DESIGN, SYNTHESIS, MOLECULAR DOCKING STUDIES OF NOVEL QUINAZOLINE HYBRIDS AS POTENTIAL ANTICANCER AGENTS

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Abstract

A series of ten novel aniline linked quinazoline derivatives **(5a-5j)** were synthesized in good to excellent yields by multicomponent reaction. Compound 4-(2-chloroethyl)-2-methylquinazoline **(3)** was generated by coupling reaction of N-phenylacetamide **(1)** and 3-chloropropanenitrile **(2)** in presence of trifluoromethanesulfonic anhydride,2-chloropyridine and carbon tetra chloride under reflux. Compound **(3)** up on condensation with different substituted anilines in the presence of trimethyl amine as a catalyst and DMF yielded the final product. The synthesized derivatives were characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS. Subsequently, the compounds were screened for their cytotoxicity potential against HeLa, LoVo, PA-1 and MCF-7 cancer cell lines and one normal human fibroblast using the MTT method. Designed quinazolines derivatives were studied for their molecular interaction with the target EGFR kinase (2ITY) using the molecular docking tool GLIDE. Among them, most of the compounds showed good to

excellent anticancer activity, especially 5e, 5g, 5h and 5i exhibited the most potent cytotoxic activity against cancer cell lines. Compounds 5c and 5h displayed highest interactions with the target EGFR kinase. These findings directly identify the aniline linked quinazoline derivatives as novel anticancer agents and further verify the importance of the quinazoline pharmacophore in drug design.

Keywords: Aniline linked quinazoline, characterization, anticancer, MTT method.

Introduction

Cancer is one of the leading causes of the death globally. Numerous anti-cancer drugs and treatment regimens are already used for effective treatment of cancer, and numerous drugs are in various stages of clinical trials. There are wide ranges of anticancer medications available on the market, and studies indicate that these compounds may cause a variety of adverse effects. The lowering of a drug's cytotoxicity to healthy cells is a significant issue in the treatment of cancer. As a result, scientists from all over the world are working to develop anticancer medications that are both effective and safe. As a result of intensive research, it is now possible to examine the quinazoline scaffold and its numerous derivatives as a novel class of cancer chemotherapeutic drugs that have already demonstrated promising activity against diverse tumours. Quinazoline derivatives already hold a significant position in contemporary medicinal chemistry. Quinazoline and its derivatives has been the subject of interest in numerous studies because of their potential

to treat cancer (Rina et al., 2021). Anticancer drugs which contain quinazoline in its structural motif are lapatinib, erlotinib, gefitinib, canertinib and vandetanib (Asmaa et al., 2014; Mostafa et al., 2016).

Quinazoline (1,3-diazanaphthalene) is an aromatic heterocyclic compound, it has bicyclic structure that contains a benzene ring fused to pyrimidine (Alagarsamy et al., 2018). Quinazolines and its derivatives possess an extensive range of biological activities like anti-cancer (Sasada et al., 2008), antiviral (Jafari et al., 2016), antibacterial, anti-tubercular (Al-Salahi et al., 2016), antihypertensive (Selvam et al., 2014), anti-inflammatory, anti-oxidant (Solomon et al., 2004), anti-convulsant (Rakesh et al., 2015), antifungal (Ugale et al., 2014), analgesic (Ji et al., 2014), sedative-hypnotic (Alagarsamy et al., 2002), and anti-histaminic (Raju et al., 1999).

The current research endeavours on the synthesis of novel quinazoline-aniline derivatives and their subsequent evaluation for anticancer activity is driven by the pressing demand for the discovery and development of highly efficacious and safer anti-cancer agents. The primary objective of this study is to contribute to the advancement of novel compounds that possess enhanced therapeutic potential, there by augmenting the available arsenal of cancer treatment options and ultimately leading to improved clinical outcomes for patients.

