122.1, 120.1, 119.1 (CH-triazole), 64.7 (CH_2). ESI-MS: *m/z* 475.5 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{26}H_{18}Cl_2N_8O_2$: C, 57.26 (57.21); H, 3.33 (3.29); N, 20.55 (20.52).

1,4-*Bis*((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1,4-dihydroquinoxaline-2,3-dione (6c): Pale yellow solid; yield: 81%; m.p.: 224-226 ºC; IR (KBr, ν_{max}, cm^{-1}): 3200 (C=C-H) 3096 (C-C-H), 1686 (CO), 1430 (N-CH_2), 874 (C-H, Ar), 792 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 9.03 (s, CH-triazole, 2H), 8.02 (d, *J* = 4.0 Hz, 2H), 7.98-7.96 (m, 4H), 7.85 (d, *J* = 8.0 Hz, 4H), 7.70-7.68 (m, 2H), 7.46-7.43 (m, 4H), 5.41 (s, 4H, $2CH_2$); ^{13}C NMR (DMSO, 101 Hz) δ ppm: 192.7 (CO), 152.7 (=C= triazole), 148.6, 143.1, 135.3, 129.7, 121.5, 120.8, 119.6, 118.1 (CH-triazole), 62.5 (CH_2). ESI-MS: *m/z* 475.5 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{26}H_{18}Cl_2N_8O_2$: C, 57.26 (57.20); H, 3.33 (3.27); N, 20.55 (20.51).

1,4-*Bis*((1-(2-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1,4-dihydroquinoxaline-2,3-dione (6d): Pale green solid; yield: 77%; m.p.: 238-240 ºC; IR (KBr, ν_{max}, cm^{-1}): 3357 (OH), 3066 (C=C-H, *str.*) 2979 (C-C-H *str.*), 1765 (CO), 1455 (N-CH_2, *str.*), 807 (C-H, Ar), 739 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm 10.27 (s, 1H, OH), 9.17-9.18 (m, CH-triazole, 2H), 8.42 (d, *J* = 8.0 Hz, 2H), 8.21 (d, *J* = 8.0 Hz, 2H), 8.09-8.07 (m, 2H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.77-7.71 (m, 4H), 5.50 (s, 2H, CH_2), 5.45 (s, 2H, CH_2). ^{13}C NMR (DMSO, 101 Hz) δ ppm: 189.4 (CO), 149.9 (=C= triazole), 143.0, 140.8, 139.2, 135.2, 133.1, 129.8, 124.0, 123.4, 122.0, 121.9 (CH-triazole), 61.8 (CH_2). ESI-MS: *m/z* 509.5 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{26}H_{20}N_8O_4$: C, 61.41 (61.38); H, 3.96 (3.90); N, 22.04 (22.01).

1,4-*Bis*((1-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1,4-dihydroquinoxaline-2,3-dione (6e): Pale brown solid; yield: 74%; m.p.: 220-222 ºC; IR (KBr, ν_{max}, cm^{-1}): 3352 (OH), 3060 (C=C-H) 2972 (C-C-H), 1749 (CO), 1450 (N-CH_2), 815 (C-H, Ar), 745 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm 9.97 (s, 2H, OH), 8.81 (s, CH-triazole, 2H), 8.00 (s, 4H), 7.85 (d, *J* = 8.0 Hz, 4H), 7.67-7.68 (d, *J* = 8.0 Hz, 2H), 6.94-6.92 (d, *J* = 8.0 Hz, 2H), 5.38 (s, 4H, $2CH_2$); ^{13}C NMR (DMSO, 101 MHz) δ ppm 189.0 (CO), 157.7 (=C= triazole), 153.8, 128.5, 121.9, 121.8, 121.6, 121.5, 115.9, 110.5 (CH-triazole), 64.6 (CH_2). ESI-MS: *m/z* 509.5 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{26}H_{20}N_8O_4$: C, 61.41 (61.37); H, 3.96 (3.91); N, 22.04 (22.00).

