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6,7-disubstituted-4-anilinoquinazoline: design, synthesis and anticancer activity as a novel series of potent anticancer agents

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Running title: Novel 4-anilinoquinazoline derivatives as potent anticancer agents

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Abstract

Background: Epidermal Growth Factor Receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) are responsible for several pathological conditions such as the development of different kinds of tumors. The combined inhibition of both signal transduction pathways seems to be a promising novel approach for cancer treatment.

Methods: In this study, novel 4-anilinoquinazoline derivatives with various substituents on C-7 position of quinazoline moiety were designed, synthesized, and evaluated for their anti-proliferative activity against A431 and HU02 cell lines.

Results: Compounds **8a**, **8d**, and **8f** displayed the most potent anticancer activities against A431 ($IC_{50} = 1.78 \mu M$, $8.25 \mu M$, and $7.18 \mu M$, respectively) in comparison with reference standards (erlotinib $IC_{50}=8.31 \mu M$ and vandetanib $IC_{50}=10.62 \mu M$). Molecular docking studies proved that **8a** as the most potent compound could be efficiently accommodated in the ATP binding site of EGFR and VEGFR-2 through the formation of essential hydrogen bonds between quinazoline N1 atom and the Met796 backbone of EGFR as well as the Cys919 backbone of VEGFR-2 with a distance of 1.94 \AA and 1.398 \AA , respectively.

Conclusion: Compound **8a** as the most potent compound with morpholine and 3-bromoaniline at the 7 and 4 positions of quinazoline scaffold, respectively, deserves more study and structural optimization as an anticancer agent.

Keywords: Synthesis, Antiproliferative activity, 4-anilinoquinazoline, Molecular modeling

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۲۷ **Introduction**

۲۸ Most of the cellular functions including proliferation, angiogenesis, differentiation, and migration
۲۹ are regulated by protein kinases and their overexpression plays a critical role in formation of cancer
۳۰ cells.¹⁻³ Abnormal protein kinases signaling, particularly receptor tyrosine kinases (RTKs), which
۳۱ mediates numerous signal transduction pathways may cause both the proliferation of cancer cells
۳۲ and angiogenesis as well as tumor development.^{4,5} The ErbB family of RTK consists of four
۳۳ distinct members, including the epidermal growth factor receptor (EGFR; ErbB1; HER1 in
۳۴ humans), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4).^{6,7} Activation of the cytoplasmic
۳۵ tyrosine kinase domains and downstream signaling pathways happen by binding ligands such as
۳۶ growth factors to the extracellular domain of EGFR. It consequently induces dimerization and
۳۷ autophosphorylation.⁸ The activation of various signal transduction pathways (Jak/STAT,
۳۸ Ras/MAPK, PI3K/Akt) and intracellular processes due to EGFR dysregulation by upactivation,
۳۹ overexpression or mutation can cause tumorigenesis and is usually associated with a poor
۴۰ prognosis in cancerous disorders.^{1,2} Thus, EGFR can be considered as a useful therapeutic target
۴۱ for developing novel anticancer agents.⁹ Angiogenesis, through which new blood and lymphatic
۴۲ vessels form from pre-existing vasculature, is a vital process in both normal physiological
۴۳ development as well as continued tumor growth and metastasis-facilitating effect.¹⁰ The activation
۴۴ of vascular endothelial growth factor receptor (VEGFR-2) also known as kinase insert domain
۴۵ receptor (KDR), a member VEGFRs family, by vascular endothelial growth factor (VEGF)
۴۶ induces downstream signaling transduction pathways. It can lead to angiogenesis, high vascular
۴۷ permeability, and tumor growth and progression.¹¹⁻¹³ Thus, by inhibition of VEGFR-2, the process
۴۸ of angiogenesis and tumor growth can be blocked.¹⁴ EGFR and VEGFR-2 are involved in various
۴۹ pathological conditions and the development of several types of cancers. Also, it has been proven
۵۰ that blockade of VEGFR-2 can boost the anticancer effect of EGFR inhibitors. In contrast,
۵۱ activation of VEGFR-2 without any impact on EGFR signaling pathway may contribute to EGFR
۵۲ inhibitors-resistance.^{8,15} So, concurrent inhibition of EGFR and VEGFR signaling pathways seems
۵۳ to be a great method of cancer treatment.¹⁶ Among different kinds of synthesized derivatives,
۵۴ quinazoline nucleus, in special 4-anilinoquinazolines, have drawn a lot of interest over the last

decade because of their diverse biological activities, particularly as EGFR and VEGFR-2 inhibitors.¹⁷ There are several drugs as EGFR and VEGFR-2 blocking agents that reveal this structure, including erlotinib (Tarceva), gefitinib (Iressa), vandetanib (Caprelsa), lapatinib (Tykerb), icotinib (Conmana), and afatinib (Tovok). Some potent drugs inhibiting the EGFR kinase have been designed with alkoxy, especially ethoxy at the C-7 position such as erlotinib, pelitinib, and gefitinib. Vandetanib, a dual tyrosine kinase inhibitor, targets both EGFR and VEGFR-2 (Figure 1).¹⁸

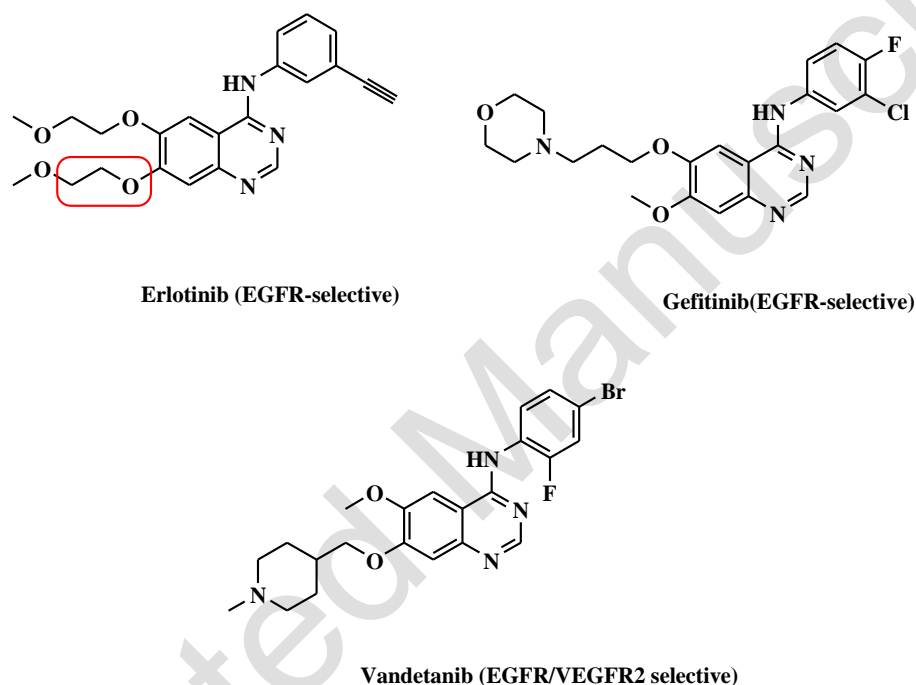


Figure 1. Structures of EGFR tyrosine kinase inhibitors

In this work, some novel compounds designed and synthesized with various substituted anilines and basic side chains at C-4 and C-7 positions of the quinazoline core, respectively, based on the structures of erlotinib and vandetanib (Figure 2). The cytotoxicity of synthesized compounds was also evaluated against A431 human skin cancer (Epidermoid carcinoma) cells as an EGFR overexpressed cancer cell line as well as HU02 (Foreskin fibroblast) as a normal cell line using MTT assay. Besides, docking studies were performed by AutoDock software on the crystal structure of EGFR (PDB ID: 1M17) and VEGFR (PDB ID: 2RL5) tyrosine kinase domains.

