Asian Journal of Chemistry; Vol. 37, No. 11 (2025), 2819-2825
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2025.34518

Synthesis and Biological Evaluation of Quinoline-1,2,3-Triazole-Azo Hybrids as Potential EGFR Inhibitors: *in vitro* and *in silico* Approaches

RAJU MANNOORI[1], SIDDHARTHA MARUPATI[2], ASHOK KUMAR BAPURAM[3], SAIDI REDDY MODUGU[1] and RAVINDER MANCHAL[1,*]

[1]Department of Chemistry, Chaitanya (Deemed to be University), Hyderabad-500075, India
[2]Department of Chemistry, Vardhaman College of Engineering (Autonomous), Hyderabad-501218, India
[3]Department of Chemistry, Guru Nanak Institutions Technical Campus (GNITC B-Tech -I), Ibrahimpatnam, Hyderabad-501506, India

*Corresponding author: E-mail: visitravi76@gmail.com

Received: 23 July 2025 *Accepted*: 1 October 2025 *Published online*: 27 October 2025 AJC-22171

In this work, the synthesis and characterization of quinolime-1,2,3-triazoles (**7a-n**) *via* azocoupling, O-propargylation and copper(I) catalyzed azide alkyne cycloaddition reactions is reported. All the synthesized compounds were evaluated for *in vitro* anticancer activity against human breast cancer cell lines like MDA-MB-468, MDA-MB-231 and MCF-7 and 5-fluorouracil (5-FU) as reference drug. Among all, only five compounds **7d**, **7j**, **7k**, **7l** and **7n** exhibited greater activity than the standard drug 5-fluorouracil against three breast cancer cell lines with IC_{50} values ranging from 1.1 μM to 9.3 μM. Similarly, *in vitro* tyrosine kinase EGFR inhibition for the same compounds revealed that compound **7l** has exhibited 2.6 times more inhibitory activity with IC_{50} value of 0.15 μM compared to erlotinib. Finally, *in silico* molecular docking on EGFR shown good binding interactions with target protein (pdb id 4HJO).

Keywords: 1,2,3-Tiazole, Quinolone, Azo bond, EGFR, Docking studies, ADMET.

INTRODUCTION

Chemotherapy remains a cornerstone in cancer treatment and continues to advance with the development of novel anticancer agents. However, major limitations such as non-selective cytotoxicity, suboptimal safety profiles and insufficient therapeutic efficacy continue to hinder the clinical success of many currently available chemotherapeutic drugs [1]. One of the best alternatives for these defects is available in the form of targeted therapy, where working mechanism involves the targeting specific proteins, which will inhibit the growth and spread of the cancer cells [2]. For that purpose, epidermal growth factor receptor (EGFR) will be one of the remarkable and required targeted therapies which also given by previous reports [3]. The protein EGFR is one of the members in tyrosine kinase receptor family and will be active mediator in cell proliferation, apoptosis angiogenesis and metastatic spread [4-6].

The quinoline nucleus plays a crucial role in the development of anticancer agents, as its derivatives have shown remarkable effectiveness through various mechanisms, including the inhibition of angiogenesis, cell cycle arrest, disruption of cell migration and induction of apoptosis. Quinoline is a vital scaffold that has contributed numerous drugs, including topotecan, camptothecin and irinotecan (anticancer drugs), sparfloxacin, ciprofloxacin, gatifloxacin (antibacterial drugs); saquinavir (antiviral), dibucaine (local anesthetic), clioquinol (antifungal) and cardiotonic agents like vesnarinone, along with several anti-malarial drugs such as quinine, quinidine, chloroquine, primaquine, mefloquine and amodiaquine [7-11]. The biological relevance of quinoline and its derivatives in relation to anticancer activity has been extensively documented in various reviews articles [12-15]. Notably, the quinolone based anticancer agents such as lenvatinib, bosutinib, cabozantinib and tipifarnib are currently undergoing clinical trials (Fig. 1) [2-4].

Interestingly, 1,2,3-triazole is also widely recognized as a key scaffold in drug discovery and development due to its versatile biological activities. For example, Mahmoud *et al.* [16] synthesized a series of 1,2,3-triazole-carboximidamide derivatives that exhibited potent inhibition of EGFR, with IC_{50} values ranging from 83 to 112 nM, comparable to the reference

