Pharmaceutical Sciences, 2025, 31(1), 43-64
doi:10.34172/PS.024.40740
https://ps.tbzmed.ac.ir/

Review Article

A Review of Quinazoline-Based EGFR/VEGFR-2 Dual Inhibitors as Potent Anticancer Agents: Structure-Activity Relationship and Docking Studies

Fatemeh Yousefbeyk[1], Saeed Ghasemi[2*]

[1]*Department of Pharmacognosy, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.*
[2]*Department of Medicinal Chemistry, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.*

Article Info

Article History:
Received: 31 Jul 2024
Accepted: 10 Nov 2024
ePublished: 21 Dec 2024

Keywords:
-Dual inhibitors
-EGFR
-Kinase
-Quinazolines
-Structure-activity relationship
-VEGFR-2

Abstract

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase (RTK) that initiates various signaling pathways resulting in processes such as gene expression, proliferation, angiogenesis, and inhibition of apoptosis. Dysregulation of EGFR signaling causes tumor development and metastasis. Therefore, targeting EGFR can be introduced as a promising way for cancer treatment. Angiogenesis, the formation and growth of new capillaries from pre-existing vasculature, is a key process in many physiological and pathological processes, including embryonic development, tissue growth, wound healing, cancer, rheumatoid arthritis, diabetic retinopathy, axon growth, and inflammatory diseases. Vascular endothelial growth factor receptors (VEGFRs), as receptor tyrosine kinases, especially VEGFR-2, have been introduced as the main mediators of angiogenesis. Therefore, VEGFR-2 inhibitors could be attractive agents for blocking angiogenesis and tumor growth. Due to the common downstream signaling pathways of EGFR and VEGFR-2, simultaneous inhibition of both receptor tyrosine kinases can be used as a valuable method in cancer therapy. Targeting the ATP-binding site of the tyrosine kinase domain using small molecules, either reversibly or irreversibly, is one way to inhibit EGFR and VEGFR-2. Different drugs with various scaffolds such as quinazoline (Vandetanib) and pyrimidine (Regorafenib) have been approved by the FDA for the treatment of various malignancies. Among them, the quinazoline skeleton is an attractive core with a wide range of activities. Vandetanib, a quinazoline-based EGFR/VEGFR-2 dual inhibitor, is an orally administered drug for the treatment of locally advanced or metastatic medullary thyroid cancer. Due to the limited number of multitarget kinases, as well as limitations in their clinical efficacy, adverse effects, and drug resistance, there is a vital need to introduce novel inhibitors with superior selectivity and efficacy compared to existing ones to overcome these challenges. Therefore, we reviewed the structure-activity relationship (SAR), EGFR/VEGFR-2 inhibitory activities, anticancer effects, and docking studies of synthesized quinazoline-based EGFR/VEGFR-2 dual inhibitors.

Introduction

Cancer is one of the most significant threats facing humanity. After cardiovascular diseases, cancer is the second leading cause of death worldwide.[1,2] There are various issues associated with current anticancer drugs, including drug resistance, lack of selectivity, and toxicity. Given these challenges, it is imperative to discover chemotherapeutic agents that are effective and specific simultaneously.[3,4] In order to discover new anticancer agents, it is crucial to understand the role of various cellular and molecular mechanisms in the initiation and progression of cancer particularly proteins involved in the signal transduction pathways.[5-7] Protein kinases (PKs) reversibly catalyze the transfer of a γ-phosphate group from purine nucleotide triphosphates (ATP and GTP) to the hydroxyl groups of serine and threonine residues, or the phenolic hydroxyl group of tyrosine residues. They act as phosphate acceptors for their proteins, forming phosphate monoesters. Based on the amino acids phosphorylated, PKs are divided into two main families: tyrosine kinases or serine and threonine kinases. Phosphorylation leads to conformational changes in proteins or disrupts their surface, influencing the protein-protein interactions that control the downstream signaling cascades and potentially impact gene transcription. The human PK gene family comprises 518 members and 106 pseudogenes, highlighting intricate internal and external interactions. Among PKs, 90 genes are related to protein tyrosine kinases (PTKs), including 58 receptor tyrosine kinases (RTKs) (divided into 20 groups) and 32 nonreceptor tyrosine kinases (divided into 10 groups).

*Corresponding Author: Saeed Ghasemi, E-mail: ghasemi_saeed@yahoo.com, ghasemi_s@gums.ac.ir

The RTKs feature an extracellular domain at the protein's N-terminal with ectodomains serving as recognition sites for extracellular signaling ligands, a single transmembrane alpha-helix, and an intracellular domain containing a tyrosine kinase domain at the protein's C-terminal. The binding of ligands to the extracellular ligand-binding domain (ECD) of the RTKs results in conformational changes, often leading to dimerization, activating the kinase domain, and transmitting signals within the cell. RTKs are essential enzymes in the signal transduction pathways, and several physiological processes, including proliferation, differentiation, migration, and angiogenesis, rely on them.[8-11] Additionally, they contribute to the development of several diseases, including inflammatory diseases, malignancies, and metabolic disorders.[12,13]

The Epidermal Growth Factor Receptor (EGFR)

EGFR is a member of the ErbB family of receptor tyrosine kinases (RTKs) that initiate signaling pathways, mediating the actions of epithelial cells. The ErbB family consists of four closely associated members: ErbB1(HER-1/EGFR), ErbB2(HER-2/neu), ErbB3(HER-3), and ErbB4(HER-4) with close similarities in structure and function.[14,15] EGFR is a 170-kd glycoprotein that comprises three main parts, including an extracellular ligand-binding domain (ECD), a short transmembrane sequence (TMS), and an intracellular domain (ICD) with a tyrosine kinase domain. Approximately 40,000 to 100,000 EGFR receptors are expressed in each normal cell.[16] More than 40 ligands are involved in the binding, activation, and further signaling control of EGFR. These include high-affinity ligands such as EGF, transforming growth factor alpha (TGF-α), heparin-binding EGF-like growth factor (HB-EGF), and betacellulin, as well as low-affinity ones like amphiregulin and epiregulin.[17,18] The ECD of EGFR consists of four domains, including two homologous I and III domains as the ligand-binding site, and two cysteine-rich domains, comprising II and IV.[18] Binding of a ligand to EGFR results in homo- or heterodimerization of the receptor at the extracellular region, followed by internalization and autophosphorylation of the tyrosine kinase domains.[19] Phosphorylated tyrosine kinase domains serve as binding sites for recruiting signal transducers and activators of intracellular proteins, like Ras, which in turn stimulates intracellular signaling pathways. The Ras-Raf mitogen-activated protein kinase (MAPK) and the phosphatidyl inositol 3 kinase (PI-3K)/Akt pathways are the two main signaling cascades for the HER family.[20-23] Several biological processes are regulated by these signaling pathways, like gene expression, proliferation, angiogenesis, and apoptosis inhibition, all of which play a crucial role in the cancer progression.[24,25] Overexpression of EGFR is observed in a large number of solid tumors, including colon cancer, breast cancer, non–small-cell lung cancer (NSCLC), head and neck cancer, renal cancer, and ovarian cancer.[26] This activation can subsequently trigger downstream signaling cascades.[27] Overactivation, overexpression, or mutation of EGFR may cause dysregulated EGFR signaling, leading to tumor growth and metastasis.[28,29] The significant beneficial role of targeting EGFR in several types of cancers, including non-small cell lung cancer, colorectal cancer, squamous cell carcinoma of the head and neck, pancreatic cancer, and breast cancer has been well-established by a number of research studies.[30] Therefore, the development of new antitumor compounds that specifically target EGFR can be considered for cancer therapy.[31,32]

EGFR Inhibitors

Monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs) are two approaches for targeting and blocking the activation of EGFR, each working through different mechanisms. Binding of mAbs to the ECD can prevent EGFR activation, while TKIs target the tyrosine kinase of ICD. Additionally, in non-small cell lung cancer (NSCLC), colorectal cancer (CRC), pancreatic cancer, breast cancer, and squamous cell carcinoma of the head and neck (SCCHN) overall survival, progression-free survival (PFS), and overall response are all extended.[30] Small molecules targeting TK interact reversibly or irreversibly with the ATP-binding pockets of the tyrosine kinase domain, blocking autophosphorylation and activation of several downstream signaling pathways. Gefitinib and erlotinib, as first-generation reversible TKIs, are clinically associated with a better prognosis for patients with non-small cell lung cancer with EGFR-activating mutations. Second-generation irreversible inhibitors, such as afatinib and dacomitinib, exhibit increased activity against EGFR oncogenic cells. However, patients who respond to these therapies eventually develop acquired resistance within 9-14 months. In nearly 60% of these cases, the secondary point mutation T790M (substitution of methionine with threonine at amino acid position 790) is observed. Third-generation inhibitors, like osimertinib, olmutinib, and rociletinib, have been introduced to overcome the aforementioned resistance (Figure 1).[33] Also, cetuximab and panitumumab are two anti-EGFR mAbs that are currently available for clinical use. Several researches have shown the significant effects of EGFR-targeted drugs in numerous types of cancers, including metastatic KRAS-negative SCCHN /CRC, metastatic or locally advanced NSCLC, HER2-overexpressing breast cancer, and metastatic or advanced pancreatic cancer.[30] Figure 2 shows the pharmacophoric features of Erlotinib, an FDA-approved quinazoline-based EGFR inhibitor.[34]

Vascular Endothelial Growth Factor Receptor (VEGFR) and Angiogenesis

The formation of new blood vessels, known as vasculogenesis and angiogenesis, is involved in several physiological and pathological processes. These processes include embryonic development, tissue growth, wound healing, cancer, rheumatoid arthritis, diabetic retinopathy, axon growth, and inflammatory diseases. These signaling routes are mediated through a variety of factors such

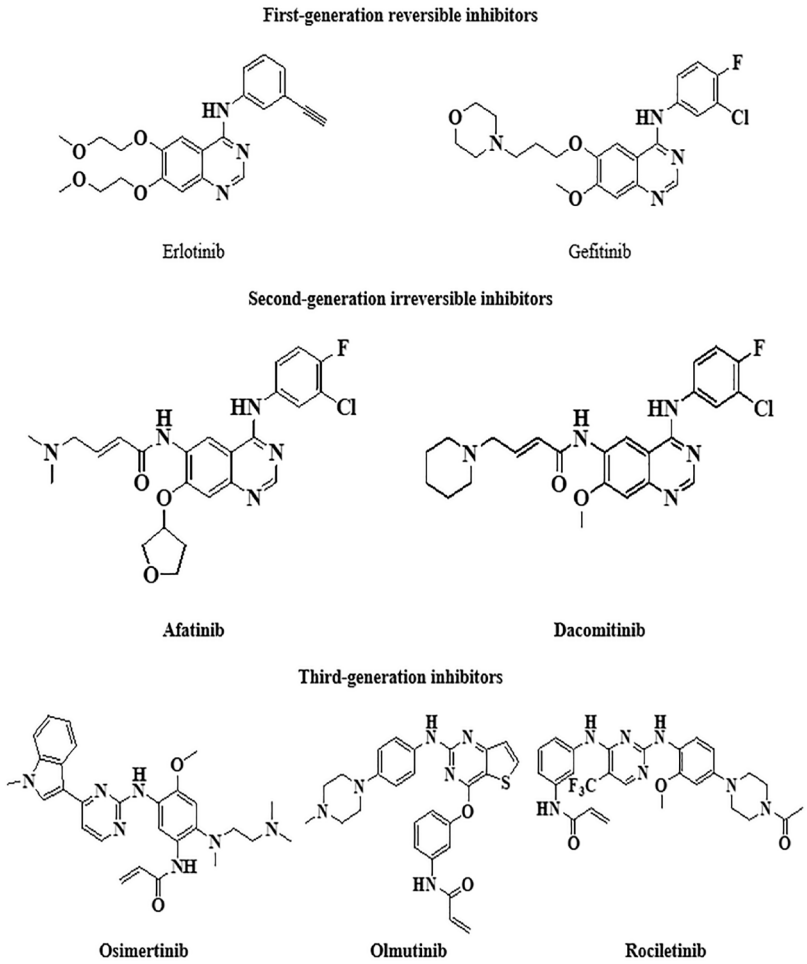


Figure 1. The structures of EGFR tyrosine kinase inhibitors.

as vascular endothelial growth factors (VEGFs), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), ephrin-Eph receptors, angiopoietin-1, hepatocyte growth factor (HGF), transforming growth factor-β (TGF-β), and interleukin 6 (IL-6), etc.[35] Vasculogenesis is a fundamental process in the formation of the blood vessel system in embryos. It occurs through the de novo production and differentiation of endothelial precursor cells into endothelial cells, representing the initial stage of the vascular network formation. Angiogenesis is a vital physiological process that involves the growth of new capillaries from the pre-existing vasculatures developed during the earlier stage of vasculogenesis. This process is characterized by continuous growth, sprouting, splitting, and further growth of vessels.[36] It plays a crucial role in providing oxygen and nutrients to cells, as well as removing waste materials, and is a vital step in the development of tumors and metastasis.[37,38] The human VEGF/VEGFR system consists of five main ligands, including VEGF-A (also known as VEGF), VEGF-B, VEGF-C, VEGF-D, and PGF (placental growth factor). It also includes three main VEGF receptors VEGFR-1 (Flt-1), VEGFR-2 (KDR), and VEGFR-3 (Flt-4). Additionally, there are two non-protein kinase co-receptors, neuropilin-1 and neuropilin-2 (NRP-1 and -2). VEGFR-1 and VEGFR-2 are involved in angiogenesis, while VEGFR-3 is a main regulator of lymphangiogenesis. Among all mentioned receptors, VEGFR-2 is primarily found in vascular endothelial cells and serves as the major signal transducer for angiogenesis. It functions through multiple pathways, such as PLCγ-PKC-MAPK, PLCγ-PKCeNOS-NO, TSAd-Src-PI3K-Akt, SHB-FAK-paxillin, SHB-PI3KAkt, and NCK-p38-MAPKAPK2/3. As a result, VEGFR-2 is a key target for controlling angiogenesis in malignancy. It also plays a critical role in the management of neuronal degeneration and ischemic diseases due to its participation in proangiogenesis.[39]

VEGFR-2 Inhibitors

Vascular endothelial growth factor receptors (VEGFRs) and their ligands, vascular endothelial growth factors (VEGFs), have a key role in regulating both physiological and pathological angiogenesis. The binding of VEGF to VEGFR-2 is the critical pathway that activates angiogenesis. Similar to EGFR, when dimeric VEGFs bind to monomeric VEGFR-2, it results in receptor dimerization and activation.[40,41] The activation of vascular endothelial growth factor receptor 2 (VEGFR-2/KDR)

