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Quinazoline Based Dual Inhibitors of EGFR and VEGFR as Potential Anti-proliferative Agents-A Review

Airin Benny, Aravind A* and Krishna Priya A
Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Govt. Medical College Thiruvananthapuram- 695011.

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***Corresponding Author Email:** aravindaphd@gmail.com

Abstract
It has been established that the overexpression of tyrosine kinase inhibitors, EGFR and VEGFR is the primary factor contributing to the development and spread of carcinomas, including pancreatic, lung, and breast cancers. Quinazoline nucleus is a distinct and flexible scaffold with a broad range of pharmacological action, particularly in the area of tyrosine kinase inhibitors, where the US Food and Drug Administration has approved more than twenty small molecule inhibitors in the past 20 years. Quinazoline derivatives are effective inhibitors of EGFR and VEGFR, which are key targets in cancer therapy. Their ability to inhibit the action of these receptors aids in the control of tumour growth and metastasis, making them essential tools in the treatment of many cancers. Some novel quinazoline and quinazolinone derivatives which act as EGFR and VEGFR dual inhibitors are discussed in this review.

Keywords
EGFR, VEGFR, cancer, quinazoline, tyrosine kinase.

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INTRODUCTION:
Cancer is regarded as the most dangerous disease afflicting humanity today. It is a collection of diseases characterized by aberrant cell proliferation that may infiltrate or disperse to different regions of the body. About 4% of all investigations are focused on cancer research, which is a reflection of the enormous amount of resources that has been spent on this multifaceted disease globally. Over the past decade, the output of cancer research has increased annually and numerous notable advancements in the diagnosis, comprehension, and treatment of this condition have been established [1-3]. Cancer is a very complex condition and treating it comes with numerous challenges. The majority of cancer patients are treated by various chemotherapeutic drugs, either on their own or in combination with other drugs. Understanding the biology and metabolism of proliferating cells has led to the invention of more than 100 FDA-approved anticancer medicines over the past 50 years and many more are in progress. Alkylators, antifolates and other antimitotic drugs were previously used to treat lymphomas and leukemia. However, tumor recurrence prompted the development of targeted therapy, which entailed focusing on particular molecular changes linked to different pathways in the progression of cancer [4]. Chemotherapy has several limitations, including poor selectivity, high organ toxicity, reduced specificity, and innate or acquired resistance multidrug resistance [5]. Even if there have been significant breakthroughs in the treatment of cancer, the search for novel anticancer agents is still generally crucial. The development of

safer anticancer medications has long been a priority because growing numbers of patients are being treated for disseminated tumours, which requires new medications. The quest for novel anticancer moieties has been greatly aided by small molecule targeted therapy as the underlying causes of cancers and their mechanisms have come to light [6].

Receptor tyrosine kinases (RTKs) are a family of membrane-bound receptors that play a crucial to the efficient functioning of cells. They phosphorylate tyrosine residues on key intracellular substrate proteins, acting as signal transducers that facilitate cell-to-cell communication. Basically, they are at the centre of intricately linked signalling pathways and actively participate in the control of several processes such as cell migration, proliferation, differentiation, and metabolism in order to maintain cellular homeostasis [7]. It has been frequently noticed and recognised that altered activation of RTKs serves an integral part in the development of a numerous malignancies [8]. Several tyrosine kinase inhibitors (TKIs) have been developed as cancer therapies and have demonstrated remarkable anti-tumour properties. Two of these tyrosine kinases that are widely targeted are the vascular endothelial growth factor receptor (VEGFR) and the epidermal growth factor receptor (EGFR) [9].

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

Epidermal Growth Factor Receptor or HER-1 or ErbB-1 belong to the ERBB family of tyrosine kinases (RTKs). The other receptors in this family include ErbB-2(HER-2), ErbB-3(HER-3) and ErbB-4(HER-4). These receptors have an extracellular ligand-binding domain, a hydrophobic transmembrane domain and a cytoplasmic tyrosine kinase binding domain. This receptor is activated by the binding of specific ligands like Epidermal Growth Factor (EGF) and Transforming Growth Factor (TGF-α) which is produced by the same cells which express ErbB-1 receptor or by the surrounding cells. These ligands bind to the receptor via the extracellular domain and the result is receptor dimerization, tyrosine kinase activation and transphosphorylation. These activated receptors interact with different signalling molecules that transmit signals in the cell. Activation of the signalling cascade triggers DNA synthesis in cells that express the EGFR.

The Epidermal Growth Factor Receptor plays an important role in initiating the signalling cascade which controls the cell proliferation, survival and metastasis by regulating diverse cellular pathways. Over expression of EGFR is reported in many tumours supporting the hypothesis that dysregulation of EGFR gene expression and signalling could play a major biological role in cancer. Ligand binding leads to receptor activation and then the downstream activation of RAS-RAF-MEK-ERK pathway and PI3K/AKT pathways therefore exert an effect on cell proliferation, survival and the metastatic potential of tumour cell. Mutations lead to EGFR over expression and these somatic mutations involving EGFR results in its constant activation and thus causes uncontrolled proliferation [10].