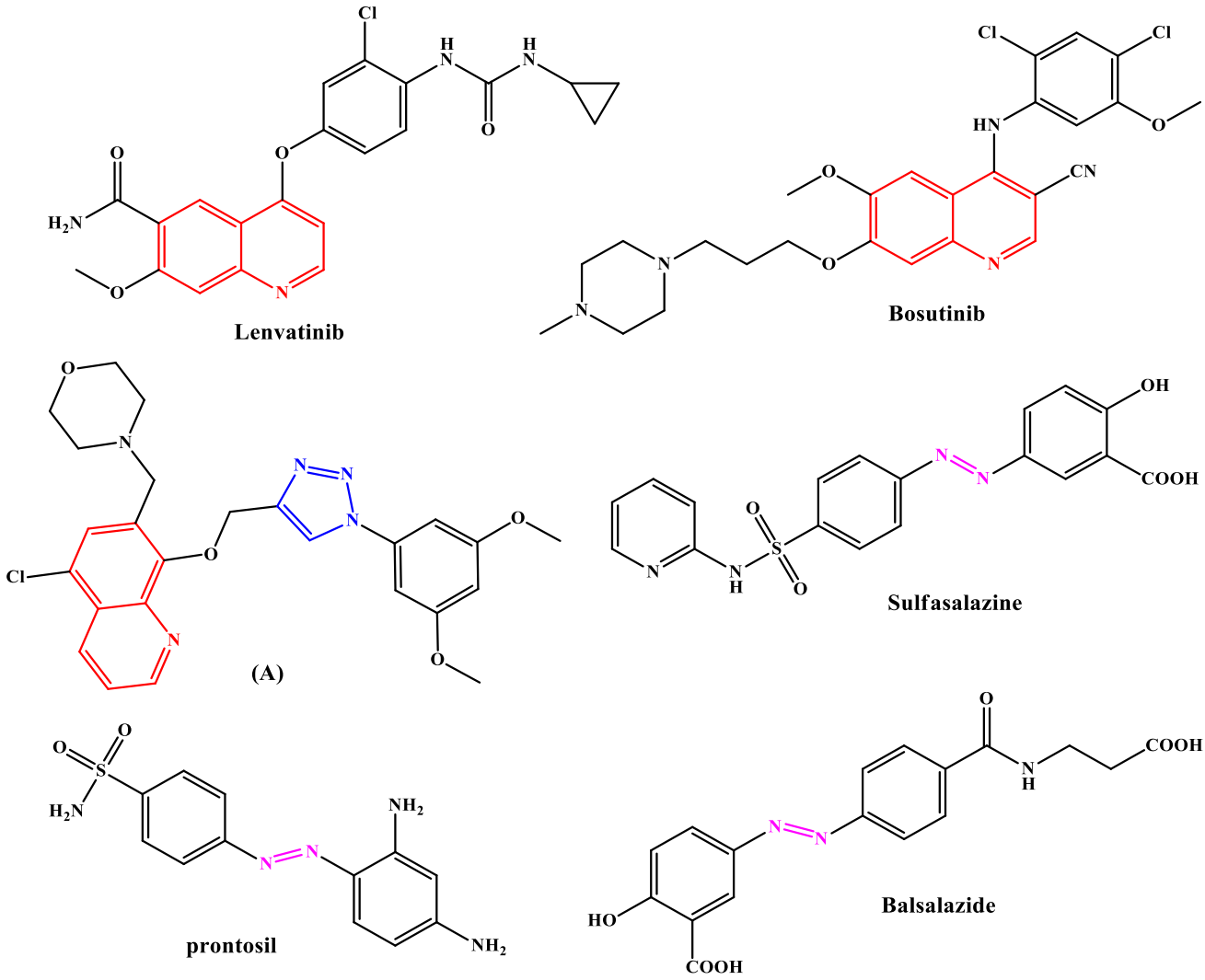


Fig. 1. Some of the biologically active compounds and drugs with quinoline, 1,2,3-triazole and azo bonds

drug erlotinib, which showed an IC_{50} of 80 nM. Similarly, Mamidala *et al*. [17] reported quinoline-morpholine coupled 1,2,3-triazole hybrids (compound A; Fig. 1), some of which demonstrated up to three-fold stronger EGFR tyrosine kinase inhibition than erlotinib, with an IC_{50} of 0.14 μM. The ability of 1,2,3-triazoles to participate in hydrogen bonding, dipole–dipole interactions, hydrophobic contacts and van der Waals forces with target proteins underscores their significance in medicinal chemistry, particularly in the development of anti-cancer agents [18,19]. On the other hand, the azo compounds, having the azo bond (-N=N-), are intriguing motifs in organic chemistry. These vibrant compounds have found extensive use as dyes in various industries including printing, food, paper, lasers, cosmetics, electronics, optics and material sciences [20]. An antibacterial drug prontosil (Fig. 1) discovery, brought azo compounds to the forefront of medicinal chemistry. This was followed by the development of additional drugs such as basalazide, phenazopyridine and sulfasalazine, solidifying the significant role of azo compounds in the pharmaceutical market [21,22].

Considering the importance of targeted therapy and the pharmacological potential of quinoline, 1,2,3-triazole and the azo scaffolds, in this work, the novel hybrid compounds were synthesized incorporating all these pharmacophores (Fig. 2). This approach leverages the benefits of pharmacophore hybridization to enhance therapeutic efficacy and biological activity [23,24].

EXPERIMENTAL

All chemicals were procured from Sigma-Aldrich, USA and used without further purification. Thin layer chromatography (TLC) was performed using Merck silica gel $60F_{254}$ precoated plates and column chromatography was carried out with silica gel in the 60-120 mesh range. Melting points of the compounds were determined using a Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 400 MHz spectrometer, while ^{13}C NMR spectra were obtained using a Bruker 100 MHz spectrometer. DMSO-d_6 was used as solvent, with TMS as reference. Electron impact (EI) mass spectra, taken at an ionizing voltage of 70 eV, were measured on a Shimadzu QP5050 A quadrupole-based mass spectrometer.

Synthesis of 5-((4-methoxyphenyl)diazenyl)quinolin-8-ol (3): Compound quinolin-8-ol (**1**, 10 mmol) dissolved in

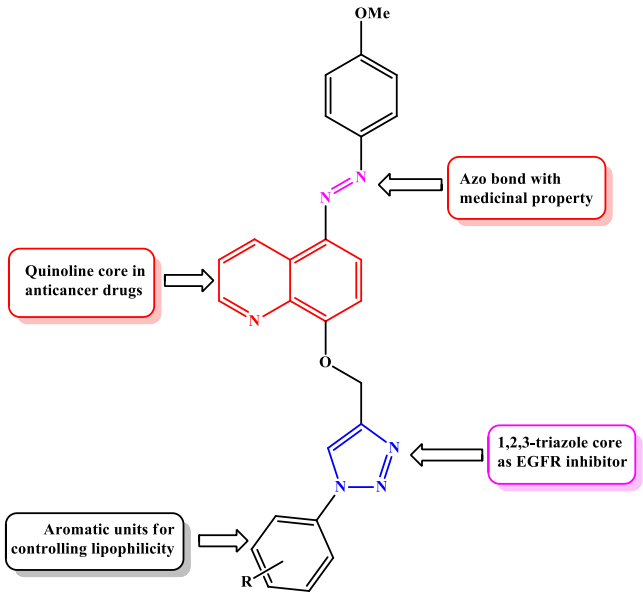


Fig. 2. Designing strategy

KOH(aq) solution (5%, 80 mL) was transferred dropwise to the round bottom flask at 0-5 ºC containing 4-methoxybenzene diazonium chloride (**2**, 10 mmol) with vigorous stirring and then ethanol (100 mL) was added after additional 1 h stirring. The obtained suspension was acidified by 1% HCl(aq) solution and the resulting precipitate was collected and washed with water (3 × 50 mL). Finally, it was purified with column chromatography using ethyl acetate/hexane eluent. Yield: 81%.

