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Synthesis And Characterization Of 1,2,4-Triazole Fused Quinoline Derivatives: Molecular Docking Studies, Antibacterial, Anticancer Activity

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Abstract:
*The development of hit and lead medications with novel molecular structures can be accomplished by the hybridization of bioactive natural and synthetic molecules. A variety of different novel structures of 1,2,4-triazole fused quinoline moieties have been developed and produced for this study. Spectroscopy techniques such as IR,[1]H NMR, [13]C NMR, and ESI-MS were utilized to confirm all the developed substances. Its antibacterial and anti-cancer effects were assessed in vitro. A wide range of methods were used to examine the interaction between drugs and proteins. The compounds were evaluated against human breast tumor cell lines in vitro to figure out whether they had anticancer properties. The MCF-7 breast cancer cell line has been utilized to investigate a few drugs' growth-inhibiting characteristics. Compounds **3a, 3e, 3h**, and **3j** have been found to have active properties. However, if compared to other compounds, compound II-3h is more active than others. Scaffolds **3b, 3e**, **3f**, and **3j** exhibit the potential antibacterial effects when used with the standard medication streptomycin. Biological activity was also confirmed by the molecular docking research studies. Most of the molecules satisfied the Lipinski rules. Whenever paired together, these compounds can be recognized as strong antiproliferative agents with further modification.*

Keywords: *Quinoline, 1,2,4-triazole, anticancer and antibacterial activities, molecular docking studies, MCF-7 Cell lines.*

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I. Introduction

In current medicinal chemistry, the discovery of innovative drug-like small compounds based on pharmacologically active scaffolds is a logical and exciting approach. Biologically active pharmacophores have been combined to create a variety of compounds that medicinal chemists use to create new therapeutic medicines with a wide range of pharmacological activity 1-3]. Uncontrolled cell development without differentiation and an increase in cells that are abnormal that result in tumor formation make cancer an extremely complicated disease. Approximately 10 million people died from cancer in 2020, accounting for one out of every six deaths worldwide. The most prevalent malignancies in the world are those of the breast, lungs, colon, rectum, and prostate. According on the cancer's stage and type, the primary cancer therapies are hormone therapy, radiation, chemotherapy, and surgery. However, among the primary drawbacks of these medicines are multidrug resistance (MDR) and harm to healthy cells during cancer treatment. Finding new and specific chemicals to act as antiproliferative agents is therefore urgently needed. One of quinazolinone analogs' primary modes of action as anticancer medicines is thought to be the inhibition of the epidermal growth factor receptor (EGFR) enzyme. Tyrosine kinase (TK)-active receptor EGFR plays an essential role in the cycle of division of cells, proliferation, metastasis, and survival. Some malignant tissues, including lung, brain, ovarian, colon, breast, and prostate cancers, have been demonstrated to have over-activation of EGFR. Hence, EGFR targeting may be considered as a desirable and sensible strategy for cancer treatment [4-6]. The two most effective and selective EGFR inhibitors that included quinazoline frameworks in their structures are erlotinib and gefitinib.

In view of their biological activity, heterocyclic molecules that are play a vital role in medical chemistry. Quinazoli-none is a critical structural motif among compounds that are biologically active and is also crucial for the combinatorial assembly of heterocyclic scaffolds because it is a heterocyclic aromatic molecule including oxygen and nitrogen atoms that are rich in electrons. Quinazolines are necessary nitrogen-containing heterocycles in medicinal chemistry [7]. Quinazoline derivatives, such as quinazolinone, have an array of biological properties. A wide range of therapeutic uses [8-11] appear for quinazolinone derivatives, particularly 4(3H)-quinazolinone, including antibacterial and antifungal, anti-HIV, antimalarial, antiviral, anti-inflammatory,

anticancer, analgesic, anticonvulsant, central nervous system depressant, antioxidant, and anti-leukemic. 1,2,4-triazoles are heterocyclic compounds that contain nitrogen and are used in an array of domains, notably organic catalysts, material sciences, and medicinal chemistry.