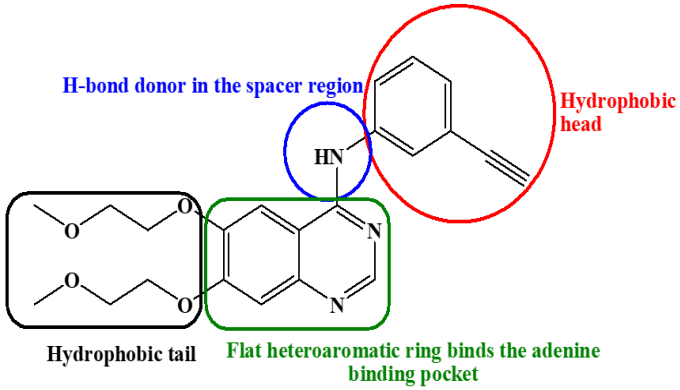


Figure 2. Pharmacophoric features of Erlotinib as FDA-approved EGFR inhibitor.

can initiate a number of signaling pathways, leading to downstream signal transduction, biological responses, and pathological mechanisms in angiogenesis. This enhances vascular permeability, proliferation, and metastasis of cancer cells.[35,42] Consequently, blocking the activation of VEGFR-2 is a promising strategy for inhibiting angiogenesis and improving cancer chemotherapy.[43,44] It can be achieved through different methods. Firstly, RNA interference (RNAi), antisense oligonucleotides (ASOs), and ribozyme (Rz) can block the expression of VEGF and VEGFR-2 genes.[35,45] The second approach is to block the binding of VEGF to VEGFR-2 using of neutralizing antibodies (nAbs) and soluble VEGFR-2 (sVEGFR-2). Small molecule inhibitors targeting the VEGF/VEGFR-2 tyrosine kinase domain can also effectively inhibit angiogenesis.[35,46] Vascular endothelial cells can be targeted for destruction by linking VEGF with toxic small molecules or VEGFR-2 monoclonal antibodies (mAbs) with small molecule inhibitors. This strategy effectively hinders the growth and development of vascular endothelial cells that overexpressVEGFR-2.[35,47] Several VEGFR-2 inhibitors are available, including regorafenib, sorafenib, pazopanib, sunitinib, tivozanib, and vatalanib (Figure 3).[48] Figure 4 displays the pharmacophoric features of VEGFR-2 inhibitors based on the structure of sorafenib, an FDA-approved VEGFR-2 inhibitor.

Types of Small Molecule Kinase Inhibitors

The active conformation of protein kinases is targeted by conventional kinase inhibitors, such as ceritinib, crizotinib, gefitinib, pazopanib, ruxolitinib, and vandetanib, which competitively bind to the ATP binding site. Due to the highly conserved ATP binding site of protein kinases, designing selective small molecule inhibitors that only target the ATP site is challenging. Reversible inhibitors (Type-I inhibitors) must compete with the high concentration of ATP, resulting in a decrease in their efficacy. Given that most inactive protein kinases have a DFG-out conformation, Type-II inhibitors, also known as DFG-out inhibitors, can stabilize this inactive conformation and demonstrate higher potency and selectivity compared to Type-I inhibitors. Drugs like imatinib, sorafenib, nilotinib, regorafenib, and ponatinib have been developed as Type-II inhibitors. The Type-III kinase inhibitors (pexidartinib and

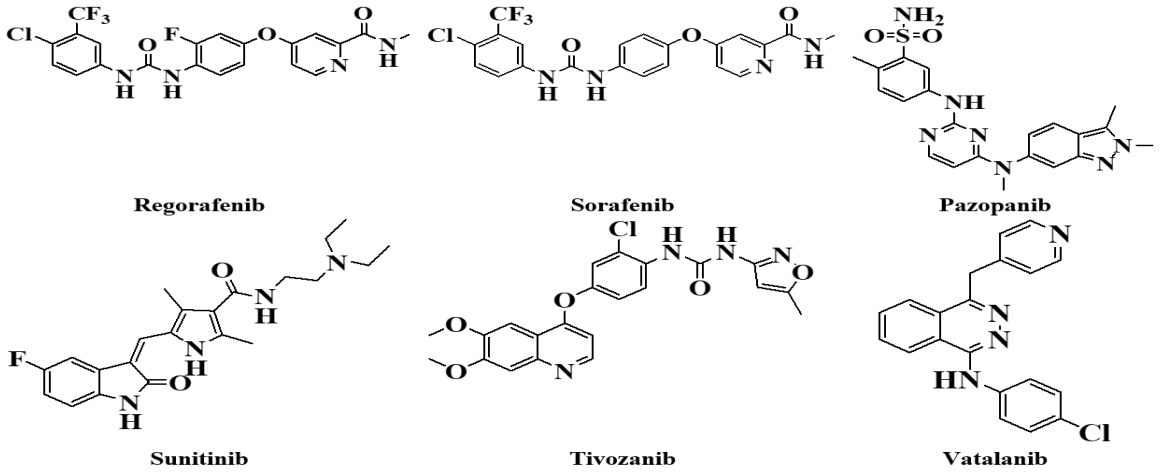


Figure 3. The structures of VEGFR-2 tyrosine kinase inhibitors.

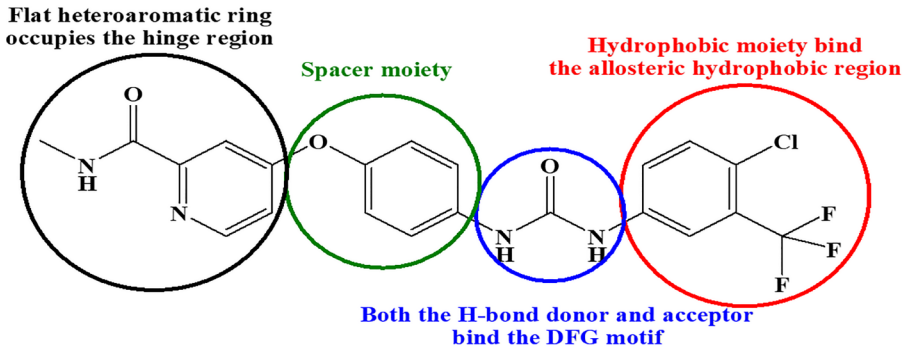


Figure 4. Pharmacophoric features of sorafenib as FDA-approved VEGFR-2 inhibitor.

selumetinib) interact outside of the ATP-binding site and act as allosteric inhibitors, making them noncompetitive inhibitors of kinases. Their interaction near the ATP-binding sites allows for highly selective inhibition. Type-IV kinase inhibitors, also known as substrate-directed inhibitors, like ON012380, reversibly bind outside the ATP binding site, which is distinct from the ATP-binding site. They are noncompetitive with ATP, making them highly selective kinase inhibitors. Lastly, the covalent kinase inhibitors (afatinib, ibrutinib, and dacomitinib) are Type-V inhibitors that irreversibly bind to the kinase active site through a covalent bond with the cysteine residue.[49,50]

EGFR/VEGFR-2 Dual Inhibitors

EGFR and VEGFR-2, which share common downstream signaling pathways and functional relationships, can contribute to the progression of several malignancies. Additionally, blocking EGFR leads to a decline in VEGF expression, which is a key inducer of angiogenesis. Furthermore, inhibiting VEGFR-2 improves the effectiveness of EGFR inhibitors.[51,52] Therefore, simultaneously inhibiting both signaling pathways can be considered a valuable method in cancer treatment.[53] Figure 5 illustrates dimerization, activation, shared signaling pathways, and some small molecules TKIs.

Vandetanib (ZD6474), a 4-anilinoquinazoline, is one of the FDA-approved drugs in 2012 that acts as an inhibitor of multiple receptor tyrosine kinases, targeting both EGFR and VEGFR-2 (Figure 6). Developed and successfully marketed as a new therapeutic agent by AstraZeneca in 2010, it is taken orally once a day for the treatment of unresectable locally advanced or metastatic medullary thyroid cancer.[54]

Figure 7 shows the pharmacophoric features of vandetanib as EGFR/VEGFR-2 dual inhibitors with a quinazoline scaffold.[1,28]

Development of quinazoline-based EGFR/VEGFR-2 dual inhibitors: SAR and docking studies

Currently, various EGFR/VEGFR-2 dual inhibitors have been designed and synthesized with different scaffolds. This review aims to highlight the SAR, biological activities, including cytotoxic activity and inhibition of EGFR/VEGFR-2, and the binding modes of quinazoline-based

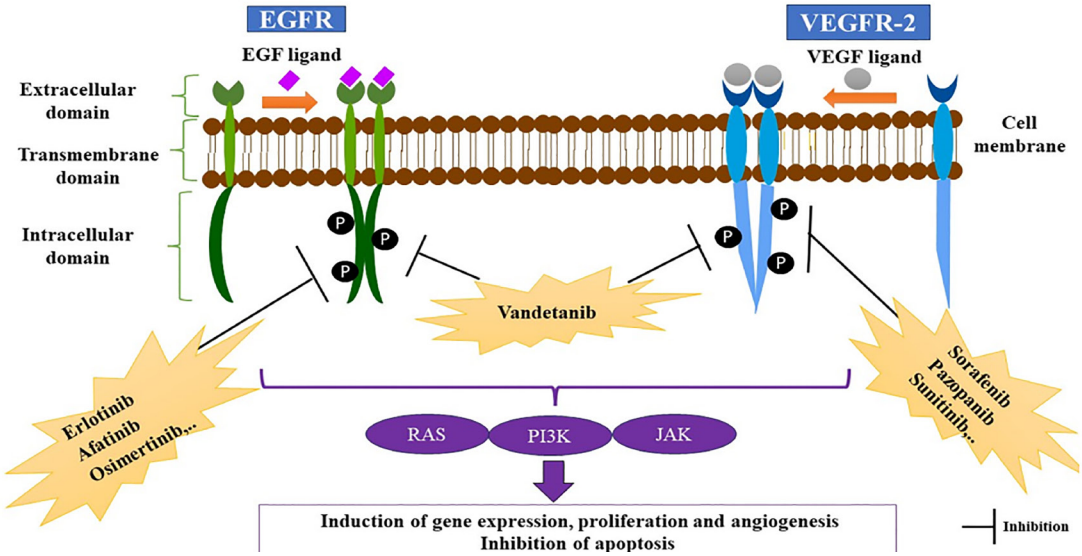


Figure 5. EGFR/VEGFR-2 common downstream signaling pathways and their small molecules TKIs.

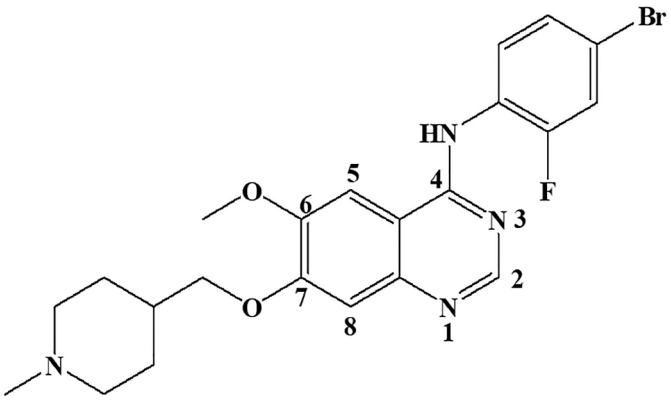


Figure 6. The structures of vandetanib

compounds in docking studies, focusing on the recent progress in design and synthesis of novel quinazoline-based dual inhibitors.

Several 4-anilinoquinazoline derivatives were designed and synthesized by Garofalo *et al.*[52] (Figure 8). These new compounds were assessed for their cytotoxic effects on hormone-independent prostate cancer cell lines (PC3) and their ability to inhibitor EGFR and VEGFR-2. Urea containing derivatives (compounds 2d-2j) showed slightly lower cytotoxicity than the reference drug gefitinib, while other compounds (compounds 2a-2c) did not exhibit significant cytotoxic activity, with IC_{50}s greater than 10 μM. Urea derivatives bearing a phenyl group (compound 2d) or 2,4-difluorophenyl group (compound 2f) were more cytotoxic than compounds with the bulky steric substituents. Replacing one of the methoxy groups at the 6- or 7-position of the quinazoline skeleton with a diethylaminoethyl group (groups A and C) led to a significant drop in enzymatic activities. Also, aliphatic-urea derivatives (compounds 2h-2j) and carbamic acid ester derivatives (compounds 2b and 2c) were not effective cytotoxic agents. All of the compounds showed mild to moderate inhibitory activity against EGFR and VEGFR kinases. Most alkyl or arylurea derivatives exhibited promising results against VEGFR-2, making them selective VEGFR inhibitors with IC_{50} values ranging from 4.31 to 6.62 μM. This may be due to the hydrogen bond formation of urea oxygen and the NH moiety with the active site of VEGFR-2. Replacing the urea with a carbamic acid methyl ester moiety (compound 2b) resulted in a dual EGFR/VEGFR active agent (IC_{50} values of 6.87 and 5.79 μM on EGFR and VEGFR-2, respectively), whereas its ethyl ester (compound 2c) did not show activity on EGFR. These results indicate that bulky groups such as ethyl carbamate (compound 2c) with an IC_{50} of 5.62 μM or urea derivatives (compounds 2d, 2e, 2g, 2h, and 2i) with IC_{50} values ranging from 4.31 to 6.18 μM resulted in increased inhibitory activity on VEGFR-2 kinase. However, those modifications on the aniline ring led to a complete loss of activity on EGFR.

Riadi and colleagues[55] reported the preparation of a novel quinazolinone molecule, ethyl 2-((3-(4-fluorophenyl)-6-methyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetate (Figure 9). They also studied its cytotoxicity towards three cell lines: HeLa (human cervix cancer), A549 (human lung adenocarcinoma), and MDA-MB-231(triple-negative breast cancer). In addition, its inhibitory activities against EGFR and VEGFR-2 tyrosine kinases were studied. Compound 4 showed significant cytotoxicity towards A549, MDA-MB-231, and HeLa cells (IC_{50} values of 0.57, 1.19, and 1.26 μM, respectively) compared to the reference drug, docetaxel. (IC_{50} values of 10.08, 3.98,

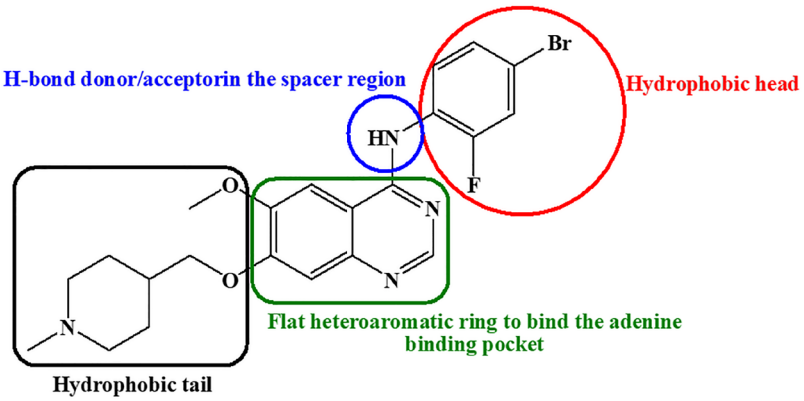


Figure 7. Pharmacophoric features of vandetanib as FDA-approved EGFR/VEGFR-2 dual inhibitor.