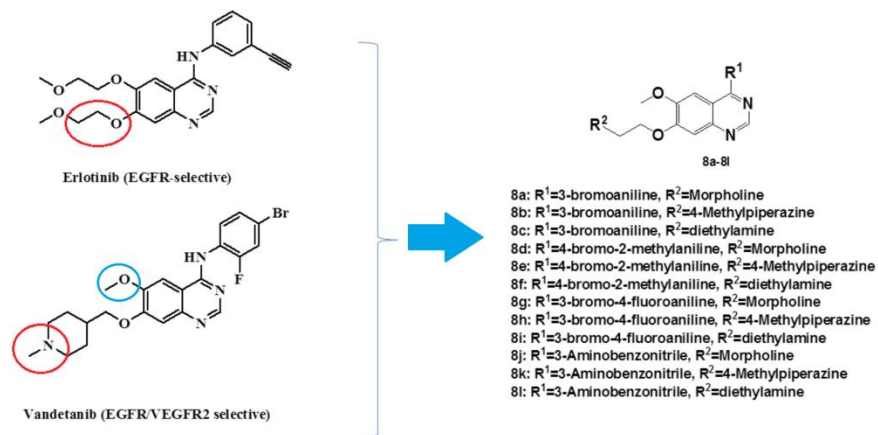


Figure 2. The structural design strategy of the targeted 6,7-disubstituted-4-anilino-quinazoline derivatives 8a-l.

Materials and methods

Chemistry

All commercially available solvents and reagents were prepared from Merck and Sigma Aldrich and used without further purification. The A431 human skin cancer (Epidermoid carcinoma) cells and the Hu02 (Foreskin fibroblast) as a normal cell line were purchased from the Iranian Biological Resource Center (IBRC). The melting points were measured using an Electrothermal-9100 melting point apparatus and are uncorrected. The infrared (IR) spectra were obtained on Perkin-Elmer Spectrum Two FT-IR spectrophotometer equipped with the universal attenuated total reflectance (UATR) with a ZnSe–Diamond composite crystal. ¹HNMR and ¹³CNMR spectra were determined in DMSO-*d*₆ on Bruker FT-500 and 400 MHz spectrometers and chemical shifts (δ) are given in parts per million (ppm) by tetramethylsilane (TMS) as an internal standard. Elemental analysis was done by a Perkin Elmer 2400 (automatic elemental analyzer) and results were within ±0.5 of the calculated values. The mass spectra were recorded on an Agilent 5973 mass spectrometer (70 eV). Merck silica gel 60 F254 plates were used for analytical TLC and monitoring the reactions. Column chromatography was performed on silica gel 60 (Merck, particle size 0.06–0.20 mm).

Methyl 4-hydroxy-3-methoxybenzoate (1)

To a solution of vanillic acid (1.68 g, 10 mmol) in dry methanol (15 mL), thionyl chloride (1.09 mL, 15 mmol) was added dropwise at 0°C, over 10 minutes. After the mixture stirring at room temperature overnight, the solvent and excess thionyl chloride were evaporated under reduced

pressure. Then, the residual was eluted by H₂O (10 mL) before the mixture was extracted by EtOAc (3×10 mL). The EtOAc layer was dried over anhydrous sodium sulfate (Na₂SO₄) and concentrated to give **1** as a white precipitate.

Yield: 69%; mp=125-127 °C; IR (KBr, cm⁻¹) v_{max}: 3537(OH), 1698(C=O), 3025(C-H benzene). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 7.45 (d, J=8.5, 1H, H-C₆ phenyl), 7.42(d, J=2 Hz, 1H, H-C₂ phenyl), 6.8 (d, J=8 Hz, 1H, H-C₅ phenyl), 3.80(s, 3H, OCH₃), 3.78 (s, 3H, COOCH₃). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 166.5, 152.1, 147.8, 123.8, 120.7, 115.6, 112.8, 56.0, 52.13. MS (ESI): m/z 182.2 [M+H]⁺.

Methyl 4-(2-chloroethoxy)-3-methoxybenzoate (2)

The mixture of **1** (1.52 g, 10 mmol), potassium carbonate (2.76 g, 20 mmol), and a catalytic amount of tetrabutylammonium bromide (TBAB) was refluxed in methanol (20 ml) for 20 min. Then, 1-bromo-2-chloroethane (2.86 g, 20 mmol) was added to the mixture. The obtained mixture was refluxed for 4 hours and cooled to room temperature. The precipitate was filtered, washed with acetonitrile (3×5 ml), and the solvent was evaporated under reduced pressure and dried to give **2**. Yield: 82%; mp=115-117.3 °C; IR (KBr, cm⁻¹) v_{max}: 1705(C=O), 1512(CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 7.56 (d, J=8.5 Hz, 1H, H-C₆ phenyl), 7.46 (s, 1H, H-C₂ phenyl), 7.09 (d, J=8.5, 1H, H-C₅ phenyl), 4.31 (t, J=5 Hz, 2H, OCH₂), 3.97 (t, J=5 Hz, 2H, ClCH₂), 3.82(s, 3H, OCH₃), 3.81 (s, 3H, COOCH₃). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 166.3, 152.0, 148.9, 123.4, 122.8, 112.9, 112.5, 69.1, 56.0, 52.3, 43.2. MS (ESI): m/z 244.2 [M+H]⁺.

Methyl 4-(2-chloroethoxy)-5-methoxy-2-nitrobenzoate (3)

A mixture of HNO₃ (5 mL, 65%) and H₂SO₄ (5 mL, 98%) was added to a solution of compound **2** (0.61 g, 2.5 mmol) in dry acetonitrile (5 ml) dropwise at 0-5 °C. After this mixture was stirred below 10 °C for about 6 h, it was slowly poured into ice water (5 mL). The organic layer was washed using saturated sodium bicarbonate (2 × 5 mL) and brine (2 × 5 mL) and dried over anhydrous Na₂SO₄. The acetonitrile was concentrated under reduced pressure, and the residue was dried to give **3** as a yellow solid.

Yield: 86%; mp=119-120 °C; IR (KBr, cm⁻¹) v_{max}: 1715 (C=O), 1520(NO₂). ¹H NMR (DMSO-*d*₆, 500 MHz): δppm 7.67(s, 1H, H-C₃ phenyl), 7.34 (s, 1H, H-C₆ phenyl), 4.42 (t, J=5 Hz, 2H, OCH₂), 3.98 (t, J=5 Hz, 2H, ClCH₂), 3.93 (s, 3H, OCH₃), 3.82(s, 3H, COOCH₃). ¹³CNMR

(DMSO-*d*₆, 125 MHz) δ ppm 165.8, 152.8, 149.2, 141.0, 121.4, 111.9, 109.2, 69.8, 57.0, 53.5, 43. MS (ESI): *m/z* 289.1 [M+H]⁺.

Methyl 4-(2-chloroethoxy)-2-amino-5-methoxybenzoate (4)

A mixture of compound **3** (0.19 g, 0.667 mmol), powdered iron (0.13 g, 2.33 mmol), and NH₄Cl (0.177 g, 3.34 mmol) in MeOH:H₂O (5 mL: 2.5 mL) was refluxed for about 4.5 h. The obtained mixture was filtered while hot; the filter cake was washed with chloroform (2 × 5 mL). The filtrate was extracted with chloroform (3 × 15 mL), and dried over anhydrous Na₂SO₄. The solvent was evaporated and the column chromatography was used for purification of the residue using ethyl acetate/hexane as eluent (30:70, v:v) to give **4** as a yellowish-brown compound.

Yield: 68%; mp=108-107 °C; IR (KBr, cm⁻¹) *v*_{max}: 3473, 3360(NH₂), 1514(CH₂O), 1203 (CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δ ppm 7.15(s, 1H, H-C₆ phenyl), 6.37 (s, 1H, H-C₃ phenyl), 4.19 (t, J=5 Hz, 2H, OCH₂), 3.96 (t, J=5 Hz, 2H, ClCH₂), 3.74 (s, 3H, OCH₃), 3.66 (s, 3H, COOCH₃). ¹³CNMR (DMSO-*d*₆, 125 MHz) δ ppm 165.8, 152.8, 149.0, 121.4, 111.9, 109.1, 69.8, 57.0, 53.5, 43.0. MS (ESI): *m/z* 259.1 [M+H]⁺.