Synthesis of 5-((4-methoxyphenyl)diazenyl)-8-(prop-2-yn-1-yloxy)quinoline (5): A mixture of 5-((4-methoxyphenyl)-diazenyl)quinolin-8-ol (**3**, 0.0125 mol), K_2CO_3 (0.03 mol) and 3-bromoprop-1-yne (**4**, 0.018 mol) in 30 mL of DMF was allowed to stirring at room temperature for 12 h. The progress of reaction as monitored by TLC, excess of cold water was added to the reaction mixture. The obtained crude product was filtered and further purified by 60-120 mesh size silica gel column chromatography using (3:7) ethyl acetate/hexane eluent. Yield: 78%.

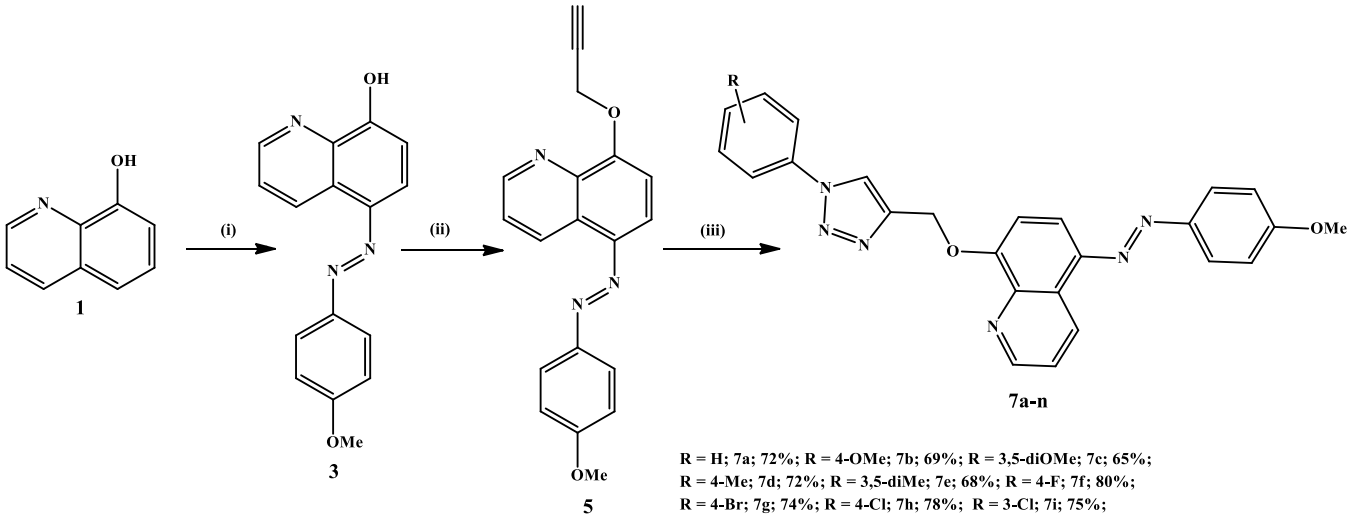
Synthesis of diazenyl-1,2,3-triazol-4-yl-quinoline derivatives (7a-n): A mixture of intermediate **5** (0.6 mmol) and aryl azides (**6a-n**, 0.9 mmol) and CuI (0.06 mmol) in THF (10 mL) was stirred at 60 ºC for 14 h. The progress of reaction as monitored by TLC, the reaction mixture was extracted with ethyl acetate (2 × 15 mL) and combined organic layers were reduced under vacuum (**Scheme-I**). Finally, the crude products were purified by 60-120 mesh size silica gel column chromatography using (1:1) ethyl acetate/hexane eluent.

5-((4-Methoxyphenyl)diazenyl)quinolin-8-ol (3): Colourless solid; m.p.: 152-154 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.54 (br s, 1H), 8.82 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.03 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.80-7.75 (m, 3H), 7.42 (t, *J* = 7.6 Hz, 1H), 6.97-6.92 (m, 3H) 3.85 (s, 3H) ppm.

5-((4-Methoxyphenyl)diazenyl)-8-(prop-2-yn-1-yloxy)-quinoline (5): Colourless solid; m.p.: 156-158 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.81 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.04 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.79-7.74 (m, 3H), 7.42 (t, *J* = 7.6 Hz, 1H), 6.96-6.91 (m, 3H), 4.95 (s, 2H), 3.85 (s, 3H), 2.34 (s, 1H) ppm.

5-((4-Methoxyphenyl)diazenyl)-8-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)quinoline (7a): Cream solid; m.p.: 190-192 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.82 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.43 (s, 1H), 8.01 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.81-7.73 (m, 5H), 7.49-7.41 (m, 4H), 6.96-6.91 (m, 3H), 5.11 (s, 2H), (3.86, s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.3, 158.9, 148.3, 147.5, 144.4, 142.1, 141.2, 137.6, 136.2, 129.8, 128.6, 125.2, 124.3, 122.3, 121.7, 121.1, 120.6, 114.6, 111.2, 64.5, 56.3; MS (ESI): *m/z* 437 $[M+H]^+$; CHN analysis for $C_{25}H_{20}N_6O_2$; Calcd. (found) %: C, 68.80 (68.83); H, 4.62 (4.64); N, 19.25 (19.28).

8-((1-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7b): Colourless solid; m.p.: 197-199 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.80 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.41 (s, 1H), 8.01



Scheme-I: Synthesis of 5-((4-methoxyphenyl)diazenyl)quinolin-8-ol linked 1,2,3-triazole hybrids (**7a-n**)

(dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.80-7.75 (m, 3H), 7.60 (d, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 1H), 6.97-6.92 (m, 3H), 6.86 (d, *J* = 7.7 Hz, 2H), 5.10 (s, 2H), 3.84 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.3, 158.8, 158.2, 148.4, 147.5, 144.5, 142.2, 141.2, 136.3, 132.1, 125.3, 124.3, 123.9, 122.1, 121.2, 120.6, 114.5, 114.1, 111.1, 64.4, 56.3; MS (ESI): *m/z* 467 $[M+H]^+$; CHN analysis for $C_{26}H_{22}N_6O_3$; Calcd. (found) %: C, 66.94 (66.97); H, 4.75 (4.73); N, 18.02 (18.06).