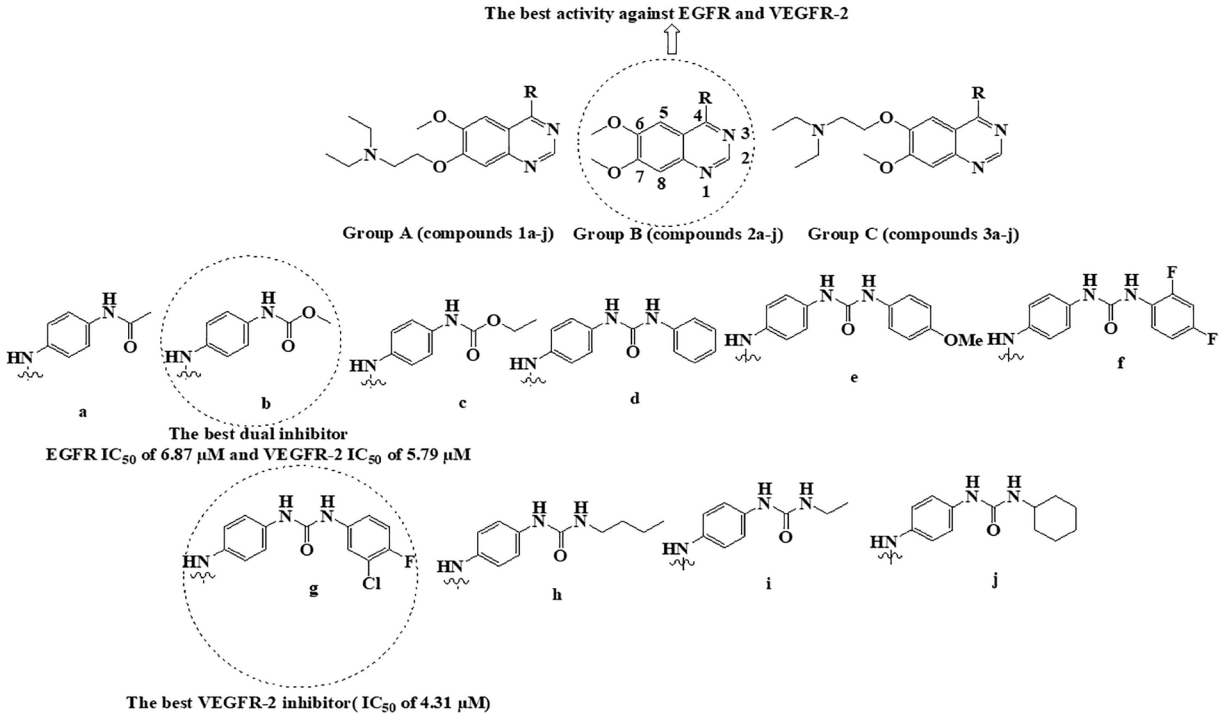


Figure 8. Structure of 4-anilinoquinazoline derivatives containing amide, carbamic acid ester, and urea moieties.

and 9.65 µM, respectively). This compound also showed IC_{50} values of 61.2 and 192 nM on EGFR and VEGFR-2 kinases, respectively. However, docetaxel, as the standard drug, exhibited higher inhibitory activity on EGFR and VEGFR-2 (IC_{50} values of 56.1 and 89.3 nM, respectively). Notably, the inhibitory activity against EGFR was better than that against VEGFR-2 tyrosine kinase. Furthermore, the results of the docking study were in line with the in vitro tests, showing that the compound had better binding energy with EGFR (-6.63 kcal/mol) than with VEGFR-2(-5.97 kcal/mol). This stronger affinity for EGFR, in comparison with VEGFR-2, may be related to two hydrogen bonds formed between the carbonyl of the quinazoline core and the ethoxy group of this compound, as well as Met793 and Cys797 of EGFR, respectively. Also, a halogen bond was formed between the fluorine and Pro794 of EGFR. Conversely, this compound did not show strong interactions with the VEGFR-2 complex and only displayed some moderate intermolecular interactions, which were in line with the activity on the VEGFR-2.

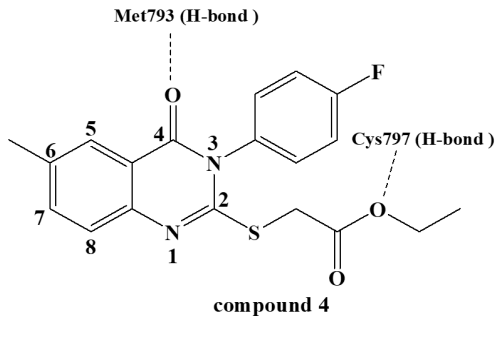


Figure 9. Structure of new quinazolinone molecule containing thioacetate group.

In a study by Bang *et al.*[56], a group of novel dual inhibitors targeting EGFR and VEGFR-2 was designed and synthesized (Figure 10). Also, the most potent inhibitor against EGFR and VEGFR was evaluated for its toxicity against four cell lines containing A431(human epidermoid squamous carcinoma), HUVEC (human umbilical vein endothelial cells), H1975 (human non-small cell lung cancer cells), and Hs27 (human skin fibroblast cells). In this study, different chain lengths (n) ranging from 1 to 3 carbons at the 7-position of the quinazoline core were utilized. All the synthesized analogs presented better IC_{50} values for EGFR ranging from 2 to 10 nM than ZD-6474 as the standard compound (IC_{50} of 800 nM). However, compounds with a one-carbon chain length (compounds 5c-5i) exhibited better VEGFR-2 inhibitory activity than derivatives with two- (compound 5a) or three-carbon (compound 5b) chain lengths. Also, the impact of R-substituted derivatives was investigated, which were substituted with various alkyl and heteroalkyl groups for the one-carbon chain length. The analogs containing heteroalkyl groups (compounds 5g-5i) displayed better inhibitory activities than the alkyl substitutes. Among the analogs, compounds bearing dimethylaminomethyl (compound 5g) and methoxymethyl (compound 5i) substitutions presented stronger inhibition of both EGFR and VEGFR-2. In addition, the compound with dimethylaminomethyl and a one-carbon chain length (compound 5g) demonstrated potent cytotoxicity against A431, HUVEC, and H1975 (IC_{50} values of 14, 93, and130 nM, respectively) compared to ZD-6474 and gefitinib as

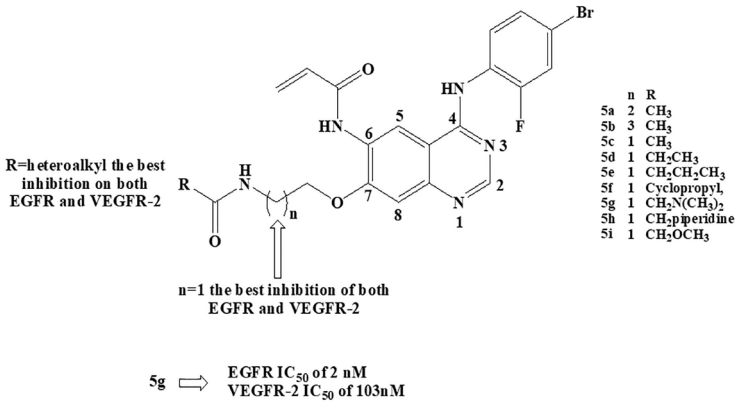
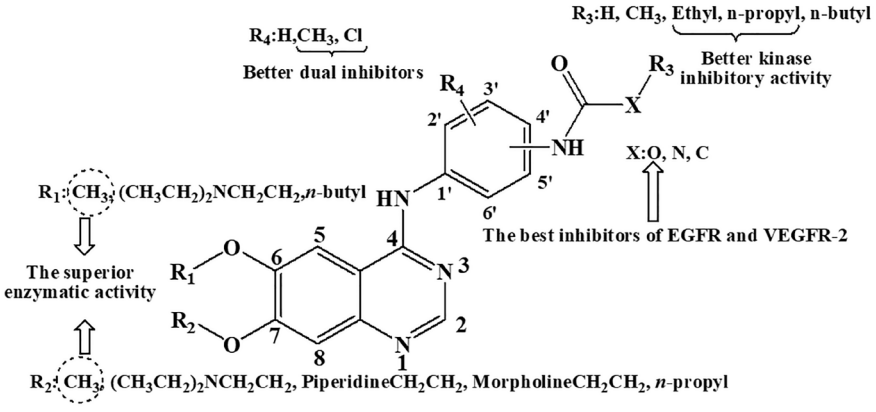


Figure 10. Structure of the synthesized 4-anilinoquinazoline acrylamide derivatives.

standard drugs (IC_{50} values ranging from 43 to >1000 nM). However, this compound did not show any cytotoxicity on Hs27 (IC_{50} value of >1000 nM). Therefore, this compound revealed strong cytotoxic activities against cell lines, which overexpressed EGFR (A431) and VEGFR-2 (HUVEC) as well as a 1st generation EGFR inhibitor-resistant cell line (H1975). Additionally, this compound significantly blocked the angiogenesis in mice in a dose-dependent manner due to the inhibitory activity on VEGFR-2.

Antonio Garofalo and his colleagues reported on a study involving a series of anilinoquinazoline derivatives that contain carbamic acid esters on the aniline ring. The synthesized compounds were evaluated for their in vitro inhibitory activity on EGFR and VEGFR-2 kinases, as well as their cytotoxic activity against various cancer cell lines, including PC3, HT29 (colon cancer cell line), MCF-7 (breast cancer cells), and EAHY926 (umbilical immortalized cancer cell line with overexpressed VEGFR-2 glycoprotein) (Figure 11).

The carbamic acid esters (X:O) showed the highest activity against both EGFR and VEGFR-2 kinases among all synthesized compounds. Introduction of urea (X: NH) or amide (X: CH_2) derivatives resulted in a decrease in activity on the two kinases. Compounds with a carbamic acid ester at the para-position of the aniline ring (compounds 6a, 6b, and 6c) showed better activity on EGFR and VEGFR than those with this group at the meta-position. Compounds bearing methyl or chlorine groups at the ortho position of the carbamate group showed dual inhibitory activity against both EGFR and VEGFR-2 with submicromolar IC_{50} values. Ethyl and propyl carbamates (compounds 6a, 6b, and 6c), in comparison with methyl and butyl ones, indicated better kinase inhibition targets. The substitution of two chlorides on the ortho and meta positions of the aniline ring led to a significant reduction in inhibitory activity on EGFR and VEGFR-2 due to the steric hindrance. Substitution of the methoxy groups located at the 6- or -positions of the quinazoline scaffold with alkylamino groups as a basic side chain led to an improvement in the cytotoxicity. Substitution of the methoxy group at the 6- or



6a: R_1:CH_3; **R_2:**CH_3; **R_3:**Ethyl; **R_4:**3-Cl; **X:**O; **4' carbamic acid ester** (IC_{50} values of 0.9 and 0.5 μM against EGFR and VEGFR-2)
The most potent dual inhibitor
6b: R_1:CH_3; **R_2:**CH_3; **R_3:**Ethyl; **R_4:**3-CH_3; **X:**O; **4' carbamic acid ester** (IC_{50} values of 0.9 and 0.65 μM against EGFR and VEGFR-2)
6c: R_1:CH_3; **R_2:**CH_3; **R_3:**Propyl; **R_4:**3-CH_3; **X:**O; **4' carbamic acid ester** (IC_{50} values of 0.9 and 0.85 μM against EGFR and VEGFR-2)

Figure 11. Structure of 4-aniliquinazoline carboxamide ester, urea, and amide derivatives.

7-position of the quinazoline skeleton by a diethylamino side chain resulted in a considerable reduction in enzymatic activities. It is worth mentioning that substitution of the methoxy group with alkylamino at the 7-position of the quinazoline core is better tolerated than at the 6-position. Piperidine and morpholine side chains at the 7-position of the quinazoline core exhibited a significant reduction in EGFR inhibition, with an IC_{50} value of more than 1 μM. However, they showed increased activity on VEGFR-2. None of the synthesized compounds displayed significant cytotoxicity on all evaluated cell lines in comparison with gefitinib and vandetanib as the standard compounds. Piperidine ethoxy at the 7-position of the quinazoline scaffold derivatives showed promising antiproliferative activity on HT29 cells (IC_{50} <6 μM). A butoxy side chain at the 6-position revealed significant cytotoxicity on PC3 and EAHY926 cells (IC_{50} <3 μM), surpassing the effects of gefitinib and vandetanib. These findings confirmed that these modifications caused an increase in antiproliferative activity. Compounds with a propoxy side chain at the 7-position of the quinazoline skeleton also represented notable inhibition of VEGFR-2 with an IC_{50} <1 μM. In the molecular docking study into the ATP active site of EGFR, the N1 nitrogen of the quinazoline interacted with the Met769 NH of EGFR through a hydrogen bond. Also, the N3 nitrogen of quinazoline formed a hydrogen bond with the Thr766 hydroxy group. Furthermore, the NH and the carbonyl oxygen of the carbamate group showed interactions with NH of Glu738 and Thr830, respectively. In the ATP active site of VEGFR-2, the quinazoline core indicated various interactions between the CO carbamate group and Asp1046 as well as the NH carbamate group and the Lys866 and Glu883. The essential hydrogen bond interaction was observed between the N1 of the quinazoline core and the Cys919.[57]

4-Anilinoquinazoline urea derivatives were synthesized and evaluated for their inhibition of EGFR and VEGFR-2. A molecular docking study was carried out for investigating the interactions with kinases (Figure 12). The 4-anilinoquinazoline scaffold plays a critical role in activity against EGFR. So, sorafenib with a pyridine ring instead of a quinazoline core lacks activity on EGFR. The 3'-urea linker (compound 7a) showed increased inhibitory activity toward EGFR compared to the 4'-urea substitute (compound 7b), whereas the opposite was observed for VEGFR-2. Substitution of 4-chloro-3-(trifluoromethyl)-phenyl with 3-chloro-4-fluorophenyl on phenyl urea (R_3) led to an inactive compound against both EGFR and VEGFR-2. Using 3-methyl (R_3) on the phenyl urea resulted in a substantial loss of activity against EGFR and VEGFR-2. Also, some compounds containing aliphatic tertiary amino substitutes with an ether linked to the 7-position of the quinazoline skeleton were synthesized. Removing the substitute at the 6-position of the quinazoline core decreases molecular weight and prevents potential metabolic liability. Introduction of the more polar hydroxyl-containing group, 4-(2-hydroxyethyl)piperazin-1-yl or ethyl(2-hydroxyethyl)amino at the 7-position of the quinazoline core, resulted in improved inhibitory activity against EGFR and VEGFR-2. It has been proven that the inhibitory activity toward kinases is affected by both the substituted urea group (R_3) and substituent at the 7-position of the quinazoline (R_2). Docking was done in the ATP binding site of the DFG-out conformation of VEGFR-2 (PDB code 2OH4). The quinazoline core resides in a hydrophobic pocket containing Leu838, Ala864, Phe916, and Leu1033. Quinazoline N1 forms a hydrogen bond with the Cys917 backbone in the hinge region. The para-substituted aniline ring occupies a hydrophobic pocket near the ATP binding site. The carbonyl and two

