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Original article

Inhibition of tumor cell growth and angiogenesis by 7-Aminoalkoxy-4-aryloxy-quinazoline ureas, a novel series of multi-tyrosine kinase inhibitors

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ABSTRACT

Several regulatory and signaling molecules governing angiogenesis are targets of interest for the development of drugs in the cancer, including growth factors such as Vascular Endothelial Growth Factor (VEGF) and Platelet-Derived Growth Factor (PDGF). A series of 4-aryloxy-6,7-dimethoxyquinazolines, previously synthesized in our laboratory, has shown a nanomolar inhibition of kinase enzymatic activity of VEGFR, PDGFR and c-Kit. We have therefore studied the impact of the variation in the 7-position substitution of the quinazoline core. Substitution by aminoalkoxy chains led to new highly potent ATP-competitive inhibitors of VEGFR, PDGFR and c-Kit enzyme with IC_{50} values in the nanomolar range and this substitution has increased greatly antiproliferative activity on cancer cell lines (PC3, MCF7, HT29) and HUVEC (human umbilical vein endothelial cells). One of the most promising compounds (**36**) was assessed for its ability to limit the induction of web like network of capillary tubes by the human umbilical vascular endothelial cells (HUVEC) and for its ability to inhibit invasion.

1. Introduction

Hypoxia is a salient feature of many types of solid cancers and arises as the result of spatial disorganization and flow based disruption of abnormal microvasculature initiated by the growing tumor. The impact of tumor hypoxia is multifaceted, with effects on several aspects of tumor biology, including genetic instability, angiogenesis, invasiveness, survival and metabolism [1]. Angiogenesis, the formation of new blood vessels from the pre-existing vasculature, is critical for development and subsequent growth of tumors and is a fundamental process for the formation of metastases [2]. Therefore, the inhibition of angiogenesis is an attractive therapeutic approach to treat many human tumors [3].

Vascular endothelial growth factor (VEGF) has been identified as the most common regulator of tumor angiogenesis, vascular permeability, endothelial cell activation, proliferation, and migration [4]. VEGF is a member of a family including of five homodimeric glycoprotein members (VEGF-A; VEGF-B; VEGF-C; VEGF-D and placenta growth factor, PlGF) and exerts its activity through binding to three high-affinity transmembrane receptors: VEGFR-1, VEGFR-2 (also known as KDR) and VEGFR-3 [5]. These tyrosine kinase receptors (TKRs) consist of an extracellular ligand-binding domain connected to an intracellular tyrosine kinase domain through a segment in the plasma membrane. Binding of VEGF to VEGFR results in receptor dimerization and stimulation of the receptor associated tyrosine kinase activity, which leads to phosphorylation of tyrosine residues and initiates a signaling cascade (such as ERK (extracellular-signal regulated kinase)/MAPK (mitogen-activated protein kinase) or PI3K/Akt pathways) [6].

Preventing activation of VEGF receptors has been shown to inhibit angiogenesis, tumor progression, and dissemination [7,8]. Bevacizumab, a recombinant humanized monoclonal antibody against VEGF, has been approved as first-line therapy for metastatic colorectal cancer [9]. In addition, small synthetic molecules that act with an ATP-competitive mechanism by binding the ATP pocket of

Abbreviations: ATP, adenosine 5′-triphosphate; DMSO, dimethyl sulfoxide; EGFR, epidermal growth factor receptor; EtOAc, ethyl acetate; FC, flash chromatography; HUVEC, human umbilical vein endothelial cell; MeOH, methanol; MKI, multi-kinase inhibitor; PDGFR, platelet-derived growth factor receptor; RCC, renal cell carcinoma; rt, room temperature; TKR, tyrosine kinase receptor; VEGFR, vascular endothelial growth factor receptor.

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the protein kinase domain have been approved for treatment of several cancers [10].

As a result of the substantial sequence homology shared by three isoforms of VEGFR and other RTKs (such as platelet-derived growth factor receptor, PDGFR and c-Kit) in their catalytic domains, VEGFR inhibitors are not selective for one kinase [11]. However, their poor selectivity for VEGFRs may be an advantage because PDGFR and c-Kit must be also inhibited in order to prevent cancer evolution. Additionally, during VEGF inhibition, a hypoxic environment can drive a shift from VEGF dependent signaling to other proangiogenic pathways. For this reason, the use of multi-targeted inhibitors should be of great therapeutic benefit. Multi-kinase inhibitors (MKIs) are a new class of antiangiogenic therapeutics that inhibit kinase activities of several receptors, and recent trial data have demonstrated the efficacy of MKIs in the treatment of metastatic renal cell carcinoma (RCC), brain cancer or hepatocellular carcinoma [12,13,14]. Based on these positive clinical trial results, various oral anti-angiogenic MKIs, like sunitinib, sorafenib or pazopanib, have gained regulatory approval for use in RCC (Fig. 1).

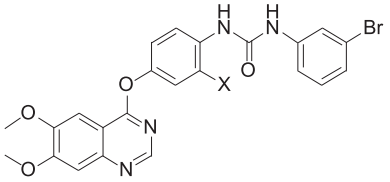
Previously, our group has described structure-activity relationships (SAR) studies and preliminary biological evaluation of *N*-(aromatic)-*N*′-{4-[(6,7-dimethoxyquinazolin-4-yl)oxy]-phenyl} urea derivatives which inhibited VEGFR-2 at nanomolar concentrations [15,16,17].

More recently, to determine the kinase selectivity profile of three selected compounds of this series (**1**–**3**), several kinases were chosen for screening. The kinase panel consists of members of VEGFR family (VEGFR-1, -2 and -3), PDGFR-ß, c-Kit, EGFR, c-Met, Src and Raf. The data of kinase inhibition are summarized in Table 1.

The compounds (**1**–**3**) show low (or no) inhibitory efficacy against four tested kinases of the panel (EGFR, c-Met, Src and Raf) but inhibit VEGFR (-1, -2 and -3), PDGFR-ß and c-Kit at nanomolar values. However, their excellent kinase inhibitory effect against VEGFR (-1, -2 and -3), PDGFR-ß and c-Kit is not associated with an antiproliferative activity on cell-based assays on PC3, HT29 and MCF7 ($IC_{50} > 10$ μM). In this study, we aimed to improve both the enzymatic and proliferative inhibitions of these *N*-(aromatic)-*N*′-{4-[(6,7-dimethoxyquinazolin-4-yl)oxy]-phenyl}urea derivatives.

The effect of varying the *O*-substituent at the 7-position on the quinazoline core was studied in the laboratory on 4-anilino derivatives [18]. Enzymatic and antiproliferative activities were influenced by the side chain at the 7-position of quinazoline. Indeed, introduction of aminoalkyl such as piperidinoethoxy or diethylaminoethoxy side chains, increased inhibitory activity against VEGFR-2 and antiproliferative activity on cancer cell lines. With regard to these observations, we designed a novel series of 7-aminoalkoxy-4-aryloxy-quinazoline ureas and we report here synthesis and biological evaluation. Inhibitory activity against nine tyrosine kinases was determined and these compounds were evaluated for their antiproliferative activity towards five cell lines (PC3, HT29, MCF7, HUVEC and MRC5) by MTS test.

Table 1
IC_{50} values (nM) of kinase inhibition.