Materials and Methods

Analytical grade chemicals were purchased from sigma Aldrich, Bangalore, India for the study. Monitored the reaction by merck-precoated aluminium TLC plates of silica gel 60 F254. Visualized the spots with iodine vapours and in the UV chamber. For the isolation and purification of the compounds, column chromatography was used. The melting points of the compounds were determined by Remi electronic melting point apparatus. ^{1}H NMR spectra was recorded on BRUKER DRX – 500 MHz. Expressed chemical shift values (δ) in ppm with reference to TMS (tetramethyl silane)- internal standard. Designated the splitting patterns as: s,

singlet; d, doublet; t, triplet; q, quartet; m, multiplet. MASS spectra recorded on BRUKER ESI-IT MS.

General procedure for the synthesis of substituted Quinazolines

The N-vinyl amides used as the starting materials for the two-step synthesis of the quinazoline derivatives. This approach relies on electrophilic amide activation, employing the reagent combination of 2-chloropyridine (2-ClPyr) and trifluoromethanesulfonic anhydride (Tf_2O). Additionally, the quinazoline moiety linked to different substituted anilines in an amination reaction with the previously generated quinazoline alkyl chloride (Figure 1) (Henry, 1925; Shubankhar et al., 2014).

Step 1: Procedure for the synthesis of 4-(2-chloroethyl)-2-methylquinazoline (3)

Equimolar mixtures of starting materials substituted N-phenylacetamide **(1)** were mixed with a substituted 3-chloropropanenitrile **(2)** in CCl_4, 0.5 equivalents of Tf_2o (trifluoromethanesulfonic anhydride) and 2-chloropyridine were added to reaction mixture and kept under reflux (Henry., 1925). Constantly monitored the reaction by TLC under UV- light. After completion of reaction, acetic acid was used for acidic work up process, to get the crude product of quinazoline **(3)**. The crude product was isolated by using suitable mobile phase system from the column chromatography and purified the product by recrystallization in suitable solvent.

Step 2: Procedure for the synthesis of aniline linked quinazoline derivatives (5a-5j)

4-(2-chloroethyl)-2-methylquinazoline **(3)** and various substituted anilines **(a-j)** dissolved in DMF and agitated the mixture at room temperature using triethylamine as a catalyst. After completion of the reaction, the mixture subjected to acidic work up with dilute CH_3COOH and the crude product was purified using column chromatography with suitable solvent to get the final product **(5a-5j)**.

Cytotoxic activity: MTT Assay

Cervical (HeLa), breast (MCF7), ovarian (PA-1), colorectal carcinoma (LoVo) and one normal human dermal fibroblast (NHDF) cell lines were grown in Dulbecco's modified Eagle's medium added with 10% fetal bovine serum and 1% penicillin and 10 mg of streptomycin and incubated in a humidified atmosphere of 5% CO_2 at 37 °C. The cells were seeded and maintained in triplicate in a 96-well plate in 150 μL of the culture medium for study purposes and permitted to attach for 24 h prior to treatment. The cells were then treated with the synthesized compounds (5a-j) and doxorubicin (standard) at 0, 1, 5, 10, 25, and 50 μM and incubated for 24 h. DMSO was used as a control, as all compounds were solubilized in 50% DMSO. Maintained less than 0.1% concentration of DMSO in each well. After 24 h, the growth medium was removed and replaced with 100 μl of serum-free medium containing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), (5 mg/mL)) and incubated for 4 h at 5% CO_2 and 37 °C. Yellow, water-soluble tetrazolium salt -MTT, is converted to purple, water-insoluble formazan, by mitochondrial dehydrogenases. The old medium was then discarded, and the formazan was dissolved by adding 100 μl of isopropanol. The absorbance was measured at 570 nm using a monochromator multimode reader against the blank (Hatem et al., 2017).