Currently two basic approaches are undertaken for EGFR targeted cancer therapy;

1) Use of monoclonal antibodies: acts on extracellular domain and create a ligand competitive inhibition, thus preventing receptor dimerization, auto-phosphorylation and downstream signalling.
   E.g. Cetuximab, Panitumumab
2) Small molecule tyrosine kinase inhibitors: acts on intracellular domain by binding to catalytic ATP binding sites analogous to adenosine triphosphates (ATP) and thereby prevents downstream signalling.
   E.g. Gefitinib, Erlotinib, Lapatinib etc

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (VEGFR)

Vascular Endothelial Growth Factor receptors are signalling proteins that are present only in vascular endothelial cells which help in angiogenesis or production of new blood vessels and vascular permeability that are essential for cancer growth. They are categorized into three; VEGFR-1, VEGFR-2, and VEGFR-3. Different ligands that can bind these receptors are VEGFA/B/C/D/E and placental growth factors (PIGF). On ligand binding, VEGF-A, VEGFR-2 dimerizes, resulting in kinase activation and autophosphorylation of tyrosine residues that leads to the activation of signal transduction molecules phospholipase C-γ (PLC-γ), PI3K, Akt, Ras, Src, and MAPK. Phosphorylation of tyrosine residue (Tyr1175) results in the binding and phosphorylation of PLC-γ, which subsequently stimulates the release of Ca^{2+} from internal stores and activation of protein kinase C (PKC). Activation of PKC stimulates the *Raf/MEK/ERK* pathway, which promotes cell proliferation. Ca_2 mobilization and PKC activation are thought to be key signalling events in VEGF-A-induced vascular permeability via activation of endothelial nitric oxide synthase activity [11].

Several methods of inhibiting VEGFR are follows:

a) Monoclonal antibodies: Bevacizumab
b) Small molecule kinase inhibitors: Sorafenib, Sunitinib
c) Proteins that binds VEGF: VEGF Trap.

Angiogenesis, the production of new blood vessels from existing vasculature, is crucial for both normal physiological development and tumour growth and

metastasis. VEGFR-2 belongs to the VEGFR family. VEGF stimulation triggers downstream signal transduction, leading to angiogenesis, increased vascular permeability, and tumour growth. Inhibiting VEGFR-2 has been shown to effectively prevent angiogenesis [12].

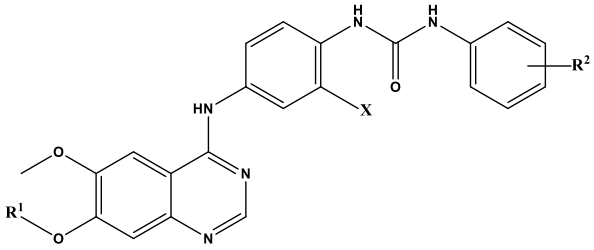
COMPOUND 1

Quinazoline derivatives, particularly 4-anilinoquinazolines, have been studied for their biological properties, including their ability to inhibit EGFR [13]. A group of 4-anilinoquinazoline compounds with glycine methyl ester moiety or a substituted diaryl urea were developed, based on previous work and they were found to be dual inhibitors of both VEGFR-2 and EGFR [14].

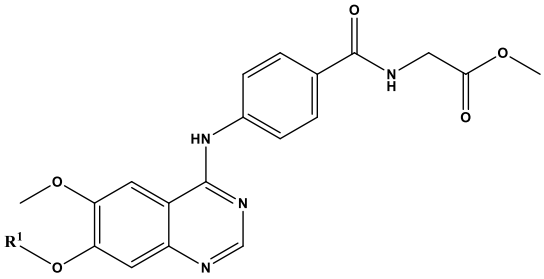
The enzymatic activity of all the synthesized quinazolin-4-amine derivativesand1a–r against EGFR and VEGFR-2 were assessed. As a positive control, the approved EGFR and VEGFR-2 inhibitor drug vandetanib was used. Table 1 provided a summary of the findings. Compounds1g and 1i had the strongest inhibitory action against EGFR, with an IC_{50} of 1 nM, whereas compounds 1j and 1l demonstrated the strongest activity against VEGFR-2, with an IC_{50} of 14 nM.

The MTT assay was used to assess the antiproliferative properties of the compounds against the human colorectal adenocarcinoma cell line HT-29, the human breast cancer cell line MCF-7, and the human lung cancer cell line H460. Table 1 illustrates that the majority of these compounds displayed moderate to good antiproliferative activity. Compounds 1i, 1j, 1l, 1n, and 1o outperformed the reference medication vandetanib in its actions against three cell lines.

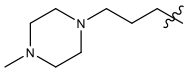
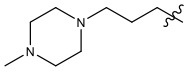
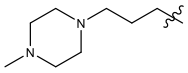
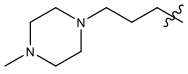
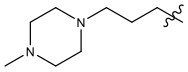
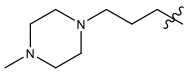
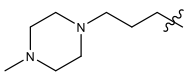
Table 1. Enzymatic and cellular results for 4-anilinoquinazoline derivatives.