7-(2-chloroethoxy)-6-methoxyquinazolin-4(3H)-one (5)

A solution of **4** (0.1 g, 0.33 mmol) and formamidine acetate (57 mg, 0.55 mmol) was dissolved in absolute ethanol (10 ml) and refluxed for 6 h. Then, the mixture was cooled to 0°C, and the precipitated crystals were filtered, washed with cold ethanol, and dried to give **5** as a white solid.

Yield: 81%; mp=252-254 °C; IR (KBr, cm⁻¹) *v*_{max}: 1675(C=O), 1491(CH₂O), 1262 (CH₃O). ¹HNMR (DMSO-*d*₆, 400 MHz): δ ppm 8.00 (s, 1H, H-C₂ Quinazoline), 7.48 (s, 1H, H-C₈ Quinazoline), 7.17 (s, 1H, H-C₅ Quinazoline), 4.42 (t, J=4.8 Hz, 2H, OCH₂), 4.02 (t, J=4.8 Hz, 2H, ClCH₂), 3.90 (s, 3H, OCH₃). ¹³CNMR (DMSO-*d*₆, 100 MHz) δ ppm 160.5, 153.5, 148.9, 145.1, 144.4, 116.4, 109.5, 105.7, 69.3, 56.2 43.2. MS (ESI): *m/z* 254.1 [M+H]⁺.

7-(2-chloroethoxy)-4-chloro-6-methoxyquinazoline (6)

To a solution of **4** (0.136 g, 0.527 mmol) in dichloromethane (2 mL), oxalyl chloride (0.33g, 2.63 mmol) and dimethyl formamide (DMF) (0.2 mL) were added dropwise and stirred at room temperature for 2 days. Chloroform (10 mL) was added to the mixture and then, it was neutralized with saturated sodium bicarbonate, and dried over anhydrous Na₂SO₄. The chloroform was evaporated to give the product **6** as a yellow solid.

Yield: 88%; mp=142-144 °C; IR (KBr, cm⁻¹) v_{max}: 1675(C=O), 1491(CH₂O), 1262 (CH₃O).
¹HNMR (DMSO-*d*₆, 400 MHz): δppm 8.88 (s, 1H, H-C₂ Quinazoline), 7.48 (s, 1H, H-C₈ Quinazoline) 7.38 (s, 1H, H-C₅ Quinazoline), 4.54(t, J=4.8 Hz, 2H, OCH₂), 4.08 (t, J=4.8 Hz, 2H, ClCH₂), 4.02 (s, 3H, OCH₃). ¹³CNMR (DMSO-*d*₆, 100 MHz) δppm 158.4, 155.7, 152.6, 151.6, 148.8, 119.2, 108.2, 102.9, 69.8, 56.6, 43.0. MS (ESI): 273 [M+H]⁺.

General procedure for the synthesis of 7a-7d

A solution of **6** (0.1 g, 0.36 mmol) and appropriate aniline derivatives (0.73 mmol), including 3-bromoaniline (**7a**), 4-bromo-2-methylaniline (**7b**), 3-bromo-4-fluoroaniline (**7c**), and 3-aminobenzonitrile (**7d**) in isopropanol (5 mL) was heated to reflux for 3 h. The reaction mixture was cooled to 0°C, and the resulting precipitate was filtered, washed with cold isopropanol (5 ml), and dried to afford **7a-7d** products.

7-(2-chloroethoxy)-N-(3-bromophenyl)-6-methoxyquinazolin-4-amine (7a)

Yield: 94%; mp=248.7-250.1 °C; IR (KBr, cm⁻¹) v_{max}: 3250(NH-aniline), 1451(CH₂O), 1280(CH₃O), 776(C-Cl). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 11.68 (s, 1H, H-N aniline), 8.90 (s, 1H, H-C₂ quinazoline), 8.48 (s, 1H, H-C₈ quinazoline), 7.06 (s, 1H, H-C₂ aniline), 7.81 (d, J=10 Hz, 1H, H-C₄ aniline), 7.52 (t, J=10 Hz, 1H, H-C₅ aniline), 7.47 (s, 1H, H-C₅ quinazoline), 7.43(d, J=7 Hz, 1H, H-C₆ aniline), 4.48 (t, J=8.4 Hz, 2H, OCH₂) , 4.12(t, J=8.4 Hz, 2H, ClCH₂), 4.06 (s, 3H, OCH₃). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 158.6, 155.4, 150.6, 149.2, 139.1, 136.0, 131.0, 129.2, 127.6, 124.0, 121.6, 108.2, 104.9, 101.1, 69.8, 57.6, 42.8. MS (ESI): m/z 408.0 [M+H]⁺.

7-(2-chloroethoxy)-N-(4-bromo-2-methylphenyl)-6-methoxyquinazolin-4-amine (7b)

Yield: 92%; mp=243.4-245 °C; IR (KBr, cm⁻¹) v_{max}: 3250(NH-aniline), 1451(CH₂O), 1280(CH₃O), 776(C-Cl). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 11.72 (s,1H, H-N aniline), 8.74 (s, 1H, H-C₂ quinazoline), 8.49 (s, 1H, H-C₅ quinazoline), 7.64 (s, 1H, H-C₈ quinazoline), 7.52 (d, J=5.10 Hz, 1H, H-C₆ aniline), 7.51 (s, 1H, H-C₃ aniline), 7.32 (d, J=5.10 Hz, 1H, H-C₅ aniline), 4.49 (t, J=5.5 Hz, 2H, OCH₂), 4.11 (t, J=6 Hz, 2H, ClCH₂), 4.05 (s, 3H, OCH₃), 2.23 (s, 3H, CH₃-C₂ aniline). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 159.5, 155.4, 150.6, 149.3, 138.4, 135.5, 133.6, 130.3, 129.8, 120.8, 107.6, 107.6, 105.1, 101.0, 69.8, 57.5, 42.8, 18.1. MS (ESI): m/z 423.1 [M+H]⁺.

7-(2-chloroethoxy)-N-(3-bromo-4-fluorophenyl)-6-methoxyquinazolin-4-amine (7c)

Yield: 87%; mp=247-249.3 °C; IR (KBr, cm⁻¹) v_{max}: 3232(NH-aniline), 1493(CH₂O), 1282(CH₃O), 775(C-Cl). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 11.66 (s, 1H, H-N aniline), 9.06 (s, 1H, H-C₂ quinazoline), 8.55 (s, 1H, H-C₅ quinazoline), 8.33(bris, 1H, H-C₆ aniline), 7.99(bris, 1H, H-C₂ aniline), 7.70(t, J= 8.5Hz, 1H, H-C₅ aniline), 7.56 (s, 1H, H-C₈ quinazoline), 4.68 (bris, 2H, OCH₂), 4.28 (bris, 2H, ClCH₂), 4.23 (s, 3H, OCH₃). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 157.7, 155.8, 149.7, 143.0, 136.5, 130.5, 129.8, 126.4, 117.7, 116.1, 111.8, 11.7, 108.9, 94.8, 70.0, 57.6, 43.0. MS (ESI): m/z 429.1 [M+H]⁺.