Molecular Docking

The X-ray crystal structures of EGFR kinase (2ITY) was obtained from the Protein Data Bank. The Protein Preparation Wizard module of Schrödinger software was used to prepare the protein complex by introducing hydrogen atoms and allocating bond orders to the protein's 3D structure. The LigPrep module of Schrödinger software was used to prepare ligands with defined chirality and optimize their 3D structures using the OPLS 2005 force field. The receptor sites for 2LY4 and 4QR9 were analyzed using the SITEMAP ANALYSIS TOOL of Maestro 11.8, and receptor grids were generated using the grid generation tool of Schrödinger suite. Molecular

docking was performed using the Glide program's extra-precision docking modes (Glide XP), and the XP Glide score was calculated using the binding interaction energy, van der Waals energy, electrostatic potential energy, and strain energy. The binding interaction of the ligands to the active site of EGFR kinase was examined using Schrödinger Maestro interface (Choodamani et al., 2021).

Results

Synthesis

The structural and physicochemical data of the synthesized quinazoline derivatives were enumerated in Table 1.

Spectral characteristics of the synthesizedquinazoline derivatives

N-(2-(2-methylquinazolin-4-yl)ethyl)aniline (5a)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.2 Hz, 1H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.1, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 7.14 – 7.07 (m, 2H), 6.73 (tt, J = 6.8, 1.0 Hz, 1H), 6.63 – 6.57 (m, 2H), 5.95 (t, J = 5.5 Hz, 1H), 3.48 (q, J = 5.3 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 2.41 (s, 3H).^{13}C NMR: δ 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 119.9 (2C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 129.4 (1C, s), 130.4 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 159.3 (1C, s), 166.4 (1C, s). Mass: *m/z:* $(M+H)^{+}$ found 264.25 for $C_{17}H_{17}N_3$.

4-fluoro-N-(2-(2-methylquinazolin-4-yl)ethyl)aniline (5b)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.3 Hz, 1H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.1, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 6.97 – 6.89 (m, 2H), 6.80 – 6.74 (m, 2H), 5.74 (t, J = 5.6 Hz, 1H), 3.48 (q, J = 5.3 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 2.46 (s, 3H).^{13}C NMR: δ 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 115.6 (2C, s), 118.2 (2C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 129.4 (1C, s), 130.4 (1C, s), 147.3 (1C, s),

148.4 (1C, s), 159.3 (1C, s), 162.5 (1C, s), 166.4 (1C, s).Mass: *m/z:* $(M+H)^+$ found 282.15 for $C_{17}H_{16}FN_3$.

4-chloro-N-(2-(2-methylquinazolin-4-yl)ethyl)aniline (5c)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.2 Hz, 1H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.1, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 7.14 – 7.08 (m, 2H), 6.65 – 6.59 (m, 2H), 5.78 (t, J = 5.5 Hz, 1H), 3.48 (q, J = 5.3 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 2.49 (s, 3H).^{13}C NMR: δ 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 120.5 (2C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 128.9 (2C, s), 129.4 (1C, s), 130.4 (1C, s), 133.7 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 159.3 (1C, s), 166.4 (1C, s).Mass: *m/z:* $(M+H)^+$ found 298.05 for $C_{17}H_{16}ClN_3$.

N-(2-(2-methylquinazolin-4-yl)ethyl)-4-nitroaniline (5d)

Light brown Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.2 Hz, 1H), 8.03 – 7.97 (m, 2H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.1, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 6.84 – 6.78 (m, 2H), 6.30 (t, J = 5.6 Hz, 1H), 3.43 (q, J = 5.3 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 2.50 (s, 3H). ^{13}C NMR: δ 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 116.6 (2C, s), 125.0 (2C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 129.4 (1C, s), 130.4 (1C, s), 147.2-147.4 (2C, 147.3 (s), 147.3 (s)), 148.4 (1C, s), 159.3 (1C, s), 166.4 (1C, s). Mass: *m/z:* $(M+H)^+$ found 309.15 for $C_{17}H_{16}N_4O_2$.