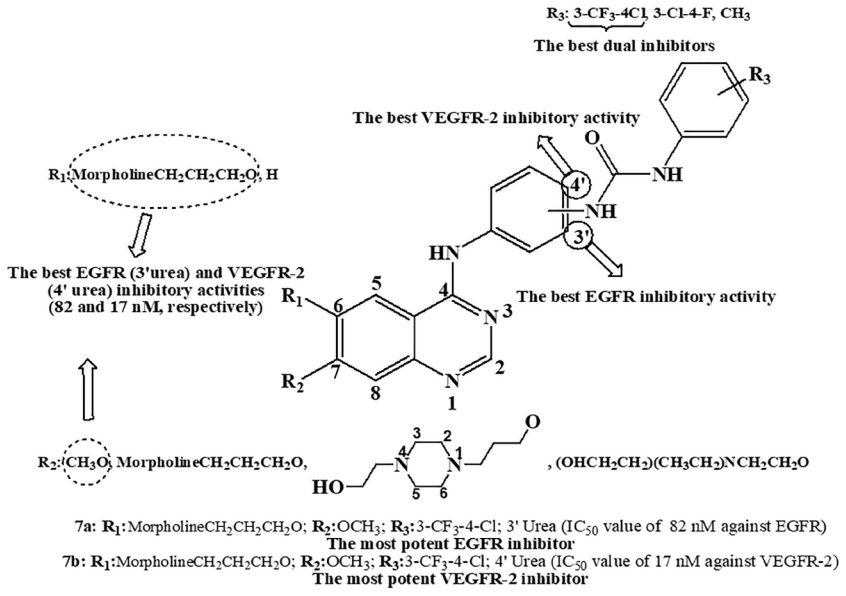


Figure 12. Structure of 4-aniliquinazoline urea derivatives.

NHs of urea interact with the backbone of Asp1044 and the side chain of Glu883 through hydrogen bonds, respectively. The terminal 4-chloro-3-(trifluoromethyl)phenyl occupies another hydrophobic pocket lined with Ile866, Leu887, Ile890, Leu1017, and His1024.[58]

In another study by Zhang *et al.*[59], some 4-anilinoquinazolines with unsymmetrical diaryl urea derivatives were synthesized and tested for their inhibitory activity on EGFR and VEGFR-2. The cytotoxicity of the compounds was also evaluated against three cell lines: HT-29, MCF-7, and H460 (human lung cancer) (Figure 13).

A benzyloxy group at the 7-position (R_1) of the quinazoline core resulted in a significant reduction in inhibitory activity towards EGFR and VEGFR-2 (IC_{50} > 10 μM). Also, substitution of chlorine at the ortho position of the urea (X) on the aniline ring led to an increase in inhibition on both kinases. Compounds containing a 4-methylpiperazine moiety at the 7-position of the quinazoline scaffold showed better activity on both EGFR and VEGFR-2 in comparison with the compounds with a morpholine and piperidine group at this position. However, morpholine (compound 8b) or piperidine substitutes at the 7-position of the quinazoline core caused a significant decrease in inhibitory activity on EGFR, but increased activity on VEGFR-2 compared to the 4-methylpiperazine group (compound 8a) at the mentioned position, considering that all compounds contained meta and para-dimethyl substituents on the phenyl urea (R_2) (1 and 14 nM against EGFR and VEGFR-2, respectively). Substitution of phenylurea with a glycine methyl ester group (compound 8c) exhibited moderate inhibition against EGFR and VEGFR-2. Cellular cytotoxicity evaluation showed that chlorine in the ortho position of the urea group is critical for cytotoxic activity (compounds 8a and 8b). The compound bearing piperidine at the 7-position of the quinazoline core, as well as meta and para methyl substitutes (R_2), exhibited the best cytotoxicity on all tested cancer cell lines. It showed IC_{50} values of 1.85 μM for HT-29, 1.27 μM for MCF-7, and 2.90 μM for H460. Additionally, this compound displayed moderate inhibitory effects on EGFR and VEGFR-2. Similarly, the compound bearing a piperidine substitute at the 7-position of the quinazoline scaffold and p-chlorine on the phenyl urea ring (R_2) exhibited high cytotoxic activity against three cancer cell lines (IC_{50} <5 μM). Using glycine methyl ester (compound 8c) instead of phenylurea led to a complete loss of cytotoxicity (IC_{50} > 50 μM) in most of the compounds. Docking studies showed that the protonated N4 of piperazine formed an ionic bond with Asp1003 in the ATP binding site of EGFR. N1 of the quinazoline and NHs of urea moiety interacted with Met793 and Asp855 with hydrogen bonds, respectively. Furthermore, Val726, Leu718, and Leu844 played a role in forming hydrophobic interactions with the compounds. The hydrophobic pocket containing Leu838, Ala864, Phe916, and Leu1033 amino acids interacted with the quinazoline nucleus. In VEGFR-2 docking studies, quinazoline N1 as well as the carbonyl and NHs of the urea formed hydrogen bonds with the Cys917 backbone as well as the Asp1044 backbone and Glu883 side chain, respectively. The phenyl of aniline occupied the hydrophobic pocket near the ATP binding site. The 3,4-dimethylphenyl urea located into another hydrophobic pocket lined with Leu887, Ile886, Leu1017, and Ile890.

4-anilinoquinazoline-acylamino derivatives were synthesized and evaluated for the cytotoxicity against three cell lines, including HT-29, MCF-7, and H460, as well as inhibitory activities towards EGFR and VEGFR-2 (Figure 14). Also, a docking study was carried out to investigate the binding mode of the synthesized compounds.

Compounds with a benzyloxy chain at the 7-position of the quinazoline core presented a reduction in inhibitory activity against EGFR and VEGFR-2 (IC_{50}>10μM). On the other hand, compounds with a 4-methylpiperazinyl propoxy group at the 7-position of the quinazoline scaffold (compounds 9a and 9b) showed better potency against both

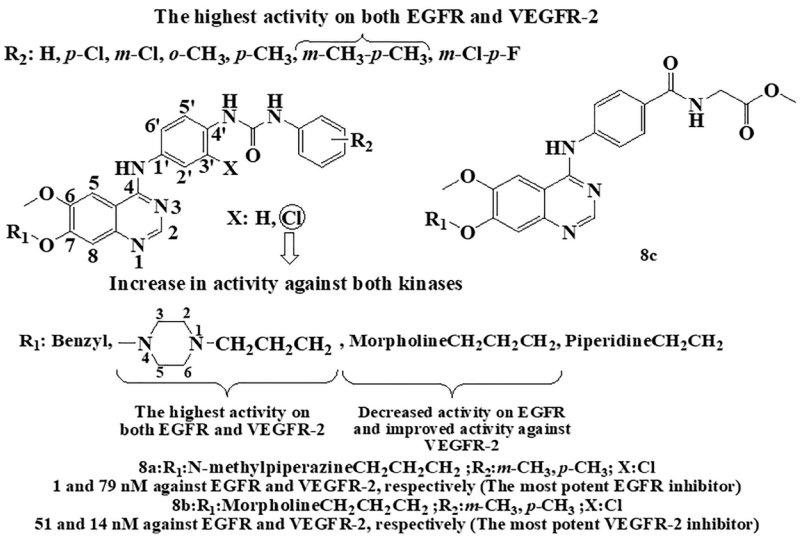


Figure 13. Structure of 4-anilinoquinazoline derivatives containing phenylurea and a glycine methyl ester groups.

kinases in comparison with the compounds with piperidin-1-yl ethoxy and morpholin-4-yl propoxy substitutes at this position. Most of the compounds demonstrated moderate to good anti-proliferative activities. The compound with 4-methylpiperazin-1-yl propoxy at the 7-position of the quinazoline core and a m-Cl group on the phenylamide displayed the best cytotoxicity against HT-29, MCF-7, and H460 cells with IC_{50} values of 5.27, 4.41, and 11.95μM, respectively. Interestingly, the compound containing piperidin-1-yl ethoxy at the 7-position of the quinazoline skeleton showed high selectivity towards MCF-7 (IC_{50} of 0.10 μM on MCF-7 versus IC_{50} values of 5.12 μM and 16 μM against HT-29 and H460, respectively).

In the ATP binding site of EGFR, the protonated N4 of piperazine interacted with Glu804 via an ionic bond. A hydrogen bond formed between N3 of the quinazoline core and the Cys797 backbone. Also, hydrophobic interactions were observed with a pocket lined with Val726, Leu718, and Leu844. Within the ATP-binding cavity of VEGFR-2 kinase (PDB:4ase), N1 of the quinazoline core, N4 of piperazine, and the NH of the acylamino interacted through H-bonds with Cys919, Leu840, and Glu885, respectively. Fitting into the DFG-out conformation of VEGFR-2 (PDB:2OH4) was comfortable, with the quinazoline skeleton occupying a hydrophobic pocket created by Leu838, Ala864, Phe916, and Leu1033 amino acids. N1 of the quinazoline core accepted a hydrogen bond from the backbone of Cys917 in the hinge region. The methoxy at the 6-position of the quinazoline formed hydrogen bonds with two NHs of Arg1049. The phenylamide occupied the hydrophobic pocket neighboring the ATP binding site. The carbonyl and NH of the acylamino interacted with the backbone of Asp1044 and the side chain of Glu883 through hydrogen bond interactions. The terminal phenyl was located in a second hydrophobic pocket lined by Leu887, Ile886, Leu1017, and Ile890.[60]

Sun and colleagues designed and synthesized quinazoline- and thiourea-containing sorafenib derivatives. They evaluated the inhibitory activities of these derivatives against EGFR and VEGFR-2 tyrosine kinases, as well as against three cancer cell lines: HCT116, MCF-7, and B16 (Figure 15). Moreover, the most potent compounds were investigated for their in vivo antitumor activity by a B16 melanoma xenograft model test. A docking study was conducted to clarify the mode of interaction at the ATP binding site of EGFR and VEGFR-2.

Compounds with two strong electron-withdrawing groups on the terminal aromatic ring (Ar) connected to the thiourea displayed strong inhibitory activity on EGFR (IC_{50} ranging from 0.01 to 0.05 μM) and VEGFR-2 (IC_{50} ranging from 0.05 to 0.19 μM). However, compounds containing electron-donating groups presented a noticeable decline in activity (IC_{50}>10 μM). Compounds bearing chlorine or bromine at the para position and trifluoromethyl at the meta position of the terminal phenyl (Ar) with sulfur as the linker (X: S) (compound 10a) showed the best activity against EGFR (IC_{50} of 0.01μM). Conversely, the compound with chlorine at the para position and trifluoromethyl at the meta position of the terminal phenyl (Ar) with oxygen as the linker (X: O) (compound 10b) presented the most potent activity against VEGFR-2 (IC_{50} of 0.05μM). This may be attributed to several reasons. First, compounds with an electron-deficient phenyl ring can form hydrophobic interactions with specific amino acid residues. Also, some electron-withdrawing groups (-F, $-CF_3$) can interact with the ATP binding site through the formation of hydrogen bonds. Additionally, compounds bearing diaryl thioether moieties (X: S) showed more potent activity against both

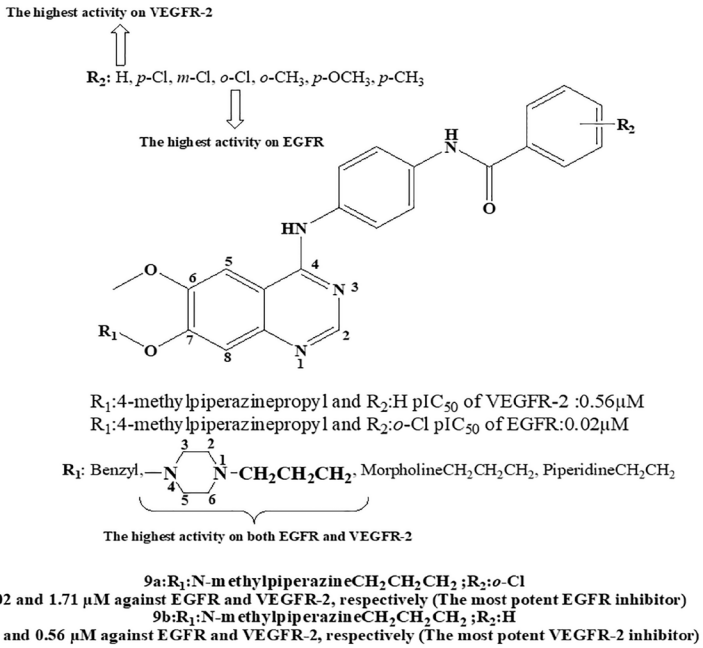


Figure 14. Structure of 4-anilinoquinazoline acylamino derivatives.