3-(7-(2-chloroethoxy)-6-methoxyquinazolin-4-ylamino)benzonitrile (7d)

Yield: 87%; mp=247-249.3 °C; IR (KBr, cm⁻¹) v_{max}: 3229 (NH-aniline), 2227 (CN), 1451 (CH₂O), 1217 (CH₃O), 776 (C-Cl). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 11.48 (s, 1H, H-N aniline), 8.88(s, 1H, H-C₂ quinazoline), 8.36 (s, 1H, H-C₅ quinazoline), 8.28 (s, 1H, H-C₂ aniline), 8.08 (d, J=8 Hz, 1H, H-C₆ aniline), 7.76 (d, J=7.5 Hz, 1H, H-C₄ aniline), 7.70 (t, J=7.5 Hz, 1H, H-C₅ aniline), 7.36 (s, 1H, H-C₈ quinazoline), 4.49 (bris, 2H, OCH₂), 4.08 (s, 2H, OCH₃), 4.05 (bris, 2H, ClCH₂). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 158.6, 155.6, 149.9, 142.7, 138.7, 137.4, 130.7, 129.9, 129.8, 129.6, 128.1, 119.0, 112.1, 104.8, 104.7, 70.0, 57.6, 43.0. MS (ESI): m/z 355[M+H]⁺.

General procedure for the synthesis of 8a-8l

A mixture of compound **7** (0.26 mmol), potassium iodide (10 mg), DMF (1 mL), and an appropriate secondary amine (morpholine, N-methylpiperazine, and diethylamine) was heated to reflux for 2 h. The mixture was cooled to ambient temperature, and crushed ice was added to it. Then, the reaction mixture was extracted using chloroform (3 × 5), washed with saturated sodium bicarbonate and brine, and dried over anhydrous Na₂SO₄. The chloroform was evaporated under reduced pressure. Silica gel column chromatography (elution with ethyl acetate/hexane (4 : 6; v : v)) was used for purification of final products.

7-(2-morpholinoethoxy)-N-(3-bromophenyl)-6-methoxyquinazolin-4-amine (8a)

Yield: 40%; mp=173.3-174.8 °C; IR (KBr, cm⁻¹) v_{max}: 3450 (NH aniline), 1495 (CH₂O), 1290 (CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.67 (s, 1H, H-N aniline), 8.50(s, 1H, H-C₂ quinazoline), 8.18 (s, 1H, H-C₅ quinazoline), 7.92 (s, 1H, H-C₂ aniline), 7.90 (s, 1H, H-C₄ aniline), 7.32 (t, J=10 Hz, 1H, H-C₅ aniline), 7.25(d, J=8.5 Hz, 1H, H-C₆ aniline), 7.21(s, 1H, H-C₈

quinazoline), 4.24 (t, J=5 Hz, 2H, OCH₂), 3.96 (s, 3H, OCH₃), 3.57 (brs, 4H, H-C_{3,5} morpholine), 2.75 (brs, 2H, NCH₂), 2.49 (brs, 4H, H-C_{2,6} morpholine). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 157.5, 154.2, 153.5, 147.5, 130.1, 128.0, 128.1, 120.0, 119.8, 109.3, 108.5, 108.4, 102.7, 102.8, 67.0, 66.8, 57.3, 56.8, 54.2. MS (ESI): m/z 458.2 [M+H]⁺. Anal. Calcd for C₂₁H₂₃BrN₄O₃: C, 54.91; H, 5.05; N, 12.20. Found: C, 55.05; H, 5.06; N, 12.25.

7-(2-(4-methylpiperazin-1-yl)ethoxy)-N-(3-bromophenyl)-6-methoxyquinazolin-4-amine (8b)

Yield: 38%; mp=226.3-228 °C; IR (KBr, cm⁻¹) v_{max}: 3410 (NH aniline), 1490 (CH₂O), 1250(CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.54 (s, 1H, H-N aniline), 8.51 (s, 1H, H-C₂ quinazoline), 8.15 (s, 1H, H-C₅ quinazoline), 7.83 (d, J=8 Hz, 1H, H-C₄ aniline), 7.77 (s, 1H, H-C₂ aniline), 7.34 (t, J=9 Hz, 1H, H-C₅ aniline), 7.28 (d, J=7.5 Hz, 1H, H-C₆ aniline), 7.22 (s, 1H, H-C₈ quinazoline), 4.23 (brs, 2H, OCH₂), 3.96 (s, 3H, OCH₃), 2.75 (s, 2H, NCH₂), 2.50 (brs, 4H, H-C_{3,5} piperazine), 2.33 (brs, 4H, H-C_{2,6} piperazine), 2.14 (s, 3H, NCH₃). ¹³CNMR (DMSO-*d*₆, 125 MHz) 156.6, 154.2, 153.2, 149.7, 147.7, 141.9, 130.9, 126.1, 124.5, 121.7, 121.1, 109.5, 108.7, 102.6, 67.2, 56.9, 56.8, 55.3, 53.6, 46.3. MS (ESI): m/z 471.2 [M+H]⁺. Anal. Calcd for C₂₂H₂₆BrN₅O₂: C, 55.94; H, 5.55; N, 14.83. Found: C, 56.09; H, 5.57; N, 14.76.

7-(2-(diethylamino)ethoxy)-N-(3-bromophenyl)-6-methoxyquinazolin-4-amine (8c)

Yield: 38%; mp=219-221 °C; IR (KBr, cm⁻¹) v_{max}: 3415 (NH aniline), 1435 (CH₂O), 1219 (CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.64 (s, 1H, H-N aniline), 8.52 (s, 1H, H-C₂ quinazoline), 8.26 (s, 1H, H-C₅ quinazoline), 7.92 (d, J=11 Hz, 1H, H-C₆ aniline), 7.87 (s, 1H, H-C₂ aniline), 7.62 (d, J=8 Hz, 1H, H-C₄ aniline), 7.42 (t, J=6.5 Hz, 1H, H-C₅ aniline), 7.23 (s, 1H, H-C₈ quinazoline), 4.20 (brs, 2H, OCH₂), 3.98 (s, 3H, OCH₃), 2.88 (brs, 2H, NCH₂), 2.61 (d, J=7 Hz, 4H, H-CH₂ diethylamine), 1.01 (t, J=8.4 Hz, 6H, H-CH₃ diethylamine). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 158.6, 157.3, 145.5, 141.8, 139.6, 134.6, 130.9, 126.2, 124.6, 121.2, 119.4, 115.3, 112.5, 107.9, 91.2, 65.3, 57.0, 54.8, 48.0, 9.2. MS (ESI): m/z 442.2 [M+H]⁺. Anal. Calcd for C₂₁H₂₅BrN₄O₂: C, 56.63; H, 5.66; N, 12.58. Found: C, 56.89; H, 5.67; N, 12.63.

7-(2-morpholinoethoxy)-N-(4-bromo-2-methylphenyl)-6-methoxyquinazolin-4-amine (8d)

Yield: 34%; mp=196.8-197.9 °C; IR (KBr, cm⁻¹) v_{max}: 3468 (NH aniline), 1451 (CH₂O), 1227 (CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.37 (s, 1H, H-N aniline), 8.26 (s, 1H, H-C₂ quinazoline), 7.79 (s, 1H, H-C₅ quinazoline), 7.53 (s, 1H, H-C₃ aniline), 7.41 (d, J=9 Hz, 1H, H-

۲۴۰ C₅ aniline), 7.27 (d, J=8 Hz, 1H, H-C₆ aniline), 7.19 (s, 1H, H-C₈ quinazoline), 4.24(t, J=3 Hz, 2H,
 ۲۴۱ OCH₂), 3.92 (s, 3H, OCH₃), 3.58 (brs, 4H, H-C_{3,5} morpholine), 2.77 (brs, 2H, NCH₂), 2.49 (brs,
 ۲۴۲ 4H, H-C_{2,6} morpholine), 2.16 (s, 3H, H-CH₃ aniline). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 160.1,
 ۲۴۳ 157.4, 147.8, 141.4, 138.5, 132.1, 130.3, 123.0, 112.1, 109.1, 106.2, 80.9, 79.3, 66.8, 64.6, 54.2,
 ۲۴۴ 52.9, 50.5, 21.0. MS (ESI): m/z 472.2 [M+H]⁺. Anal. Calcd for C₂₂H₂₅BrN₄O₃: C, 55.82; H, 5.32;
 ۲۴۵ N, 11.84. Found: C, 55.64; H, 5.33; N, 11.78.