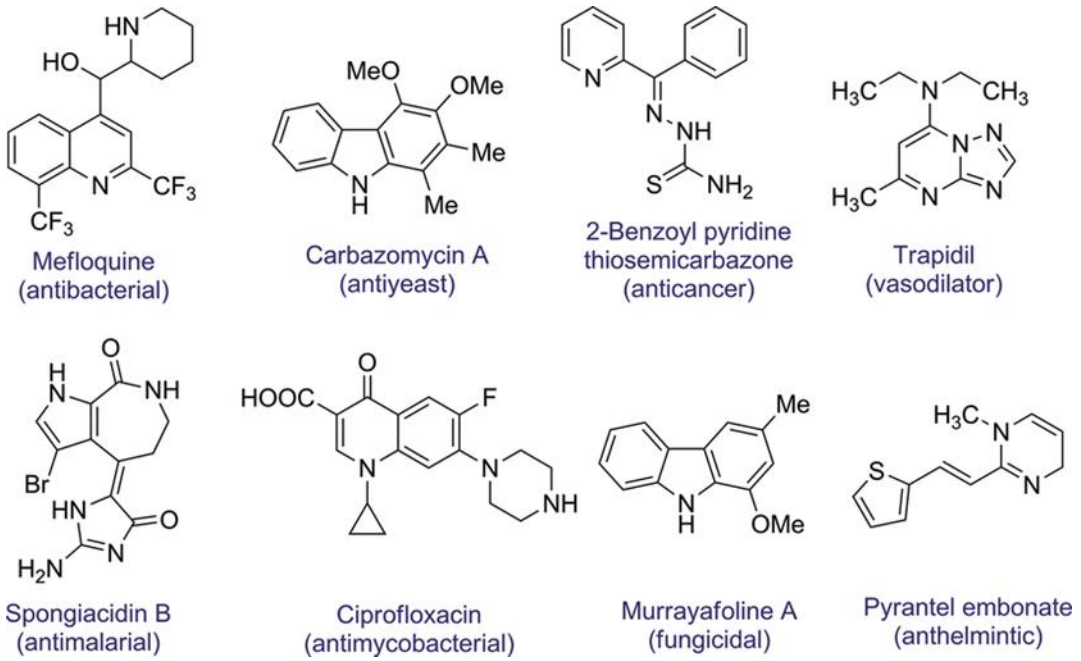

Figure-1 Biologically active molecules containing Quinazoline moiety

The antibacterial abilities of 1,2,4-triazole compounds are widely recognized, and medications with 1,2,4-triazole groups, namely fluconazole, flupoxam, and anastrozole, are known to be readily accessible on the market. Studies have shown that particular 1,2,4-triazole compounds display superior antibacterial, anticancer, anti-inflammatory, and antihypertensive properties [12]. The properties of 1,2,4-triazole-containing compounds are common and well-known, researchers are still interested in them because of their excellent pharmacokinetics, broad spectrum of activity, and low toxicity, as demonstrated by numerous studies. New analogs containing different substituents are also still being synthesized. In this study, we synthesized different 1,2,4-triazole fused quinzoline derivatives II-3(a-l) in order to create more potent anticancer drugs, taking into consideration the significance of quinazoline and triazole base structures in cancer treatment [13].

II. Materials And Methods:

Experimental:

Using conventional methods, all of the solvents and reagents were dried and purified. The AR and LR grade chemicals and reagents used in the present study were supplied by S.D. Fine Chem. Ltd., Sigma Hi-media, Rolex, Reachem, Aldrich, and Rolex. The uncorrected melting points in open capillary tubes were determined using a Veergo digital melting point analyzer. TLC has been carried out on 0.25 mm thick silica gel GF254 coated plates using ethyl acetate and n-hexane (1:2). The BRUKER AVANCE II 400MHz NMR Spectrometer instrument was put to use to record the ^{1}HNMR spectra of the made compounds in deuterated DMSO, using TMS acting as the internal standard. KBr pellets were used to record the infrared spectra using the Perkin Elmer FTIR spectrophotometer. The LC-MSD Tranp-SL2010A SHIMADZU uses dimethyl sulfoxide (DMSO) as a solvent for collecting mass spectra.

Preparation of 1,2,4-triazole fused novel quinazoline derivatives: The study in discussion is based on Schiff's base reactions and cyclization, which entail the reaction of TCH with benzoic acid followed by its reaction with quinoline-3-carbaldehyde to produce derivatives with the names II-(3a-3j).

Step-I: Synthesis of Thiocarbohydrazide (TCH). 85% hydrazine hydrate (24 ml) and water (75 ml) were combined together, and carbon disulphide (13 ml, 0.22 mol) was added dropwise while being agitated. The mixture was then allowed to shake for 30 minutes at room temperature. Later then, the reaction temperature was rapidly raised to 100–1100 ^{0}C. The reaction mixture had been cooled in an ice bath and filtered after refluxing for two hours. Pure thiocarbohydrazide crystals have been produced by recrystallizing the residue from water following it had been washed with ethanol and ether.

Step-II: Synthesis of 4-amino-5-phenyl-3-thiol-1,2,4-Triazole (1a-1d): A round-bottom flask has been filled with a solution of thiocarbohydrazide (0.015 mol) and benzoic acid (0.01 mol). For five minutes at a time, the mixture was heated in a sand bath. Employing TLC, the reaction's completeness was determined. Following cooling, the addition of frigid water to the reaction mixture, the resultant precipitate was filtered and treated a water wash. Utilizing ethanol, the product was recrystallized.

Step-II:5-phenyl-4-(((E)-4-((E)-(quinolin-3-ylimino)methyl)benzylidene)amino)-4H-1,2,4-triazole-3-thiol-II-3(a-l). In a round-bottom flask, 30-ml of ethanol, glacial acetic acid (2–5 ml), and aromatic amines (0.01 mol) were mixed using 4-amino-5-phenyl-3-thiol-1,2,4-triazole (1a–d) and terephthaldehyde (0.01 mol). The reaction mixture was then refluxed for a period of two to three hours. TLC (n-hexane: Ethyl acetate) (8:2) was employed for monitoring the reaction's progression. During cooling, the reaction mixture approached room temperature. A crystalline solid has been generated by filtering out the solid, washing it with hexane, and then recrystallizing it from methanol.