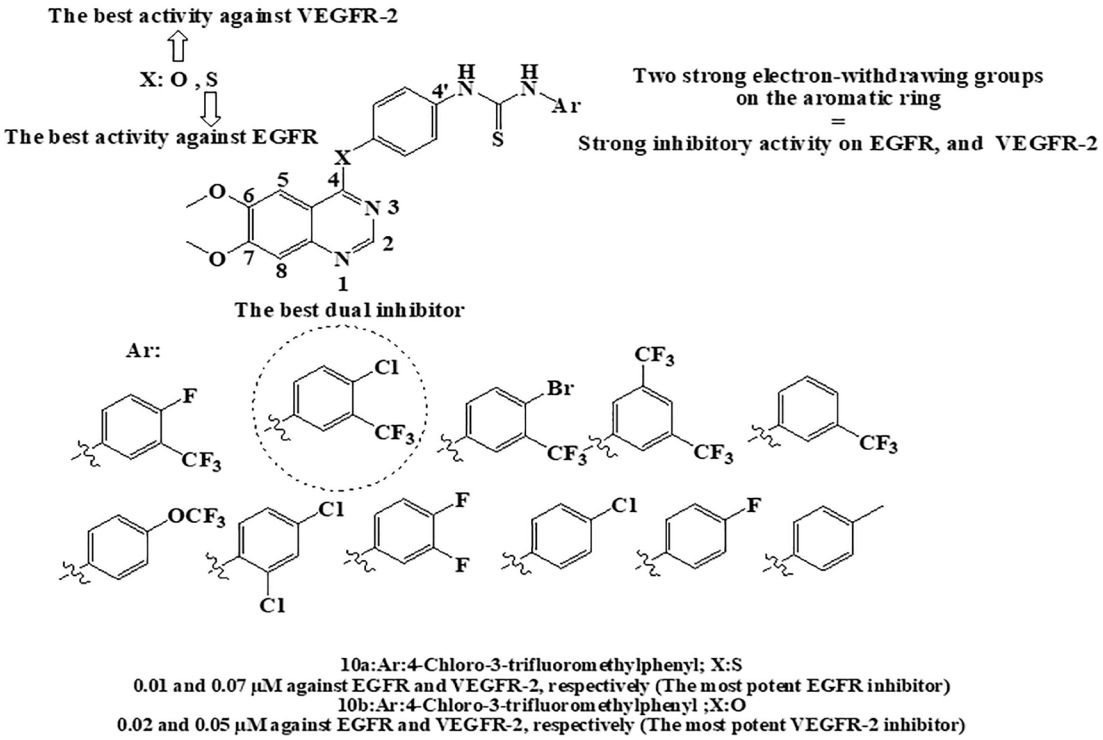
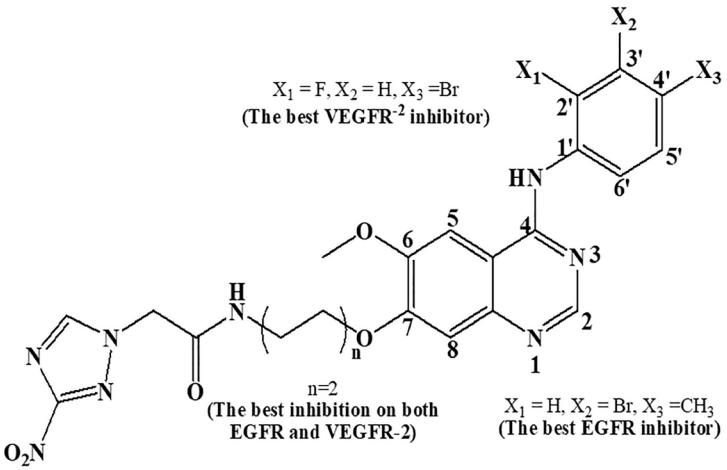


Figure 15. Structure of quinazoline-and thiourea-containing sorafenib derivatives.

EGFR and VEGFR-2 in comparison with the diaryl ether compounds (X: O). Moreover, the introduction of a chlorine substituent at the ortho position of the thiourea group on the terminal phenyl (Ar) resulted in a decrease in activity against both EGFR and VEGFR-2. The same compounds with the best inhibitory activities toward EGFR and VEGFR-2 also showed the most potent in vitro and in vivo antiproliferative activities, surpassing sorafenib as the standard. Furthermore, molecular docking of the compound containing bromine at the para position and trifluoromethyl at the meta position of the terminal phenyl (Ar) with sulfur as the linker (X: S) showed that this compound could effectively bind with EGFR (PDB: 2ity). The protonated N3 of quinazoline interacted with the Met793 in the ATP binding site of EGFR using an ionic bond. The NH of the thiourea could form two H-bonds with Pro794 and Met793. Also, oxygen atoms of the 6 and 7 methoxy groups of the quinazoline core interacted with Lys745 through a hydrogen bond. Hydrophobic interactions were also formed with the hydrophobic pocket in the active site of EGFR lined with Phe795, Met793, and Leu718. This compound could also interact with the inactive DFG-out conformation of the VEGFR-2 ATP active site (PDB: 4asd). The quinazoline and disubstituted phenyl formed hydrophobic interactions with Phe795, Leu844, Met793, Val726, and Leu718. The two hydrogen bonds were formed between the NH of the thiourea moiety, as well as the 6 and 7 methoxy groups with Asp800 and Lys745, respectively.[61]

In a study, Wei *et al.*[62] reported the synthesis of several 4-anilinoquinazoline derivatives containing a 3-nitro-1,2,4-triazole moiety to achieve potent EGFR/VEGFR-2 inhibitors (Figure 16). The cytotoxicity of these compounds was assessed on the A549 cell line and human small cell lung cancer cells (H446) under normoxic and hypoxic conditions.

The structure-activity relationship (SAR) showed that the length of the linker between the triazole and quinazoline moiety (n) significantly influenced inhibitory activity. The optimal length for EGFR/VEGFR-2 inhibitory activity was found to be 2 carbons (n=2). The substituent on the aniline ring is crucial for VEGFR-2 selectivity, with bulky and heavy halogens on the aniline ring favoring the inhibition of EGFR and VEGFR-2. The compound 11j, with bromine and methyl at the meta and para positions of the aniline ring, respectively, showed the best EGFR inhibitory activity with an IC_{50} of 0.37 nM. On the other hand, substitution of fluorine and bromine at the ortho and para positions of the aniline ring (compound 11h) displayed the most potent inhibition of VEGFR-2 (IC_{50} value of 36.78 nM). In cytotoxicity assays, compounds with the highest inhibitory activity against EGFR and VEGFR-2 displayed the strongest inhibition in hypoxia against A549 and H446 cells in comparison with vandetanib, the standard compound. The highest anti-proliferative activities of these compounds in hypoxia may be related to their superior inhibitory activity against VEGF gene expression in the A549 cell line. Additionally, these compounds exhibited noteworthy inhibitory activity and a favorable safety profile against tumor development in dose-dependent anticancer



11a: X_1 = F, X_2 = H, X_3 =Br, n=1
11b: X_1 = Cl, X_2 = H, X_3 =F, n=1
11c: X_1 = H, X_2 = Br, X_3 =CH_3, n=1
11d: X_1 = F, X_2 = H, X_3 =Br, n=3
11e: X_1 = Cl, X_2 = H, X_3 =F, n=3
11f: X_1 = H, X_2 = Br, X_3 =CH_3, n=3
11g: X_1 = H, X_2, X_3 = F, n=3
11h: X_1 = F, X_2 = H, X_3 =Br, n=2
11i: X_1 = Cl, X_2 = H, X_3 =F, n=2
11j: X_1 = H, X_2 = Br, X_3 =CH_3, n=2
11k: X_1 = H, X_2, X_3 = F, n=2
11l: X_1, X_3 = H, X_2 = F, n=2
11m: X_1, X_2 = H, X_3 = F, n=2
11n: X_1 = F, X_2, X_3 =Cl, n=2

Figure 16. Structure of 4-anilinoquinazoline derivatives containing 3-nitro-1,2,4-triazole moieties.

tests using A549 xenograft models. Also, the molecular docking of 10h with EGFR (PDB code 4I23) showed that the N1 of the quinazoline core formed a critical H-bond with Met793. Additionally, the aniline ring interacted with the EGFR active site through hydrophobic interactions, whereas the 3-nitro-1,2,4-triazole moiety did not show any additional hydrogen bond with EGFR. Furthermore, the docking study of 10j inVEGFR-2 active site (PDB code 2RL5) demonstrated the aniline ring was positioned in a narrow and deep hydrophobic pocket of VEGFR-2. Also, a crucial hydrogen bond was formed between the backbone Cys919 and N1 of the quinazoline atom. 10j showed a coplanar binding mode to EGFR, while the folding of the 3-nitro-1,2,4-triazole moiety of 10j formed a U shape, which resulted in a π-π stacking interaction with the Phe1047 amino acid of VEGFR-2. Furthermore, an additional hydrogen bond was formed between the N3 of the quinazoline skeleton of 10j and the Thr916 amino acid side chain hydroxyl group of VEGFR-2, and two hydrogen bonds were formed between the side chain carbonyl oxygen atom of 10j and the Asn923 backbone residue.

In different studies by Moghadam *et al.*[3,4,63,64] a new series of 4-anilinoquinazoline were designed, synthesized, and evaluated for their toxicity against A431, HUVEC, and HU02 (normal human foreskin fibroblast cells) (Figure 17). Different aniline derivatives (R_1) as well as aromatic and aliphatic amines (R_2) were used to evaluate the cytotoxicity of compounds. Most of the synthesized compounds showed significant cytotoxicity against both cancer cell lines, including A431 and HUVEC, while they did not show any activity against HU02. Among the tested compounds, the compound bearing m-toluidine at the 4-position of the quinazoline (R_1) and imidazole at the 7-position of the quinazoline (R_2) (compound 12) showed the best

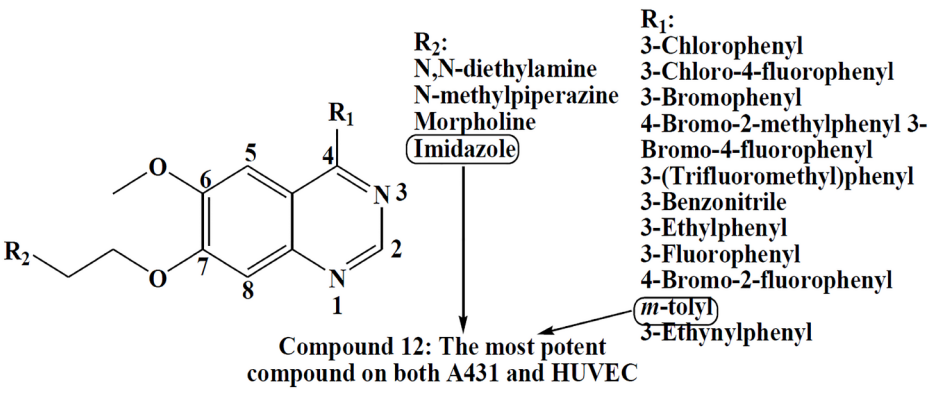


Figure 17. Structure of 6,7-disubstituted 4-anilinoquinazoline derivatives.

activity against both cancer cell lines with IC_{50} values of 0.11 and 5.01 μM against A431 and HUVEC, respectively, which was better than vandetanib as the standard drug (10.62 and 5.75 μM on A431 and HUVEC, respectively). Also, incorporating 3-ethynylaniline at the R_1 position and imidazole at the R_2 position resulted in a potent compound with IC_{50} of 0.2697 and 5.243 μM on A431 and HUVEC, respectively. In the imidazole-containing compounds at the 7-position of the quinazoline core, compounds with 3-(Trifluoromethyl) aniline, 3-aminobenzonitrile, 3-bromoaniline and 3-chloro-4-fluoroaniline at the 4-position of the quinazoline skeleton were other potent compounds that showed activity lower than vandetanib. In compounds with aliphatic substituents at the R_2 position, N, N-dimethyl at 7-position of the quinazoline core with 3-ethylaniline and 3-chloro-4-fluoroaniline at 4-position of the quinazoline core were the most potent compounds with IC_{50} of 8.2 and 9.7 μM on A431and 0.87 and 3.57 μM on HUVEC, respectively, which were better than vandetanib. Within this group, the compound with 3-bromoaniline and morpholine was the most potent compound on A431 with an IC_{50} value of 1.78 μM. Docking studies showed that the N1 of the quinazoline core of compound 12 formed a vital hydrogen bond with Met769 of the EGFR ATP-binding site. Additionally, hydrophobic interactions were formed with amino acids in the hydrophobic pocket lined with Val702, Ala719, Lys721, Met742, Leu764, and Thr766. Moreover, docking analysis with VEGFR-2 ATP-binding site exhibited the formation of one H-bond between N3 of imidazole and Cys919 residue. Also, the m-tolyl of this compound interacted with hydrophobic interactions with the hydrophobic pocket comprising Val848, Ala866, Lys868, Thr916, and Phe1047.

Ghorab *et al.*[65] reported the synthesis of novel 6-iodoquinazolinone compounds containing a benzenesulfonamide group with different acetamide moieties (Figure 18). The compounds were evaluated on HepG2, MCF-7, HCT-116, and A549 cell lines as well as against EGFR-mutant and VEGFR-2. A docking study was carried out to clarify the interactions of compounds.

In this study, the focus of the SAR study was on the effect of substituting the pyridine ring and two methoxy ethoxy moieties in two FDA-approved drugs, Sorafenib and Erlotinib, with a 6-iodoquinazolin-4-one moiety. The investigation also included replacing the urea and amino linkers in Sorafenib and Erlotinib with alternative long spacers. Additionally, various substituents with diverse electronic and lipophilic properties were introduced to evaluate their influence on anticancer activity. Overall, factors such as the iodoquinazolinone ring, spacer, linker (HBA-HBD), lipophilicity, and electronic properties of the substituents played a significant role in the anticancer activity. Compounds bearing naphthalene (compound 13n), 3,3-dimethylbutyl (compound 13b), phenethyl (compound 13f), and 3,4-dimethoxyphenethyl (compound 13g) presented the highest potency against MCF-7, HepG2, A549, and HCT116, respectively. Moreover, compound 13n with naphthalene-1-yl exhibited the highest inhibitory activities towards $EGFR^{T790M}$ (IC_{50} of 0.0728 μM) and VEGFR-2 (IC_{50} of 0.0523μM) tyrosine kinases compared to Erlotinib (IC_{50} against EGFR =0.24 μM) and Sorafenib (IC_{50} against VEGFR-2 =0.14 μM). The HepG2 cell line was most susceptible to the compound bearing neohexyl substitute (compound 13a), while derivatives with naphthalene-1-yl (compound 13n), 3,3-dimethoxyphenylethyl (compound 13g), and phenylethyl (compound 13f) displayed more cytotoxicity against MCF-7, HCT-116, and A549. Compound 8 arrested the growth of MCF-7 cells at the radio-sensitive G2/M phase. Flow cytometric studies confirmed the induction of early and late apoptosis. Likewise, evaluating the selectivity index of compound 13n using the HEK-293 normal cell line showed a favorable safety profile of this compound. The docking studies of compound 13n into the active site of EGFR formed four hydrogen bonds, including the NH group of the acetamide linker, carbonyl, quinazoline N1, and the terminal NH of sulfonamide moiety with Gln791, Met793, Val726, and Pro794, respectively. The naphthyl