۲۴۶

۲۴۷ **7-(2-(4-methylpiperazin-1-yl)ethoxy)-N-(4-bromo-2-methylphenyl)-6-methoxyquinazolin-4-**
 ۲۴۸ **amine (8e)**

۲۴۹ Yield: 38%; mp=219-221 °C; IR (KBr, cm⁻¹) v_{max}: 3209 (NH aniline), 1493 (CH₂O), 1205
 ۲۵۰ (CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.41 (s, 1H, H-N aniline), 8.27 (s, 1H, H-C₂
 ۲۵۱ quinazoline), 8.20 (s, 1H, H-C₅ quinazoline), 7.84 (s, 1H, H-C₃ aniline), 7.53 (s, 1H, H-C₈
 ۲۵۲ quinazoline), 7.42 (d, J=10 Hz, 1H, H-C₆ aniline), 7.27 (d, J=9.5 Hz, 1H, H-C₅ aniline), 4.23 (brs,
 ۲۵۳ 2H, OCH₂), 3.93 (s, 3H, OCH₃), 2.77 (brs, 2H, NCH₂), 2.55 (brs, 4H, H-C_{3,5} piperazine), 2.42 (brs,
 ۲۵۴ 4H, H-C_{2,6} piperazine), 2.21 (s, 3H, CH₃-C₂ aniline), 2.17 (s, 3H, NCH₃). ¹³CNMR (DMSO-*d*₆,
 ۲۵۵ 125 MHz) δppm 159.7, 152.7, 151.7, 146.8, 135.6, 128.2, 126.3, 122.8, 119.4, 119.3, 116.0, 110.2,
 ۲۵۶ 94.7, 66.9, 54.6, 54.1, 52.1, 52.0, 51.8, 18.4. MS (ESI): m/z 385.0 [M+H]⁺. Anal. Calcd for
 ۲۵۷ C₂₃H₂₈BrN₅O₂: C, 56.79; H, 5.80; N, 14.40. Found: C, 56.60; H, 5.82; N, 14.43.

۲۵۸ **7-(2-(diethylamino)ethoxy)-N-(4-bromo-2-methylphenyl)-6-methoxyquinazolin-4-amine (8f)**

۲۵۹ Yield: 38%; mp=219-221 °C; IR (KBr, cm⁻¹) v_{max}: 3413 (NH aniline), 1501 (CH₂O), 1300
 ۲۶۰ (CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.48 (s, 1H, H-N aniline), 8.26 (s, 1H, H-C₂
 ۲۶۱ quinazoline), 8.24(s, 1H, H-C₅ quinazoline), 7.88 (s, 1H, H-C₃aniline), 7.53 (s, 1H, H-C₈
 ۲۶۲ quinazoline), 7.42 (d, J=11 Hz, 1H, H-C₅ aniline), 7.27 (d, J=9.5 Hz, 1H, H-C₆ aniline), 4.20 (brs,
 ۲۶۳ 2H, OCH₂), 3.93 (s, 2H, OCH₃), 2.90 (brs, 2H, NCH₂), 2.63 (d, J=6.5 Hz, 4H, H-CH₂
 ۲۶۴ diethylamine), 2.17 (s, 3H, H-CH₃-C₂ aniline), 1.01 (t, J=7 Hz, 6H, H-CH₃ diethylamine).
 ۲۶۵ ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 158.0, 153.7, 148.4, 147.4, 138.3, 133.4, 130.2, 129.5,
 ۲۶۶ 118.9, 108.4, 104.5, 103.0, 67.6, 56.8, 51.5, 47.6, 18.5, 12.2. MS (ESI): m/z 458.4 [M+H]⁺. Anal.
 ۲۶۷ Calcd for C₂₂H₂₇BrN₄O₂: C, 57.52; H, 5.92; N, 12.20. Found: C, 57.54; H, 5.94; N, 12.16.

۲۶۸ **7-(2-morpholinoethoxy)-N-(3-bromo-4-fluorophenyl)-6-methoxyquinazolin-4-amine (8g)**

Yield: 34%; mp=217-220 °C; IR (KBr, cm⁻¹) v_{max}: 3543 (NH aniline), 1499 (CH₂O), 1289 (CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.77 (s, 1H, H-N aniline), 8.47 (s, 1H, H-C₂ quinazoline), 8.27 (d, J=6 Hz, 1H, H-C₅ quinazoline), 7.94(s, 1H, H-C₂ aniline), 7.93 (brs, 1H, H-C₅ aniline), 7.39 (t, J=9 Hz, 1H, H-C₆ aniline), 7.23 (s, 1H, H-C₈ quinazoline), 4.24(t, J=5.5 Hz, 2H, OCH₂), 3.96(s, 3H, OCH₃), 3.57 (brs, 4H, H-C_{3,5} morpholine), 2.76(brs, 2H, NCH₂), 2.49 (brs, 4H, H-C_{2,6} morpholine). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 153.2, 149.6, 147.5 (d, ¹J_{CF}=210.1 Hz), 137.7, 137.7, 133.7, 126.7, 123.6 (d, ²J_{CF}=22.2 Hz), 116.8 (d, ²J_{CF}=24.6 Hz), 113.1, 102.9, 96.6, 84.2, 75.0, 66.7, 57.3, 57.1, 57.0, 54.2. MS (ESI): m/z 476.2 [M+H]⁺. Anal. Calcd for C₂₁H₂₂BrFN₄O₃: C, 52.84; H, 4.65; N, 11.74. Found: C, 53.04; H, 4.63; N, 11.79.

7-(2-(4-methylpiperazin-1-yl)ethoxy)-N-(3-bromo-4-fluorophenyl)-6-methoxyquinazolin-4-amine (8h)

Yield: 45%; mp=219.3-222.1 °C; IR (KBr, cm⁻¹) v_{max}: 3410 (NH aniline), 1424(CH₂O), 1282(CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.60 (s, 1H, H-N aniline), 8.49 (s, 1H, H-C₂ quinazoline), 8.13 (s, 1H, H-C₅ quinazoline), 7.82 (s, 1H, H-C₂ aniline), 7.80 (brs, 1H, H-C₆ aniline), 7.44 (brs, 1H, H-C₅ aniline), 7.22 (s, 1H, H-C₈ quinazoline), 4.23 (brs, 2H, OCH₂), 3.96 (s, 3H, OCH₃), 2.75 (brs, 2H, NCH₂), 2.50 (brs, 4H, H-C_{3,5} piperazine), 2.32 (brs, 4H, H-C_{2,6} piperazine), 2.14 (s, 3H, NCH₃). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 156.6, 154.7, 153.98, 149.9, 147.6 (d, ¹J_{CF}=207.3 Hz), 137.4, 124.0, 122.8 (d, ²J_{CF}=23.8 Hz), 119.2, 117.1 (d, ²J_{CF}=21.2 Hz), 117.0, 109.4, 108.7, 102.6, 67.2, 56.8, 56.9, 55.3, 53.6, 46.3. MS (ESI): m/z 491.0 [M+H]⁺. Anal. Calcd for C₂₂H₂₅BrFN₅O₂: C, 53.89; H, 5.14; N, 14.28. Found: C, 54.13; H, 5.15; N, 14.21.