Dimethyl 4,4'-(((2,3-dioxo-2,3-dihydroquinoxaline-1,4-diyl)*bis*(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))diben-

zoate (6f): Off-white solid; yield: 79%; m.p.: 235-237 ºC; IR (KBr, ν_{max}, cm^{-1}): 3055 (C=C-H, *str.*) 2967 (C-C-H *str.*), 1742 (CO), 1439 (N-CH_2, *str.*), 822 (C-H, Ar), 740 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 8.64 (s, CH-triazole, 2H), 7.65 (d, *J* = 8.0 Hz, 4H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 4H), 7.15 (t, *J* = 7.6 Hz, 2H), 5.39 (s, 4H, 2CH_2), 3.85 (s, 6H, 2OCH_3); ^{13}C NMR (DMSO, 101 Hz) δ ppm: 190.4 (CH_3CO), 187.6 (CO), 151.5 (=C= triazole), 139.2, 130.8, 125.7, 124.1, 122.1, 120.8, 112.9 (CH-triazole), 61.8 (CH_2), 56.1 (CH_3). ESI-MS: *m/z* 562 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{30}H_{24}N_8O_6$: C, 60.81 (60.77); H, 4.08 (4.05); N, 18.91 (18.88).

4,4'-(((2,3-Dioxo-2,3-dihydroquinoxaline-1,4-diyl)*bis*-(methylene))*bis*(1H-1,2,3-triazole-4,1-diyl))dibenzonitrile (6g): Off-white solid; yield: 68%; m.p.: 243-245 ºC; IR (KBr, ν_{max}, cm^{-1}): 3050 (C=C-H), 2959 (C-C-H), 1737 (CO), 1433 (N-CH_2), 1251 (CN), 822 (C-H, Ar), 740 (C-H, aliph.). ^{1}H NMR (DMSO, 400 MHz) δ ppm: 9.03 (s, CH-triazole, 2H), 8.02 (s, 4H), 7.92-7.90 (m, 2H), 7.86-7.81 (m, 4H), 7.45 (d, *J* = 8.0 Hz, 2H), 5.41 (s, 4H, 2CH_2); ^{13}C NMR (DMSO, 101 MHz) δ ppm: 196.2 (CO), 165.6 (=C= triazole), 159.5, 150.5, 132.5, 131.2, 130.3, 122.8, 120.0, 114.0 (CH-triazole), 61.8 (CH_2), 56.10 (CN). ESI-MS: *m/z* 527.16 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{28}H_{18}N_{10}O_2$: 63.87 (63.83); H, 3.45 (3.41); N, 26.60 (26.55).

1,4-*Bis*((1-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione (6h): Green yellow solid; yield: 73%; m.p.: 247-249 ºC; IR (KBr, ν_{max}, cm^{-1}): 3043 (C=C-H, *str.*) 2942 (C-C-H *str.*), 1726 (CO), 1430 (N-CH_2, *str.*), 1262 (CF_3), 817 (C-H, Ar), 731 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 8.89 (s, CH-triazole, 2H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.83-7.81 (m, 8H), 7.15 (d, *J* = 8.0 Hz, 2H), 5.39 (s, 4H, 2CH_2); ^{13}C NMR (DMSO, 101 MHz) δ ppm 190.6 (CO), 159.2 (=C= triazole), 151.8, 147.1, 129.6, 123. 6 (CF_3), 122.2, 121.7, 114.7, 113.2 (CH-triazole), 61.5(CH_2). ESI-MS: *m/z* 613 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{28}H_{18}F_6N_8O_2$: 54.91 (54.88); H, 2.96 (2.92); N, 18.29 (18.25).

1,4-*Bis*((1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione (6i): White solid; yield: 75%; m.p.: 215-217 ºC; IR (KBr, ν_{max}, cm^{-1}): 3027 (C=C-H, *str.*), 2928 (C-C-H *str.*), 1722 (CO), 1426 (N-CH_2, *str.*), 824 (C-H, Ar), 735 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 9.15 (s, CH-triazole, 2H), 8.19-8.11 (m, 4H), 8.02-7.92 (m, 2H), 7.86-7.84 (d, *J* = 8.0 Hz, 4H), 7.45 (d, *J* = 8.0 Hz, 2H), 5.43 (s, 4H, 2CH_2), 2.73 (s, 6H, 2CH_3); ^{13}C NMR (DMSO, 101 MHz) δ ppm: 191.8 (CO), 145.7 (=C= triazole), 143.1, 142.4, 135.2, 133.0, 131.7, 129.8, 122.1, 119.0 (CH-triazole), 60.9 (CH_2), 20.68 (CH_3). ESI-MS: *m/z* 505 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{28}H_{24}N_8O_2$: 66.65 (66.61); H, 4.79 (4.75); N, 22.21 (22.18).