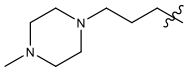
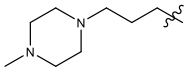
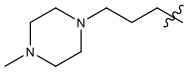
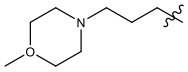
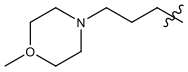
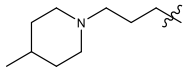
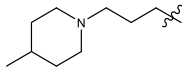
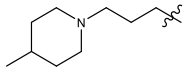
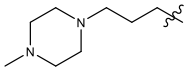
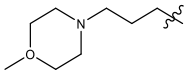
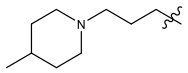


1a-o

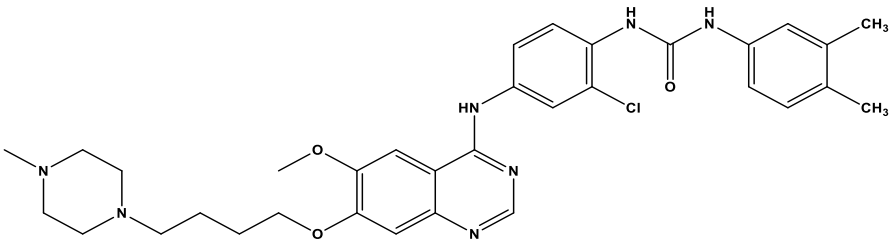


1p-r

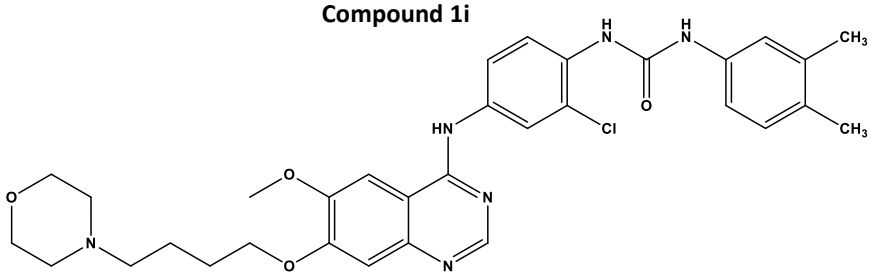
Compd No.	X	R^1	R^2	Enzymaticinhibition (IC_{50}, nM)		Proliferativeinhibition (IC_{50}, µM)		
				EGFR	VEGFR-2	HT-29	MCF-7	H460
1a	H		H	194	576	49.44	28.39	28.39
1b	H		*p*-Cl	235	815	28.68	>50	17.80
1c	H		*m*-Cl	500	588	22.86	24.72	8.16
1d	H		*o*-CH_3	502	1986	>50	32.17	22.29
1e	H		*m*-CH_3, *p* -CH_3	668	214	25.94	36.47	22
1f	Cl		H	14	261	17.33	>50	33
1g	Cl		*p*-Cl	1	279	20.65	>50	28

1h	Cl		*o*-CH_3	15	178	4.81	6.21	>50
1i	Cl		*m*-CH_3, *p* -CH_3	1	79	1.76	7.28	26
1j	Cl		*m*-Cl, *p*-F	78	14	6.41	3.20	12.10
1k	Cl		*p*-Cl	17	528	21.99	39.23	28
1l	Cl		*m*-CH_3, *p* -CH_3	51	14	7.29	2.63	3.52
1m	Cl		H	212	1873	>50	>50	>50
1n	Cl		*p*-Cl	>10000	>10000	4.38	1.89	5
1o	Cl		*m*-CH_3, *p* -CH_3	817	18	1.85	1.27	2.90
1p				611	275	>50	>50	>50
1q				763	404	>50	>50	>50
1r				9941	966	>50	8.39	>50
Vandetanib				11	15	18.95	11.83	17.10

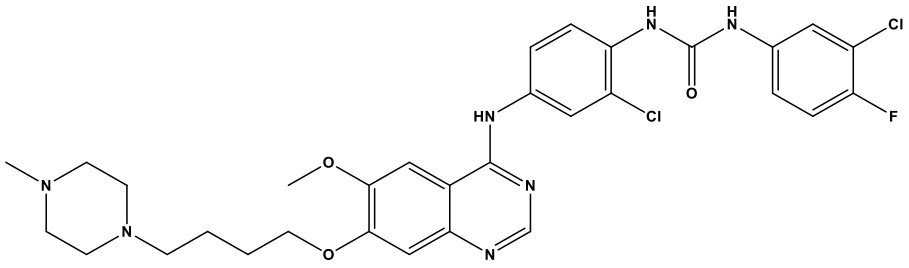
The most effective inhibitory compounds against EGFR (IC_{50} = 1 nM, 78 nM, and 51 nM, respectively) and VEGFR-2 (IC_{50} = 79 nM, 14 nM, and 14 nM, respectively) were compounds 1i, 1j, and 1l. They also had strong antiproliferative properties [15].