Enzymatic inhibitory (IC_{50}, nM)			
Compound	**1**	**2**	**3**
X	H	CH_3	Cl
EGFR[a]	>10,000	>10,000	>10,000
VEGFR-1[b]	46	35	18
VEGFR-2[c]	6	5	8
VEGFR-3[b]	9	15	15
PDGFR-ß[b]	5	89	20
c-Kit[b]	16	102	38
c-Met[b]	2310	>10,000	2000
Src[b]	5470	>10,000	3130
Raf[b]	934	2810	112

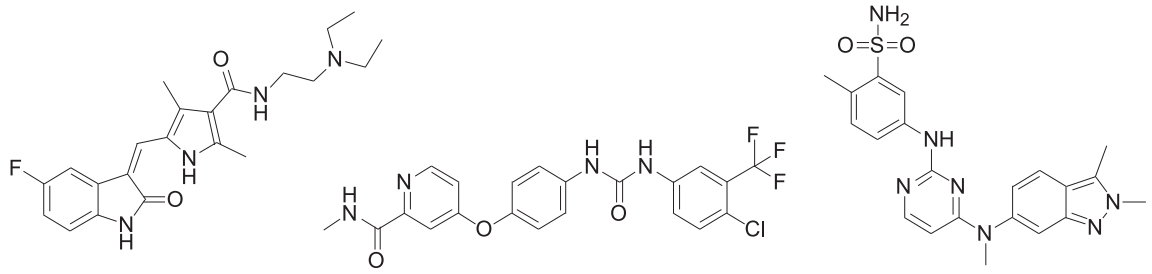
[a] Inhibition of EGFR (purified from human carcinoma A431 cells) tyrosine kinase activity.
[b] Inhibition of VEGFR-1, VEGFR-3, PDGFR-ß, c-Kit, c-Met, Src and Raf was performed at ProQinase GmbH (Freiburg. Germany).
[c] Inhibition of VEGFR-2 (recombinant human protein) tyrosine kinase activity.

2. Chemistry

We have synthesized new quinazoline compounds which were divided in five groups distinguished by the nature of the ether linker at the C-6 and C-7 positions of the quinazoline core: 6-butoxy-7-diethylaminoethoxy- (series A); 6-methoxy-7-diethylaminoethoxy- (series B); 6-methoxy-7-piperidino-ethoxy- (series C); 6-methoxy-7-pyrrolidinoethoxy- (series D) and 6-methoxy-7-piperidinopropoxyquinazoline (series E) (Table 2).

The key intermediates 4-chloro-7-aminoalkoxyquinazolines (**16**–**18**) were obtained in five steps for series C, D and E (Scheme 1).

Compounds (**4**–**6**) were synthesized from methyl vanillate by alkylation reaction in the presence of appropriate commercial chloroalkane and potassium carbonate in acetone. Nitration was realized in the presence of a mixture of nitric acid and tin tetrachloride in dichloromethane [19]. Nitration occurs selectively at the 2-position due to electronic effects of different substituents on the benzene ring. The position of nitro group was confirmed by ^1H NMR. The nitro derivatives (**7**–**9**) were obtained with yields



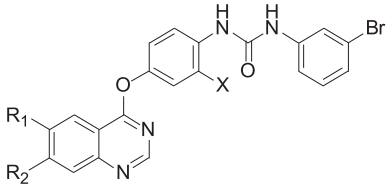
sunitinib, Sutent®
(VEGFR, PDGFR, c-Kit, Flt-3, Ret)

sorafenib, Nexavar®
(VEGFR, PDGFR, c-Kit, Flt-3, Raf)

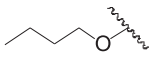
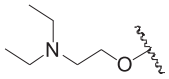
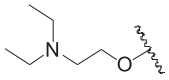
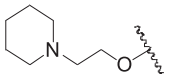
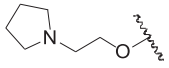
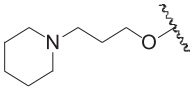
pazopanib, Votrient®
(VEGFR, PDGFR, c-Kit)

Fig. 1. Examples of multi-tyrosine kinase inhibitors marketed.

Table 2
Target compounds



X = H, CH_3 or Cl

Series	*R*1	*R*2
A		
B		
C		
D		
E		

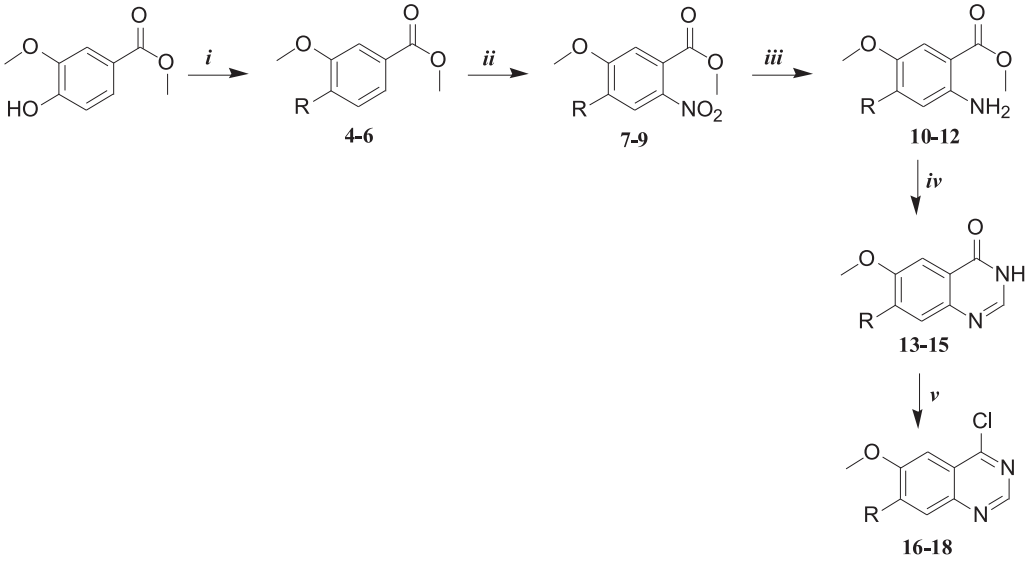
between 24 and 78%. Catalytic hydrogenation of the nitro group in the presence of Raney® nickel led to amino derivatives (10–12) (41–79% of yields) which were converted by cyclization with formamide in the presence of ammonium formate to obtain the cyclised compounds (13–15) with yields between 59 and 75% [20]. Finally, cyclised compounds were reacted with phosphorus oxychloride to obtain the key intermediates 4-chloroquinazolines (**16**–**18**). [21]

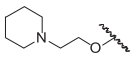
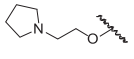
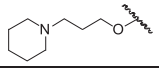
As depicted in Scheme 2, the amino groups of commercial 4-aminophenols were converted by condensation with commercial 3-bromophenylisocyanate in pyridine at room temperature to obtain the corresponding ureas (**21**–**23**) in high yields and short time reaction. [22]

The synthesis of the final products is illustrated in Scheme 3. The 4-chloroquinazolines **19** and **20**, belonging respectively at series A and B, were prepared according to described procedure using respectively methyl vanillate and methyl 3,4-dihydroxybenzoate as starting materials [23]. Reaction of chloride derivatives (**16**–**20**) with appropriate phenolurea (**21**–**23**) in presence of tetra-*N*-butylammonium bromide in 2-butanone and 20% solution of sodium hydroxide mixture allowed the obtaining of desired products of various series with low yields (**24**–**37**).