1,4-*Bis*((1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1,4-dihydroquinoxaline-2,3-dione (6j): Off-white solid; yield: 78%; m.p.: 242-244 ºC; IR (KBr, ν_{max}, cm^{-1}): 3033 (C=C-H, *str.*), 2941 (C-C-H *str.*), 1731 (CO), 1539 (OCH_3), 1420 (N-CH_2, *str.*), 838 (C-H, Ar), 732 (C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 8.67 (s, CH-triazole, 2H), 7.86-8.05 (m, 6H), 7.49 (d, *J* = 8. 0Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 5.41 (s, 4H, 2CH_2), 3.89 (s, 6H, 2OCH_3); ^{13}C NMR (DMSO, 101 MHz) δ ppm: 191.5 (CO), 159.8 (=C= triazole), 145.7, 142.8, 142.4, 138.4, 134.2, 131.7, 130.2, 121.9, 119.9, 119.0, (CH-triazole), 61.7 (CH_2), 43.9 (OCH_3). ESI-MS: *m/z* 537 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{28}H_{24}N_8O_4$: 62.68 (62.64); H, 4.51 (4.46); N, 20.88 (20.85).

1,4-*Bis*((1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1,4-dihydroquinoxaline-2,3-dione (6k): Brown solid; yield: 82%; m.p.: 228-230 ºC; IR (KBr, ν_{max}, cm^{-1}) 3038 (C=C-H, *str.*) 2934 (C-C-H *str.*), 1728 (CO), 1427 (N-CH_2, *str.*), 841 (C-H, Ar), 737(C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 8.94 (s, CH-triazole, 2H), 8.00 (d, *J* = 8.0 Hz, 2H), 7.86-7.78 (m, 6H), 7.46-7.40 (m, 4H), 5.40 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 101 MHz) δ ppm: 185.7 (CO), 150.0 (=C= triazole), 142.9, 140.8, 129.9, 128.9, 124.1, 123.3, 122.1, 120.2 (CH-triazole), 61.9 (CH_2). ESI-MS: *m/z* 513 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{26}H_{18}N_8O_2F_2$: 60.94 (60.90); H, 3.54 (3.51); N, 21.87 (21.84).

1,4-*Bis*((1-(4-bromophenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-1,4-dihydroquinoxaline-2,3-dione (6l): Off-white solid; Yield: 74%; m.p.: 218-220 ºC; IR (KBr, ν_{max}, cm^{-1}): 3034 (C=C-H, *str.*), 2930 (C-C-H *str.*), 1737 (CO), 1421 (N-CH_2, *str.*), 835 (C-H, Ar), 732(C-H, aliph.); ^{1}H NMR (DMSO, 400 MHz) δ ppm: 8.43-8.39 (m, 2H), 8.16 (d, *J* = 8.0 Hz, 4H), 7.98-7.96 (m, 2H), 7.68-7.65 (m, 2H), 7.45 (d, *J* = 8.0 Hz, 4H), 5.58 (s, 2CH_2); ^{13}C NMR (DMSO, 101 MHz) δ ppm: 185.3 (CO), 159.8 (=C= triazole), 145.7, 143.1, 142.4, 140.8, 135.6, 132.8, 131.7, 122.1, 119.0 (CH-triazole), 62.3 (CH_2). ESI-MS: *m/z* 635 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{26}H_{18}N_8O_2Br_2$: 49.23 (49.19); H, 2.86 (2.82); N, 17.67 (17.63).