Compound 1i



Compound 1j



Compound 1l

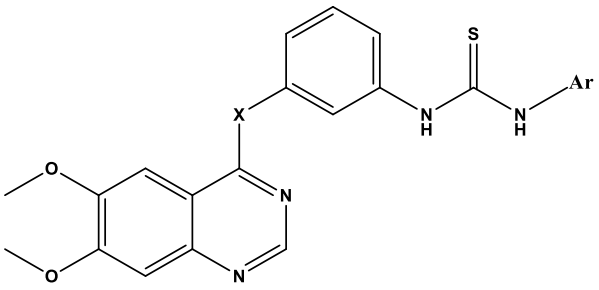
COMPOUND2

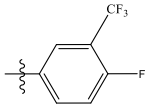
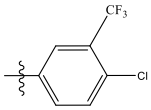
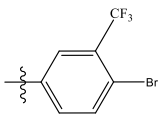
As dual TK inhibitors for EGFR and VEGFR-2, a series of novel quinazoline and thiourea-containing sorafenib analogs (2a–q) were developed.

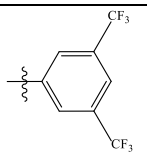
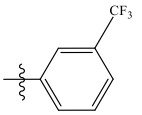
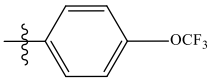
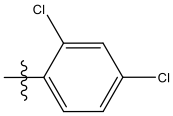
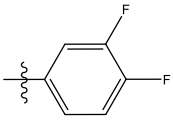
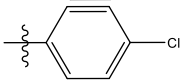
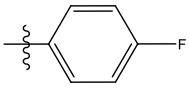
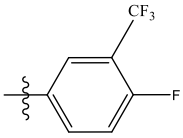
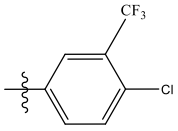
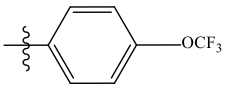
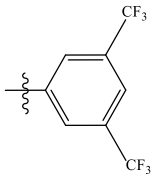
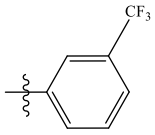
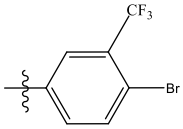
Using sorafenib as a positive control, the enzymatic activity of all the synthesized compounds (2a–q) against EGFR and VEGFR-2 was determined. The majority of the tested compounds had strong inhibitory effects against both EGFR and VEGFR-2, as seen in Table 2. Compounds 2m, 2q, and 2b had the highest potency among them, with IC_{50}s of 0.01 µM and 0.05 µM, respectively, against EGFR and VEGFR-2, which is similar to sorafenib's IC_{50}s of 0.02 µM and 0.08 µM against EGFR and VEGFR-2, respectively.

Initially, sorafenib was used as a positive control in the MTT assay to assess the *in vitro* cell cytotoxicities of all the novel compounds against the HCT116, MCF-7, and B16 cell lines. Table 2 also includes a summary of the findings. Strong antiproliferative effects were shown by the majority of the target compounds against each of the three cell lines. Among the compounds that were examined, compounds 2b, 2c, 2e, 2l, 2m, 2o, and 2q showed selective inhibitory actions against various cell lines and antiproliferative activities that were comparable to those of sorafenib. In addition to having stronger antiproliferative effects against HCT-116, MCF-7, and B16 cell lines than sorafenib, compounds 2b and 2q also exhibited the strongest EGFR/VEGFR-2 inhibitory activity.

Table 2. Enzymatic and cellular response of the target compounds



Compd No.	Substituent		IC_{50}(µM)		IC_{50}(µM)		
	X	Ar	EGFR	VEGFR-2	HCT-116	MCF-7	B16
2a	O		0.04	0.19	37.36	38.15	16.95
2b	O		0.02	0.05	9.13	17.72	6.11
2c	O		0.05	0.18	10.03	22.36	9.68

2d	O		N.D	N.D	15.02	18.88	8.49
2e	O		0.14	0,35	12.16	13.30	14.76
2f	O		>10	>10	58.61	18.79	53.95
2g	O		7.71	>10	19.83	17.09	24.47
2h	O		4.88	7.06	49.51	15.99	19.38
2i	O		4.17	>10	>100	22.14	27.35
2j	O		5.90	7.11	89.66	23.59	34.13
2k	O		>10	>10	>100	26.48	>100
2l	S		N.D	N.D	8.79	24.96	9.33
2m	S		0.01	0.07	8.11	20.91	7.89
2n	S		>10	>10	64.47	32.43	68.37
2o	S		0,02	0.09	9.57	19.02	12.25
2p	S		0.07	0.27	9.03	21.04	12.94
2q	S		0,01	0.08	8.35	15.06	5.57
Sorafenib			0.02	0.08	10.55	17.87	9.29