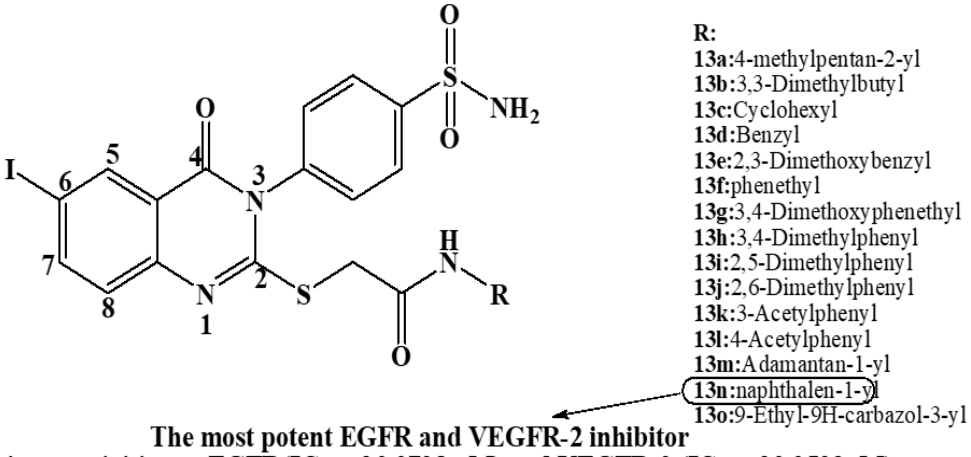
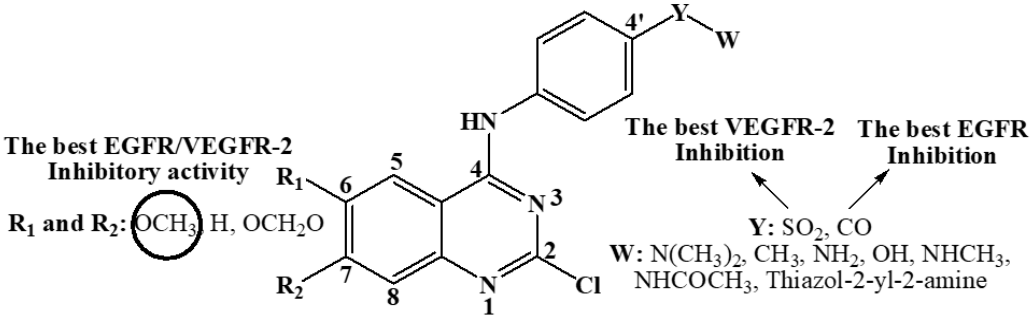


Figure 18. Structure of quinazoline sulfonamide derivatives.

ring occupied the hydrophobic pocket I lined with Gln791, Met790, Asp855, Thr854, Leu777, Leu788, Lys745, Val726, Gly724, and Phe723. Also, the phenyl ring at the 3-position of the quinazoline core placed in the hydrophobic pocket II comprising Val845, Leu844, Pro794, Phe795, Met793, and Leu718. The binding mode of this compound showed five hydrogen bonds with the VEGFR-2 active site. The carbonyl group of quinazoline and NH of the acetamide linker formed two H-bonds with Glu917 and Glu885, respectively. Additionally, the sulfonamide group interacted with the active site through three hydrogen bonds with Asp1046, Cys1045, and His1026. The quinazoline skeleton occupied the hydrophobic pocket created by Leu1035, Cys919, Phe918, Glu917, Lys868, Val848, and Leu840. Moreover, the naphthyl ring located in the hydrophobic region lined by Asp1046, Cys1045, His1026, Ile892, Ile888, and Glu885.

Novel 2-chloro-4-anilino-quinazolines designed as EGFR and VEGFR-2 dual inhibitors were synthesized and assessed for their inhibitory activities by de Castro Barbosa *et al.*[27] (Figure 19). Among the synthesized compounds bearing sulfonamide, amine, and acetamide substituents at the 4-position of the aniline ring, a direct relationship was observed between the inhibitory activities against EGFR and VEGFR-2. However, this correlation did not hold true for the acetamide derivative bearing acetamide at the 4-position of the aniline ring as well as methoxy at the 6- and 7-positions of the quinazoline core which showed an IC_{50} of 37.6 µM for EGFR and 1.99 µM for VEGFR-2. Moreover, the lack of inhibition on both enzymes for compounds without any substitutes at the 6- and 7-positions of the quinazoline scaffold showed the importance of the dioxygenated quinazoline skeleton in interacting with the ATP binding site. Conversely, replacing the 6,7-dimethoxy group with a dioxolane ring was not suitable for interacting with the ATP binding sites of both kinases. The compounds bearing a hydrogen bond donor at the para position of the aniline ring showed an increase in activities on both the EGFR and VEGFR-2 tyrosine kinases. The primary and secondary sulfonamides (Y: SO_2; W: NH_2 and $NHCH_3$, respectively) resulted in an increase in potency in comparison with the corresponding dimethyl-sulfonamide (Y: SO_2; W: $NH(CH_3)_2$) and methyl sulfone (Y: SO_2; W: CH_3). The results showed that the hydrogen bond donating ability in the primary sulfonamide is an important structural property. The methyl-sulfonamide derivatives displayed similar potency in the inhibition of EGFR and VEGFR-2 in comparison with the primary sulfonamide. Additionally, the primary amide (Y: CO; W: NH_2) showed significant inhibitory activity against both tyrosine kinases, whereas the corresponding sulfonic acid (Y: SO_2; W: OH) and carboxylic acid (Y: CO; W: OH) were not inhibitors. It could be concluded that binding to the ATP binding site of the EGFR and VEGFR-2 tyrosine kinases was not dependent on the acidity of the para substituents. The docking studies of potent compounds (compounds 14a, 14b, and 14c) into the active site of EGFR showed the formation of five hydrogen bonds. These include N1 of quinazoline with Met793, the sulfone group with Lys745 and NH of the Asp855 backbone, and the sulfonamide moiety with Glu762 and Asp855. The quinazoline ring occupied a hydrophobic pocket lined with Leu718, Val726, Leu792, and Leu844. Also, the aniline ring at the 4-position of the quinazoline core interacted with Lys745 side chain through a cation-π interaction. Similarly, these compounds also formed five hydrogen bonds with the VEGFR-2 active site, including the N1 of quinazoline with Cys919, the sulfone group with Lys868 and NH from the Asp1046, and the primary sulfonamide with Glu855 and Asp1046. The quinazoline core was located into a hydrophobic pocket created by Leu840, Val848, Phe918, and Leu1035. Furthermore, the aniline ring formed a cation-π bond with Lys868.[27]

A group of quinazoline-based derivatives was designed, synthesized, and evaluated against EGFR and VEGFR-2



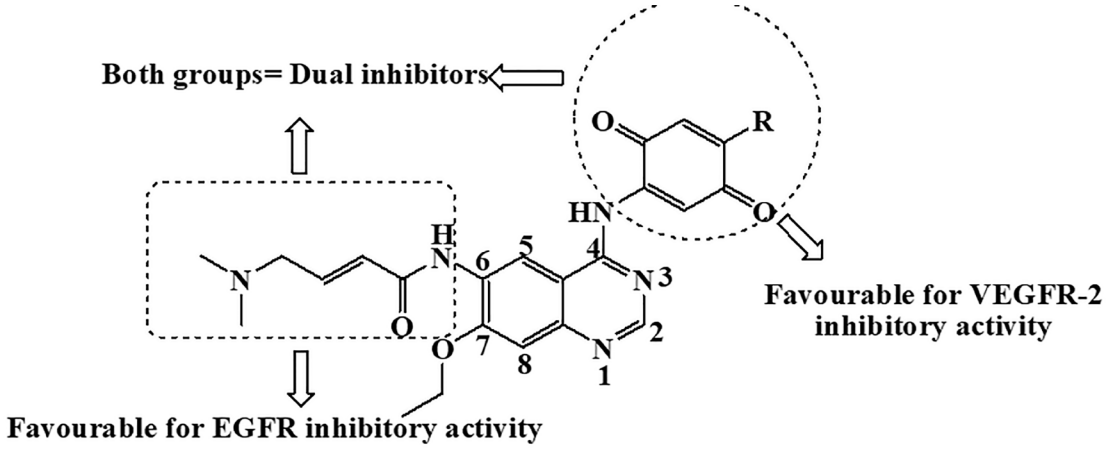
14a:R_1 and R_2: OCH_3; **Y:** CO; **W:** NH_2 (**The best EGFR Inhibitor**)
(EFGR IC_{50}=0.9 µM and VEGFR-2 Inhibitor IC_{50}=1.17 µM)
14b:R_1 and R_2: OCH_3; **Y:** SO_2; **W:** $NHCH_3$ (**The best VEGFR-2 Inhibitor)**
(EFGR IC_{50}=1.63 µM and VEGFR-2 Inhibitor IC_{50}=0.85 µM)
14c:R_1 and R_2: OCH_3; **Y:** SO_2; **W:** NH_2
(EFGR IC_{50}=2.37 µM and VEGFR-2 Inhibitor IC_{50}=1.02 µM)

Figure 19. Structure of 2-chloro-4-anilinoquinazoline derivatives.

tyrosine kinases using various ATP concentrations ranging from 1μM to 1mM (Figure 20). Compounds bearing a quinone ring at the 4-position of the quinazoline scaffold (compounds 15a-15d) could be inhibitors of VEGFR-2, with IC_{50} values ranging from 46.1 to53.7 nM, without any significant activity on EGFR. On the other hand, the compounds with the 4-(dimethylamino)crotonamide Michael acceptor moiety at the 6-position of the quinazoline skeleton but without a quinone ring at the 4-position of the quinazoline core showed inhibitory activity on EGFR with weak activity against VEGFR-2 tyrosine kinase. Compounds with both groups could block the activity of both enzymes. It is evident that the presence of the 4-(dimethylamino)crotonamide Michael acceptor group has modified the inhibitory profiles of these compounds, leading to enhanced EGFR activity. Additionally, the inclusion of the quinone ring imparts VEGFR-2 inhibitory activity. Reversible EGFR inhibitors lose all inhibition on EGFR kinase when the ATP concentration is raised from 1 μM to 1 mM. As dual irreversible inhibitors, compounds showed an average increase in IC_{50} values when the ATP concentration was raised from 1 μM to 1 mM. For reversible VEGFR-2 inhibitors, the inhibitory activity on VEGFR-2 kinase significantly decreased as the ATP concentration increased from 1 μM to 1 mM. Docking study of compound 15a into the ATP binding site of EGFR showed that the carbonyl group of a quinone interacted with the NH of aniline through an intramolecular H-bond. Also, an H-bond formed between the N1 of quinazoline and NH of Met-769. The N3 of the quinazoline formed a water bridge H-bond with the hydroxyl group of Thr-766. A benzyloxy quinone ring formed a π–stacking interaction with the phenyl ring of Phe-832. Docking of compound 15a in ATP-binding site of VEGFR-2 revealed that N1 of the quinazoline formed an H-bond with the NH of Cys-919. Also, a π–stacking interaction between the benzyloxy quinone ring and the phenyl ring of Phe-1047 was observed.[53]

Binding site and docking analysis

Based on the interactions with ATP, the EGFR tyrosine kinase ATP-binding site consists of the following regions: the hinge region, which serves as the adenine binding site, the solvent-accessible region where sugar and phosphate bind, hydrophobic pocket I, hydrophobic pocket II, and the back pocket known as the allosteric pocket. Two regions that typically display the active and inactive conformations of EGFR TK are the αC helix and the DFG motif. In the active conformation, the αC helix located at residues 753–767 in EGFR, rotates inward against the N-lobe and towards the active site. This movement results in a decrease in the distance between Glu762 of the αC helix and Lys745 of the β3 strands, leading to the formation of a salt bridge and additional interactions with the α- and β-phosphate groups of ATP.[66,67] The C-terminal domain consists of a flexible activation loop typically with 20-30 residues in length, characterized by a conserved Asp-Phe-Gly ("DFG") motif. In the active conformation of EGFR, the Asp855 residue points into the ATP-binding pocket, known as DFG-in. However, in the inactive form, flipping



15a: R = OCH_2Ph-3-F (The best EGFR inhibitor)
(EGFR IC_{50} of 4.1 nM; VEGFR-2 IC_{50} of 113.4 nM)
15b:R=OCH_2-2-thiophene (The best VEGFR-2 inhibitor)
(EGFR IC_{50} of 47.5 nM; VEGFR-2 IC_{50} of 76.2 nM)
15c:R=OCH_2Ph-3-Cl (The best dual inhibitor)
(EGFR IC_{50} of 12.2 nM; VEGFR-2 IC_{50} of 80.2 nM)
15d:R=OCH_2Ph
(EGFR IC_{50} of 18.7 nM; VEGFR-2 IC_{50} of 102.3 nM)

Figure 20. Structure of quinazoline derivatives containing quinone and 4-(dimethylamino)crotonamide Michael acceptor groups.

the DFG motif by approximately180° relative to the active conformation moves the Asp855 away from the ATP binding pocket, known as DFG-out. Docking studies of the quinazoline-based EGFR TKIs (vandetanib as the standard drug and synthesized compounds) have shown that the Met793 or Met769 residues form H-bonds with the N1 of the quinazoline core. Additionally, the Thr766 or Thr830 amino acids can interact with the N3 of the quinazoline skeleton through water bridges. Also, the N3 of the quinazoline ring can interact with the Asp1003 or Met 793 through an ionic bond. The 4-aniline group is located in hydrophobic pocket I, where the quinazoline ring extends into hydrophobic pocket II. The groups at the 6- and 7-positions of the quinazoline ring extend into the solvent-accessible region and interact through an additional ionic bond with Asp800 (Figure 21).[27,53,55,57,59-65,68]

The VEGFR-2 binding site is comprised four main regions. The hinge region is lined with crucial residues, including Cys919 and Glu917, which are crucial for forming H-bonds. The conserved DGF motif is made up three residues, including Asp1046, Phe1047, and Gly1048. Based on the arrangement of the Phe1047 residue in the kinase domain, there are two states: active (DFG-in) and inactive (DFG-out). In the DFG-in conformation, Phe1047 is deep within the allosteric site, whereas in the DFG-out conformation, Phe1047 amino acid flips out of the hydrophobic pocket, creating an empty hydrophobic pocket to accommodate the aromatic ring of inhibitors. The DFG-motif region, rich in H-bonds, contains residues Glu883, Asp1046, and Phe1047, which occupied with H-bond donors and acceptors. The hydrophobic pocket I is comprised of Leu840, Phe918, and Gly992 residues, while hydrophobic pocket II consists of Leu889, Ile892, Val898, and Ile1044 residues. In synthesized compounds and vandetanib as the standard drug, the N1 or N3 of the quinazoline skeleton forms one to three critical H-bonds with Cys919, Cys917, or Glu917 in the hinge region. These bonds are critical for fitting the quinazoline skeleton properly in the VEGFR-2 ATP-binding site and ensuring the proper inhibitory activity of the inhibitors. Additionally, the quinazoline core of the compounds interacts with hydrophobic pocket I. In the DFG domain, the amide, urea, sulfonamide, carboxamide, thiourea, and acylamino groups interact with Asp1046 and Glu885 through two or three H-bonds. The substituted or unsubstituted aniline rings of the compounds occupy hydrophobic pocket II (Figure 22).[27,53,57-65,69-71]