4-methoxy-N-(2-(2-methylquinazolin-4-yl)ethyl)aniline (5e)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.2 Hz, 1H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.1, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 6.83 – 6.77 (m, 2H), 6.64 – 6.58 (m, 2H), 5.94 (t, J = 5.6 Hz, 1H), 3.77 (s, 3H), 3.43 (q, J = 5.2 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 2.43 (s, 3H). ^{13}C NMR: δ 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 56.0 (1C, s), 114.5 (2C, s), 120.5 (2C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 129.4 (1C, s), 130.4 (1C,

s), 147.3 (1C, s), 148.4 (1C, s), 159.3 (1C, s), 159.8 (1C, s), 166.4 (1C, s). Mass: *m/z:* $(M+H)^+$ found 294.25 for $C_{18}H_{19}N_3O$.

2,5-dichloro-N-(2-(2-methylquinazolin-4-yl)ethyl)aniline (5f)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.3 Hz, 1H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.1, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 7.34 (d, J = 7.2 Hz, 1H), 7.04 – 6.95 (m, 2H), 6.85 (d, J = 2.2 Hz, 1H), 3.56 (q, J = 5.4 Hz, 2H), 3.05 (t, J = 5.3 Hz, 2H), 2.43 (s, 3H). ^{13}C NMR: δ 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 121.6 (1C, s), 121.8 (1C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 129.3-129.4 (2C, 129.3 (s), 129.4 (s)), 130.2 (1C, s), 130.4 (1C, s), 132.3 (1C, s), 134.4 (1C, s), 147.3 (1C, s), 159.3 (1C, s), 166.4 (1C, s). Mass: *m/z:* $(M+H)^+$ found 333.10 for $C_{17}H_{14c}l_2N_3$.

N-(2-(2-methylquinazolin-4-yl)ethyl)-4-(trifluoromethyl)aniline (5g)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.2 Hz, 1H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.1, 1.3 Hz, 1H), 7.54 (dq, J = 7.5, 1.4 Hz, 2H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 6.77 – 6.71 (m, 2H), 5.76 (t, J = 5.6 Hz, 1H), 3.48 (q, J = 5.3 Hz, 2H), 3.03 (d, J = 5.2 Hz, 2H), 2.46 (s, 3H).^{13}C NMR: δ 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 117.9 (2C, s), 123.8 (1C, s), 125.7 (1C, s), 126.4-126.5 (3C, 126.4 (s), 126.5 (s)), 126.9 (1C, s), 129.4 (1C, s), 130.3-130.5 (2C, 130.3 (s), 130.4 (s)), 147.3 (1C, s), 148.4 (1C, s), 159.3 (1C, s), 166.4 (1C, s). Mass: *m/z:* $(M+H)^+$ found 332.15 for $C_{18}H_{16}F_3N_3$.

4-methyl-N-(2-(2-methylquinazolin-4-yl)ethyl)aniline (5h)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.2 Hz, 1H), 7.88 (dd, J = 7.6, 1.3 Hz, 1H), 7.72 (td, J = 7.2, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 7.03 – 6.98 (m, 2H), 6.64 – 6.58 (m, 2H), 5.61 (t, J = 5.6 Hz, 1H), 3.48 (q, J = 5.3 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 2.46 (s, 3H), 2.33 (d, J = 1.2 Hz, 3H). ^{13}C NMR: δ 21.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 117.9 (2C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 129.4 (1C, s), 129.6

(2C, s), 130.4 (1C, s), 141.5 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 159.3 (1C, s), 166.4 (1C, s). Mass: *m/z:* $(M+H)^+$ found 278.20 for $C_{18}H_{19}N_3$.