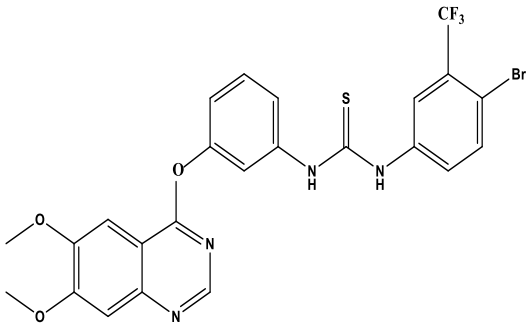
The B16 melanoma xenograft model was developed in C57BL/6J mice, and the compounds 2b, 2m, and 2q were selected to evaluate their anticancer activity *in vivo* with sorafenib serving as a positive control. Table 3 illustrates the efficacy of compounds 2b, 2m, 2q, and sorafenib to induce tumor regression. Oral

administration of these compounds at doses of 90 mg/kg, respectively, suppressed the growth of B16 tumors at 31.25%, 49.22%, 20.31%, and 64.06%. When it comes to inhibiting B16 melanoma, compounds 2b and 2q outperformed sorafenib. In every treatment group, there was no noticeable reduction in weight.

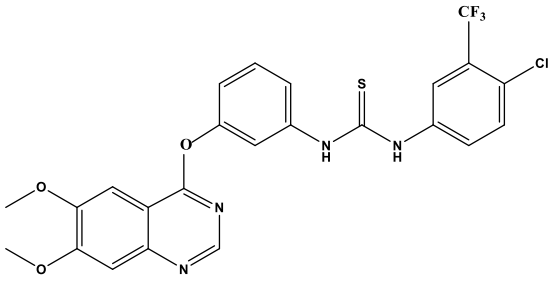
Table 3. The effect of 2b, 2m, 2q and sorafenib on the growth of B16 xenograft model.

Groups	Dosage (mg/kg)	Number initial/end	Body weight (g)		Tumor weight (g)	Inhibition rate (%)
			Initial	End		
Control	0	6/6	19.6 ± 2.3	21.8 ±2.3	1.28 ±0.56	
Sorafenib	90	6/6	20.7 ± 2.1	21.3 ±2.4	0.88 ±0.46	31.25%
2b	90	6/6	18.9 ±1.8	19.5 ± 3.0	0.65 ±0.22	49.22%
2m	90	6/6	19.2 ±2.1	20.9 ±1.6	1.02 ±0.36	20.31%
2q	90	6/6	19.1 ±2.7	20.2 ±2.7	0.46 ±0.14	64.06%

In the future, compounds 2b and 2q may be developed as potent anticancer drugs [16].



Compound 2b



Compound 2q

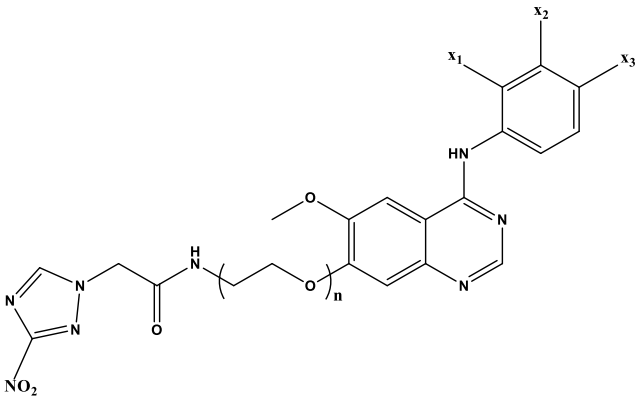
COMPOUND 3

A number of new 4-anilinoquinazoline derivatives by altering the aniline moiety of vandetanib and introducing a 3-nitro-1,2,4-triazole group to the side chain were designed and synthesized. Lead compounds 3a and 3g demonstrated strong inhibition of VEGFR-2 kinase and EGFR.

By using vandetanib and LYL-10 as positive controls in a kinase inhibitory assay, the targeted compounds' potential to inhibit EGFR and VEGFR2 were assessed. As shown in Table 4, the results of the tests indicate that (LYL-10, 3a, 3c, 3d, 3e, 3g,) had more effective EGFR inhibitory actions than vandetanib (IC_{50} ¼ 19.76 nM), with IC_{50} values ranging from 0.37 to 12.93 nM. The majority of the compounds that were targeted and had a long link chain between 4-anilinoquinazoline and 3-nitro-1,2,4-triazole (n = 2 or 3; 3a, 3c, 3d, 3e, 3g,; IC_{50} ranging from 0.37 to 12.93 nM) effectively inhibited EGFR more than the compounds that had a short side chain.It suggested that the EGFR inhibitory action of specific compounds could be significantly impacted by the length of the linker separating the quinazoline and trizole moiety. Compared to vandetanib, the

majority of the target compounds exhibited comparatively stronger inhibitory effects against VEGFR-2 kinase (IC_{50} ranging from 36.78 to 4082.09 nM).The inhibitory activity of 3a against VEGFR-2 kinase (IC_{50} ¼ 36.78 nM) was comparable to that of vandetanib (IC_{50} ¼ 33.26 nM).