MTT assay procedure: Cells were seeded at 5×10^4 cells per well in 96-well plates and cultured to 90-95% confluences. Solutions of the synthesized derivatives were added to the wells, which were then incubated with 100 µL of media for 48 h. Following incubation, 20 µL of MTT solution (5 mg/mL) was added and incubated for an additional 4 h. After removing the medium, 200 µL of DMSO was added to dissolve the formazan crystals. The optical densities were measured at 490 nm and 630 nm using a microplate reader and cell growth inhibition was calculated using the appropriate formula [27].

$$\text{Inhibition (\%)} = \left(1 - \frac{(\text{Sample group OD}_{490} - \text{Sample group OD}_{630})}{(\text{Conrol group OD}_{490} - \text{Control group OD}_{630})}\right) \times 100$$

GraphPad Prism was used to determine the IC_{50} values, with at least three independent standard deviations reported.

RESULTS AND DISCUSSION

In **Scheme-I**, the synthetic route of 1,4-*bis*((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione derivatives (**6a-l**) is described. The initial step involved the cyclization reaction of *o*-phenylenediamine (**1**) with oxalic acid dehydrate by treatment of 4 N HCl to obtain 1,4-dihydroquinoxaline-2,3-dione (**2**). The NH protons on compound **2** were treated with propargyl bromide (**3**) in presence of K_2CO_3 get 1,4-di(prop-2-yn-1-yl)-1,4-dihydroquinoxaline-2,3-dione (**4**). Finally, the terminal alkynes of compound **4** were allowed to undergo copper catalyzed click reaction by addition of substi-

tuted aromatic azides **5a-l** in presence of catalytic amounts of copper sulphate pentahydrate and sodium ascorbate to obtain 1,4-*bis*((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydro-quinoxaline-2,3-dione derivatives (**6a-l**). The structure of title compounds was elucidated by analyzing NMR, Mass and IR spectroscopy data. For example, compound **6a**, IR spectrum confirmed the C=O absorption stretching frequency peak near 1718 cm^{-1}. The ^1H NMR spectrum of compound **6a**, presented a singlet at δ 9.21 ppm corresponding to two triazole ring protons. Another singlet at δ 5.45 ppm confirmed the two -CH_2- groups planked between quinoxaline and triazole rings. The other 16 protons were integrated in aromatic region ranging between δ 8.48 ppm and δ 7.21 ppm. In ^{13}C NMR of **6a**, the C=O carbons signal appeared at δ 192.4 ppm, the -CH_2- carbons signal at δ 60.9 ppm. Finally, the ESI-MS spectrum reported it *m/z* 546.15 $[M+H]^+$ peak.

Cytotoxicity: The *in vitro* anticancer activity of quinoxaline based *bis*-1,2,3-triazole derivatives screened against human breast cancer (MCF-7), human prostate cancer (PC-3) and human cervical cancer (HeLa) cell lines at concentrations of 5 µM and 10 µM, Doxorubicin was used as standard reference and the growth inhibitory activities of compounds **6a-l** against MCF-7 cell presented in Fig. 2, PC-3 cell line in Fig. 3 and HeLa cell line in Fig. 4. Generally, compounds **6d**, **6g**, **6h**, **6i** and **6k** exhibited high inhibitory activity, comparable to or exceeding that of doxorubicin, particularly at higher concentration (10 µM). The findings highlight the promising capacity of these compounds to serve as potent inhibitors against the examined cancer cell lines.

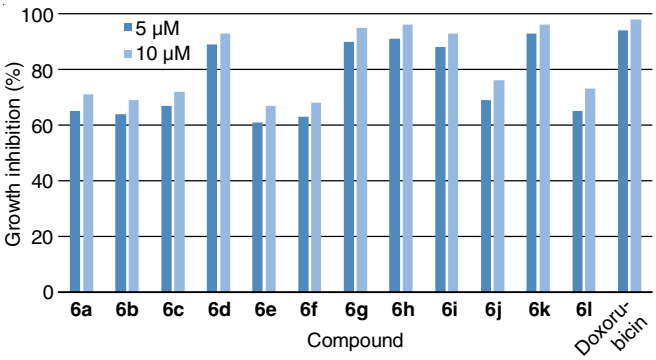


Fig. 2. Percentage of growth inhibition of compound **6a-l** against MCF-7 cell line