4-ethyl-N-(2-(2-methylquinazolin-4-yl)ethyl)aniline (5i)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.2 Hz, 1H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.1, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 7.12 (dt, J = 7.9, 1.0 Hz, 2H), 6.64 – 6.58 (m, 2H), 5.66 (t, J = 5.5 Hz, 1H), 3.48 (q, J = 5.3 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 2.67 – 2.59 (m, 2H), 2.46 (s, 3H), 1.20 (t, J = 7.3 Hz, 3H). ^{13}C NMR: δ 14.6 (1C, s), 25.8 (1C, s), 28.7 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 117.9 (2C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 129.4 (1C, s), 129.9 (2C, s), 130.4 (1C, s), 144.2 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 159.3 (1C, s), 166.4 (1C, s). Mass: *m/z:* $(M+H)^+$ found 292.35 for $C_{19}H_{21}N_3$.

3,4-dimethyl-N-(2-(2-methylquinazolin-4-yl)ethyl)aniline (5j)

Pale Yellow Solid; ^{1}H NMR (500 MHz, DMSO-d6) δ 8.09 (dd, J = 9.4, 1.2 Hz, 1H), 7.88 (dd, J = 7.5, 1.3 Hz, 1H), 7.72 (td, J = 7.2, 1.3 Hz, 1H), 7.40 (ddd, J = 9.6, 7.0, 1.3 Hz, 1H), 6.94 (dt, J = 7.7, 1.1 Hz, 1H), 6.51 – 6.44 (m, 2H), 6.20 (t, J = 5.6 Hz, 1H), 3.51 (q, J = 5.3 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 2.46 (s, 3H), 2.21 (d, J = 0.7 Hz, 6H). ^{13}C NMR: δ 19.9-20.1 (2C, 20.0 (s), 20.0 (s)), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 117.9 (1C, s), 119.9 (1C, s), 125.7 (1C, s), 126.4 (1C, s), 126.9 (1C, s), 129.4 (1C, s), 130.0 (1C, s), 130.4 (1C, s), 130.9 (1C, s), 133.4 (1C, s), 147.3 (1C, s), 148.5 (1C, s), 159.3 (1C, s), 166.4 (1C, s). Mass: *m/z:* $(M+H)^+$ found 292.35 for $C_{19}H_{21}N_3$.

Cytotoxic activity

The synthesized quinazoline derivatives (5a-5j) were evaluated for their cytotoxicity potential against cervical (HeLa), breast (MCF7), ovarian (PA-1), colorectal carcinoma (LoVo) and one normal human dermal fibroblast (NHDF) cell lines and compared with the standard anticancer drug Doxorubicin. The cytotoxic activities of titled compounds were measured as IC_{50} (in µM)

value. The results of MTT assay were mentioned in Table 2. The assay results showed that the tested compounds exhibited good to moderate anti-proliferative activities against the tested cell lines compared to normal cells with reference to the standard anticancer drug Doxorubicin.

The assay results revealed that among the all-synthesised compounds **5e** showed potent cytotoxicity against four cancer cell lines employed in the study such as HeLa (IC_{50} 11.26± 0.162 µM), MCF7 (IC_{50} 12.82±1.05µM), PA-1 (IC_{50} 17.29±1.03µM), LoVo(IC_{50} 14.25±0.99 µM). Compound **5h** showed good cytotoxic activity against HeLa (IC_{50} 9.15±1.83 µM), MCF7 (IC_{50} 9.66±1.01 µM), PA-1 (IC_{50} 7.03±1.49 µM) and **5i** showed good activity against HeLa (IC_{50} 11.92±0.51µM), MCF7 (IC_{50} 11.23±2.32 µM), LoVo(IC_{50} 11.56±1.72 µM) cancer cell lines. Compound **5g** displayed good activity against HeLa (IC_{50} 6.60±1.33µM), PA-1 (IC_{50}13.62±2.32 µM), LoVo (IC_{50} 14.60±1.01µM) cancer cell lines. Compound **5d** exhibited toxicity against HeLa (IC_{50} 12.13± 0.147µM), MCF7 (IC_{50} 12.41±0.45µM), and compound **5j** PA-1 (IC_{50} 13.13±4.25 µM), LoVo(IC_{50} 12.15±0.65 µM) cancer cell lines.Compound **5c** showed good activity against MCF-7 cancer cell line with IC_{50}12.66±1.02 µM.