8-((1-(3,5-Dimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)-methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7c): Colourless solid; m.p.: 203-205 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.82 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.45 (s, 1H), 8.02 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.79-7.74 (m, 3H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.20 (s, 2H), 6.96-6.91 (m, 3H), 6.76 (s, 1H), 5.09 (s, 2H), 3.88 (s, 6H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.2, 160.4, 158.9, 148.2, 147.6, 144.3, 142.2, 141.2, 140.5, 136.2, 125.3, 124.4, 122.5, 121.1, 120.5, 114.5, 111.1, 99.7, 97.8, 64.2, 56.7, 56.3; MS (ESI): *m/z* 519 $[M+Na]^+$; CHN analysis for $C_{27}H_{24}N_6O_4$; Calcd. (found) %: C, 65.31 (65.33); H, 4.87 (4.90); N, 16.93 (16.91).

5-((4-Methoxyphenyl)diazenyl)-8-((1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl)methoxy)quinoline (7d): Colourless solid; m.p.: 193-195 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.81 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.43 (s, 1H), 8.01 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.84-7.74 (m, 5H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 2H), 6.95-6.90 (m, 3H), 5.09 (s, 2H), 3.84 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.4, 158.9, 148.5, 147.6, 144.2, 142.3, 141.2, 138.4, 137.3, 136.4, 129.6, 125.3, 124.8, 124.2, 122.2, 121.2, 120.6, 114.4, 111.3, 64.2, 56.4, 21.5; MS (ESI): *m/z* 451 $[M+H]^+$; CHN analysis for $C_{26}H_{22}N_6O_2$; Calcd. (found) %: C, 69.32 (69.33); H, 4.92 (4.95); N, 18.66 (18.64).

8-((1-(3,5-Dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7e): Grey solid; m.p.: 196-198 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.80 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.46 (s, 1H), 8.02 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.80-7.75 (m, 3H), 7.50 (s, 2H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.21 (s, 1H), 6.98-6.93 (m, 3H), 5.11 (s, 2H), (3.85, s 3H), 2.40 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.3, 158.8, 148.2, 147.5, 144.3, 142.2, 141.3, 140.2 (2C), 138.5, 136.3, 127.5, 126.1, 125.2, 124.4, 122.5, 121.3, 120.5, 114.6, 111.3, 64.4, 56.4, 21.8 ppm; MS (ESI): *m/z* 465 $[M+H]^+$; CHN analysis for $C_{27}H_{24}N_6O_2$; Calcd. (found) %: C, 69.81 (69.83); H, 5.21 (5.25); N, 18.09 (18.07).

8-((1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7f): Colourless solid; m.p.: 192-194 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.81 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.50 (s, 1H), 8.01 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.79-7.74 (m, 3H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.01-6.91 (m, 5H), 5.14 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 162.7, 161.4, 160.1, 158.9, 148.5, 147.4, 144.5, 142.1, 141.4, 136.3, 134.2, 125.3, 124.8, 124.3, 122.7, 121.2, 120.6, 118.6, 118.2, 114.5, 111.2, 64.7, 56.4 ppm; MS (ESI): *m/z* 477 $[M+Na]^+$; CHN analysis for $C_{25}H_{19}FN_6O_2$; Calcd. (found) %: C, 66.07 (66.05); H, 4.21 (4.24); N, 18.49 (18.47).

8-((1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7g): Yellow solid; m.p.: 204-206 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.81 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.46 (s, 1H), 8.04 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.80-7.75 (m, 5H), 7.49-7.41 (m, 3H), 6.96-6.91 (m, 3H), 5.11 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.2, 158.7, 148.4, 147.3, 144.3, 142.2, 141.2, 136.1, 135.7, 132.4, 125.3, 124.4, 122.4, 121.9, 121.3, 120.9, 120.5, 114.6, 111.3, 64.5, 56.4; MS (ESI): *m/z* 515 $[M+H]^+$; CHN analysis for $C_{25}H_{19}BrN_6O_2$; Calcd. (found) %: C, 58.26 (58.28); H, 3.72 (3.75); N, 16.31 (16.27).

8-((1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7h): Colourless solid; m.p.: 195-197 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.82 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.48 (s, 1H), 8.02 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.83-7.74 (m, 5H), 7.42-7.36 (m, 3H), 6.97-6.92 (m, 3H), 5.13 (s, 2H), 3.85 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.2, 158.7, 148.2, 147.4, 144.3, 142.3, 141.1, 137.1, 136.3, 133.3, 130.1, 125.2, 124.4, 123.5, 122.8, 121.3, 120.5, 114.6, 111.3, 64.7, 56.4; MS (ESI): *m/z* 471 $[M+H]^+$; CHN analysis for $C_{25}H_{19}ClN_6O_2$; Calcd. (found) %: C, 63.76 (63.74); H, 4.07 (4.10); N, 17.85 (17.88).

8-((1-(3-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7i): Colourless solid; m.p.: 196-198 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.81 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.47 (s, 1H), 8.01 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.78-7.73 (m, 3H), 7.52 (s, 1H), 7.41-7.32 (m, 2H), 7.08-7.01 (m, 2H), 6.96-6.91 (m, 3H), 5.12 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.2, 158.7, 148.5, 147.6, 144.2, 142.2, 141.3, 140.5, 136.4, 134.2, 131.2, 126.3, 125.3, 124.4, 122.6, 121.8, 121.2, 120.6, 119.1, 114.6, 111.2, 64.6, 56.3; MS (ESI): *m/z* 471 $[M+H]^+$; CHN analysis for $C_{25}H_{19}ClN_6O_2$; Calcd. (found) %: C, 63.76 (63.73); H, 4.07 (4.09); N, 17.85 (17.88).