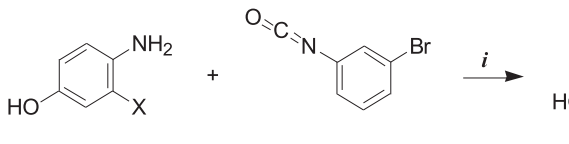
3. Results and discussion

In this section, we describe the biological evaluation of the synthesized 7-aminoalkoxyquinazoline derivatives concerning their inhibitory efficacy on several protein kinases and their antiproliferative activity. Vandetanib (EGFR/VEGFR inhibitor) and/or antiangiogenic inhibitor cediranib (VEGFR, PDGFR and c-Kit



Series	R	Yield				
		i	*ii*	*iii*	*iv*	*v*
C		**4**, 95%	**7**, 58%	**10**, 79%	**13**, 59%	**16**, 71%
D		**5**, 89%	**8**, 24%	**11**, 51%	**14**, 75%	**17**, 49%
E		**6**, 94%	**9**, 78%	**12**, 41%	**15**, 75%	**18**, 70%

Scheme 1. Reagents and conditions: (*i*) aminoalkyl chloride, K_2CO_3, acetone, reflux; (*ii*) HNO_3, $SnCl_4$, CH_2Cl_2, −70 °C; (*iii*) Raney® Ni, H_2, MeOH/CH_2Cl_2, rt; (*iv*) $HCONH_2$, $HCOONH_4$, reflux; (*v*) $POCl_3$, reflux.



X	Yield
H	**21**, 95%
CH_3	**22**, 89%
Cl	**23**, 94%

Scheme 2. Reagents and conditions: (*i*) pyridine, rt, nitrogen atmosphere.

inhibitor) were used as the reference substances in the following evaluations.

3.1. Kinase selectivity

The synthesized compounds were tested on a panel of nine isolated kinase receptors including: EGFR, VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-ß, c-Kit, c-Met, Src and Raf. Inhibitory activity against EGFR and VEGFR-2 tyrosine kinases was determined by our team by measuring the levels of phosphorylation of the tyrosine-specific peptides (poly(Glu Tyr)substrate) *in vitro* using [γ-^{32}P] ATP. Inhibition of VEGFR-1, VEGFR-3, PDGFR-ß, c-Kit, c-Met, Src and Raf was performed at ProQinase GmbH (Freiburg, Germany) by a radiometric protein kinase assay (^{33}PanQinase® activity assay). The inhibition of kinase data are summarized in Table 3.

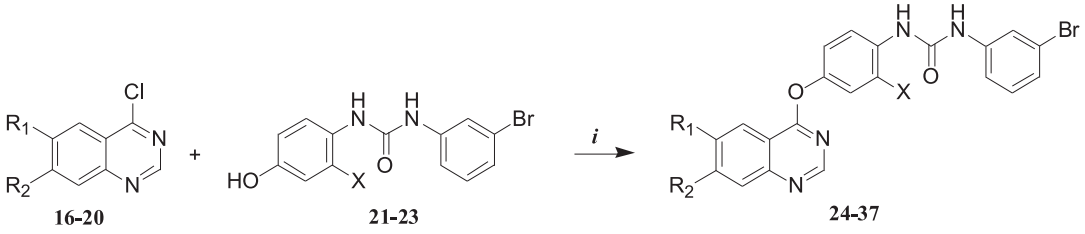
Compounds of series A (**24**, **25**), with a butoxy chain in C-6 position, show a decrease in activity against VEGFR, PDGFR-ß and c-Kit compared to the reference compounds (**1**–**3**). Series B (**26**–**28**), with a methoxy chain in C-6 position and a diethylaminoethoxy group in C-7 position, were more efficient than their 6-butoxy-analogs (**24**, **25**) with IC_{50} values at nanomolar range against VEGFR, PDGFR-ß and c-Kit. Cyclisation of diethylaminoethoxy group in piperidino- (series C, compounds **29**–**31**) or pyrrolidinoethoxy (series D, compounds **32**–**34**) chain is reasonably well tolerated. Replacement of piperidinoethoxy chain by piperidinopropoxy (series E, compounds **35**–**37**) led to an excellent inhibitory activity against VEGFR, PDGFR-ß and c-Kit with IC_{50} values < 12 nM. In addition, the length of the linker shows a significant difference in activity against EGFR. Indeed, compounds (**35**–**37**) of series E show an EGFR inhibition with micromolar IC_{50} while the compounds of other series, with aminoethoxy group, cause no EGFR inhibition at 10 μM concentration.

For kinases c-Met, Src and Raf, the inhibition values are low for all compounds. However, no-substitution of the aromatic group by a chlorine or methyl group (X = H: **26**, **29**, **32**) is tolerated and causes a low increase in activity against c-Met and Src. Unlike, inhibition of Raf is improved when the aromatic group is substituted by a chlorine (X = Cl: **28**, **31**, **34**, **37**).

All synthesized compounds show higher affinity for VEGFR (1, 2 and 3), PDGFR-ß and c-Kit (IC_{50} values at nanomolar range) than for c-Met, Src, Raf and EGFR, like reference cediranib. The molecules interfering simultaneously with multiple RTKs might be more effective than single target agents. With the approval by FDA of sorafenib and sunitinib – targeting VEGFR, PDGFR, FLT-3 and c-Kit – a new generation of anti-cancer drugs, able to inhibit more than one pathway, would probably play a major role.

3.2. Cellular inhibitory

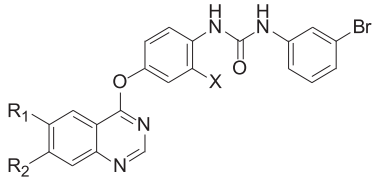
Antiproliferative activities of compounds were evaluated toward the hormone-independent PC3 prostate cancer cells, MCF7 breast cancer cells and HT29 colon cancer cells by MTS test. The



Series	R_1	R_2	Yield		
			X = H	X = CH_3	X = Cl
A			**24**, 22%	**25**, 52%	-
B			**26**, 15%	**27**, 28%	**28**, 13%
C			**29**, 15%	**30**, 12%	**31**, 13%
D			**32**, 24%	**33**, 25%	**34**, 9%
E			**35**, 26%	**36**,18%	**37**, 21%

Scheme 3. Reagents and conditions: (*i*) nBu$_4$N$^+$Br$^-$, 2-butanone/20% NaOH, 100 °C.

Table 3
IC_{50} values (nM) of enzymatic inhibition (EGFR, VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-ß, c-Kit, c-Met, Src and raf) .



Series	*R1*	*R2*	*X*	Compd	Enzymatic inhibitory (IC_{50}, nM) EGFR[a]	VEGFR-1[b]	VEGFR-2[c]	VEGFR-3[b]	PDGFR-ß[b]	c-Kit[b]	c-Met[b]	Src[b]	Raf[b]
Vandetanib					800	150	69	260	5300	N.D.	N.D.	N.D.	N.D
Cediranib					2100	39	14	11	38	1530	967	156	>10,000
A			H	**24**	>10,000	122	12	199	61	55	1770	1090	308
			CH_3	**25**	>10,000	59	44	112	395	168	3320	2130	273
B			H	**26**	>10,000	7	1	22	7	7	963	232	245
			CH_3	**27**	>10,000	19	8	12	98	91	1100	1900	400
			Cl	**28**	>10,000	4	4	13	15	9	1420	283	60
C			H	**29**.HCl	>10,000	11	4	26	8	12	977	262	356
			CH_3	**30**	>10,000	6	2	19	51	25	3440	604	114
			Cl	**31**	>10,000	5	3	17	64	22	4710	654	119
D			H	**32**	>10,000	5	3	23	4	6	706	239	205
			CH_3	**33**	>10,000	5	2	14	36	13	2870	548	103
			Cl	**34**	>10,000	5	1	8	17	13	1850	254	63
E			H	**35**	9710	4	2	12	4	6	350	268	155
			CH_3	**36**.HCl	2300	5	4	8	9	9	1390	120	126
			Cl	**37**	1930	3	1	6	5	5	619	73	66

N.D. Not determined.
[a] Inhibition of EGFR (purified from human carcinoma A431 cells) tyrosine kinase activity.
[b] Inhibition of VEGFR-1, VEGFR-3, PDGFR-ß, c-Kit, c-Met, Src and Raf was performed at ProQinase GmbH (Freiburg, Germany).
[c] Inhibition of VEGFR-2 (recombinant human protein) tyrosine kinase activity.

synthesized compounds were evaluated also for their anti-proliferative activity towards the human umbilical vein endothelial cells (HUVEC) because of their role in angiogenesis (Table 4).