Molecular Docking

Results of the molecular docking experiments of designed compounds with the target EGFR kinase were reported in Table 1. The molecular docking results of quinazoline derivatives with EGFR kinase (2ITY) show that compounds 5c, 5h, and doxorubicin have the lowest binding energies. This suggests that these compounds are the most likely to inhibit the activity of EGFR kinase.

Discussion

A novel series of aniline linked quinazoline derivatives were synthesized in good to excellent yields through a feasible multicomponent reaction. Synthesized compounds were characterized and assessed for anticancer activity against four cancer cell lines (HeLa, Lovo, PA-1 and MCF-

7) and one normal (NHDF) cell lines. Relatively most of the compounds showed good to moderate cytotoxic activity against the tested cell lines compared to normal cells with reference to the standard anticancer drug Doxorubicin.

Among the series of compounds synthesized, molecule **5g** (4-CF_3) was found to be more potent against HeLa cell lines, followed by molecule **5h** (4-CH_3), and the molecule **5e** (4-OCH_3), displaying IC_{50} values of 6.60±1.33, 9.15±1.83, and 11.26± 0.162 µM respectively. The substance 5f (2,5-dichloro) with an IC_{50} of 26.18± 4.73 µM exhibited the least cytotoxic activity against HeLa cell lines. Weak electron-withdrawing group substituted **5f** (2,5-diCl) demonstrated the least activity while strong electron-withdrawing group substituted **5g** and weak and moderate electron releasing group bearing **5h** and **5e** showed significant activity against HeLa cell lines.

The molecules **5h** (4-CH_3), **5i** (4-OC_2H_5) and **5f** (2,5-diCl), were found to be active against MCF7 cell lines with IC_{50} = 9.66±1.01, 11.23±2.32 and 12.15±0.52µM respectively. Least potency (IC_{50} = 28.39±1.01µM) was exhibited by the compound 5j(3,4-dimethyl) against these cell lines.Weak and moderate electron-releasing groups containing compounds **5h** and **5i**, as well as moderate electron-withdrawing group possessing compound **5f**, showed potent cytotoxicity, while weak electron-releasing group substituted compound **5j** (3,4 dimethyl) exhibited the least cytotoxicity.

The molecule **5h** (4-CH_3) showed significant cytotoxicity with IC_{50} being 7.03±1.49 µMagainst PA-1 cell lines. In addition, to 5h, the molecules **5j** (3,4 dimethyl), and **5g**(4-CF_3), also displayed good cytotoxicity against PA-1 cell lines, with IC_{50} values of 13.13±4.25 and 13.62±2.32 µM respectively. The substance **5f** (2,5-diCl), showed least activity against PA-1 cell lines. The weak electron-releasing substituent's bearing molecules 5h and 5j and strong electron withdrawing group substituted compound 5g showed potent activity, while moderate electron withdrawing group possessing 5f displayed least cytotoxicity.

The compounds **5i** (4-OC_2H_5), **5j** (3,4 dimethyl), and **5e** (4-OCH_3) showed potent cytotoxicity against LoVo cell lines, with IC_{50}values of 11.56±1.72, 12.15±0.65 and 14.25±0.99 µM, respectively.The compounds bearing moderately electron-releasing ethoxy and methoxy groups, i.e., **5i** and **5e,** and the weak electron-releasing methyl group substituted compound **5j,** demonstrated good activity against LoVo cell lines. Compound 5h showed the least activity against LoVo cell lines. The addition of another methyl group at the 3-position of 5h leads to increased cytotoxicity, as seen with compound 5j against LoVo cell lines.