8-((1-(3,5-Dichlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7j): Colourless solid; m.p.: 202-204 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.82 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.49 (s, 1H), 8.02 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.82-7.75 (m, 5H), 7.52 (s, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 6.97-6.92 (m, 3H), 5.09 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.4, 158.9, 148.2, 147.4, 144.4, 142.3, 141.2, 139.8, 136.4, 135.8, 125.4, 124.9, 124.3, 122.8, 121.3, 120.7, 119.3, 114.7, 111.4, 64.3, 56.3; MS (ESI): *m/z* 506 $[M+H]^+$; CHN analysis for $C_{25}H_{18}Cl_2N_6O_2$; Calcd. (found) %: C, 59.42 (59.44); H, 3.59 (3.62); N, 16.63 (16.65).

4-(4-(((5-((4-Methoxyphenyl)diazenyl)quinolin-8-yl)-oxy)methyl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (7k): Light yellow solid; m.p.: 196-198 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.81 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.50 (s, 1H), 8.03 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.88 (d, *J* = 7.7 Hz, 2H), 7.80-7.75 (m, 3H), 7.50 (d, *J* = 7.7 Hz, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 6.96-6.91 (m, 3H), 5.14 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.2, 158.8, 148.3, 147.6, 144.3, 142.2, 141.3, 139.9, 136.3, 126.3, 125.9, 125.3, 124.4, 122.8, 121.2, 120.5, 119.7, 117.8, 114.6, 111.3, 64.8, 56.4; MS (ESI): *m/z* 462 $[M+H]^+$; CHN analysis for $C_{26}H_{19}N_7O_2$; Calcd. (found) %: C, 67.67 (67.65); H, 4.15 (4.18); N, 21.25 (21.28).

5-((4-Methoxyphenyl)diazenyl)-8-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)quinoline (7l): Cream solid; m.p.: 198-200 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm:

8.81 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.54 (s, 1H), 8.26 (d, *J* = 7.4 Hz, 2H), 8.01 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.82-7.74 (m, 5H), 7.45 (t, *J* = 7.6 Hz, 1H), 6.97-6.92 (m, 3H), 5.12 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.4, 158.9, 148.4, 147.6, 146.5, 144.5, 142.3, 141.3, 140.9, 136.4, 126.6, 125.2, 124.5, 122.9, 122.4, 121.3, 120.6, 114.5, 111.2, 64.7, 56.5; MS (ESI): *m/z* 482 $[M+H]^+$; CHN analysis for $C_{25}H_{19}N_7O_4$; Calcd. (found) %: C, 62.37 (62.41); H, 3.98 (3.95); N, 20.36 (20.39).

5-((4-Methoxyphenyl)diazenyl)-8-((1-(3-nitrophenyl)-*1H*-1,2,3-triazol-4-yl)methoxy)quinoline (7m): Cream solid; m.p.: 199-201 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.82 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.74 (s, 1H), 8.51 (s, 1H), 8.02 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.80-7.71 (m, 5H), 7.44-7.37 (m, 2H), 6.98-6.92 (m, 3H), 5.14 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.3, 158.8, 148.3, 147.3, 146.8, 144.4, 142.2, 141.4, 138.2, 136.3, 132.1, 128.3, 125.4, 124.4, 122.6, 121.8, 121.1, 120.7, 120.2, 114.5, 111.3, 64.8, 56.3; MS (ESI): *m/z* 504 $[M+Na]^+$; CHN analysis for $C_{25}H_{19}N_7O_4$; C, 62.37 (62.40); H, 3.98 (3.95); N, 20.36 (20.34).

8-((1-(4-Chloro-3,5-dimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-((4-methoxyphenyl)diazenyl)quinoline (7n): Colourless solid; m.p.: 202-204 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.80 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 8.48 (s, 1H), 8.01 (dd, *J* = 7.6 Hz, 1.7 Hz, 1H), 7.80-7.75 (m, 3H), 7.43 (t, *J* = 7.6 Hz, 1H), 6.98-6.93 (m, 3H), 6.80 (s, 2H), 5.10 (s, 2H), 3.88 (s, 6H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 158.7, 152.3, 148.4, 147.4, 144.3, 142.3, 141.2, 139.2, 136.4, 125.3, 124.5, 122.7, 121.3, 120.6, 118.5, 114.7, 111.2, 107.3, 64.5, 56.8, 56.4; MS (ESI): *m/z* 531 $[M+H]^+$; CHN analysis for $C_{27}H_{23}ClN_6O_4$; Calcd. (found) %: C, 61.08 (61.06); H, 4.37 (4.40); N, 15.83 (15.81).

MTT assay: Each well of a 96-well tissue culture plate was inoculated with 100 μL of complete medium containing 1×10^4 cells. The plates were incubated at 37 ºC in a humidified 5% CO_2 incubator for 18 h before the experiment. After removing the medium, 100 μL of fresh medium containing the test compounds and 5-fluorouracil at varying concentrations (0.5, 1 and 2 μM) were added to each well and incubated at 37 ºC for 24 h. The medium was then discarded and 10 μL of MTT dye was added to each well. The plates were incubated at 37 ºC for 2 h. The resulting formazan crystals were solubilized in 100 μL of extraction buffer. Optical density (OD) was measured at 570 nm using a Multi-mode Varioskan microplate reader (Thermo-Scientific). The concentration of DMSO in the medium never exceeded 0.25%.

Tyrosine kinase EGFR inhibitory activity: The tyrosine kinase EGFR inhibitory activity was assessed using the EGFR Kinase Assay Kit (PBS Bioscience, catalog # 40321), with erlotinib serving as standard. All three compounds were tested in triplicate. The IC_{50} values for the compounds and the reference were calculated by averaging the results of the three experiments, with the standard deviation from the triplicates also considered.

Molecular docking studies: Molecular docking studies were conducted using AutoDock 4.2 tools. The protein structure was downloaded from the Protein Data Bank with PDB ID 4HJO. The ligand and water molecules were removed from the protein and Gasteiger charges were calculated after the addition of polar hydrogens. The ligands were drawn using ChemDraw 12, saved as .mol files and energy minimized. These files were then converted into PDB format using Discovery Studios. A grid box was generated with 60 points along each of the three coordinate axes. The Lamarckian Genetic Algorithm (4.2) was used to generate the DPF file.