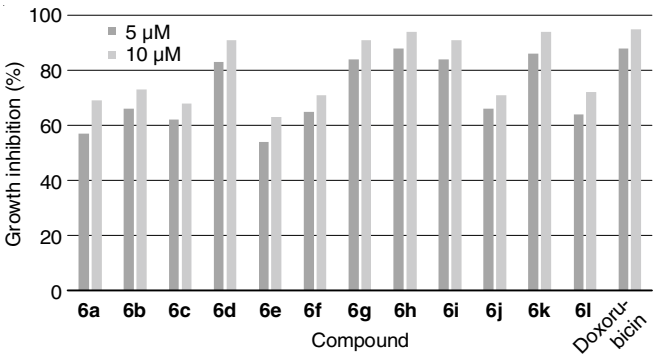


Fig. 3. Percentage of growth inhibition of compounds **6a-l** against PC-3 cell line

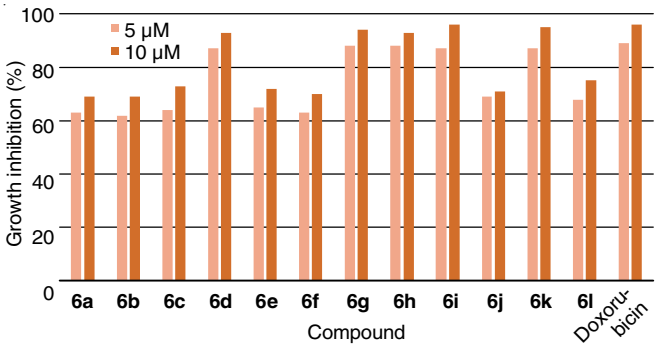


Fig. 4. Percentage of growth inhibition of compounds **6a-l** against HeLa cell line

Based on these results, the IC_{50} value (Table-1) of selected compounds (**6b**, **6c**, **6d**, **6f**, **6g**, **6h**, **6i** and **6l**) among MCF-7, PC-3 and HeLa cancer cell lines were determined. Interestingly, compounds **6g** with 4-cyano substituent and compound **6l** with 4-bromo substituent presented potent activity against MCF-7 cell with IC_{50} value of 2.62 ± 0.06 µM and 2.71 ± 0.02 µM, compared to doxorubicin IC_{50} value of 2.82 ± 0.03 µM against MCF-7 cells. The same compounds presented potent activity against PC-3 (**6g** = 3.70 ± 0.06 µM, **6l** = 3.73 ± 0.05 µM) and HeLa (**6g** = 3.21 ± 0.07 µM, **6l** = 3.09 ± 0.10 µM) cell line. Additionally, the best activity was reported by compounds **6c** and **6i** against all the three cell lines. The IC_{50} value of 4-chloro substituent compound **6c** presented as 3.12 ± 0.05 µM, 3.98 ± 0.12 µM and 3.56 ± 0.06 µM against MCF-7, PC-3 and HeLa cell lines, respectively. Similarly, the IC_{50} value of methyl substituted compound **6i** displayed as 3.06 ± 0.11 µM (MCF-7), 3.96 ± 0.02 µM (PC-3) and 3.39 ± 0.08 µM (HeLa). The other compounds IC_{50} value seen to be good to moderate. These data elucidate the comparative efficacy of these compounds as prospective anticancer pharmaceuticals across various cell types. The activity of these compounds can be ascribed to the presence of pharmacophores, namely quinoxaline and two triazole rings within the core structure and attached electron withdrawing or electron donating groups such as chloro, bromo, methyl functions to triazole linked phenyl group.