From the SAR of the synthesized compounds, it was found that cytotoxicity of the compounds depends on the substituent group present on the benzene ring. It was discovered that weak electron-releasing groups such as 4-CH_3 and 4-C_2H_5, as well as moderate electron-releasing groups 4-OCH_3 and strong electron-withdrawing groups 4-CF_3 on the benzene ring, enhanced cytotoxic activity, whereas molecules containing groups such as weak electron with drawing and electron releasing i.e., 2,5-diCl, 3,4-dimethyl, and 4-CH_3 had the least cytotoxicity. The 4-CH_3 on the benzene ring showed potent activity against three cell lines HeLa, MCF-7, and PA-1, while it displayed the least activity against LoVo cell lines. The results revealed that the phenyl ring is essential for cytotoxic activity.

The binding energy is a measure of the strength of the interaction between a ligand and a receptor. In the case of the quinazoline derivatives, the lower binding energies of compounds 5c, 5h, and doxorubicin are likely due to their favorable interactions with the key residues in the EGFR kinase binding pocket. Compound 5c has a hydrogen bond with Thr766 and a hydrophobic interaction with Phe873 (Figure 2(a)). Compound 5h has a hydrogen bond with Thr766 and a π-π stacking interaction with Phe873 (Figure 2(b)). Doxorubicin has a hydrogen bond with Thr766 and a hydrophobic interaction with Tyr872. The lower binding energies of these compounds suggest that they are more likely to inhibit the activity of EGFR kinase. EGFR

kinase is a key enzyme in the signaling pathway that leads to the growth and proliferation of cancer cells. By inhibiting the activity of EGFR kinase, these compounds can potentially prevent the growth and spread of cancer cells.

Conclusion

A novel series of aniline linked quinazoline derivatives were synthesized in good to excellent yields through a feasible multicomponent reaction. Synthesized compounds were characterized and assessed for anticancer activity against three selected cancer cell lines and one normal cell line. Relatively most of the compounds showed good to moderate cytotoxic activity against the tested cell lines compared to normal cells with reference to the standard anticancer drug Doxorubicin. The compound 5e (4-OCH_3) exhibited highest potency against all the cell lines followed by compounds 5g (4-CF_3), 5h (4-CH_3) and 5i (4-C_2H_5).The results revealed that the phenyl ring is essential for cytotoxic activity. Molecular docking of quinazoline derivatives revealed that the compounds have the potential to interact with the target EGFR kinase (2ITY). This suggests that the compounds may be cytotoxic, or cell-killing, and that the cytotoxicity may be due to the molecular interactions between the compounds and the EGFR kinase. Further investigations are needed to establish the detailed mechanism of action of the developed novel quinazoline derivatives.