RESULTS AND DISCUSSION

The synthesis of anticipated diazenyl-1,2,3-triazol-4-yl-quinoline derivatives (**7a-n**) was achieved in three main steps (**Scheme-I**). In the first step, the synthesis of 5-((4-methoxyphenyl)diazenyl)quinolin-8-ol (**3**) was achieved by azocoupling reaction [25] between quinolin-8-ol (**1**) and 4-methoxybenzenediazonium chloride (**2**) in presence of KOH. In the second step, intermediate **3** was reacted with 3-bromoprop-1-yne (**4**) by means of K_2CO_3 in DMF at room temperature for 12 h to afford 5-((4-methoxyphenyl)diazenyl)-8-(prop-2-yn-1-yloxy)quinoline (**5**). Finally, the third step involves Cu(I) catalyzed [3+2]-cycloaddition between terminal alkyne intermediate **5** and several aryl azides (**6a-n**) in THF at 60 ºC for 14 h gave the desired products **7a-n** in moderate to good yields.

***In vitro* anticancer activity:** *In vitro* anticancer activity screening was performed for all the newly synthesized compounds (**7a-n**) by considering three breast cancer cell lines like MDA-MB-468, MDA-MB-231 and MCF-7 using MTT assay and 5-fluorouracil (5-FU) was used as reference drug. Among all the compounds that were tested, five compounds **7d**, **7j**, **7k**, **7l** and **7n** were exhibited greater activity than the standard drug 5-fluorouracil against three breast cancer cell lines with IC_{50} values ranging from 1.1 μM to 9.3 μM (Table-1). Especially compound **7l** has displayed predominant anticancer activity towards three cell lines (MDA-MB-468, MDA-MB-231 and MCF-7) with IC_{50} values 1.1 μM, 3.1 μM and 3.5 μM, respectively. Similarly, compound **7d** has shown remarkable activity against same cell lines with IC_{50} values 2.3 μM, 4.5 μM and 4.9 μM, respectively.

The structure-activity relationship-SAR *i.e.* effect of different substituents on phenyl ring attached to 1,2,3-triazole unit on anticancer activity was analyzed and presented. In case of compounds having electron donating substituents, the compound **7d** having 4-methyl substituent has shown greater activity against three cell lines compared to 5-FU. The compound **7e** with two methyl groups at 3rd and 5th position has exhibited less activity than compound **7d** with one methyl group at 4th position. The compounds with methoxy substituents **7b** (4-OMe) and **7c** (3,5-di-OMe) have displayed lesser activity than the compounds with methyl substituents (**7d, 7e**). Compound **7a** without any substituent has shown less activity than reference and also compounds **7b**, **7d** and **7e**. Introducing chlorine at 4th position in addition to two methoxy groups at 3rd and 5th position leads to compound **7n** which exhibited greater activity than 5-FU against three cell lines. Similarly, in case of compounds with electron-withdrawing substituents on phenyl ring, compound **7l** containing 4-nitro group has exhibited highest activity than standard 5-fluorouracil against three cell lines. Compound **7j** with two chlorine substituents at 3rd and 5th position has exhibited second highest anticancer

TABLE-1
In vitro ANTICANCER ACTIVITY OF COMPOUNDS (**7a-n**) AND TYROSINE KINASE EGFR INHIBITION

Entry	MDA-MB-468	MDA-MB-231	MCF-7	MCF-10A	EGFR
7a	72.5 ± 1.7	85.4 ± 2.1	84.8 ± 1.8	NT	NT
7b	68.2 ± 1.5	57.2 ± 1.3	65.2 ± 1.4	NT	NT
7c	87.8 ± 2.1	92.4 ± 2.2	94.8 ± 2.4	NT	NT
7d	2.3 ± 0.1	4.5 ± 0.1	4.9 ± 0.1	> 100	0.21 ± 0.01
7e	25.1 ± 1.1	19.2 ± 1.1	27.4 ± 3.1	NT	NT
7f	18.2 ± 1.2	15.4 ± 2.1	22.6 ± 1.1	NT	NT
7g	19.5 ± 1.3	14.7 ± 1.7	18.6 ± 1.4	NT	NT
7h	34.6 ± 1.7	45.4 ± 1.1	34.8 ± 1.6	NT	NT
7i	67.8 ± 1.4	71.6 ± 1.8	62.1 ± 1.4	NT	NT
7j	4.1 ± 0.1	6.7 ± 0.1	5.2 ± 0.1	> 100	0.32 ± 0.03
7k	6.5 ± 0.2	5.8 ± 0.1	7.7 ± 0.2	> 100	0.28 ± 0.02
7l	1.1 ± 0.1	3.1 ± 0.1	3.5 ± 0.1	> 100	0.15 ± 0.03
7m	27.5 ± 1.2	37.2 ± 1.4	45.7 ± 1.4	NT	NT
7n	7.1 ± 0.2	8.9 ± 0.3	9.3 ± 0.2	> 100	0.38 ± 0.01
5-FU	7.7 ± 0.2	10.9 ± 0.1	12.5 ± 0.3	NT	NT
Erlotinib	NT	NT	NT	NT	0.40 ± 0.01

Average of triplicates ± standard deviation; NT = Not tested

activity in the series. Similarly, the compound **7k** with cyano group at 4th position has also displayed superior activity than reference drug 5-FU. On the other hand, in case of compounds having one halogen the activity was in the order of 4-F > 4-Br > 4-Cl > 3-Cl. Further, compounds **7d**, **7j**, **7k**, **7l** and **7n** were shown very less toxicity (IC_{50} > 100 μM) towards normal cell line MCF-10A. Overall, it was observed that compounds bearing electron-withdrawing substituents on the phenyl ring exhibited enhanced anticancer activity compared to those containing electron-donating groups.