TABLE-1
IC_{50} VALUES OF SELECTED COMPOUNDS AGAINST MCF-7, PC-3 AND HeLa CELL LINES

Entry	IC_{50} (µM ± SEM)		
	MCF-7	PC-3	HeLa
6b	6.84 ± 0.07	6.39 ± 0.09	6.01 ± 0.11
6c	3.12 ± 0.05	3.98 ± 0.12	3.56 ± 0.06
6d	4.37 ± 0.07	4.61 ± 0.04	4.89 ± 0.08
6f	6.68 ± 0.09	8.76 ± 0.08	6.98 ± 0.12
6g	2.62 ± 0.06	3.70 ± 0.06	3.21 ± 0.07
6h	4.88 ± 0.12	4.32 ± 0.07	4.56 ± 0.12
6i	3.06 ± 0.11	3.96 ± 0.02	3.39 ± 0.08
6l	2.71 ± 0.02	3.73 ± 0.05	3.09 ± 0.10
Doxorubicin	2.82 ± 0.03	3.86 ± 0.02	3.31 ± 0.07

Conclusion

The synthesis of 1,4-*bis*((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-1,4-dihydro quinoxaline-2,3-dione derivatives (**6a-**

l) accomplished successfully *via* click chemistry protocol. These compounds were screened for their *in vitro* anticancer activity against MCF-7, PC-3 and HeLa cancer cell lines. The compounds **6g**, **6l**, **6c** and **6i** demonstrated promising percentage growth inhibition against all the three cell lines. The determined IC_{50} value proved their efficacy against all cell lines when compared to standard reference doxorubicin. Therefore, the quinoxaline appended *bis*-1,2,3-triazole analogues reported may serve as effective inhibitors of MCF-7, PC-3 and HeLa cancer cells and should be considered in the development of chemotherapeutics during the drug discovery process.

ACKNOWLEDGEMENTS

The authors thank the Head, Department of Chemistry, Osmania University, Hyderabad, India for providing research facilities.