7-(2-(diethylamino)ethoxy)-N-(3-bromo-4-fluorophenyl)-6-methoxyquinazolin-4-amine (8i)

Yield: 33%; mp=232-234 °C; IR (KBr, cm⁻¹) v_{max}: 3508 (NH aniline), 1500 (CH₂O), 1203 (CH₃O). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.58 (s, 1H, H-N aniline), 8.50 (s, 1H, H-C₂ quinazoline), 8.21 (s, 1H, H-C₅ quinazoline), 7.87 (brs, 1H, H-C₆ aniline), 7.84 (s, 1H, H-C₂ aniline), 7.42 (t, J=9 Hz, 1H, H-C₅ aniline), 7.24 (s, 1H, H-C₈ quinazoline), 4.27 (brs, 2H, OCH₂), 3.96 (s, 3H, OCH₃), 3.04 (brs, 2H, NCH₂), 2.75 (brs, 4H, H-CH₂ diethylamine), 1.06 (t, J=5 Hz, 6H, H-CH₃ diethylamine). ¹³CNMR (DMSO-*d*₆, 125 MHz) δppm 163.9, 157.9, 156.5, 152.4, 151.3 (d, ¹J_{CF}=212.1 Hz), 140.0, 137.7, 126.7 (d, ²J_{CF}=25.7 Hz), 123.5, 122.5, 117.0 (d, ²J_{CF}=19.6 Hz), 110.9, 109.9, 91.3, 67.9, 66.9, 56.9, 47.8, 12.30. MS (ESI): m/z 462 [M+H]⁺. Anal. Calcd for C₂₁H₂₄BrFN₄O₂: C, 54.44; H, 5.22; N, 12.09. Found: C, 54.66; H, 5.23; N, 12.05.

۳۰۰ **3-(7-(2-morpholinoethoxy)-6-methoxyquinazolin-4-ylamino)benzonitrile (8j)**

۳۰۱ Yield: 30%; mp=210-213 °C; IR (KBr, cm⁻¹) v_{max}: 3459 (NH aniline), 1442 (CH₂O), 1228
 ۳۰۲ (CH₃O), 2228 (CN). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.90 (s, 1H, H-N aniline), 8.53(s, 1H,
 ۳۰۳ H-C₂ quinazoline), 8.42 (s, 1H, H-C₅ quinazoline), 8.21(d, J=7 Hz, 1H, H-C₆ aniline), 7.95 (s, 1H,
 ۳۰۴ H-C₂ aniline), 7.58 (t, J=7.5 Hz, 1H, H-C₅ aniline), 7.53 (d, J=7.5 Hz, 1H, H-C₄ aniline), 7.24 (s,
 ۳۰۵ 1H, H-C₈ quinazoline), 4.26(t, J=5 Hz, 2H, OCH₂), 3.98 (s, 3H, OCH₃), 3.58(brs, 4H, H-C_{3,5}
 ۳۰۶ morpholine), 2.77 (t, J=5 Hz, 2H, NCH₂), 2.50 (brs, 4H, H-C_{2,6} morpholine). ¹³CNMR (DMSO-
 ۳۰۷ *d*₆, 125 MHz) δppm 155.9, 153.6, 152.4, 149.3, 147.1, 140.6, 129.4, 126.2, 124.4, 118.8, 111.1,
 ۳۰۸ 109.0, 108.0, 108.01, 102.2, 66.3, 66.1, 56.7, 56.4, 53.6. MS (ESI): m/z 405.3 [M+H]⁺. Anal. Calcd
 ۳۰۹ for C₂₂H₂₃N₅O₃: C, 65.17; H, 5.72; N, 17.27. Found: C, 65.05; H, 5.74; N, 17.32.

۳۱۰
 ۳۱۱ **3-(7-(2-(4-methylpiperazin-1-yl)ethoxy)-6-methoxyquinazolin-4-ylamino)benzonitrile (8k)**

۳۱۲ Yield: 35%; mp=214.3-216 °C; IR (KBr, cm⁻¹) v_{max}: 3415 (NH aniline), 1484 (CH₂O), 1289
 ۳۱۳ (CH₃O), 2220 (CN). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm 9.86 (s, 1H, H-N aniline), 8.53 (s, 1H,
 ۳۱۴ H-C₂ quinazoline), 8.40 (s, 1H, H-C₅ quinazoline), 8.18 (s, 1H, H-C₂ aniline), 7.92 (d, J=7.5 Hz,
 ۳۱۵ 1H, H-C₆ aniline), 7.57 (t, J=8.5 Hz, 1H, H-C₅ aniline), 7.51 (s, 1H, H-C₈ quinazoline), 7.23 (d,
 ۳۱۶ J=9 Hz, 1H, H-C₄ aniline), 4.24 (brs, 2H, OCH₂), 3.97 (s, 3H, OCH₃), 2.76 (brs, 2H, NCH₂), 2.50
 ۳۱۷ (brs, 4H, H-C_{3,5} piperazine), 2.33 (brs, 4H, H-C_{2,6} piperazine), 2.24 (s, 3H, NCH₃). ¹³CNMR
 ۳۱۸ (DMSO-*d*₆, 125 MHz) δppm 159.7, 157.1, 149.7, 145.9, 140.2, 128.2, 126.3, 122.8, 119.3, 117.0,
 ۳۱۹ 116.0, 112.5, 110.2, 94.4, 66.0, 56.2, 54.6, 53.2, 52.1, 51.8. MS (ESI): m/z 418.0 [M+H]⁺. Anal.
 ۳۲۰ Calcd for C₂₃H₂₆N₆O₂: C, 66.01; H, 6.26; N, 20.08. Found: C, 66.20; H, 6.25; N, 20.14.

۳۲۱ **3-(7-(2-(diethylamino)ethoxy)-6-methoxyquinazolin-4-ylamino)benzonitrile (8l)**

۳۲۲ Yield: 38%; mp=218.5-220 °C; IR (KBr, cm⁻¹) v_{max}: 3415 (NH aniline), 1435 (CH₂O), 1219
 ۳۲۳ (CH₃O), 2226 (CN). ¹HNMR (DMSO-*d*₆, 500 MHz): δppm (DMSO-*d*₆) 9.73 (s, 1H, H-N aniline),
 ۳۲۴ 8.55 (s, 1H, H-C₂ quinazoline), 8.38 (s, 1H, H-C₅ quinazoline), 8.17 (d, J=11 Hz, 1H, H-C₆
 ۳۲۵ aniline), 7.88 (s, 1H, H-C₂ aniline), 7.61 (d, J=8 Hz, 2H, H-C_{4,5} aniline), 7.27 (s, 1H, H-C₈
 ۳۲۶ quinazoline), 4.20 (t, J=6.5 Hz, 2H, OCH₂), 3.98 (s, 3H, OCH₃), 2.86 (s, 2H, NCH₂), 2.58 (brd,
 ۳۲۷ J=7 Hz, 4H, H-CH₂ diethyleamine), 1.01 (t, J=8.4 Hz, 6H, H-CH₃ diethyleamine). ¹³CNMR
 ۳۲۸ (DMSO-*d*₆, 125 MHz) δppm 158.9, 157.3, 145.5, 141.8, 139.6, 134.6, 130.9, 126.2, 124.6, 121.2,

119.4, 115.3, 112.5, 107.9, 91.2, 65.3, 57.0, 54.8, 48.0, 9.2. MS (ESI): m/z 391.2 $[M+H]^+$. Anal. Calcd for $C_{22}H_{25}N_5O_2$: C, 67.50; H, 6.44; N, 17.89. Found: C, 67.73; H, 6.46; N, 17.81.