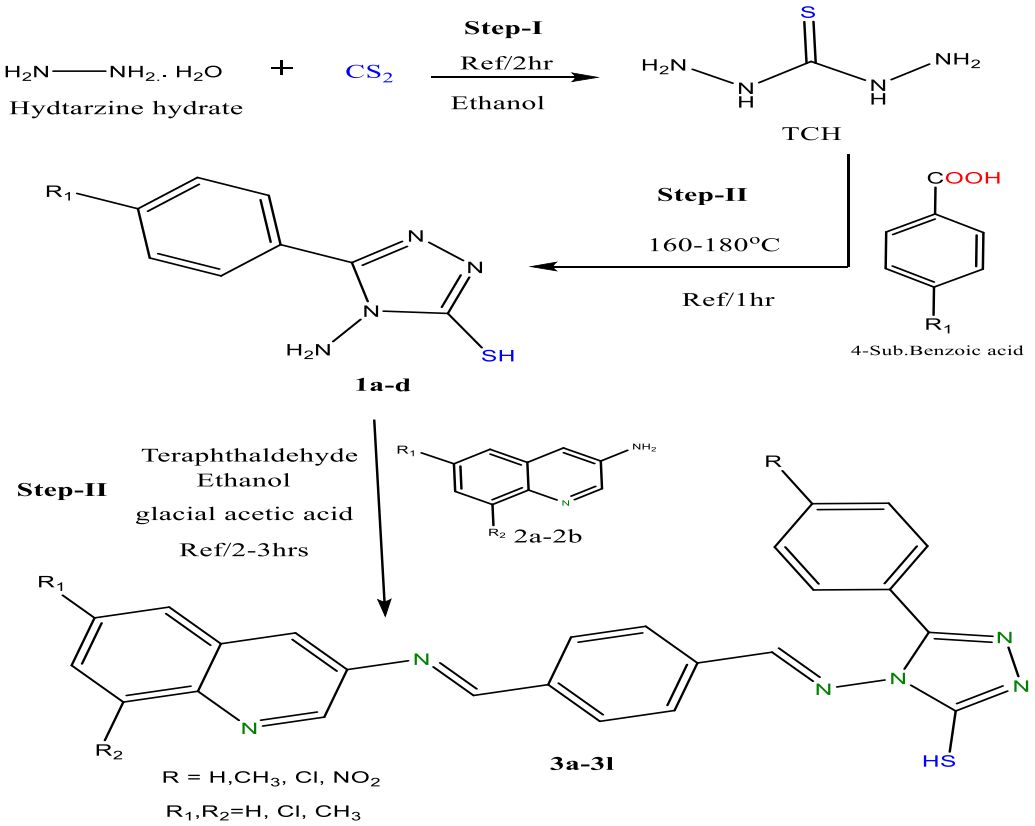

Figure-2. Synthetic pathway of novel 1,2,4-triazole fused Quinoline derivatives (3a-3l)

Table-1. Physical Characterization of quinazoline fused 5-mercapto 1,2,4-trizole derivatives

Compound	R, R_1 and R_2	Mol. Formula	Mol.Wt (g/mole)	M.P(°C)	%Yield
3a	H, H, H	$C_{25}H_{18}N_6S$	434.13	203-205	92
3b	CH_3, H, H,	$C_{26}H_{20}N_6S$	448.15	187-189	80
3c	H, H, CH_3	$C_{26}H_{20}N_6S$	448.15	213-215	79
3d	Cl, H, H	$C_{25}H_{17}ClN_6S$	468.09	243-245	85
3e	CH_3, H, Cl	$C_{26}H_{19}ClN_6S$	482.11	166-168	87
3f	Cl, H, Cl	$C_{25}H_{16}Cl_2N_6S$	502.05	231-233	85
3g	H, CH_3, Cl	$C_{26}H_{19}ClN_6S$	482.11	215-217	78
3h	CH_3, CH_3, H	$C_{27}H_{22}N_6S$	462.16	187-189	83
3i	CH_3, CH_3, Cl	$C_{27}H_{21}ClN_6S$	496.12	209-211	91
3j	Cl, CH_3, H	$C_{26}H_{19}ClN_6S$	482.11	221-223	80
3k	NO_2, CH_3, H	$C_{26}H_{19}N_7O_2S$	493.13	237-239	75
3l	NO_2, H, Cl	$C_{25}H_{16}ClN_7O_2S$	513.08	261-263	78

Antibacterial activity [14]: Both Gram-positive (Bacillus subtilis, Staphylococcus aureus) and Gram-negative (Escherichia coli, Klebsiella pneumoniae) microorganisms have been identified using the Agar diffusion assay, which was used to screen the entire series of newly synthesized triazole linked quinoline derivatives for antimicrobial properties. To obtain the same concentration of 100 μg/ml, the test compounds were dissolved in methanol and a minimum of 1-2 ml of DMSO. The same amount of streptomycin, a widely employed antibiotic, has been produced. The diameter of the growth inhibition zones (in mm) was computed to measure the percentage of antibacterial activity during incubation; outcomes can be found in Table-2, Figure-3.

Anticancer activity [15-16]*:* Novel 1,2.4-triazole attributed quinoline compounds have been evaluated for putative anticancer activities employing the MTT assay on human breast cancer les-MCF-7. The effects of a compound can be determined by this invitro assay, which measures cell viability and proliferation. Six distinct concentrations of each substance were tested in the experiment, which was performed three times to ensure reproducibility. Doxorubicin was used as a standard for comparison, and the resulting cytotoxic data is summarized in Table-3, Figure-5.

Molecular Docking Studies [17-20]*:* It was employed the in silico molecular modeling tool AutoDock Vina [16] to investigate potential drug candidate against EGFR. To capture the structure and characteristics of the receptor through many fields for accurate ligand posture scoring, a receptor grid was created. This grid is essential for locating the protein's active site and getting it ready for ligand docking. Open Babel was used to convert ten compounds that were created with ACD/ChemSketch and generated in MDL Molfile format following 3D optimization to PDBQT format. The MGL tool was used to enhance the three-dimensional structure of Epidermal Growth Factor Receptor tyrosine kinase (PBD ID: 1M17), which was taken from the protein data bank, through eliminating far-off water molecules, adding hydrogen atoms and missing residues, and decreasing energy. Table 5 displays the binding energies and important interacting amino acids for the dataset ligands, offering valuable information for upcoming medication development.

III. Results And Discussion

Spectral characterization of (3a-3l)

Compound-3a: 5-phenyl-4-(((E)-4-((E)-(quinolin-3-ylimino)methyl)benzylidene)amino)-4H-1,2,4-triazole-3-thiol. IR(vcm^{-1}): Triazole-SH stretching at 3528cm^{-1}; Aromatic-CH stretching at 3031cm^{-1}; Aliphatic-CH stretching absorption at 2985, 2787cm^{-1}; Imine-CH=N stretch at 1614cm^{-1}; Aromatic-C=C stretch at 1485cm^{-1}; C-S stretching at 1283cm^{-1}; C-N stretch at 1026cm^{-1}. **^{1}H-NMR (DMSO) δ ppm:** singlet proton (1H, Triazole-SH) at 11.0565ppm; singlet (Imine-H, 2H) at 9.5986, 9.5483ppm; singlet (2H, Aromatic-H) at 8.3235, 8.2658ppm; Aromatic-H with doublet and triplet protons (13H) at 7.7697-7.0482. **^{13}C-NMR(DMSO) δ ppm:** 158.34, 152.05, 143.33, 140.98, 135.23, 132.67, 130.05, 128.56, 126.94, 124.34, 121.92, 118.23, 115.03, 114.56, 53.09, 48.94. **Mass (m/z, %):**434.13(M^+); 435.32(M^++1, 100).