Table 4. Target compound results for the *in vitro* EGFR and VEGFR-2 kinase inhibitory activity assay.



Compound 3a-3g

Compounds	substituents				IC_{50}(nmol/L)	
	N	X_1	X_2	X_3	EGFR	VEGFR-2
3a	2	F	H	Br	5.90	36.78
3b	2	Cl	H	F	20.83	4082.09
3c	2	H	Br	CH_3	0.37	407.47
3d	2	H	F	F	3.70	1104.22
3e	2	H	F	H	3.11	1196.59
3f	2	H	H	F	16.43	864.18
3g	2	F	Cl	Cl	0.69	67.84
LYL-10		F	H	Br	3.23	27.13
Vandetanib		F	H	Br	19.76	33.26

Target compounds were tested for their *in vitro* anti-proliferative activities on human small cell lung cancer cells (H446) and adenocarcinomic human alveolar basal epithelial cells (A549) lines under normoxic or hypoxic circumstances using the tetrazolium salt (WST-8) assay.With an exposure dose of 8 Gy, the potential radiosensitizing properties of the targeted compounds were also assessed in hypoxic conditions. Vandetanib inhibited 21.06% and 25.58% of cell growth in A549 and H446 cells (Table 5), but it was less powerful than the target compounds, 3a, 3b, 3c, 3f, 3g, which inhibited around 5-20% of cell growth for both cancer cell lines under normoxic conditions. Target compound 3a demonstrated greater anti-proliferative activities in both cell lines under hypoxic conditions (75.86% of inhibition in A549 cells and 84.37% of inhibition in H446 cells) compared to vandetanib (67.26% of inhibition in A549 cells and 77.67% of inhibition in H446 cells) (Table 5).

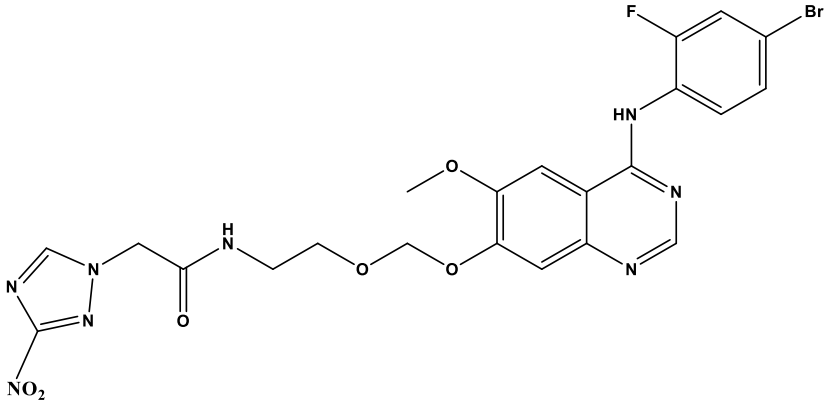
Table 5. *In vitro* anti-proliferative activity assay results (inhibition ratios) in normoxia and hypoxia.

Compounds	Inhibition Ratios (%)			
	A549 Cell line		H446 Cell line	
	Normoxia	Hypoxia	Normoxia	Hypoxia
Vandetanib	21.06	67.26	25.58	77.67
3a	6.75	75.86	18.95	84.37
3b	10.33	69.01	13.87	80.31
3c	5.48	65.39	18.65	77.71
3f	8.43	72.53	11.08	78.70
3g	13.76	82.25	20.52	77.90

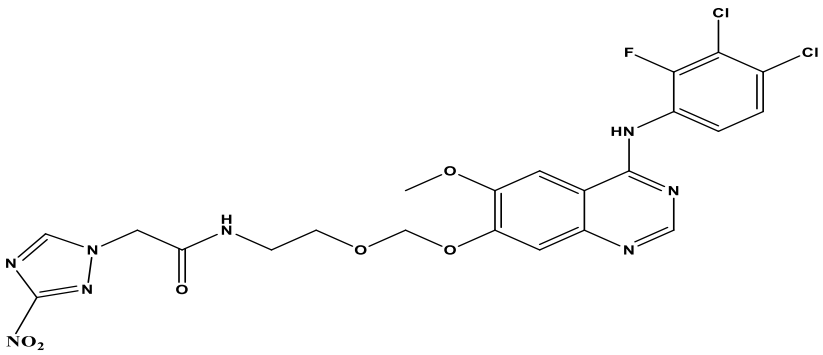
Based on their strong antiproliferative activity from *in vitro* studies, the targeted compounds, 3a and 3g, were chosen to be further studied for their anticancer activities in mouse models. A549 tumor cell xenograft models were used to test the efficacy of 3a and 3g against vandetanib in terms of tumor growth suppression. An equal number of male BALB/c-nu mice were randomly assigned to the vehicle control group, experimental groups (3a or 3g treated), and positive control group (vandetanib treated). Each cohort had five mice that bore tumors, respectively. Tumor size and mouse body weight were measured every day during the 17-day treatment of the mice with 3a, 3g, and vandetanib given orally at a dose of 10 mg/kg. Each set of three tumor-bearing mice participated in *in vivo* dose-dependent anti-proliferation tests. During the course of 18 days, the mice were given oral doses of 5 mg/kg, 15 mg/kg, and 50 mg/kg of 3a and 3g. The tumor growth was assessed on days 4, 6, 8, 11, 13, 15, and 18.