Antiproliferation assay

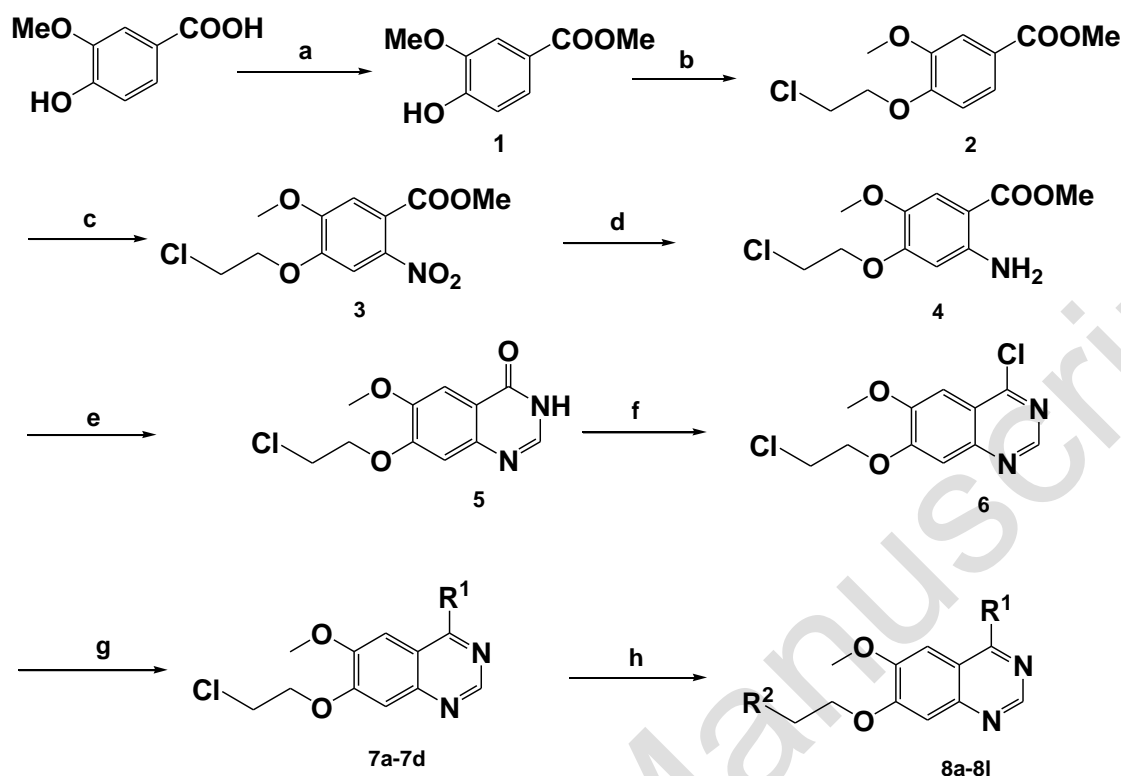
The antiproliferative activities of the synthesized 4-anilinoquinazoline derivatives on A431 (Human carcinoma cell) and HU02 (Foreskin fibroblast) cell lines were evaluated using MTT assay. Briefly, cells were plated in 96-well plates (cell density of 5×10^3 /well) and incubated at 37°C under a humidified atmosphere with 5% CO_2 for 24h. The cells were treated with erlotinib and vandetanib as reference standards and test compounds at different concentrations (1, 2, 5, 10, 20, 50, 100, and 200 μ M). MTT solution (Sigma; 5mg/ml of PBS) was added to each well, and the cells were incubated for 3h at 37°C, after 72h of treatment. Then, the supernatant was removed, 150 μ l DMSO was added into each well for solubilization. Absorbance was recorded using Biotek Epoch™ microplate reader at 570 nm. All experiments were performed in triplicate. The IC_{50} value [the concentration needed for 50% inhibition of cell viability] for reference compounds, vandetanib and erlotinib, and each synthesized compound was determined in μ M by GraphPad Prism (GraphPad Software., Inc. San Diego, CA).

Molecular Docking

Compound **8a** was selected as the most potent agent for molecular docking with EGFR and VEGFR2. AutoDock 4.2 and AutoDock Tools 1.5.4 (ADT) was used for docking study. The X-ray crystal structures of the EGFR tyrosine kinase domain in complex with 4-anilinoquinazoline inhibitor erlotinib (PDB ID: 1M17) and the VEGFR2 kinase domain with a 2,3-dihydro-1,4-benzoxazine inhibitor (PDB ID: 2RL5) were downloaded from RCSB Protein Data Bank (<http://www.rcsb.org>). Ligand and water molecules, except for ones that were necessary for binding with the active site, were removed from crystal structures of receptors followed by adding hydrogens and Kollman charges and merging non-polar hydrogens. Structures of test compounds were sketched, and optimization of their molecular geometries was done by molecular mechanics MM+ and then semi-empirical AM1 methods by HyperChem 8.0 software. Grid box dimensions were set to $x=90$, $y=90$, $z=90$, and grid spacing of 0.375 Å. The Lamarckian genetic search algorithm was utilized for conformational search with 100 GA runs. Co-crystallized ligands were used for validation of the docking procedure based on the method as mentioned above.

Results and discussion

The synthetic pathway was depicted in Scheme 1. Vanillic acid was converted to its methyl ester (intermediate **1**) using thionyl chloride and methanol. The reaction of **1** with 1-bromo-2-chloroethane in the presence of potassium carbonate and TBAB gave **2**. Sulfuric acid and nitric acid were used for the nitration of **2** to afford compound **3**. The reduction of intermediate **3** to **4** was performed using powdered iron and ammonium chloride and followed by the ring-closure reaction in the presence of formamidine acetate and ethanol to achieve quinazoline skeleton (Compound **5**).^{19,20} Intermediate **5** was chlorinated using oxalyl chloride to yielded **6** which was then coupled with appropriate aniline groups to afford the desired intermediate **7a-7d**.^{20,21} Finally, the desired compounds (**8a-8l**) were generated through the reaction of **7a-7d** and various secondary amines using anhydrous potassium iodide.¹⁹



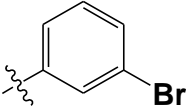
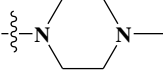
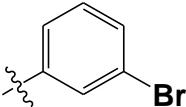
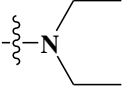
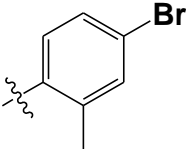
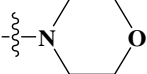
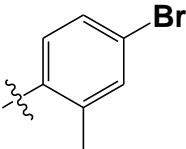

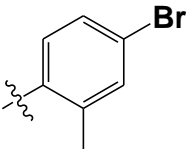
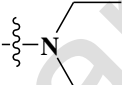
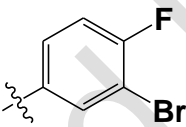
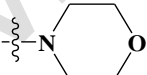
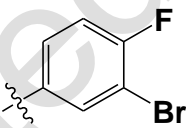
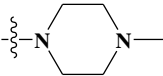
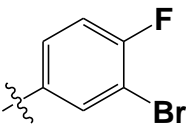
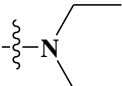
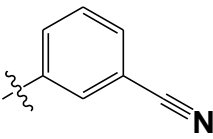
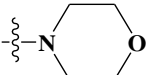
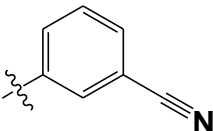

Scheme 1. Synthetic route for the synthesis of the target compounds 8a–8l. Reagents and conditions: (a) SOCl₂, MeOH, Reflux; (b) 1-Bromo-2-chloroethane, TBAB, K₂CO₃, Reflux; (c) H₂SO₄, HNO₃, 0–5 °C; (d) Fe, NH₄Cl, MeOH/H₂O, Reflux; (e) formamidine acetate, Ethanol, Reflux; (f) Oxalyl chloride, DMF, Dichloromethane, rt; (g) Aniline Derivatives, i-PrOH, Reflux; (h) KI, DMF, Secondary amine, Reflux.