Compound-3b: 4-(((E)-4-((E)-(quinolin-3-ylimino)methyl)benzylidene)amino)-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol. IR(vcm-1): Stretching at 3597cm^{-1}due to Triazole-SH; Stretching of aromatic-CH absorption at 3087cm^{-1}, stretching aliphatic-CH at 2997, 2825, 2730cm^{-1}; Imine stretching (CH=N) at 1617cm^{-1}; C=C stretching (Aromatic) at 1465cm^{-1}; stretching at 1285cm^{-1} due to S-C group; stretching of C-N at 1026cm^{-1} respectively. **^{1}H-NMR(DMSO) δ ppm:** singlet hydrogen (Triazole-SH, 1H) at 11.4143ppm; singlet (Imine-H, 2H) at 9.8098 and 9.6842ppm; aromatic singlet protons(2H) 8.3876, and 8.3098ppm; aromatic protons(12H) with doublet and triplet at 7.9132-7.0983ppm; singlet protons(3H) at 2.0423ppm due to Aromatic-CH_3. **^{13}C-NMR(DMSO) δ ppm:** 157.34, 149.03, 147.34, 145.32, 140.21, 137.98, 136.24, 134.02, 128.45, 126.72, 124.67, 123.21, 120.91, 117.83, 115.35, 113.23, 59.98, 53.43, 27.98. **Mass(m/z,%):** 448.15(M^+); 449.23(M^++1, 100).

Compound-3c: 4-(((E)-4-((E)-((8-methylquinolin-3-yl)imino)methyl)benzylidene)amino)-5-phenyl-4H-1,2,4-triazole-3-thiol. IR(vcm-1): Triazole-SH stretching at 3544cm^{-1}; stretching at 3012cm^{-1} due to Aromatic-CH; aliphatic-CH stretching at 2913, 2882cm^{-1}; Imine-CH stretching at 1613cm^{-1}; aromatic-C=C stretching at 1417cm^{-1}; C-S stretching at 1265cm^{-1}; C-N stretching at 1012cm^{-1}. **^{1}HNMR(DMSO) δ ppm:** Triazole-SH(1H, singlet)stretching at 11.5092ppm; Imine-H(2H) singlet at 9.9245, 9.8453ppm; aromatic-H(2H)with singlet at 8.3987, 8.3903ppm; doublet and triplet protons(14H) at between 7.97567.3001ppm; singlet protons(3H) at 2.0654ppm respectively. **^{13}C-NMR(DMSO) δ ppm:** 155.34, 154.38, 147.73, 145.02, 142.12, 136.76, 132.34, 129.04, 126.45, 123.04, 120.45, 118.23, 116.04, 114.32, 53.87, 50.99, 30.45. **Mass (m/z, %):** 448.15(M^+); 449.03(M^++1, 100).

Compound-3d: 5-(4-chlorophenyl)-4-(((E)-4-((E)-(quinolin-3-ylimino)methyl)benzyl idene)amino)-4H-1,2,4-triazole-3-thiol. IR(vcm-1): Stretching at 3510cm^{-1} due to triazole-SH; aromatic-CH stretch at 3103cm^{-1};

aliphatic-CH stretch at 2951, 2891cm^{-1}; stretching of imine-CH at 1617cm^{-1}; Aromatic-C=C stretch at1461cm^{-1}; stretching at 1299cm^{-1} due to C-S; C-N stretching at 1037cm^{-1}; Aromatic-Cl stretch at 847cm^{-1}. **^{1}H-NMR (DMSO) δ ppm**: 12.0564ppm with singlet proton(1H) due to triazole-SH; singlet hydrogens (2H, Imine-H) at 9.4532 and 9.4013ppm. All aromatic protons are showing with singlet(2H) at 8.3674, 8.3012ppm, doublet(10H) at 7.9453-7.4536 and triplet(2H) at 7.2985-7.1985ppm respectively. **^{13}C-NMR (DMSO) δ ppm:** 150.94, 146.34, 142.76, 140.90, 137.23, 135.56, 132.92, 129.89, 126.43, 123.98, 120.45, 119.34, 117.54, 114.76, 113.98, 51.76, 47.89, 27.45. **Mass(m/z, %):** 468.09(M^+); 469.32(M^++1, 100); 470.24 (M^++2,30).

Compound-3e: 4-(((E)-4-((E)-((6-chloroquinolin-3-yl)imino)methyl)benzylidene)amino)-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol. IR(vcm^{-1}): stretching at 3521cm^{-1} due to triazole-SH; stretching at 3031cm^{-1} due to aromatic-SH; aliphatic stretching at 2986, 2789cm^{-1}; stretching at 1614cm^{-1} due to Imine-CH; aromatic-C=C stretch at 1485cm^{-1}; C-S stretching at 1283cm^{-1}; C-N stretch at 1038cm^{-1}. **^{1}H-NMR(DMSO) δ ppm:** singlet at 11.9854ppm due to triazole-SH; singlet protons(2H) at 9.5634 and 9.4192ppm; aromatic singlet protons(2H) at 8.4201, 8.3802, 8.1976ppm. Aromatic protons with double (10H) at between 7.8533-7.2034ppm; singlet protons(3H) at 2.1432ppm due to Aromatic-CH_3. **^{13}C-NMR(DMSO) δ ppm:** 157.43, 148.43, 145.22, 144.21, 143.20, 139.91, 137.24, 136.76, 132.24, 129.82, 126.76, 124.21, 123.01, 119.43, 117.82, 115.05, 57.73, 55.44, 30.97. **Mass (m/z, %):** 482.11(M^+); 483.15(M^++1, 100); 484.35 (M^++2, 30).