Structure-activity relationship of EGFR/VEGFR-2 dual inhibitors

Based on the structure of different synthesized compounds, the SAR of quinazoline-based EGFR/VEGFR-2 inhibitors can be concluded as follows: The nitrogen atoms, especially N1 of the quinazoline ring, play a key role in interacting with receptor tyrosine kinases using a H-bond. The aniline ring bearing small lipophilic and electron-withdrawing groups such as fluoro, chloro, bromo, or trifluoromethyl at 2-, 3-, and 4-positions of the phenyl group, binds to the lipophilic pocket and is favorable for both kinases. Large lipophilic and electron-withdrawing groups, such as chlorine or bromine, are better tolerated at 3- and 4-positions of the aniline ring, whereas smaller substitutes, such as fluorine and hydrogen, are preferred at the 2-position. The replacement of the linker between the quinazoline and phenyl rings dramatically affects the inhibitory activity. A nitrogen linker is favorable for both kinases, while oxygen and sulfur atoms are more favorable for VEGFR-2. Substituting urea and thiourea groups on the aniline ring leads to increased potency on both kinases. The substitution of alkyl or arylurea derivatives at the para position of the aniline ring has shown promising results against VEGFR-2 (selective VEGFR inhibitors), likely because of the H-bonds formation between urea O and NH moieties with the active site of VEGFR-2. The

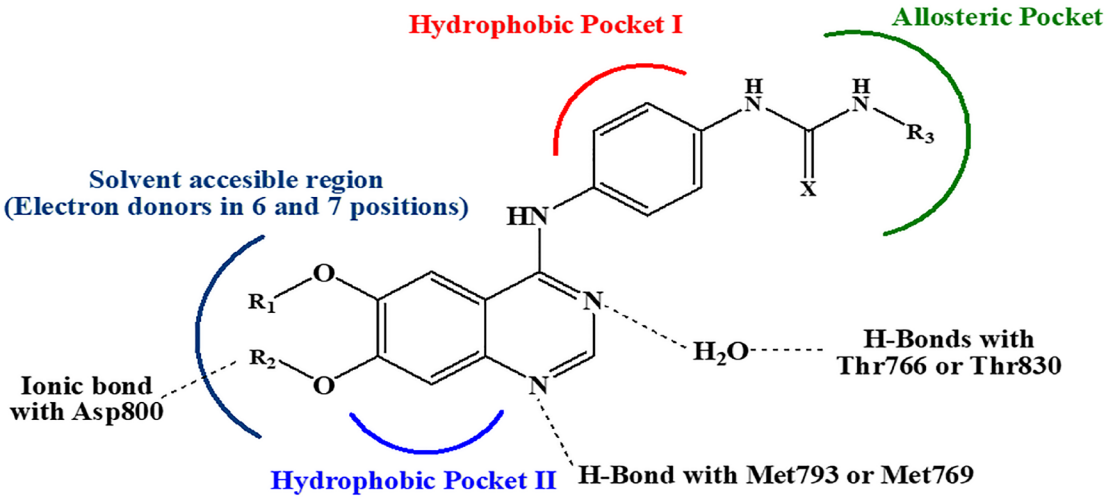


Figure 21. ATP Binding Site of EGFR.

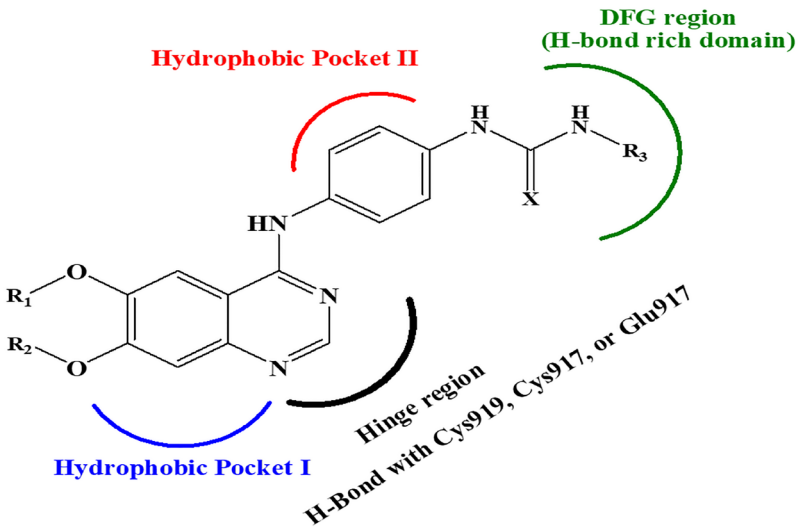


Figure 22. ATP Binding Site of VEGFR-2.

carbamic acid methyl ester substituent leads to dual EGFR/VEGFR inhibitors, especially when bearing chlorine at its ortho position, while the ethyl ester only displays activity on VEGFR-2. The two chlorine atoms on the aniline ring result in a significant reduction in inhibitory activity on both EGFR and VEGFR-2 because of the steric hindrance. The presence of hydrogen bond acceptors containing lipophilic groups such as alkyl groups at the 6- and 7-positions of the quinazoline ring, is beneficial for both kinases. Modifications at the quinazoline position 6 are more restricted, with methoxy being the preferable choice. Substituting the methoxy group with alkylamino at 7-position of the quinazoline core is better tolerated than at the 6-position. Various types of substituents, including neutral, basic, and heteroaromatic side chains at the 7-position of the quinazoline core, have resulted in potent derivatives. A two-carbon chain length (ethoxy) is the optimum distance between the substituents at the 7-position of the quinazoline core and quinazoline scaffold. The quinazoline skeleton containing basic side chains at the 7-position demonstrates potent inhibition of EGFR and VEGFR tyrosine kinases. Any substituents at the 2- or 3-positions of the quinazoline result in a significant drop in inhibitory activity against both kinases. In addition, the incorporating a Michael acceptor at the 6-position of quinazoline leads to the formation of irreversible inhibitors.[4,27,52,53,56,58-62,65,72-76]

Conclusion

Recently, blocking specific targets for cancer treatment has emerged as one of the most promising fields of research in chemotherapy due to the urgent need for drugs that offer the highest efficacy with minimal side effects. The development of multi-target design methods for creating novel potential anticancer drugs has resulted in the discovery of numerous multi-functional compounds, especially multi-kinase inhibitors. The EGFR tyrosine kinase plays a key role in gene expression, proliferation, and inhibition of apoptosis. The VEGFR-2 tyrosine kinase acts as a critical mediator in angiogenesis. Together, these proteins play a vital role in cancer progression and metastasis. Because of the synergistic effects of these kinases, dual inhibition of EGFR/VEGFR-2 tyrosine kinases could be a valuable strategy for cancer treatment. The quinazoline skeleton is one of the first synthesized scaffolds used in developing kinase inhibitors. This scaffold commonly acts by blocking the ATP binding site of kinases and there are several quinazoline-based FDA-approved drugs for cancer therapy. Vandetanib, an EGFR/VEGFR-2 inhibitor with a 4-anilinoquinazoline scaffold, is approved by the FDA for locally advanced or metastatic medullary thyroid cancer therapy. Modifications of the quinazoline core, especially at the 4-, 6-, and 7-positions, have led to developing potent inhibitors with promising anticancer activity. The aniline ring with small lipophilic/electron-withdrawing groups as well as urea, thiourea, and carbamic acid methyl ester is favorable for both kinases. The methoxy group at the 6-position and alkylamino at the 7-position, especially aminoethoxy, of the quinazoline scaffold lead to potent dual inhibitors. For both kinases, the quinazoline core and aniline ring are located in two distinct hydrophobic pockets. Also, N1 of the quinazoline core interacts with Met 793 or Met 796 of EGFR and Cys919 of VEGFR-2, which are critical for the best fitting of quinazoline with the active site of kinases. These inhibitors could serve as lead compounds for further investigation in in vivo and clinical trials.

Acknowledgements

This work was supported by Guilan University of Medical Sciences.

Author Contributions

Fatemeh Yousefbeyk: Conceptualization, Investigation, Writing–Original Draft. Saeed Ghasemi: Conceptualization, Investigation, Supervision, Writing - Review & Editing.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Al-Muntaser SM, Al-Karmalawy AA, El-Naggar AM, Ali AK, Abd El-Sattar NE, Abbass EM. Novel 4-thiophenyl-pyrazole, pyridine, and pyrimidine derivatives as potential antitumor candidates targeting both egfr and vegfr-2; design, synthesis, biological evaluations, and in silico studies. RSC Adv. 2023;13(18):12184-203. doi:10.1039/D3RA00416C
2. Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, et al. The global burden of cancer 2013. JAMA Oncol. 2015;1(4):505-27. doi:10.1001/jamaoncol.2015.0735
3. Moghadam FA, Kefayati H, Evazalipour M, Ghasemi S. Design, synthesis, biological evaluation, and docking study of novel 4-anilinoquinazolines derivatives as anticancer agents. Iran J Chem Chem Eng. 2022;41(2):353-67. doi:10.30492/ijcce.2020.132389.4271
4. Moghadam FA, Dabirian S, Dogaheh MG, Mojabi M, Yousefbeyk F, Ghasemi S. Novel 4-anilinoquinazoline derivatives as potent anticancer agents: Design, synthesis, cytotoxic activity, and docking study. Aust J Chem. 2021;74(10):730-9. doi:10.1071/CH21147
5. Gaber AA, El-Morsy AM, Sherbiny FF, Bayoumi AH, El-Gamal KM, El-Adl K, et al. Pharmacophore-linked pyrazolo [3, 4-d] pyrimidines as EGFR-TK inhibitors: Synthesis, anticancer evaluation, pharmacokinetics, and in silico mechanistic studies. Arch Pharm. 2021. doi:10.1002/ardp.202100258
6. Khattab M, Al-Karmalawy AA. Computational repurposing of benzimidazole anthelmintic drugs as potential colchicine binding site inhibitors. Future Med Chem. 2021;13(19):1623-38. doi:10.4155/fmc-2020-0273
7. Agarwal E, Brattain MG, Chowdhury S. Cell survival and metastasis regulation by akt signaling in colorectal cancer. Cell Signal. 2013;25(8):1711-9. doi:10.1016/j.cellsig.2013.03.025
8. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010;141(7):1117-34. doi:10.1016/j.cell.2010.06.011
9. Liao JJ-L. Molecular recognition of protein kinase binding pockets for design of potent and selective kinase inhibitors. J Med Chem. 2007;50(3):409-24. doi:10.1021/jm0608107
10. Bhanumathy K, Balagopal A, Vizeacoumar FS, Vizeacoumar FJ, Freywald A, Giambra V. Protein tyrosine kinases: Their roles and their targeting in leukemia. Cancers. 2021;13(2):184. doi:10.3390/cancers13020184
11. Rygiel KA, Elkins JM. Recent advances in the structural biology of tyrosine kinases. Curr Opin Struct Biol. 2023;82:102665. doi:10.1016/j.sbi.2023.102665
12. Lahiry P, Torkamani A, Schork NJ, Hegele RA. Kinase mutations in human disease: Interpreting genotype–phenotype relationships. Nat Rev Genet. 2010;11(1):60-74. doi:10.1038/nrg2707
13. Xie Z, Yang X, Duan Y, Han J, Liao C. Small-molecule kinase inhibitors for the treatment of nononcologic diseases. J Med Chem. 2021;64(3):1283-345. doi:10.1021/acs.jmedchem.0c01511
14. Sheng Q, Liu J. The therapeutic potential of targeting the egfr family in epithelial ovarian cancer. Br J Cancer. 2011;104(8):1241-5. doi:10.1038/bjc.2011.62
15. Janmaat ML, Giaccone G. Small-molecule epidermal growth factor receptor tyrosine kinase inhibitors. Oncologist. 2003;8(6):576-86. doi:10.1634/theoncologist.8-6-576
16. Herbst RS. Review of epidermal growth factor receptor biology. Int J Radiat Oncol Biol Phys. 2004;59(2):S21-S6. doi:10.1016/j.ijrobp.2003.11.041
17. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, et al. Epidermal growth factor receptor (egfr) signaling in cancer. Gene. 2006;366(1):2-16. doi:10.1016/j.gene.2005.10.018
18. Bogdan S, Klämbt C. Epidermal growth factor receptor signaling. Curr Biol. 2001;11(8):R292-R5. doi:10.1016/s0960-9822(01)00167-1
19. Franklin WA, Veve R, Hirsch FR, Helfrich BA, Bunn PA Jr. Epidermal growth factor receptor family in lung cancer and premalignancy. Semin Oncol. 2002;29(1 Suppl 4):3-14. doi:10.1053/sonc.2002.31520.
20. Alroy I, Yarden Y. The erbb signaling network in embryogenesis and oncogenesis: Signal diversification through combinatorial ligand-receptor interactions. FEBS Lett. 1997;410(1):83-6. doi:10.1016/s0014-5793(97)00412-2
21. Burgering BMT, Coffer PJ. Protein kinase b (c-akt) in phosphatidylinositol-3-oh kinase signal transduction. Nature. 1995;376(6541):599-602. doi:10.1038/376599a0
22. Liu W, Li J, Roth RA. Heregulin regulation of akt/protein kinase b in breast cancer cells. Biochem Biophys Res Commun. 1999;261(3):897-903. doi:10.1006/bbrc.1999.1144
23. Muthuswamy SK, Gilman M, Brugge JS. Controlled dimerization of erbb receptors provides evidence for differential signaling by homo-and heterodimers. Mol Cell Biol. 1999;19(10):6845-57. doi:10.1128/MCB.19.10.6845
24. Kharbanda A, Walter DM, Gudiel AA, Schek N, Feldser DM, Witze ES. Blocking EGFR palmitoylation suppresses PI3K signaling and mutant KRAS lung tumorigenesis. Sci Signal. 2020;13(621):eaax2364. doi:10.1126/scisignal.aax2364.
25. Chan TO, Rittenhouse SE, Tsichlis PN. Akt/pkb and other d3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation. Annu Rev Biochem. 1999;68(1):965-1014. doi:10.1146/annurev.biochem.68.1.965
26. Herbst RS, Langer CJ. Epidermal growth factor