Introduction of aminoalkyl in C-7 position of quinazoline allowed to a significant increase in inhibition effects on the growth of three cancer cell lines PC3, HT29 and MCF7. The cellular results of all compounds are equivalent or better than reference compounds (vandetanib and cediranib). The best results are obtained for the inhibition of colon cancer cells HT29 ($IC_{50} \approx 1$ μM). The difference between cellular and enzymatic results can be explained because cancer cell lines PC3, HT29 and MCF7 are not only dependent on the VEGFR signal.

Replacing *O*-methoxy by aminoalkoxy groups at the 7-position allowed to keep good levels of inhibition against HUVEC ($IC_{50} \leq 1$ μM). Values are similar whatever the nature of the amine (diethylamino-, piperidino-, pyrrolidino- moiety) or the length of the linker (ethoxy or propoxy). IC_{50} values of proliferation inhibitory in HUVEC are lower than cancer cell lines because normal primary HUVEC are only dependent on the VEGFR signal.

Inhibition of proliferation of compounds was evaluated on non-cancer cell lines (MRC5, human fetal lung fibroblast cells) at 1 μM. The value of 1 μM has been selected in accordance with the obtained IC_{50} on HT29. In order to assess the selectivity of our compounds for cancer cells *versus* non-cancer cells, percentages of inhibition at 1 μM on HT29 are also shown in Fig. 2. Vandetanib and cediranib were used as the reference substances.

The results showed that the inhibition of proliferation of our multi-kinase inhibitors against the cancer cell line HT29 was significantly higher than that against the nonmalignant cell line MRC5 at 1 μM, with selectivity similar to those of tyrosine kinase inhibitors marketed like vandetanib and cediranib. The inhibition values on MRC5 are always below 15%, except for the compound **37** which present a very strong proliferation inhibition on MRC5 (40% at 1 μM).

For one of most promising compounds (**36**), displaying potent enzymatic inhibitory (IC_{50} on VEGFR-(1, 2 and 3), PDGFR-ß and c-Kit < 9 nM), antiproliferative activities on several cancer cell lines (≈μM on PC3, HT29 and MCF7) and low proliferation inhibition on MRC5, follow-up studies were realized.

For the first time, kinetic study of proliferation inhibition was performed on HT29 with compound **36**. The antiproliferative activity of compound **36** was evaluated by MTS assay. The viability was determined at 24, 48 and 72 h (Fig. 3).

As outlined in Fig. 3, IC_{50} of compound **36** at 72 h is 1.00 μM on HT29. At 48 h, the IC_{50} for compound **36** is close to that IC_{50} obtained at 72 h (IC_{50} 48 h = 1.75 μM).

Compound **36** was also assessed for its ability to limit the induction of web like network of capillary tubes by the human umbilical vascular endothelial cells (HUVEC) and for its ability to inhibit cell invasion.

3.3. Endothelial tube formation

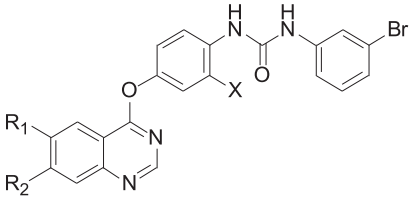
Endothelial cell tube formation assays are useful indicators of angiogenesis potential. The antiangiogenic effect was evaluated on HUVEC by treating with a reference compound (cediranib) and compound **36**. HUVEC were placed on Matrigel® in order to imitate *in vivo* HUVEC and treated with several concentrations of cediranib or of compound **36** (0.1, 1, 5 and 10 μM) (Fig. 4).

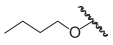
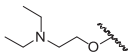
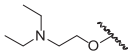
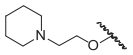
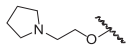
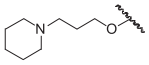
The compound **36** inhibited significantly the formation of endothelial tubes in a dose-dependent manner with lower concentrations than those of the reference compound, cediranib.

3.4. Cell invasion

We investigated also the effects of compound **36** at several concentrations (0.01, 0.1 and 1 μM) on the invasiveness of HUVEC using a Boyden chamber assay. Matrigel® was applied to the filter

Table 4
Cellular inhibition (PC3, prostate cancer cell; HT29, colon cancer cell; MCF7, breast cancer cell; HUVEC, human umbilical vein endothelial cell) results of compounds (**24**–**37**).



Series	*R1*	*R2*	*X*	Compd	Proliferation inhibitory (IC_{50}, μM)[a] PC3	HT29	MCF7	HUVEC
Vandetanib					7.30 ± 3.00	1.76 ± 0.78	9.57 ± 0.41	4.39 ± 1.69
Cediranib					>10	1.61 ± 0.49	7.94 ± 1.91	0.27 ± 0.14
A			H	**24**	3.77 ± 0	1.10 ± 0.09	0.91 ± 0.05	1.53 ± 0.73
			CH_3	**25**	3.94 ± 0.07	1.29 ± 0.54	1.45 ± 1.11	0.78 ± 0.22
B			H	**26**	2.76 ± 0.44	1.22 ± 0.66	3.83 ± 0.43	0.79 ± 0.17
			CH_3	**27**	>10	4.84 ± 0.25	>10	1.39 ± 0.60
			Cl	**28**	4.74 ± 1.00	1.19 ± 0.34	4.97 ± 1.75	0.34 ± 0.11
C			H	**29**	6.58 ± 1.17	1.60 ± 0.08	7.16 ± 1.06	0.33 ± 0.23
			CH_3	**30**	5.39 ± 1.03	1.54 ± 0.61	5.19 ± 1.09	0.49 ± 0.36
			Cl	**31**	7.03 ± 0.60	2.26 ± 0.53	5.48 ± 0	0.48 ± 0.06
D			H	**32**	5.32 ± 0.10	1.61 ± 0.08	3.90 ± 0.26	0.38 ± 0.03
			CH_3	**33**	6.59 ± 1.19	1.97 ± 1.11	6.79 ± 1.68	0.51 ± 0.26
			Cl	**34**	3.38 ± 0.16	1.46 ± 0.26	5.07 ± 1.35	0.48 ± 0.06
E			H	**35**	1.33 ± 0.20	1.08 ± 0.15	2.79 ± 1.54	0.12 ± 0.01
			CH_3	**36**	4.09 ± 0.13	1.00 ± 0.11	5.02 ± 1.19	0.33 ± 0.17
			Cl	**37**	1.94 ± 0.03	0.67 ± 0.01	2.78 ± 0.74	0.17 ± 0.02

Data are represented as the mean ± SEM of at two experiments performed in triplicate. Higher concentrations were not used to avoid precipitation of the compound in the culture medium.
[a] Cell proliferation was realized by MTS assay.

membrane, and the number of HUVEC that penetrated the Matrigel® and membrane was quantified (Fig. 5).

As shown in Fig. 5, compound **36** inhibited the invasiveness of HUVEC in a dose-dependent manner. Treatment with compound **36** at 0.01, 0.1 and 1 μM inhibited invasion by 46%, 67%, and 77%, respectively.

Considering that tube formation and invasion are highly relevant properties in the process of angiogenesis, our results showed that potent multi-kinase inhibitor **36** inhibited angiogenesis by preventing tube formation and suppressing endothelial cells invasion.