CONFLICT OF INTEREST

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

REFERENCES

1. J. Jampilek, *Curr. Med. Chem.*, **21**, 4347 (2014); https://doi.org/10.2174/0929867321666141011194825
2. A.K. Parhi, Y. Zhang, K.W. Saionz, P. Pradhan, M. Kaul, K. Trivedi, D.S. Pilch and E.J. LaVoie, *Bioorg. Med. Chem. Lett.*, **23**, 4968 (2013); https://doi.org/10.1016/j.bmcl.2013.06.048
3. E.A. Fayed, M.A. Ebrahim, U. Fathy, H.S. El Saeed and W.S. Khalaf, *J. Mol. Struct.*, **1267**, 133578 (2022); https://doi.org/10.1016/j.molstruc.2022.133578
4. M. Montana, V. Montero, O. Khoumeri and P. Vanelle, *Molecules*, **25**, 2784 (2020); https://doi.org/10.3390/molecules25122784
5. J. Cogo, J. Cantizani, I. Cotillo, D.P. Sangi, T. Ueda-Nakamura, B.P.D. Filho, A.G. Corrêa, J.J. Martín and C.V. Nakamura, *Bioorg. Med. Chem.*, **26**, 4065 (2018); https://doi.org/10.1016/j.bmc.2018.06.033
6. G. Pavale, P. Acharya, N. Korgavkar and M.M.V. Ramana, *Curr. Comput. Aided Drug Des.*, **18**, 414 (2022); https://doi.org/10.2174/1573409918666220804142753
7. A.C. Shekhar, P. Shanthan Rao, B. Narsaiah, A.D. Allanki and P.S. Sijwali, *Eur. J. Med. Chem.*, **77**, 280 (2014); https://doi.org/10.1016/j.ejmech.2014.03.010
8. M. Montana, F. Mathias, T. Terme and P. Vanelle, *Eur. J. Med. Chem.*, **163**, 136 (2019); https://doi.org/10.1016/j.ejmech.2018.11.059
9. R. Sarges, H.R. Howard, R.G. Browne, L.A. Lebel, P.A. Seymour and B.K. Koe, *J. Med. Chem.*, **33**, 2240 (1990); https://doi.org/10.1021/jm00170a031
10. P. Li, Q. Zhang, A.J. Robichaud, T. Lee, J. Tomesch, W. Yao, J.D. Beard, G.L. Snyder, H. Zhu, Y. Peng, J.P. Hendrick, K.E. Vanover, R.E. Davis, S. Mates and L.P. Wennogle, *J. Med. Chem.*, **57**, 2670 (2014); https://doi.org/10.1021/jm401958n
11. S. Tariq, K. Somakala and M. Amir, *Eur. J. Med. Chem.*, **143**, 542 (2018); https://doi.org/10.1016/j.ejmech.2017.11.064
12. G. Yashwantrao and S. Saha, *Org. Chem. Front.*, **8**, 2820 (2021); https://doi.org/10.1039/D0QO01575J
13. Y. Seqqat, B. Hafez, M. Lahyaoui, F. Toscano, R. Seqqat, M.T. Arias, B.E. Kartah, H. Elmsellem, Y.K. Rodi and F.O. Chahdi, *Mor. J. Chem.*, **12**, 1323 (2024); https://doi.org/10.48317/IMIST.PRSM/morjchem-v12i3.48982
14. A. Rani, G. Singh, A. Singh, U. Maqbool, G. Kaur and J. Singh, *RSC Adv.*, **10**, 5610 (2020); https://doi.org/10.1039/C9RA09510A
15. M. Nagamani, T. Vishnu, P. Jalapathi and M. Srinivas, *J. Iran. Chem. Soc.*, **19**, 1049 (2022); https://doi.org/10.1007/s13738-021-02365-y
16. O. Mignen, C. Brink, A. Enfissi, A. Nadkarni, T.J. Shuttleworth, D.R. Giovannucci and T. Capiod, *J. Cell Sci.*, **118**, 5615 (2005); https://doi.org/10.1242/jcs.02663
17. M. Suzuki, K. Uchibori, T. Oh-hara, Y. Nomura, R. Suzuki, A. Takemoto, M. Araki, S. Matsumoto, Y. Sagae, M. Kukimoto-Niino, Y. Kawase, M. Shirouzu, Y. Okuno, M. Nishio, N. Fujita and R. Katayama, *NPJ Precis. Oncol.*, **8**, 46 (2024); https://doi.org/10.1038/s41698-024-00542-9
18. M. Çesme, S. Onur, E. Aksakal and F. Tümer, *J. Mol. Liq.*, **409**, 125501 (2024); https://doi.org/10.1016/j.molliq.2024.125501
19. M.S. Asgari, M. Mohammadi-Khanaposhtani, M. Kiani, P.R. Ranjbar, E. Zabihi, R. Pourbagher, R. Rahimi, M.A. Faramarzi, B. Larijani, M. Biglar, M. Mahdavi, H. Hamedifar and M.H. Hajimiri, *Bioorg. Chem.*, **92**, 103206 (2019); https://doi.org/10.1016/j.bioorg.2019.103206
20. H. Elamari, R. Slimi, G.G. Chabot, L. Quentin, D. Scherman and C. Girard, *Eur. J. Med. Chem.*, **60**, 360 (2013); https://doi.org/10.1016/j.ejmech.2012.12.025
21. V. Thumma, V. Mallikanti, R. Matta, R. Dharavath and P. Jalapathi, *RSC Med. Chem.*, **15**, 1283 (2024); https://doi.org/10.1039/D3MD00479A
22. A. Paquin, C. Reyes-Moreno and G. Bérubé, *Molecules*, **26**, 2340 (2021); https://doi.org/10.3390/molecules26082340
23. S. Aitha, V. Thumma, S. Ambala, R. Matta, S. Panga and J. Pochampally, *ChemistrySelect*, **8**, e202300405 (2023); https://doi.org/10.1002/slct.202300405
24. G. Yaku, D. Ramulu, V. Thumma, A. Paluri and R. Dharavath, *ChemistrySelect*, **8**, e202300255 (2023); https://doi.org/10.1002/slct.202300255
25. F. Celik, Y. Unver, B. Barut, A. Ozel and K. Sancak, *Med. Chem.*, **14**, 230 (2018); https://doi.org/10.2174/1573406413666171120165226
26. J.F.W. Keana, S.M. Kher, S.X. Cai, C.M. Dinsmore, J. Guastella, J.-C. Huang, A.G. Glenn, V. Ilyin and Y. Lu, *J. Med. Chem.*, **38**, 4367 (1995); https://doi.org/10.1021/jm00022a003
27. V. Mallikanti, V. Thumma, K.C. Veeranki, S. Gali and J. Pochampally, *Chemistry Select*, **7**, e202204020 (2022); https://doi.org/10.1002/slct.202204020