***In vitro* tyrosine kinase EGFR inhibitory activity:** The *in vitro* tyrosine kinase EGFR inhibitory efficiency was investigated for compounds **7d**, **7j**, **7k**, **7l** and **7j** which were shown good *in vitro* anticancer activity (Table-1). Among all the compounds, **7l** has exhibited superior inhibitory activity (nearly 2.6 times more) with IC_{50} value of 0.15 μM compared to the reference drug erlotinib which has exhibited IC_{50} value of 0.40 μM. Compound **7d** has exhibited two-times more inhibition than erlotinib with IC_{50} value of 0.21 μM. On the other hand, compound **7k** has displayed 1.4 times more potency towards tyrosine kinase EGFR inhibition with IC_{50} value of 0.28 μM. Finally, compounds **7j** and **7n** also exhibited remarkable inhibitory activity towards EGFR with IC_{50} values 0.32 μM and 0.32 μM, respectively.

Molecular docking studies: Molecular docking studies were carried out using Auto dock tools on epidermal growth factor receptor which was obtained from protein data bank (PDB ID 4HJO) [26]. Five synthesized hybrid compounds **7d, 7j, 7k, 7l** and **7n**, which displayed greater *in vitro* anticancer activity were docked by taking epidermal growth factor receptor as the target protein. According to Table-2, compound **7l** (4-nitro substituent) has shown greatest binding energy *i.e.* -10.81 kcal/mol and formed two hydrogen bonds with ARG817 and LYS855 residues with bond lengths 2.04 Å and 1.85 Å, respectively (Fig. 3). Compound **7d** has exhibited second highest binding energy (-10.59 kcal/mol) and formed one hydrogen bond with ASP831 residue with bond length 2.15 Å. The remaining compounds **7j**, **7k** and **7n** have exhibited -10.32, -10.47l and -9.79 kcal/mol binding energies, respectively. Finally, the standard drug, erlotinib also docked with EGFR where it has shown -7.68 kcal/mol, binding energy and 2.30 μM, inhibition constant. It also formed one hydrogen bond with THR830 residue with bond length 2.25 Å in addition to π-cation with LYS721 residue.

Conclusion

Novel quinolone-1,2,3-triazole with azo bond hybrid compounds were synthesized, characterized and screened for *in vitro* anticancer activity against three human breast cancer cell lines including MDA-MB-468, MDA-MB-231 and MCF-7 using MTT assay and 5-fluorouracil was used as reference drug. Among the synthesized compounds, five compounds **7d**, **7j**, **7k**, **7l** and **7n** exhibited greater activity than the standard drug 5-fluorouracil. In addition to this *in vitro* tyrosine kinase

TABLE-2
MOLECULAR DOCKING RESULTS OF COMPOUNDS (**7d**, **7j**, **7k**, **7l** AND **7n**) WITH EGFR (PDB ID-4HJO)

Entry	Binding energy (kcal/mol)	Inhibition constant (nM)	No. of hydrogen bonds	Residues involved in hydrogen bonding (bond length in Å)	π-Cation & π-π stacking
7d	-10.59	17.28	1	ASP831 (2.15)	LYS721
7j	-10.32	27.19	–	–	LYS721, PHE699
7k	-10.47	21.07	–	–	–
7l	-10.81	11.98	2	ARG817 (2.04), LYS855 (1.85)	PHE699, TRP856
7n	-9.79	66.74	–	–	ARG87, PHE699, TRP856
Erlotinib	-7.68	2.30 μM	1	THR830 (2.25)	

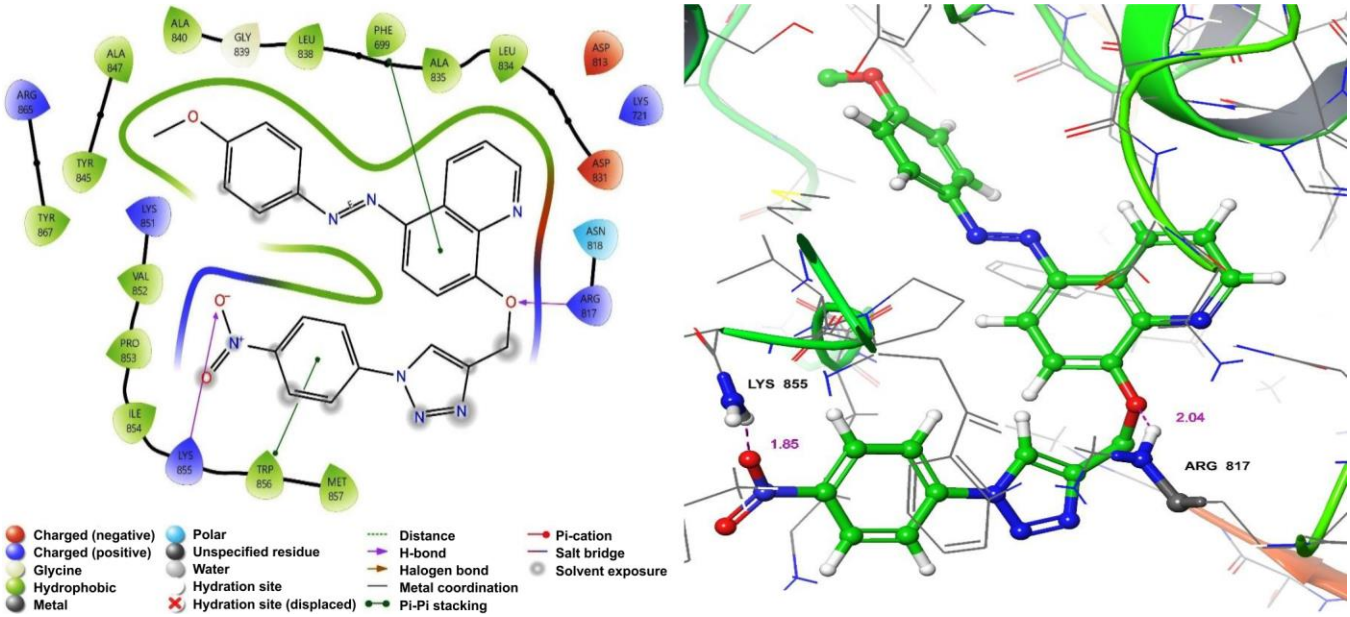


Fig. 3. 2D and 3D interaction of the compound **7l** with EGFR

EGFR inhibition also found to be good for all the same five compounds. Finally results of molecular docking studies of all five potent compounds found to be supportive with the IC_{50} data.