4. Conclusion

In summary, a series of 7-aminoalkoxy-4-aryloxy-quinazoline ureas have been synthesized. These derivatives are highly potent inhibitors of VEGFR (1, 2 and 3), PDGFR-ß and c-Kit with IC_{50} in the nanomolar range. Varying the methoxy groups on the 7-position of the quinazoline scaffold by addition of a basic side chain (diethylamino-alkoxy, piperidino-alkoxy or pyrrolidino-alkoxy) led to revealing a range of potent VEGFR, PDGFR-ß and c-Kit inhibitors which exhibited a significant antiproliferative activities on various cancer cell lines (PC3, HT29 and MCF7) and on HUVEC, but have

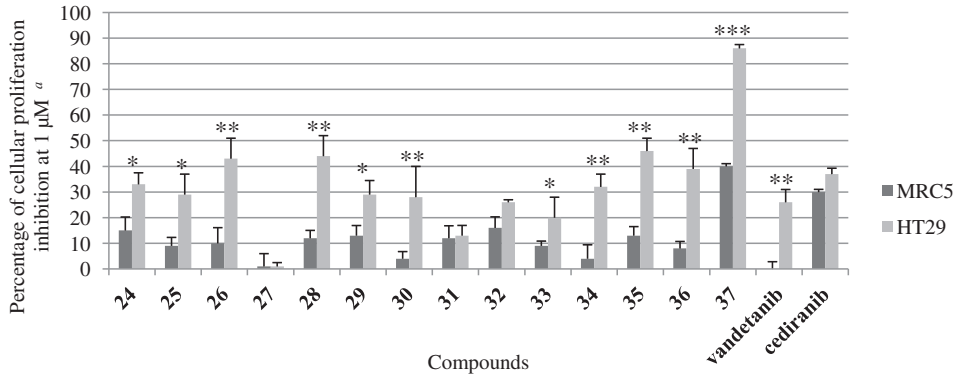
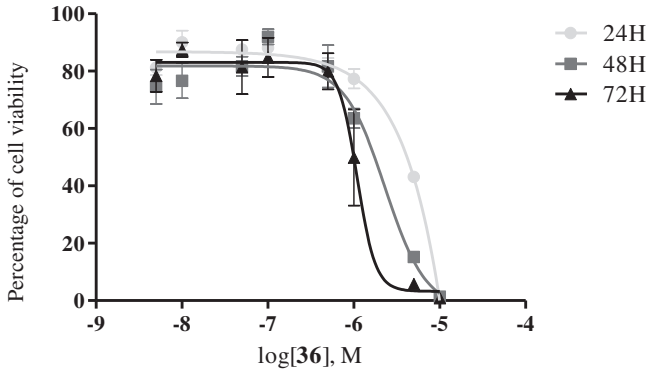


Fig. 2. Effect of compounds (**24**–**37**) on the cell proliferation of human fetal lung fibroblast cells MRC5 and colon cancer cells HT29 at 1 μM. Cell proliferation was realized by MTS assay. Data are represented as the mean ± SEM of at two experiments performed in triplicate. Higher concentrations were not used to avoid precipitation of the compound in the culture medium. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (Statistical analyzes were performed using Statistica software version 6 (StatSoft®). In order to examine the effect of our compounds, we applied Wilcoxon non-parametric matched pairs signed ranks test. Results were taken as significant if $p < 0.05$).



IC_{50} 24H	IC_{50} 48H	IC_{50} 72H
4.16 μM	1.75 μM	1.00 μM

Fig. 3. Proliferation inhibition of compound **36** on HT29 at 24 h, 48 h and 72 h. Cell proliferation was realized by MTS assay. Data are represented as the mean ± SEM of at two experiments performed in triplicate. Higher concentrations were not used to avoid precipitation of the compound in the culture medium.

lower proliferation inhibition against the normal cells MRC5. Further investigations suggested that multi-kinase inhibitor **36** strongly represses the angiogenic process by inhibiting endothelial cell invasion and preventing tube formation with lower concentrations than those of the reference compound (cediranib). The new quinazoline molecules with a highly potent inhibition of VEGFR, PDGFR-ß and c-Kit and superior antiproliferative activities on cancer cell lines compared to the reference compounds (**1**–**3**) have been described, and the objective is thus fully reached.

Further pharmacological evaluations *in vivo* with several 7-aminoalkoxy-4-aryloxy-quinazoline ureas are currently under investigation.

5. Experimental methods

5.1. General chemistry

Melting points were determined with a Büchi 535 capillary melting point apparatus and are uncorrected. Macherey Nagel Polygram® sil G/UV_{254} commercial plates were used for analytical TLC as well as UV light and/or with iodine to follow the course of the reaction. Flash chromatography (FC) was performed with silica gel Macherey Nagel Si 60, 0.015–0.040 mm (Merck). The structure of each compound was confirmed by IR (Bruker VECTOR 22 instrument) and ^{1}H NMR (300 MHz, Bruker AC300P spectrometer). Chemicals shifts (δ) are reported in parts per million downfield from TMS. *J* values are in hertz, and the splitting patterns are abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. The purity of the compounds was tested by HPLC separation followed by $APCI^+$ (atmospheric pressure chemical ionization) mass spectral detection on an LC-MS system, Thermo Electon Surveyor MSQ, and was >95%. Purity of all tested compounds is superior of 98%. HRMS experiments were performed on Q Exactive Benchtop LC-MS/MS (Thermo Scientific).

5.1.1. General procedure for aminoalkoxy derivatives (**4**–**6**)

To a solution of methyl vanillate (6 g, 32.93 mmol) in acetone (100 mL), were added the appropriate chloroalkane (39.52 mmol) and K_2CO_3 (131.72 mmol). After 5 h at reflux, the mixture was cooled to room temperature and filtered. The solvent was removed under reduced pressure and the residue dissolved in a 10% solution of K_2CO_3. The aqueous solution was extracted with EtOAc, dried over $MgSO_4$ and the solvent was removed under reduced pressure.

*5.1.1.1. Methyl 3-methoxy-4-piperidinoethoxybenzoate (**4**).* Brown oil (95%). TLC: R_f 0.8 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 2933 (piperidine), 1713 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.40–1.47 (m, 2H, CH_2), 1.57–1.65 (m, 4H, 2 CH_2), 2.51–2.54 (m, 4H, 2 CH_2), 2.84 (t, 2H, J = 6.2 Hz, CH_2), 3.90 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.21 (t, 2H, J = 6.2 Hz, CH_2), 6.90 (d, 1H, J = 8.1 Hz, ArH), 7.54 (d, 1H, J = 1.8 Hz, ArH), 7.66 (dd, 1H, J = 8.1, 1.8 Hz, ArH). LC-MS ($APCI^+$): *m/z* calcd for $C_{16}H_{23}NO_4$ 294 [$(M+H)^+$].

*5.1.1.2. Methyl 3-methoxy-4-pyrrolidinoethoxybenzoate (**5**).* Brown oil (89%). TLC: R_f 0.9 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 2950 (pyrrolidine), 1711 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.81 (m, 4H, 2 CH_2), 2.64 (m, 4H, 2CH_2), 2.99 (t, 2H, J = 6.0 Hz, CH_2), 3.89 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.21 (t, 2H, J = 6.0 Hz, CH_2), 6.90 (d, 1H, J = 8.4 Hz, ArH), 7.54 (d, 1H, J = 1.8 Hz, ArH), 7.65 (dd, 1H, J = 8.4, 1.8 Hz, ArH). LC–MS ($APCI^+$): *m/z* calcd for $C_{15}H_{21}NO_4$ 280 [$(M+H)^+$].