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Conflict of Interest

Authors declare no conflict of interest

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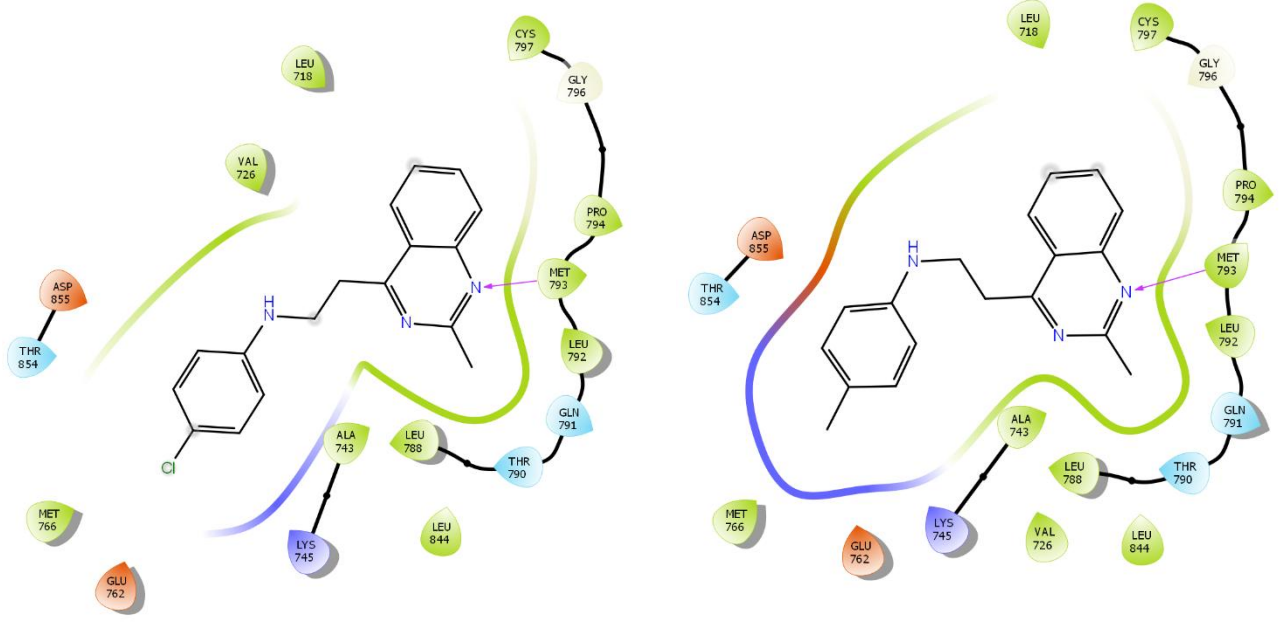
Figure1: Scheme of synthesis of substituted Quinazoline



2(a) **2(b)**

Figure 2: Molecular interaction diagram of **2(a)** Compound 5c **2(b)** Compound 5h with EGFR kinase

Table 1: Synthesized quinazoline derivatives Physical Characterization

Compound	**Structure**	**Yield (%)**	**Melting point (^{0}C)**	**Docking Score**
5a		72	218-220	-5.787
5b		72	203-204	-6.333
5c		69	215-216	-7.764
5d		78	241-241	-5.743
5e		65	233-234	-5.48
5f		70	268-269	-5.894
5g		66	246-247	-6.276

5h		68	221-222	-7.205
5i		63	236-238	-5.587
5j		61	251-252	-6.442
Doxorubicin				-8.081

Table 2: IC_{50} (µM) values of the synthesized quinazoline derivatives

Sample	**Cervical cancer (HeLa)**	**Breast cancer (MCF7)**	**Ovarian cancer (PA-1)**	**Colorectal carcinoma (LoVo)**	**Human dermal fibroblasts (NHDF)**
5a	17.96± 0.121	21.84±2.95	17.82±1.01	20.36±1.50	44.32±1.62
5b	15.09± 0.095	20.77±1.01	19.09±1.20	20.84±0.69	48.51±0.66
5c	13.27± 0.135	12.66±1.02	16.64±0.52	20.04±0.77	42.04±1.85
5d	12.13± 0.147	12.41±0.45	16.23±0.35	19.18±0.69	52.21±1.69
5e	11.26± 0.162	12.82±1.05	17.29±1.03	14.25±0.99	49.93±3.02
5f	26.18± 4.73	12.15±0.52	37.04±0.69	19.44±0.51	41.55±0.85

5g	6.60±1.33	20.15±1.33	13.62±2.32	14.60±1.01	48.79±0.55
5h	9.15±1.83	9.66±1.01	7.03±1.49	21.30±0.65	46.11±0.58
5i	11.92±0.51	11.23±2.32	18.78±0.90	11.56±1.72	47.65±2.98
5j	23.22±0.68	28.39±1.01	13.13±4.25	12.15±0.65	38.144±1.77
Doxorubicin	1.25±1.8	1.34±4.1	1.81±1.7	1.18±0.96	2.01±0.83