3a demonstrated a noteworthy suppression of A549 tumor growth, as depicted in, with a tumor growth inhibition (TGI) of 63.93% on day 17 in contrast to controls. Day 17 TGI values for 3a and vandetanib were comparable (TGI: 62.06%), suggesting that 3a's *in vivo* anticancer activity was comparable to that of vandetanib. Following a 7-day treatment period, the tumor volume gradually grew in the 3a, 3g, and vandetanib groups. Notably, the 3g group displayed a quicker rate of tumor growth than the 3a and vandetanib groups, suggesting that these compounds were not entirely effective in controlling tumor growth in A549 xenografts. Even after 17 days, 3g continued to have a noticeable inhibitory effect on tumor growth (TGI ¼ 49.05%) as compared to the vehicle control group. Therefore, in animal models, 3a and 3g continued to exhibit strong inhibitory effects on the growth of A549 tumors. Furthermore, in A549 xenografts, the tumor growth inhibitions of 3a and 3g were dose-dependent.

The foundation established by 3a and 3g will make it possible for more structural optimization and biological research to target hypoxic tumors with cancer treatment agents that are efficacious [17].



Compound 3a



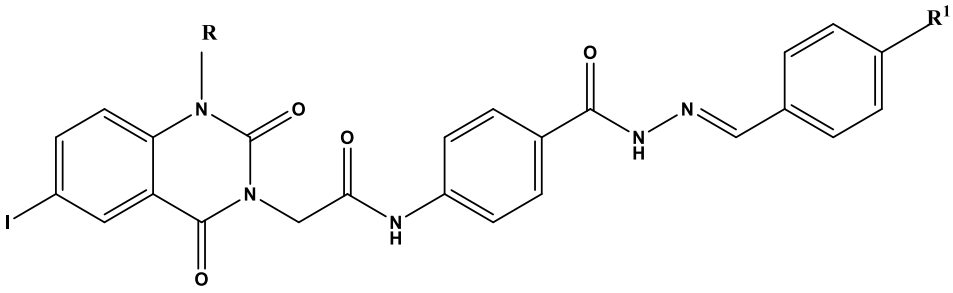
Compound 3g

COMPOUND 4

The anticancer effects of newly developed and synthesized 1-alkyl-6-iodoquinazoline derivatives 4a–e were assessed by dual targeting of EGFR and VEGFR-2 against the cancer cell lines HepG2, MCF-7, HCT116, and A549.

Once an anti-phosphotyrosine antibody was applied using the Alpha Screen technology, all derivatives were assessed for their capacity to inhibit VEGFR-2[18,19]. The IC_{50} values are given in Table7. sorafenib served as a positive control. The candidates under evaluation had excellent to low inhibitory activities, with an IC_{50} range of 0.85–2.50 µM. With IC_{50} values of 0.85, 0.90, 0.90, 1.00 and 1.2µM, respectively, compounds 4c, 4b, 4d, 4a, and 4e, significantly reduced VEGFR-2 activity. All of the compounds were evaluated for their inhibitory effects against mutant $EGFR^{T790M}$ kinases. A homogeneous time-resolved fluorescence (HTRF) assay was used in this examination [19,20]. The reference drug used was erlotinib, IC_{50} = 0.24 µM. Table7 displays a comparison of the IC_{50} values for the substances under investigation.$EGFR^{T790M}$ activity was significantly lowered by compounds 4c, 4d, 4e, and 4b, with IC_{50} values of 0.22, 0.26, 0.30, and 0.50 µM, respectively. Excellent dual $EGFR^{T790M}$/VEGFR-2 inhibitory actions were demonstrated by compound 4c.With IC_{50} values of 0.15, 0.20, and 0.25 µM, respectively, compounds 4c, 4d, and 4e significantly reduced $EGFR^{WT}$ activity.

Mosmann's MTT test [21-23] was employed to evaluate the new iodoquinazoline derivatives 4a–e. Four different human tumor cell lines (HepG2, MCF-7, HCT-116, and A549) were used to test the drugs. The half maximum effective concentration (EC_{50}) values of sorafenib and erlotinib, the study's reference cytotoxic drugs, are displayed in Table 7. The majority of the compounds displayed very good to moderate EC_{50} efficacy against the cancer cell types that were the subject of the investigation, according to the findings. Compound 4c in particular had the strongest anticancer activity against HepG2, MCF-7, HCT116, and A549 cell lines, with EC_{50} values of 5.00, 6.00, 5.17, and 5.25 µM, respectively. Even though it performed less well than sorafenib (EC_{50} = 4.00, 5.05, 5.58, and 4.04 µM) against HepG2, MCF-7, and A549, while performing subparly against HCT116, it performed better than erlotinib (EC_{50} = 7.73, 13.91, 8.20, and 5.49 µM) against the four tested cell lines.