*5.1.1.3. Methyl 3-methoxy-4-piperidinopropoxybenzoate (**6**).* Brown oil (94%). TLC: R_f 0.9 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 2935 (piperidine), 1709 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.37–1.48 (m, 2H, CH_2), 1.51–1.60 (m, 4H, 2CH_2), 2.03 (m, 2H, CH_2), 2.37–2.41 (m, 4H, 2 CH_2), 2.46 (t, 2H, J = 7.4 Hz, CH_2), 3.88 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.12 (t, 2H, J = 7.0 Hz, CH_2), 6.90 (d, 1H, J = 8.4 Hz,

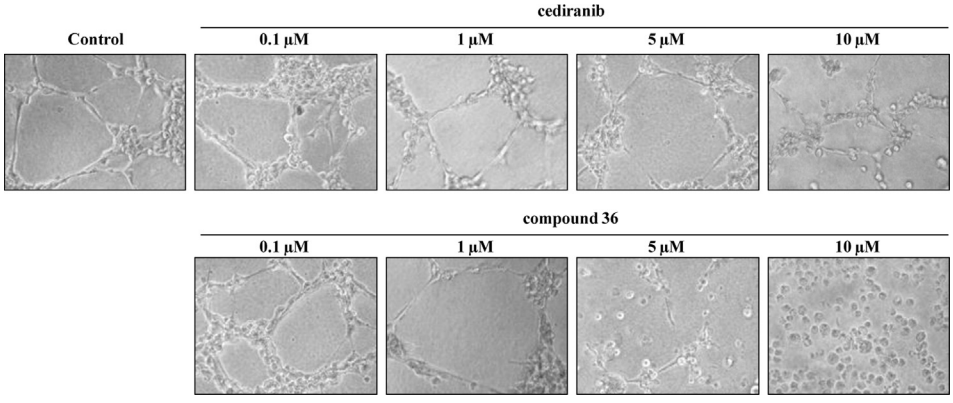


Fig. 4. Effect of cediranib and compound **36** on tube formation Images depicting the formation of HUVEC capillary-like tubular network by treatment with cediranib or compound **36** (0.1, 1, 5 and 10 μM).

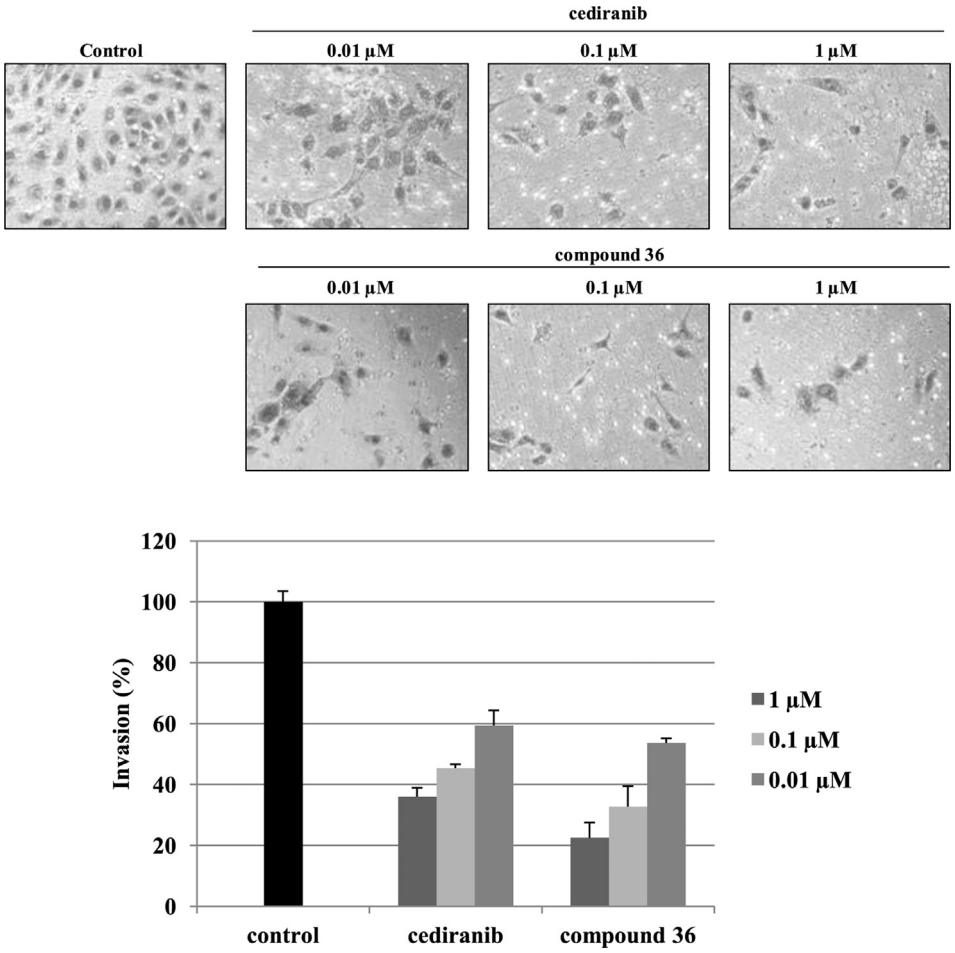


Fig. 5. Invasion inhibition on HUVEC in a Boyden chamber assay of cediranib and compound **36** Images depicting the penetration of HUVEC through the filter membrane. The number of HUVEC that penetrated the membrane was quantified.

ArH), 7.53 (d, 1H, $J = 1.9$ Hz, ArH), 7.63 (dd, 1H, $J = 8.5$, 2.0 Hz, ArH). LC–MS (APCI$^+$): *m/z* calcd for $C_{17}H_{25}NO_4$ 308 [(M+H)$^+$].

*5.1.2. General procedure for nitro derivatives (**7**–**9**)*

To a stirred mixture of aminoalkoxy derivatives (**4**–**6**) (15.30 mmol) in dichloromethane (100 mL), a mixture of nitric acid (45.90 mmol) and tin tetrachloride (45.90 mmol) diluted in 20 mL of dichloromethane was slowly added, while maintaining the temperature at −70 °C. After the addition, the mixture was stirred for an additional 4 h at room temperature. The mixture was neutralized by 10% K_2CO_3 solution and then the aqueous solution was extracted with dichloromethane, dried over $CaCl_2$ and the solvent was removed under reduced pressure.

*5.1.2.1. Methyl 2-nitro-4-piperidinoethoxy-5-methoxybenzoate (**7**).* Yellow oil (58%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 2485 (piperidine), 1730 (C=O), 1522 (NO_2). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.45 (m, 2H, CH_2), 1.62–1.69 (m, 4H, 2 CH_2), 2.55–2.59 (m, 4H, 2CH_2), 2.85 (t, 2H, $J = 9.0$ Hz, CH_2), 3.90 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.24 (t, 2H, $J = 9.0$ Hz, CH_2), 7.07 (s, 1H, ArH), 7.52 (s, 1H, ArH). LC–MS (APCI$^+$): *m/z* calcd for $C_{16}H_{22}N_2O_6$ 339 [(M+H)$^+$].

*5.1.2.2. Methyl 2-nitro-4-pyrrolidinoethoxy-5-methoxybenzoate (**8**).* Yellow oil (24%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 2470 (pyrrolidine), 1709 (C=O), 1531 (NO_2). ^{1}H NMR (DMSO-d_6): δ (ppm) 3.34 (m, 4H, 2CH_2), 3.45–3.47 (m, 2H, CH_2), 3.58–3.77 (m, 4H, 2 CH_2), 3.84 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.53 (t, 2H, $J = 5.7$ Hz, CH_2), 7.39 (s, 1H, ArH), 7.78 (s, 1H, ArH). LC-MS (APCI$^+$): *m/z* calcd for $C_{15}H_{20}N_2O_6$ 325 [(M+H)$^+$].