ACKNOWLEDGEMENTS

The authors thankful to the Chaitanya (Deemed to be University), Hyderabad, for providing the research facilities.

CONFLICT OF INTEREST

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

REFERENCES

1. A. Ayati, S. Emami, A. Asadipour, A. Shafiee and A. Foroumadi, *Eur. J. Med. Chem.*, **97**, 699 (2015); https://doi.org/10.1016/j.ejmech.2015.04.015
2. R.M. Aly, R.A.T. Serya, A.M. El-Motwally, A. Esmat, S. Abbas and D.A. Abou El Ella, *Bioorg. Chem.*, **75**, 368 (2017); https://doi.org/10.1016/j.bioorg.2017.10.018
3. D.A. Sabbah, R. Hajjo and K. Sweidan, *Curr. Top. Med. Chem.*, **20**, 815 (2020); https://doi.org/10.2174/1568026620666200303123102
4. A. Ayati, S. Emami, S. Moghimi and A. Foroumadi, *Future Med. Chem.*, **11**, 1929 (2019); https://doi.org/10.4155/fmc-2018-0416
5. S.V. Sharma, D.W. Bell, J. Settleman and D.A. Haber, *Nat. Rev. Cancer*, **7**, 169 (2007); https://doi.org/10.1038/nrc2088
6. A. Ayati, S. Moghimi, S. Salarinejad, M. Safavi, B. Pouramiri and A. Foroumadi, *Bioorg. Chem.*, **99**, 103811 (2020); https://doi.org/10.1016/j.bioorg.2020.103811
7. I.A. Bala, O.F. Al Sharif, A.M. Asiri and R.M. El-Shishtawy, *Results Chem.*, **7**, 101529 (2024); https://doi.org/10.1016/j.rechem.2024.101529
8. A. Weyesa and E. Mulugeta, *RSC Adv.*, **10**, 20784 (2020); https://doi.org/10.1039/D0RA03763J
9. S. Singh, G. Kaur, V. Mangla and M.K. Gupta, *J. Enzym. Inhib. Med. Chem.*, 30, 492 (2014); https://doi.org/10.3109/14756366.2014.930454
10. D.J. Kucharski, M.K. Jaszczak and P.J. Boratyński, *Molecules*, **27**, 1003 (2022); https://doi.org/10.3390/molecules27031003
11. R.I. Alsantali, Q.A. Raja, A.Y.A. Alzahrani, A. Sadiq, N. Naeem, E.U. Mughal, M.M. Al-Rooqi, N. El Guesmi, Z. Moussa and S.A. Ahmed, *Dyes Pigments*, **199**, 110050 (2022); https://doi.org/10.1016/j.dyepig.2021.110050
12. A. Ayati, S. Moghimi, S. Salarinejad, M. Safavi, B. Pouramiri and A. Foroumadi, *Eur. J. Med. Chem.*, **97**, 699 (2015); https://doi.org/10.1016/j.ejmech.2015.04.015
13. S. Jain, V. Chandra, P. Kumar Jain, K. Pathak, D. Pathak and A. Vaidya, *Arab. J. Chem.*, **12**, 4920 (2019); https://doi.org/10.1016/j.arabjc.2016.10.009
14. P. Panda and S. Chakroborty, *ChemistrySelect*, **5**, 10187 (2020); https://doi.org/10.1002/slct.202002790
15. A. Dorababu, *ChemistrySelect*, **5**, 13902 (2020); https://doi.org/10.1002/slct.202003888
16. M.A. Mahmoud, A.F. Mohammed, O.I.A. Salem, S.M. Rabea and B.G.M. Youssif, *J. Mol. Struct.*, **1282**, 135165 (2023); https://doi.org/10.1016/j.molstruc.2023.135165
17. A. Mamidala, K. Bokkala, N.S. Thirukovela, N. Sirassu, S. Bandari and S.K. Nukala, *ChemistrySelect*, **7**, e202203763 (2022); https://doi.org/10.1002/slct.202203763
18. K. Lal and P. Yadav, *Anticancer. Agents Med. Chem.*, **18**, 21 (2018); https://doi.org/10.2174/1871520616666160811113531
19. J. Akhtar, A.A. Khan, Z. Ali, R. Haider and M. Shahar Yar, *Eur. J. Med. Chem.*, **125**, 143 (2017); https://doi.org/10.1016/j.ejmech.2016.09.023
20. L.Z. Fekri, M. Kadivar-Kalmarzi and R.S. Varma, *Polycycl. Arom. Comp.*, **45**, 926 (2024); https://doi.org/10.1080/10406638.2024.2426631
21. D.P. Mishra, P.K. Sahu, B. Acharya, S.P. Mishra and S. Bhati, *Results Chem.*, **10**, 101712 (2024); https://doi.org/10.1016/j.rechem.2024.101712
22. Y. Ali, S.A. Hamid and U. Rashid, *Mini Rev. Med. Chem.*, **18**, 1548 (2018); https://doi.org/10.2174/1389557518666180524113111
23. C.V. Junior, A. Danuello, V.S. Bolzani, E.J. Barreiro and C.A.M. Fraga, *Curr. Med. Chem.*, **14**, 1829 (2007); https://doi.org/10.2174/092986707781058805
24. L.K. Gediya and V.C.O. Njar, *Expert Opin. Drug Discov.*, **4**, 1099 (2009); https://doi.org/10.1517/17460440903341705
25. M. Szala, J.E. Nycz, G.J. Malecki, R. Sokolova, S. Ramesova, A. Switlicka-Olszewska, R. Strzelczyk, R. Podsiadly and B. Machura, *Dyes Pigments*, **142**, 277 (2017); https://doi.org/10.1016/j.dyepig.2017.03.043
26. J.H. Park, Y. Liu, M.A. Lemmon and R. Radhakrishnan, *Biochem. J.*, **448**, 417 (2012); https://doi.org/10.1042/BJ20121513