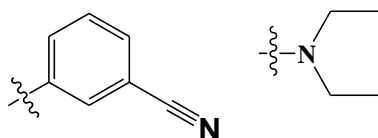
Cytotoxic activities of compounds were tested against A431 (human carcinoma cell) as an EGFR overexpressed cancer cell line as well as HU02 (Foreskin fibroblast) as a normal cell line by MTT assay.²²⁻²⁴ As is presented in table 1, most of the final compounds showed significant anti-proliferative activities on A431 (1.78 μM - >100 μM). Compound **8a** bearing 3-bromoaniline at

the 4-position and morpholine at the 7-position of quinazoline core demonstrated the best cytotoxicity on A431 ($IC_{50}=1.78 \mu M$) which was better than the reference drugs. Also, **8d** and **8f** were two other potent compounds which showed cytotoxicity more than reference standards (7.18 and 8.25 μM , respectively) with 4-bromo-2-methylaniline at C-4 position as well as morpholine and diethylamine at C-7 position of quinazoline scaffold, respectively. Likewise, compounds **8h** ($IC_{50}=10.3 \mu M$) and **8i** ($IC_{50}=10.3 \mu M$) bearing 3-bromo-4-flouroaniline at the 4-position along with N-methylpiperazine and dimethyl amine at the 7-position of quinazoline skeleton exhibited good potency against A431. Introduction of 3-bromoaniline and N-methylpiperazine at 4 and 7 positions of quinazoline skeleton in compound **8b** led to significant antitumor activity ($IC_{50}=14.3 \mu M$). Compounds with 3-aminobenzonitrile at 4 position (**8j-8l**) resulted in loss of inhibitory activities on A431. Other compounds displayed moderate to weak cellular anti-proliferative activities against A431. In order to investigate the nonspecific cytotoxic activity, the same assay was performed on the HU02 (Foreskin fibroblast) as a normal cell line. Surprisingly, these compounds showed no significant effects on the proliferation of HU02 cells. The in vitro insensitivity of this cell line to synthesized agents was most closely related to the normal EGFR expression.^{4,25} Effective antiproliferative activity of compounds against A431 cell line, and having no toxicity on the HU02 cell line are consistent with the hypothesis that these compounds may show their activities via inhibition of EGFR.

Table 1. Cytotoxic activity (IC_{50} , μM) of synthesized compounds **8a-l** on A431 and HU02 cell lines.

Compounds	R ¹	R ²	A431	HU02
8a			1.78±0.47	>100

8b			14.3 ± 3.43	>100
8c			>100	>100
8d			8.25 ± 3.56	>100
8e			21.7 ± 4.62	>100
8f			7.18 ± 2.15	>100
8g			61.97 ± 8.92	>100
8h			10.3 ± 3.22	>100
8i			12.94 ± 3.21	>100
8j			46.70 ± 5.32	>100
8k			36.69 ± 4.73	>100



>100

>100

Erlotinib hydrochloride

8.31±1.96

72.61±4.75

Vandetanib

10.62±2.54

>100

The structure-activity relationship (SAR) analysis showed that for morpholine (**8a**, **8d**, **8g**, and **8j**) and N-methylpiperazine (**8b**, **8e**, **8h**, and **8k**) substituted derivatives, the introduction of electron-donating group ($-\text{CH}_3$) at position-2 of the aniline ring in addition to moving bromo substituent from the meta site to the para position showed a moderate decrease in cytotoxicity. Also, the substitution of 3-bromine at the position-3 aniline ring with nitrile moiety as a strong electron-withdrawing substituent group (**8j** and **8k**) led to a drastically reduced anticancer activity. In morpholine derivatives, the introduction of fluorine at the para position of the aniline ring (**8g**) led to a significant decrease in anticancer activity. In contrast, analysis of N-methylpiperazine derivatives proved that the presence of fluorine as H-bond acceptor group at the para position of aniline ring (**8h**) interestingly enhanced the anticancer activity. In compounds with diethylamine group, it seems that 2, 4-disubstituted (**8f**) and 3, 4-disubstituted (**8i**) aniline groups were more potent anticancer agents than 3-monosubstituted ones (**8c** and **8l**).

Moreover, In order to study the interaction between the target compound **8a** (As the most potent compound) and vandetanib, molecular docking studies were conducted using the AutoDock 4.2 and AutoDock Tools 1.5.4 (ADT).^{19,26,27} As is presented in figure 3, N1 of the quinazoline formed one hydrogen bond with **Met769** in the ATP binding site of EGFR (PDB: 1M17) for both compounds with the distances of 1.94 Å (**8a**) and 1.638 Å (vandetanib) and bond angle values of 162.5° (**8a**) and 176.9° (vandetanib). Besides, aniline rings of **8a** and vandetanib coplanarly interacted with amino acids, including **Lys-721**, **Val-702**, and **Ala-719** into EGFR's hydrophobic pocket. Also docking study was done for compound **8a** and vandetanib into inactive DFG-out conformation of VEGFR-2 (PDB: 2RL5) (Figure 4). **8a** and vandetanib could be fitted to the DFG-out conformation of VEGFR-2 impeccably. A hydrogen bond was formed between quinazoline N1 and the backbone amide NH of **Cys919** in the hinge region (**8a**: 1.367 Å and 144.5°, vandetanib: 2.127 Å and 138.9°). The quinazoline core is located in a hydrophobic pocket

composed of **Leu840**, **Ala866**, **Phe918**, and **Leu1035**. The oxygen of morpholine formed a hydrogen bond interaction with the **Lys868** side chain.

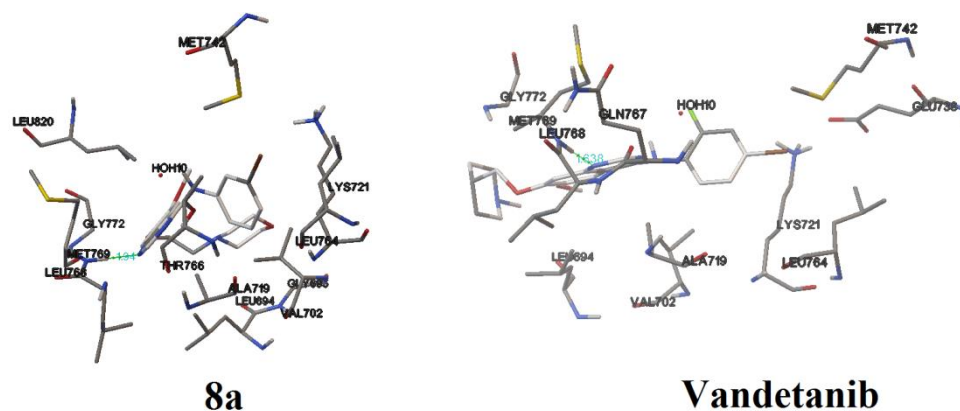


Figure 3. Docking interaction of compound **8a** and vandetanib with EGFR

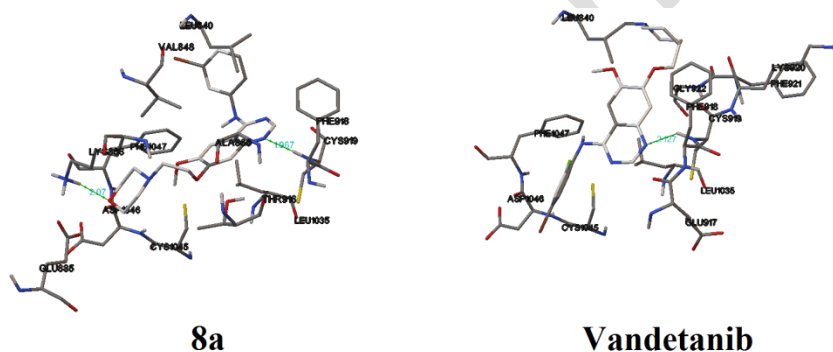


Figure 4. Docking interaction of compound **8a** and vandetanib with VEGFR

Conclusion

In conclusion, a series of 4-anilinoquinazoline derivatives containing diethylamine, morpholine, and N-methypiperazine have been designed and synthesized potent anticancer agents. Most of the tested compounds exhibited good antiproliferative activities. The most potent ones were **8a**, **8d**, and **8f** which showed better cytotoxic activity than both standards. Molecular docking proved the interaction of **8a** with the ATP binding site of EGFR and inactive DFG-out conformation of VEGFR-2. All compounds did not show any significant activities on HU02 as the normal cell line. So, **8a**, **8d**, and **8f** can be an appropriate ground for further structure optimization and evaluation of biological activities for developing novel potent cancer therapeutic agents.

Supporting Information

Supporting information contains IR, NMR, and mass spectra.

Conflict of interests

The authors confirm that this article content has no conflicts of interest.

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