Compound-3f: 5-(4-chlorophenyl)-4-(((E)-4-((E)-((6-chloroquinolin-3-yl)imino)methyl) benzylidene)amino)-4H-1,2,4-triazole-3-thiol. stretching at 3521cm^{-1} due to triazole-SH; stretching at 3031cm^{-1} due to aromatic-SH; aliphatic stretching at 2986, 2789cm^{-1}; stretching at 1614cm^{-1} due to Imine-CH; aromatic-C=C stretch at 1485cm^{-1}; C-S stretching at 1283cm^{-1}; C-N stretch at 1038cm^{-1}. **^{1}H-NMR(DMSO) δ ppm:** singlet at 11.9854ppm due to triazole-SH; singlet protons(2H) at 9.5634 and 9.4192ppm; aromatic singlet protons(2H) at 8.4201, 8.3802, 8.1976ppm. Aromatic protons with double (10H) at between 7.8533-7.2034ppm. **^{13}C-NMR(DMSO) δ ppm:** 159.05, 154.23, 149.94, 145.24, 140.21, 138.06, 135.76, 134.22, 130.76, 127.94, 126.33, 124.97, 122.03, 118.78, 116.35, 113.34, 54.35, 51.67. **Mass (m/z, %):** 482.11 (M^+); 483.15(M^++1, 100); 484.35(M^++2, 30).

Compound-3g: 4-(((E)-4-((E)-((6-chloro-8-methylquinolin-3-yl)imino)methyl)benzyl idene)amino)-5-phenyl-4H-1,2,4-triazole-3-thiol. IR(vcm^{-1}): stretching at 3542cm^{-1} due to triazole-SH; stretching at 3098cm^{-1} due to aromatic-CH; stretching at 2987, 2876cm-1 due to presence of aliphatic-CH; stretching at 1610cm^{-1} due to Imine-CH; stretch at 1498cm^{-1} due to presence of aromatic-C=C; stretch at 1298cm^{-1} due to S-C; stretch at 1087cm^{-1} due to C-N, stretching at 798cm^{-1} due to Aromatic-Cl. **^{1}H-NMR(DSO) δ ppm:** singlet proton(1H, triazole-SH) at 12.0543ppm; singlet imine protons(2H) at 9.5132-9.3987ppm; aromatic singlet protons(4H) at 8.3763, 8.3012, 8.2874 and 8.0213ppm; doublet, triplet protons(69H) at 7.954-7.3843ppm due to aromatic-H; singlet methyl protons(3H, Aromatic-CH_3). **^{13}C-NMR (DMSO) δ ppm:** 154.76, 150.87, 148.03, 146.65,142.09, 138.98, 136.54, 134.11, 130.05, 128.76, 125.76, 123.32, 120.78, 118.47, 116.45, 115.32, 113.34, 57.34, 55.24, 29.45. **Mass (m/z, %):** 482.11(M^+); 483.21(M^++1, 100); 484.32(M^++2, 30).

Compound-3h: 4-(((E)-4-((E)-((8-methylquinolin-3-yl)imino)methyl)benzylidene)amino)-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol. IR(vcm^{-1}): stretching at 3564cm^{-1} due to triazole-SH; stretching absorption at 3018cm^{-1} due to aromatic-CH; aliphatic-CH stretching at 2987, 2875cm^{-1}; stretching at 1610cm^{-1} due to Imine-CH; aromatic-C=C stretching at 1423cm^{-1}; stretching at 1298cm^{-1} due to C-S; stretching at 1065cm^{-1} due to C-N. **^{1}H-NMR(DMSO) δ ppm:** singlet proton(1H) at 11.9824ppm due to triazole-SH; Imine protons(2H) with singlet at 9.5874 and 9.5120ppm; aromatic singlet proton(2H) at 8.3201 and 8.2132ppm; aromatic doublet and triplet protons(11H) due to 8.9453-7.3433ppm, singlet proton(6H) at 2.0423-2.1983ppm due to aromatic-methyl. **^{13}C-NMR(DMSO) δ ppm:** 157.34, 154.34, 149.02, 147.35, 145.32, 143.09, 140.24, 138.76, 135.32, 129.67, 127.45, 125.34, 123.87, 120.65, 118.21, 117.23, 56.74, 53.24, 28.93, 27.34. **Mass (m/z, %):** 461.12(M^+); 462.34(M^++1, 100).

Compound-3i: 4-(((E)-4-((E)-((6-chloro-8-methylquinolin-3-yl)imino)methyl)benzyl idene)amino)-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol. IR(vcm^{-1}): Triazole-SH stretch at 3568cm^{-1}; aromatic-CH stretching at 3103cm^{-1}; aliphatic-CH stretch at 2983, 2856cm^{-1}, Imine-CH stretching at 1609cm^{-1}; aromatic-C=C stretch at 1498cm^{-1}; stretch at 1299cm-1 due to S-C; stretch at 1098cm^{-1} due to C-C; stretching at 814cm^{-1} due to Aromatic-Cl. **^{1}H-NMR(DMSO) δ ppm:** singlet proton(1H) due to triazole-SH; Imine protons(2H) at 9.6322 and 9.5463ppm; Aromatic protons with singlet(4H) at 8.3412, 8.3023, 8.1092 and 8.0342ppm. aromatic doublet protons(10H) between 7.9522-7.3512ppm; singlet protons(4H) at 2.1384 and 2.0142ppm respectively. **^{13}C-NMR(DMSO) δ ppm:** 153.23, 149.043, 147.25, 145.87, 143.24, 139.82, 136.43, 134.23, 130.21, 128.93,

125.54, 123.98, 120.15, 116.23, 115.93, 50.23, 48.32, 30.26, 27.18. **Mass (m/z, %):** 496.12(M^+); 497.43(M^++1, 100); 498.71(M^++2, 30).

Compound-3j: 5-(4-chlorophenyl)-4-(((E)-4-((E)-((8-methylquinolin-3-yl)imino)methyl) benzylidene)amino)-4H-1,2,4-triazole-3-thiol. IR(vcm^{-1}): stretching at 3598cm^{-1} due to triazole-SH; aromatic-CH stretching at 3098cm^{-1}; aliphatic-CH stretching at 2987, 2875cm^{-1}; Imine stretching at 1613cm^{-1}; aromatic-C=C stretching at 1498cm^{-1}; S-C stretching at 1299cm^{-1}; C-N stretching at 10983cm^{-1}, aromatic-Cl stretching at 812cm^{-1}. **^{1}H-NMR(DMSO) δ ppm:** triazole-H with singlet(1H) at 11.9635ppm; singlet protons(2H) at 9.6823 and 9.5324ppm due to Imine-H; singlet aromatic-H(2H) at 8.3092 and 8.2143ppm, doublet(10H), triplet(1H) protons at between 7.9854-7.4823ppm; singlet proton(3H) at 2.2873ppm due to Aromatic-CH_3. **^{13}C-NMR(DMSO) δ ppm:** 150.23, 148.34, 146.08, 138.21, 137.05, 136.28, 130.81, 128.48, 126.16, 124.04, 123.76, 119.18, 117.14, 115.27, 55.38, 52.91, 27.79. **Mass (m/z, %):** 482.11(M^+); 483.24 (M^++1, 100); 484.65(M^++2, 30).