receptors as a target for cancer treatment: the emerging role of IMC-C225 in the treatment of lung and head and neck cancers. Semin Oncol. 2002;29(1 Suppl 4):27-36. doi:10.1053/sonc.2002.31525.
27. de Castro Barbosa ML, Lima LM, Tesch R, Sant'Anna CMR, Totzke F, Kubbutat MH, et al. Novel 2-chloro-4-anilino-quinazoline derivatives as egfr and vegfr-2 dual inhibitors. Eur J Med Chem. 2014;71:1-14. doi:10.1016/j.ejmech.2013.10.058
28. El-Naggar AM, Hassan A, Elkaeed EB, Alesawy MS, Al-Karmalawy AA. Design, synthesis, and sar studies of novel 4-methoxyphenyl pyrazole and pyrimidine derivatives as potential dual tyrosine kinase inhibitors targeting both egfr and vegfr-2. Bioorganic Chem. 2022;123:105770. doi:10.1016/j.bioorg.2022.105770
29. Chilin A, Conconi MT, Marzaro G, Guiotto A, Urbani L, Tonus F, et al. Exploring epidermal growth factor receptor (egfr) inhibitor features: The role of fused dioxygenated rings on the quinazoline scaffold. J Med Chem. 2010;53(4):1862-6. doi:10.1021/jm901338g
30. Yamaoka T, Ohba M, Ohmori T. Molecular-targeted therapies for epidermal growth factor receptor and its resistance mechanisms. Int J Mol Sci. 2017;18(11):2420. doi:10.3390/ijms18112420
31. Yu H, Li Y, Ge Y, Song Z, Wang C, Huang S, et al. Novel 4-anilinoquinazoline derivatives featuring an 1-adamantyl moiety as potent egfr inhibitors with enhanced activity against nsclc cell lines. Eur J Med Chem. 2016;110:195-203. doi:10.1016/j.ejmech.2016.01.045
32. Pawar VG, Sos ML, Rode HB, Rabiller M, Heynck S, Van Otterlo WA, et al. Synthesis and biological evaluation of 4-anilinoquinolines as potent inhibitors of epidermal growth factor receptor. J Med Chem. 2010;53(7):2892-901. doi:10.1021/jm901877j
33. Yamaoka T, Kusumoto S, Ando K, Ohba M, Ohmori T. Receptor tyrosine kinase-targeted cancer therapy. Int J Mol Sci. 2018;19(11):3491. doi:10.3390/ijms19113491
34. Al-Warhi T, Al-Karmalawy AA, Elmaaty AA, Alshubramy MA, Abdel-Motaal M, Majrashi TA, et al. Biological evaluation, docking studies, and in silico adme prediction of some pyrimidine and pyridine derivatives as potential egfrwt and egfrt790m inhibitors. J Enzyme Inhib Med Chem. 2023;38(1):176-91. doi:10.1080/14756366.2022.2135512
35. Wang X, Bove AM, Simone G, Ma B. Molecular bases of vegfr-2-mediated physiological function and pathological role. Front Cell Dev Biol. 2020;8:599281. doi:10.3389/fcell.2020.599281
36. Drake CJ, LaRue A, Ferrara N, Little CD. Vegf regulates cell behavior during vasculogenesis. Dev Biol. 2000;224(2):178-88. doi:10.1006/dbio.2000.9744
37. Abdulkadir S, Li C, Jiang W, Zhao X, Sang P, Wei L, et al. Modulating angiogenesis by proteomimetics of vascular endothelial growth factor. J Am Chem Soc. 2022;144(1):270-81. doi:10.1021/jacs.1c09571
38. Basile A, Del Gatto A, Diana D, Di Stasi R, Falco A, Festa M, et al. Characterization of a designed vascular endothelial growth factor receptor antagonist helical peptide with antiangiogenic activity in vivo. J Med Chem. 2011;54(5):1391-400. doi:10.1021/jm101435r
39. Srinivasan S, Meyer RD, Lugo R, Rahimi N. Identification of pdcl3 as a novel chaperone protein involved in the generation of functional vegf receptor 2. J Biol Chem. 2013;288(32):23171-81. doi:10.1074/jbc.M113.473173
40. Chiu J, Wong JWH, Gerometta M, Hogg PJ. Mechanism of dimerization of a recombinant mature vascular endothelial growth factor c. Biochem. 2014;53(1):7-9. doi:10.1021/bi401518b
41. da Rocha-Azevedo B, Lee S, Dasgupta A, Vega AR, de Oliveira LR, Kim T, et al. Heterogeneity in vegf receptor-2 mobility and organization on the endothelial cell surface leads to diverse models of activation by vegf. Cell Rep. 2020;32(13):108187. doi:10.1016/j.celrep.2020.108187
42. Shi L, Wu T-T, Wang Z, Xue J-Y, Xu Y-G. Discovery of quinazolin-4-amines bearing benzimidazole fragments as dual inhibitors of c-met and vegfr-2. Bioorg Med Chem. 2014;22(17):4735-44. doi:10.1016/j.bmc.2014.07.008
43. Hamdi A, El-Shafey HW, Othman DIA, El-Azab AS, AlSaif NA, Abdel-Aziz AAM. Design, synthesis, antitumor, and vegfr-2 inhibition activities of novel 4-anilino-2-vinyl-quinazolines: Molecular modeling studies. Bioorganic Chem. 2022;122:105710. doi:10.1016/j.bioorg.2022.105710
44. Fontanella C, Ongaro E, Bolzonello S, Guardascione M, Fasola G, Aprile G. Clinical advances in the development of novel vegfr2 inhibitors. Ann Transl Med. 2014;2(12):123. doi:10.3978/j.issn.2305-5839.2014.08.14
45. Marchand GS, Noiseux N, Tanguay J-F, Sirois MG. Blockade of in vivo vegf-mediated angiogenesis by antisense gene therapy: Role of flk-1 and flt-1 receptors. Am J Physiol Heart Circ Physiol. 2002;282(1):H194-H204. doi:10.1152/ajpheart.2002.282.1.H194
46. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase iii clinical trials on anti-vegf therapy for cancer. Nat Clin Pract Oncol. 2006;3(1):24-40. doi:10.1038/ncponc0403
47. Stopeck A, Sheldon M, Vahedian M, Cropp G, Gosalia R, Hannah A. Results of a Phase I dose-escalating study of the antiangiogenic agent, SU5416, in patients with advanced malignancies. Clin Cancer Res. 2002;8(9):2798-80
48. Abdallah AE, Mabrouk RR, Elnagar MR, Farrag AM, Kalaba MH, Sharaf MH, et al. New series of vegfr-2 inhibitors and apoptosis enhancers: Design, synthesis and biological evaluation. Drug Des Devel Ther. 2022;16:587-606. doi:10.2147/DDDT.S344750
49. Bhullar KS, Lagarón NO, McGowan EM, Parmar I, Jha A, Hubbard BP, et al. Kinase-targeted cancer

therapies: Progress, challenges and future directions. Mol Cancer. 2018;17:1-20. doi:10.1186/s12943-018-0804-2
50. Li J, Gong C, Zhou H, Liu J, Xia X, Ha W, et al. Kinase inhibitors and kinase-targeted cancer therapies: Recent advances and future perspectives. Int J Mol Sci. 2024;25(10):5489. doi:10.3390/ijms25105489
51. El-Adl K, Sakr H, El-Hddad SSA, El-Helby A-GA, Nasser M, Abulkhair HS. Design, synthesis, docking, admet profile, and anticancer evaluations of novel thiazolidine-2,4-dione derivatives as vegfr-2 inhibitors. Arch Pharm (Weinheim). 2021;354(7):2000491. doi:10.1002/ardp.202000491
52. Garofalo A, Goossens L, Lemoine A, Farce A, Arlot Y, Depreux P. Quinazoline-urea, new protein kinase inhibitors in treatment of prostate cancer. J Enzyme Inhib Med Chem. 2010;25(2):158-71. doi:10.3109/14756360903169485
53. Wissner A, Fraser HL, Ingalls CL, Dushin RG, Floyd MB, Cheung K, et al. Dual irreversible kinase inhibitors: Quinazoline-based inhibitors incorporating two independent reactive centers with each targeting different cysteine residues in the kinase domains of egfr and vegfr-2. Bioorg Med Chem. 2007;15(11):3635-48. doi:10.1016/j.bmc.2007.03.055
54. Tsang VH, Robinson BG, Learoyd DL. The safety of vandetanib for the treatment of thyroid cancer. Expert Opin Drug Saf. 2016;15(8):1107-13. doi:10.1080/14740338.2016.1201060
55. Riadi Y, Alamri MA, Geesi MH, Anouar EH, Ouerghi O, Alabbas AB, et al. Synthesis, characterization, biological evaluation and molecular docking of a new quinazolinone-based derivative as a potent dual inhibitor for vegfr-2 and egfr tyrosine kinases. J Biomol Struct Dyn. 2022;40(15):6810-6. doi:10.1080/07391102.2021.1890221
56. Bang KC, Song TH, Park YJ, Lee JS, Jun S, Jung SH, et al. Synthesis of 4-anilinoquinazoline-derivative dual kinase inhibitors targeting egfr and vegfr-2. Bull Korean Chem Soc. 2018;39(1):123-5. doi:10.1002/bkcs.11348
57. Garofalo A, Goossens L, Lemoine A, Ravez S, Six P, Howsam M, et al. [4-(6, 7-disubstituted quinazolin-4-ylamino) phenyl] carbamic acid esters: A novel series of dual egfr/vegfr-2 tyrosine kinase inhibitors. MedChemComm. 2011;2(1):65-72. doi:10.1039/C0MD00183J
58. Zhang Q, Diao Y, Wang F, Fu Y, Tang F, You Q, et al. Design and discovery of 4-anilinoquinazoline ureas as multikinase inhibitors targeting braf, vegfr-2 and egfr. Medchemcomm. 2013;4(6):979-86. doi:10.1039/C3MD00096F
59. Zhang H-Q, Gong F-H, Ye J-Q, Zhang C, Yue X-H, Li C-G, et al. Design and discovery of 4-anilinoquinazoline-urea derivatives as dual tk inhibitors of egfr and vegfr-2. Eur J Med Chem. 2017;125:245-54. doi:10.1016/j.ejmech.2016.09.039
60. Zhang H-Q, Gong F-H, Li C-G, Zhang C, Wang Y-J, Xu Y-G, et al. Design and discovery of 4-anilinoquinazoline-acylamino derivatives as egfr and vegfr-2 dual tk inhibitors. Eur J Med Chem. 2016;109:371-9. doi:10.1016/j.ejmech.2015.12.032
61. Sun S, Zhang J, Wang N, Kong X, Fu F, Wang H, et al. Design and discovery of quinazoline-and thiourea-containing sorafenib analogs as egfr and vegfr-2 dual tk inhibitors. Molecules. 2017;23(1):24. doi:10.3390/molecules23010024
62. Wei H, Duan Y, Gou W, Cui J, Ning H, Li D, et al. Design, synthesis and biological evaluation of novel 4-anilinoquinazoline derivatives as hypoxia-selective egfr and vegfr-2 dual inhibitors. Eur J Med Chem. 2019;181:111552. doi:10.1016/j.ejmech.2019.07.055
63. Moghadam FA, Dabirian S, Tavani AE, Alipour P, Mojabi M, Evazalipour M, et al. Application of a deep eutectic solvent for the synthesis of novel imidazole-containing quinazoline derivatives as potent cytotoxic agents. Pharm Sci. 2024;30(2):215-28. doi:10.34172/PS.2023.29
64. Moghadam FA, Evazalipour M, Kefayati H, Ghasemi S. 6, 7-disubstituted-4-anilinoquinazoline: Design, synthesis and anticancer activity as a novel series of potent anticancer agents. Pharm Sci. 2020;27(2):209-18. doi:10.34172/PS.2020.72
65. Ghorab MM, Soliman AM, El-Adl K, Hanafy NS. New quinazoline sulfonamide derivatives as potential anticancer agents: Identifying a promising hit with dual egfr/vegfr-2 inhibitory and radiosensitizing activity. Bioorganic Chem. 2023;140:106791. doi:10.1016/j.bioorg.2023.106791
66. Amelia T, Kartasasmita RE, Ohwada T, Tjahjono DH. Structural insight and development of EGFR tyrosine kinase inhibitors. Molecules. 2022;27(3):819. doi:10.3390/molecules27030819
67. Vijayan R, He P, Modi V, Duong-Ly KC, Ma H, Peterson JR, et al. Conformational analysis of the dfg-out kinase motif and biochemical profiling of structurally validated type ii inhibitors. J Med Chem. 2015;58(1):466-79. doi:10.1021/jm501603h
68. Șandor A, Ionuț I, Marc G, Oniga I, Eniu D, Oniga O. Structure–activity relationship studies based on quinazoline derivatives as egfr kinase inhibitors (2017–present). Pharmaceuticals. 2023;16(4):534. doi:10.3390/ph16040534
69. Modi SJ, Kulkarni VM. Exploration of structural requirements for the inhibition of vegfr-2 tyrosine kinase: Binding site analysis of type ii,'dfg-out'inhibitors. J Biomol Struct Dyn. 2022;40(12):5712-27. doi:10.1080/07391102.2021.1872417
70. Moradi M, Mousavi A, Emamgholipour Z, Giovannini J, Moghimi S, Peytam F, et al. Quinazoline-based vegfr-2 inhibitors as potential anti-angiogenic agents: A contemporary perspective of sar and molecular docking studies. Eur J Med Chem. 2023:259;115626. doi:10.1016/j.ejmech.2023.115626

71. Klein T, Vajpai N, Phillips JJ, Davies G, Holdgate GA, Phillips C, et al. Structural and dynamic insights into the energetics of activation loop rearrangement in fgfr1 kinase. Nat Commun. 2015;6(1):7877. doi:10.1038/ncomms8877
72. Jin H, Dan H-G, Rao G-W. Research progress in quinazoline derivatives as multi-target tyrosine kinase inhibitors. Heterocycl Comm. 2018;24(1):1-10. doi:10.1021/acs.orglett.6b00691
73. Hennequin LF, Stokes ES, Thomas AP, Johnstone C, Plé PA, Ogilvie DJ, et al. Novel 4-anilinoquinazolines with c-7 basic side chains: Design and structure activity relationship of a series of potent, orally active, vegf receptor tyrosine kinase inhibitors. J Med Chem. 2002;45(6):1300-12. doi:10.1021/jm011022e
74. Xu P, Chu J, Li Y, Wang Y, He Y, Qi C, et al. Novel promising 4-anilinoquinazoline-based derivatives as multi-target rtks inhibitors: Design, molecular docking, synthesis, and antitumor activities in vitro and vivo. Bioorg Med Chem. 2019;27(20):114938. doi:10.1016/j.bmc.2019.06.001
75. Ismail RS, Abou-Seri SM, Eldehna WM, Ismail NS, Elgazwi SM, Ghabbour HA, et al. Novel series of 6-(2-substitutedacetamido)-4-anilinoquinazolines as egfr-erk signal transduction inhibitors in mcf-7 breast cancer cells. Eur J Med Chem. 2018;155:782-96. doi:10.1016/j.ejmech.2018.06.024
76. Hennequin LF, Thomas AP, Johnstone C, Stokes ES, Plé PA, Lohmann J-JM, et al. Design and structure–activity relationship of a new class of potent vegf receptor tyrosine kinase inhibitors. J Med Chem. 1999;42(26):5369-89. doi:10.1021/jm990345w