*5.1.2.3. Methyl 2-nitro-4-piperidinopropoxy-5-methoxybenzoate (**9**).* Yellow oil (78%). TLC: R_f 0.7 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 2935 (piperidine), 1731 (C=O), 1520 (NO_2). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.38–1.49 (m, 2H, CH_2), 1.54–1.65 (m, 4H, 2CH_2), 2.05 (m, 2H, CH_2), 2.37–2.45 (m, 4H, 2CH_2), 2.49 (t, 2H, $J = 7.3$ Hz, CH_2), 3.90 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.16 (t, 2H, $J = 7.3$ Hz, CH_2), 7.07 (s, 1H, ArH), 7.48 (s, 1H, ArH). LC-MS (APCI$^+$): *m/z* calcd for $C_{17}H_{24}N_2O_6$ 353 [(M+H)$^+$].

*5.1.3. General procedure for methyl-2-aminobenzoate derivatives (**10**–**12**)*

To a solution of nitro compounds (**7**–**9**) (15.30 mmol) in 100 mL of a mixture of methanol and dichloromethane (5:5), was added Raney® nickel. After 18 h at room temperature in hydrogen atmosphere, the mixture was filtered. The solvent was removed under reduced pressure and the residue was purified by FC (CH_2Cl_2/MeOH, 9:1).

*5.1.3.1. Methyl 2-amino-4-piperidinoethoxy-5-methoxybenzoate (**10**).* Brown oil (79%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 3442 (NH_2), 2935 (piperidine), 1663 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.33–1.40 (m, 2H, CH_2), 1.42–1.52 (m, 4H, 2

CH_2), 2.37–2.45 (m, 4H, 2 CH_2), 2.65 (t, 2H, J = 6.0 Hz, CH_2), 3.63 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.01 (t, 2H, J = 6.0 Hz, CH_2), 6.37 (s, 2H, NH_2), 6.40 (s, 1H, ArH), 7.12 (s, 1H, ArH). LC–MS ($APCI^+$): *m/z* calcd for $C_{16}H_{24}N_2O_4$ 309 [$(M+H)^+$].

*5.1.3.2. Methyl 2-amino-4-pyrrolidinoethoxy-5-methoxybenzoate (**11**).* Brown oil (51%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 3534 (NH_2), 2950 (pyrrolidine), 1684 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.79–2.05 (m, 4H, 2 CH_2), 2.99–3.24 (m, 4H, 2 CH_2), 3.57 (t, 2H, J = 5.5 Hz, CH_2), 3.66 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.32 (t, 2H, J = 5.1 Hz, CH_2), 6.41 (s, 1H, ArH), 6.46 (s, 2H, NH_2), 7.17 (s, 1H, ArH). LC-MS ($APCI^+$): *m/z* calcd for $C_{15}H_{22}N_2O_4$ 295 [$(M+H)^+$].

*5.1.3.3. Methyl 2-amino-4-piperidinopropoxy-5-methoxybenzoate (**12**).* Yellow oil (41%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). IR (cm^{-1}): 3424 (NH_2), 2935 (piperidine), 1678 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.35–1.83 (m, 6H, 3 CH_2), 2.15–2.24 (m, 2H, CH_2), 2.71–3.19 (m, 4H, 2 CH_2), 3.35 (m, 2H, CH_2), 3.67 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.01 (t, 2H, J = 5.8 Hz, CH_2), 6.39 (s, 1H, ArH), 6.46 (s, 2H, NH_2), 7.14 (s, 1H, ArH). LC–MS ($APCI^+$): *m/z* calcd for $C_{17}H_{26}N_2O_4$ 323 [$(M+H)^+$].

*5.1.4. General procedure for quinazolin-4-one derivatives (**13**–**15**)*

To a solution of methyl-2-amino-benzoate derivatives (**10**–**12**) (3.39 mmol) in formamide (5 mL) was added ammonium formate (10.19 mmol). The reaction mixture was stirred for 24 h under reflux conditions. The reaction was quenched by water and the mixture was alkalinized by 10% K_2CO_3 solution. The precipitated was collected by filtration, washed with H_2O and dried in vacuo.

*5.1.4.1. 6-Methoxy-7-piperidinoethoxyquinazolin-4-one (**13**).* White solid (59%). TLC: R_f 0.4 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 155–157 °C. IR (cm^{-1}): 2935 (piperidine), 1650 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.33–1.45 (m, 2H, CH_2), 1.46–1.55 (m, 4H, 2 CH_2), 2.42–2.51 (m, 4H, 2 CH_2), 2.72 (t, 2H, J = 5.4 Hz, CH_2), 3.86 (s, 3H, OCH_3), 4.20 (t, 2H, J = 5.3 Hz, CH_2), 7.15 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.94 (s, 1H, ArH), 7.98 (s, 1H, NH). LC–MS ($APCI^+$): *m/z* calcd for $C_{16}H_{21}N_3O_3$ 304 [$(M+H)^+$].

*5.1.4.2. 6-Methoxy-7-pyrrolidinoethoxyquinazolin-4-one (**14**).* Beige solid (75%). TLC: R_f 0.4 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 182–184 °C. IR (cm^{-1}): 2950 (pyrrolidine), 1658 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.82–1.87 (m, 4H, 2 CH_2), 2.70–2.79 (m, 4H, 2CH_2), 3.07 (t, 2H, J = 6.2 Hz, CH_2), 3.97 (s, 3H, OCH_3), 4.31 (t, 2H, J = 6.0 Hz, CH_2), 7.15 (s, 1H, ArH), 7.55 (s, 1H, ArH), 8.00 (s, 1H, ArH), 8.01 (s, 1H, NH). LC–MS ($APCI^+$): *m/z* calcd for $C_{15}H_{19}N_3O_3$ 290 [$(M+H)^+$].

*5.1.4.3. 6-Methoxy-7-piperidinopropoxyquinazolin-4-one (**15**).* Beige solid (75%). TLC: R_f 0.4 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 172–174 °C. IR (cm^{-1}): 2935 (piperidine), 1662 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.31–1.42 (m, 2H, CH_2), 1.43–1.53 (m, 4H, 2CH_2), 1.91 (m, 2H, CH_2), 2.33–2.36 (m, 4H, 2CH_2), 2.37 (t, 2H, J = 7.1 Hz, CH_2), 3.85 (s, 3H, OCH_3), 4.11 (t, 2H, J = 7.1 Hz, CH_2), 7.04 (s, 1H, ArH), 7.42 (s, 1H, ArH), 7.96 (s, 1H, ArH), 8.00 (s, 1H, NH). LC–MS ($APCI^+$): *m/z* calcd for $C_{17}H_{23}N_3O_3$ 318 [$(M+H)^+$].

*5.1.5. General procedure for 4-chloroquinazoline derivatives (**16**–**18**)*

A solution of quinazolin-4-one derivatives (**13**–**15**) (15.0 mmol) in phosphorus oxychloride (30 mL) was refluxed for 6 h. After removal of the solvent, the residue was dissolved in ice-water (50 mL) and the mixture was neutralized by 10% K_2CO_3 solution. The precipitate was collected by filtration and dissolved in CH_2Cl_2 (100 mL). The organic layer was washed with a 1 M solution of K_2CO_3, brine and dried over $CaCl_2$, and the solvent was removed under reduced pressure.

*5.1.5.1. 4-Chloro-6-methoxy-7-piperidinoethoxyquinazoline (**16**).* Beige solid (71%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 132–134 °C. IR (cm^{-1}): 2935 (piperidine), 1087 (C–Cl). ^1H NMR (DMSO-d_6): δ (ppm) 1.44–1.50 (m, 2H, CH_2), 1.59–1.67 (m, 4H, 2CH_2), 2.55–2.58 (m, 4H, 2CH_2), 2.93 (t, 2H, J = 6.0 Hz, CH_2), 4.06 (s, 3H, OCH_3), 4.35 (t, 2H, J = 6.0 Hz, CH_2), 7.35 (s, 1H, ArH), 7.39 (s, 1H, ArH), 8.87 (s, 1H, ArH). LC–MS ($APCI^+$): *m/z* calcd for $C_{16}H_{20}ClN_3O_2$ 321 [$(M+H)^+$ for ^{35}Cl], 323 [$(M+2+H)^+$ for ^{37}Cl].