Compound-3k: 4-(((E)-4-((E)-((8-methylquinolin-3-yl)imino)methyl)benzylidene) amino)-5-(4-nitrophenyl)-4H-1,2,4-triazole-3-thiol. IR(vcm^{-1}): 3603cm^{-1} stretching due to triazole-SH; stretch at 3019cm^{-1} due to aromatic-CH; aliphatic-CH stretch at 2985, 2875cm^{-1}; stretching at 1632cm^{-1} due to Aromatic-NO_2; Imine stretch at 16012cm^{-1}; aromatic-C=C stretch at 1487cm^{-1}; C-S stretch at 1299cm^{-1}; stretch at 1088cm-1 due to C-N. **^{1}H-NMR(DMSO) δ ppm**: singlet(1H) at 11.945ppm due to triazole-SH proton; singlet(2H) imine-H at 9.4982 and 9.3564ppm; aromatic-H(2H) singlet at 8.3921 and 8.31092ppm; aromatic doublet protons(10H) at 8.0321-7.6532ppm; triplet aromatic-H(1H) at 7.2983ppm; singlet methyl protons(3H) at 1.9987pm respectively. **^{13}C-NMR(DMSO) δ ppm:** 157.24, 150.23, 149.32, 145.81, 143.91, 142.13, 138.76, 136.29, 130.45, 127.45, 125.36, 124.02, 123.27, 117.18, 115.28, 53.23, 52.19, 31.32. **Mass (m/z, %):** 493.13(M^+); 494.34(M^++1, 100).

Compond-3l: 4-(((E)-4-((E)-((6-chloroquinolin-3-yl)imino)methyl)benzylidene)amino)-5-(4-nitrophenyl)-4H-1,2,4-triazole-3-thiol. IR(vcm-1): triazole-SH stretch at 3624cm^{-1}; aromatic-CH stretch at 3089cm^{-1}; aliphatic-CH stretch at 2986, 2876cm^{-1}; stretching at 1638cm^{-1} due to Aromatic-NO_2; imine stretch at 1602cm^{-1}; aromatic-C=C stretch at 1467cm^{-1}; -C-S stretch at 1299cm^{-1}; C-N stretch at 1098cm^{-1}; Aromatic-Cl stretch at 798cm^{-1}. **^{1}H-NMR(DMSO) δ ppm:** singlet proton (1H, triazole-SH) at 12.0432ppm; singlet hydrogen (2H, imine-H) at 9.3723 and 9.3026ppm; aromatic singlet protons(3H) at 8.3897, 8.2532 and 8.1872ppm; aromatic doublet protons(10H) at 7.9453-7.3923ppm respectively.**^{13}C-NMR(DMSO) δ ppm**: 157.92, 153.26, 147.89, 145.26, 140.02, 139.78, 136.23, 134.19, 130.46, 129.56, 126.21, 123.07, 121.72, 119.04, 116.26, 114.37, 56.47, 53.12, 31.24. **Mass (m/z, %):** 513.08(M^+); 514(M^++1, 100); 515.45(M^++2, 30**).**

Pharmacological activity

In general, novel drug development, epidemiology, and therapeutic outcomes may benefit from antibacterial and anticancer testing. Therefore, as possible antibacterial and anticancer medicines, novel quinoline compounds have been evaluated for biological testing for in vitro evaluation and comparison with pure pharmaceuticals.

Antibacterial activity: The agar cup-plate method was implemented to evaluate the antibacterial activity. Zones of inhibition, showing a reduction in bacterial growth surrounding the agar cup, were carefully inspected on the plates following the incubation period. Finally, a zone reader was employed to measure and record the zone of inhibition in millimetres (mm). The results of these testes presented in Table-3. Most of the compounds exhibited lower activity against *E. coli, S.aureus and B. subtilis* when compared to streptomycin, which was used as standard. Conversely, compounds **3b, 3e, 3f** and **3j** demonstrated higher activity and exhibited antimicrobial effects against bacteria.

Table-2. Antibacterial activity of novel 1,2,4-triazole fused Quinoline derivatives (3a-3l)

Compounds	Zone of Inhibition in mm			
	Streptococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Klebsiella pneumonia***
3a	19	09	10	13
3b	**25**	**24**	12	**23**
3c	14	10	09	16
3d	15	13	11	19
3e	**26**	16	**27**	**25**
3f	**23**	**27**	18	**26**
3g	09	14	10	19
3h	15	17	12	09
3i	09	18	09	13

3j	**24**	**25**	18	**22**
3k	18	13	09	10
3l	20	10	13	12
Streptomycin	**31**	**33**	**30**	**32**