Compound 4

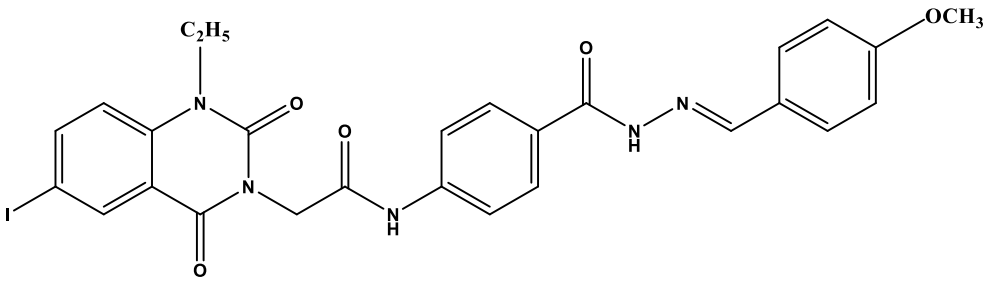
Table 6. Derivatives of compound 4

4a-e	R	R^1
A	C_2H_5	H
b	C_2H_5	CH_3
C	C_2H_5	OCH_3
D	C_3H_7	H
E	C_3H_7	Cl

Table 7. $EGFR^{T790M}$, $EGFR^{WT}$ and VEGFR-2 kinase assays and *in vitro* cytotoxic effects against the HepG2, MCF-7, HCT-116, A549 cell lines.

Compd No.	EC_{50}(μM)				IC_{50}(μM)		
	HepG2	MCF-7	HCT116	A549	VEGFR-2	$EGFR^{T790M}$	$EGFR^{WT}$
4a	6.80 ±0.7	7.50 ± 0.7	6.29 ± 0.7	6.7 ± 0.7	1.0 ± 0.10	0.75 ± 0.50	NT[d]
4b	6.20 ± 0.7	6.70 ± 0.6	5.93 ± 0.6	6.7 ± 0.6	0.9 ± 0.10	0.50 ± 0.35	0.3 ±0.03
4c	5.00 ± 0.5	6.00 ± 0.5	5.17 ± 0.5	5.2 ± 0.5	0.8 ± 0.10	0.22 ± 0.30	0.1 ± 0.03
4d	5.65 ± 0.5	6.45 ± 0.5	6.50 ± 0.5	5.4 ± 0.5	0.9 ± 0.10	0.26 ± 0.30	0.2 ± 0.03
4e	7.36 ± 0.7	7.30 ± 0.7	6.70 ± 0.7	6.3 ± 0.7	1.2 ± 0.10	0.30 ± 0.30	0.2 ± 0.03
Sorafenib	4.00 ± 0.3	5.05 ± 0.5	5.58 ± 0.5	4.0 ± 0.3	0.8 ± 0.04	NT[d]	NT[d]
Erlotinib	7.73 ± 0.6	13.9 ± 1.3	8.20 ± 0.3	5.4 ± 0.4	NT[d]	0.24 ± 0.22	0.1 ± 0.02

In the future, compounds 4c may be developed as potent anticancer drugs targeting both EGFR and VEGFR-2 [24].



Compound 4 C

CONCLUSION:

Quinazoline-based dual inhibitors provide a more complete therapeutic strategy by inhibiting both the EGFR and VEGFR signalling pathways. This dual action has the potential to overcome resistance mechanisms that arise when targeting only one of these pathways. Preclinical investigations have shown that quinazoline derivatives inhibiting both EGFR and VEGFR can significantly reduce tumour growth and increase survival rates. This shows that these compounds have a high potential for being effective in treating tumours that are resistant to single-target therapy. Many quinazoline derivatives are selective for EGFR and VEGFR over other kinases, which helps to reduce off-target effects and potential toxicity. This selectivity is critical for optimizing therapeutic results and improving the safety profile of these medications. Although the dual inhibition strategy shows considerable potential, issues like as drug resistance, optimal dose, and adverse effects must be addressed. Continuous research and clinical trials are required to optimize these compounds and validate their efficacy and safety in various cancer types. Quinazoline derivatives as dual EGFR and VEGFR inhibitors offer a substantial leap in targeted cancer therapy. Their potential to target various pathways involved in tumor growth and angiogenesis makes them promising candidates for further research and therapeutic application. More research must be done to fully grasp their potential and integrate them into effective therapy regimens.

In conclusion, the development of quinazoline-based dual inhibitors is a compelling method for improving cancer treatment, with the potential to provide patients with more effective and targeted therapeutic alternatives.

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