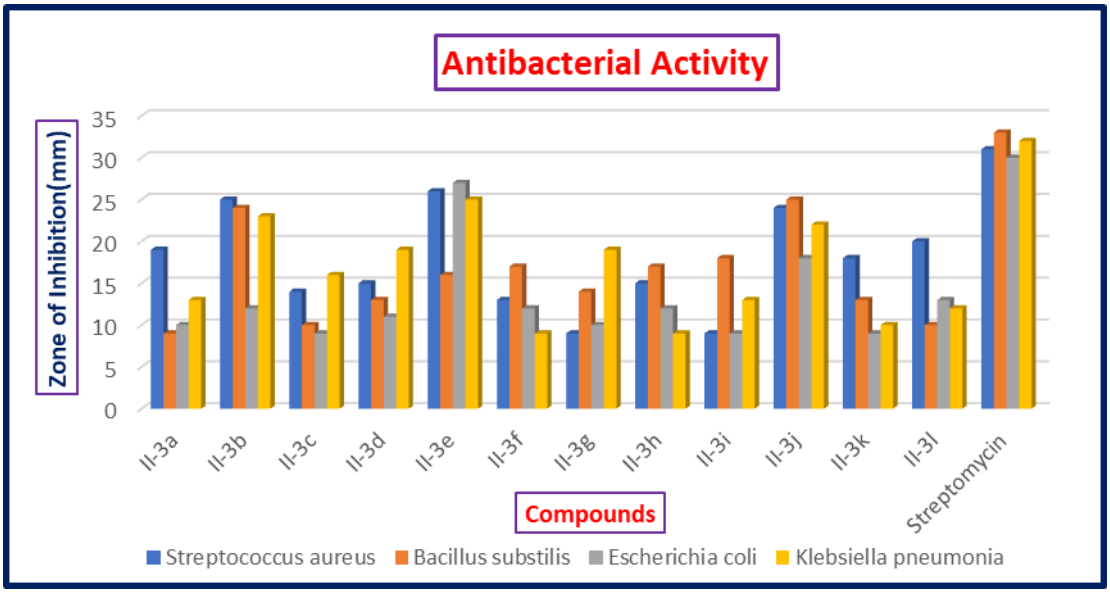

Figure-3. Graphical representation of antibacterial activity of compounds-II-3(a-l)-Zone of Inhibition

Anticancer activity:

A more thorough examination of the data in this table showed that compounds II-3(a-l) are more potent. The MTT Assay method was then employed to further evaluate the in vitro anticancer efficacy against the MCF-7 cell line, and Cisplatin was compared as a standard. From the results Table 3, the IC50 values of all test compounds were between **23.361±0.105 to 51.154±0.352µg/mL** and the **3e** and **3h** exhibited good anticancer activity against breast (MCF-7) cancer cell lines.

Table-3. Anticancer activity with IC_{50} values of 1,2,4-triazole fused Quinoline derivatives (3a, 3c, 3e, 3h, 3j, 3l)

Compound Name	MCF-7 Cell line (IC50 µg/mL)
3a	42.773±0.212
3c	46.886±0.308
3e**	**32.804±0.012**
3h**	**23.361±0.105**
3j	43.807±0.187
3l	51.154±0.352
Doxorubicin	**12.032±0.001**

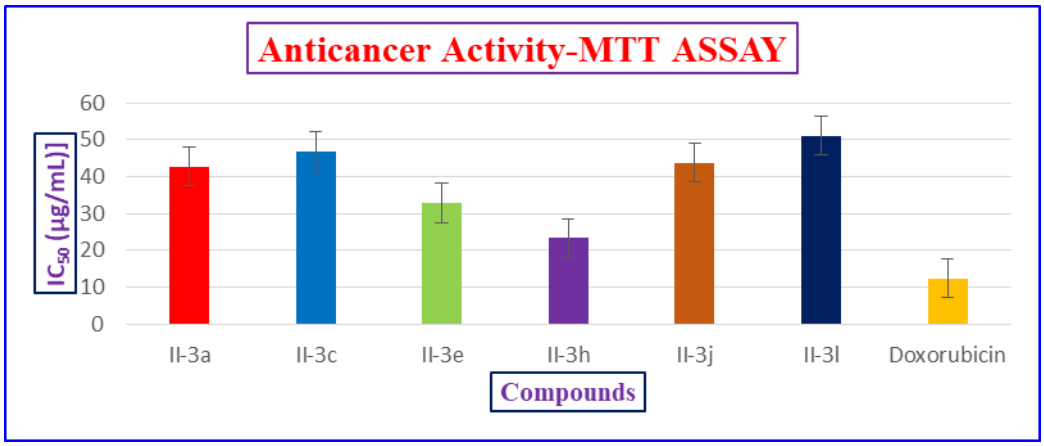

Figure-5. Graphical representation of anticancer activity by MTT Assay-IC50 vales (Mean ±S.E.M; n=6)

Molecular Docking Studies:

The binding interactions between the crystal structure of the Endothelial Growth Factor Receptor, or EGFR (PDB ID: 1M17), and new 1,2,4-triazole fused quinoline derivatives (3a-3l) were examined in molecular docking studies. Using AUTODOCK VINA, all 12 compounds were docked against the target protein's active site. In addition, these assisted to identify the ligand's conformational modifications in the protein environment. For each docked complex, the AUTODOCK suite of MGL Tools provided over 100 alternative protein-ligand complex conformations; the confirmation with the lowest binding energy was shown as the most effective binding energy. Table 4 presented the number of amino acids, interaction amino acids, and the binding energies of the dataset ligands.

Table-4. Molecular docking studies of synthesized compounds (3a-3l).

Compound No	Binding Energy (Kcal/mol)	No of H-bonds	H-bond lengths (Å)	Hydrogen bond interactions	Other Interacting amino acids (TARGET: 1M17)
3a	-10	NIL	NIL	NIL	PHE: 699, ALA:719, LYS:721, ASP:831, LEU:820, VAL:702
3b	-6.3	NIL	NIL	NIL	ASP:831, VAL:702, LEU:834, LYS:851, ASP:813
3c	-10.3	NIL	NIL	NIL	PHE: 699, ALA:719, LYS:721, ASP:831, LEU:820, VAL:702, ILE:735
3d	-6.6	NIL	NIL	NIL	PHE: 699, ALA:719, LYS:721, LEU: 768, LEU:694, LEU:820
3e	-10.2	NIL	NIL	NIL	PHE: 699, LYS:721, LEU:694, GLY: 772, ASP:831, VAL:702, LEU:834, LYS:851, ASP:813. MET:742, LEU:764
3f	-7.5	NIL	NIL	NIL	LEU:694, LEU:820, VAL:702, THR:766, PRO:853, ARG:817,
3g	-6.8	NIL	NIL	NIL	PHE: 699, ALA:719, LYS:721, ASP:831, LEU:820
3h	-10.6	NIL	NIL	NIL	PHE: 699, ALA:719, LYS:721, LEU: 768, LEU:694, LEU:820, VAL:702, THR:766, PRO:853, ARG:817, LEU:834, LYS:851, ASP:813. MET:742, LEU:764
3i	-7.3	NIL	NIL	NIL	VAL:702, THR:766, PRO:853, ARG:817, LEU:834
3j	-10.5	NIL	NIL	NIL	PHE: 699, ALA:719, LYS:721, LEU: 768, LEU:694, LEU:820, VAL:702, THR:766, PRO:853, ARG:817, LEU:834, LYS:851, ASP:813. MET:742, LEU:764
3k	-8.1	NIL	NIL	NIL	THR:766, PHE: 699, PRO:853, ARG:817, LEU:834
3l	-7.9	NIL	NIL	NIL	PHE: 699, THR:766, PRO:853, LEU: 768,

Every substance uses a mix of ionic/polar, hydrophobic, and hydrogen bonding interactions to interact with the protein target 1M17.

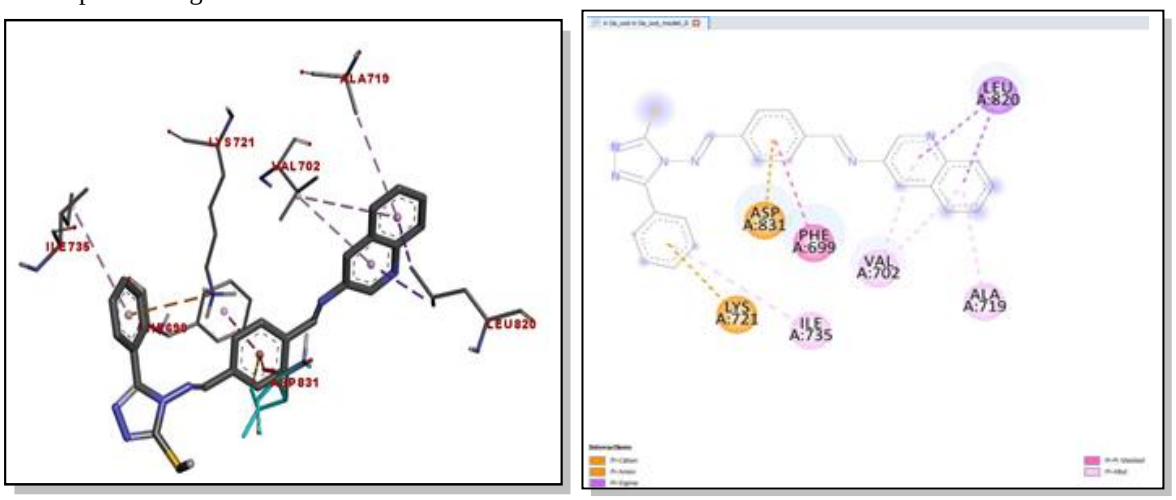

Figure-6. Compound-3a (2D and 3D)

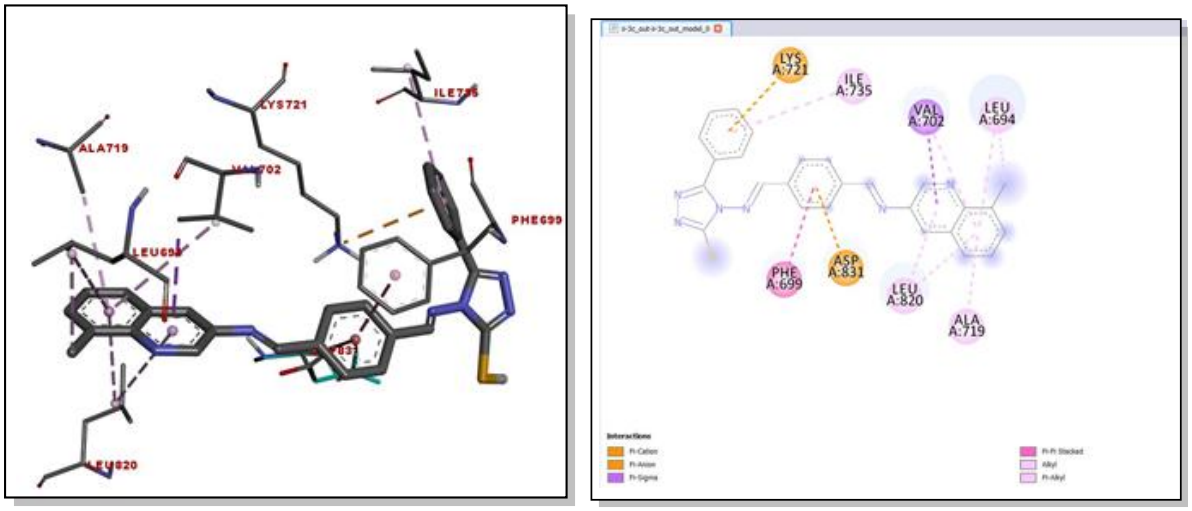

Figure-7. Compound-3c (2D and 3D)

IV. Conclusion

New 1,2,4-triazole fused quinoline derivatives (3a-3l) and FT-IR, ^{1}HNMR, ^{13}C-NMR and mass spectroscopy techniques were used to analyse all novel compounds. The MTT assay was used to evaluate the anticancer activity of the newly synthesized compounds, and almost all of them shown good to moderate activity against bacteria (Streptococcus aureus, Bacillus subtilis, Escherichia coli, and Klebsiella pneumonia). Compounds **3b, 3e, 3f** and **3j** demonstrated higher activity and exhibited antimicrobial effects against bacteria and 3**e** and **3h** exhibited good anticancer activity against breast (MCF-7) cancer cell lines. Novel compounds with electron-donating substituents showed strong antibacterial and anticancer action, and as a result, they may be used as lead molecules in developing more unique, clinically valuable entities in the future.

Aknowledgmet

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