*5.1.5.2. 4-Chloro-6-methoxy-7-pyrrolidinoethoxyquinazoline (**17**).* Beige solid (49%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 155–157 °C. IR (cm^{-1}): 2950 (pyrrolidine), 1090 (C–Cl). ^1H NMR (DMSO-d_6): δ (ppm) 1.70 (m, 4H, 2CH_2), 2.51–2.57 (m, 4H, 2CH_2), 2.90 (t, 2H, J = 6.0 Hz, CH_2), 4.00 (s, 3H, OCH_3), 4.32 (t, 2H, J = 6.0 Hz, CH_2), 7.38 (s, 1H, ArH), 7.47 (s, 1H, ArH), 8.87 (s, 1H, ArH). LC–MS ($APCI^+$): *m/z* calcd for $C_{15}H_{18}ClN_3O_2$ 307 [$(M + H)^+$ for ^{35}Cl], 309 [$(M+2+H)^+$ for ^{37}Cl].

*5.1.5.3. 4-Chloro-6-methoxy-7-piperidinopropoxyquinazoline (**18**).* Beige solid (70%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 151–153 °C. IR (cm^{-1}): 2935 (piperidine), 1086 (C–Cl). ^1H NMR (DMSO-d_6): δ (ppm) 1.35–1.42 (m, 2H, CH_2), 1.44–1.55 (m, 4H, 2CH_2), 1.92 (m, 2H, CH_2), 2.30–2.34 (m, 4H, 2 CH_2), 2.41 (t, 2H, J = 6.3 Hz, CH_2), 4.00 (s, 3H, OCH_3), 4.26 (t, 2H, J = 6.2 Hz, CH_2), 7.40 (s, 1H, ArH), 7.46 (s, 1H, ArH), 8.88 (s, 1H, ArH). LC-MS ($APCI^+$): *m/z* calcd for $C_{17}H_{22}ClN_3O_2$ 335 [$(M+H)^+$ for ^{35}Cl], 337 [$(M+2+H)^+$ for ^{37}Cl].

*5.1.6. General procedure for urea derivatives (**21**–**23**)*

To a stirred solution of aminophenol derivatives (0.10 g, 0.69 mmol) in 5 mL of pyridine was added 3-bromophenylisocyanate (0.69 mmol). After 1 h at room temperature under nitrogen atmosphere, the solvent was removed under reduced pressure. The residue was washed by CH_2Cl_2 and recrystallized.

*5.1.6.1. N-(3-Bromo-phenyl)-N′-(4-hydroxyphenyl)urea (**21**).* Crystallization from acetonitrile gave pure **21** as a white solid (71%). TLC: R_f 0.7 (CH_2Cl_2:MeOH 9:1 v/v). Mp: 227–229 °C. IR (cm^{-1}): 3301 (OH), 1635 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 6.68 (m, 2H, ArH), 7.08–7.30 (m, 5H, ArH), 7.82 (m, 1H, ArH), 8.38 (s, 1H, NH), 8.71 (s, 1H, NH), 9.10 (s, 1H, OH). LC–MS ($APCI^+$): *m/z* calcd for $C_{13}H_{11}BrN_2O_2$ 307 [$(M+H)^+$ for ^{79}Br], 309 [$(M+H)^+$ for ^{81}Br].

*5.1.6.2. N-(3-Bromo-phenyl)-N′-(3-methyl-4-hydroxyphenyl)urea (**22**).* Crystallization from acetonitrile gave pure **22** as a beige solid (89%). Mp: 204–206 °C. TLC: R_f 0.6 (CH_2Cl_2:MeOH 9:1 v/v). IR (cm^{-1}): 3288 (OH), 1635 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 2.15 (s, 3H, CH_3), 6.55 (dd, 1H, J = 2.7 Hz and J = 9.0 Hz, ArH), 6.61 (d, 1H, J = 2.7 Hz, ArH), 7.08–7.33 (m, 4H, ArH), 7.75 (s, 1H, NH), 7,89 (m, 1H, ArH), 8.95 (s, 1H, NH), 9.12 (s, 1H, OH). LC–MS ($APCI^+$): *m/z* calcd for $C_{14}H_{13}BrN_2O_2$ 321 [$(M+H)^+$ for ^{79}Br], 323 [$(M+H)^+$ for ^{81}Br].

*5.1.6.3. N-(3-Bromo-phenyl)-N′-(3-chloro-4-hydroxyphenyl)urea (**23**).* Crystallization from acetonitrile gave pure **23** as a beige solid (61%). Mp: 178–180 °C. TLC: R_f 0.6 (CH_2Cl_2:MeOH 9:1 v/v). IR (cm^{-1}): 3283 (OH), 1643 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 6.72 (dd, 1H, J = 2.4 Hz and J = 9.1 Hz, ArH), 6.84 (d, 1H, J = 2.4 Hz, ArH), 7.09–7.32 (m, 3H, ArH), 7.77 (m, 2H, ArH), 8.07 (s, 1H, NH), 9.34 (s, 1H, NH), 9.64 (s, 1H, OH). LC–MS ($APCI^+$): *m/z* calcd for

$C_{13}H_{10}BrClN_2O_2$ 340 [$(M+H)^+$ for $^{35}Cl/^{79}Br$], 342 [$(M+H)^+$ for $^{35}Cl/^{81}Br$], 342 [$(M+H)^+$ for $^{37}Cl/^{79}Br$], 344 [$(M+H)^+$ for $^{37}Cl/^{81}Br$].

*5.1.7. General procedure for final products (**24**–**37**)*

To a stirred solution of chloro derivatives (**16**–**20**) (0.10 g, 0.32 mmol) and tetrabutylammonium bromide in 10 mL of a mixture of a 20% solution of NaOH and 2-butanone (1:2) were added urea derivatives (0.32 mmol) (**21**–**23**). After 1 h at room temperature, the reaction was quenched by water, and then the aqueous solution was extracted with EtOAc (3 × 10 mL), washed with a 1 M solution of NaOH, and dried over $MgSO_4$. The solvent was removed under reduced pressure.

*5.1.7.1. N-(3-Bromo-phenyl)-N′-{4-[(6-butoxy-7-diethylaminoethoxyquinazolin-4-yl)oxy]phenyl-}urea (**24**).* Compound **24** was synthesized from **19** and **21** using the general procedure described above to afford as a white solid collected by filtration and washed with methanol (22%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 187–189 °C. IR (cm^{-1}): 1650 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 0.98–1.05 (m, 9H, $3CH_3$), 1.48–1.53 (m, 2H, CH_2), 1.82–1.85 (m, 2H, CH_2), 2.62–2.65 (m, 4H, $2CH_2$), 2.90–2.95 (m, 2H, CH_2), 4.10–4.30 (m, 4H, 2 CH_2), 7.12 (m, 1H, ArH), 7.25 (m, 3H, 3 ArH), 7.40 (m, 2H, 2 ArH), 7.52 (m, 3H, 3 ArH), 7.91 (s, 1H, ArH), 8.52 (s, 1H, ArH), 8.88 (s, 1H, NH), 8.99 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 14.24, 19.01, 19.25, 31.22, 47.84, 51.09, 68.99, 101.92, 108.02, 110.26, 117.32, 120.12, 120.35, 122.58, 123.47, 124.38, 124.62, 131.05, 131.27, 135.57, 143.59, 148.45, 149.85, 150.04, 152.70, 153.22, 155.26, 165.47. LC–MS (APCI$^+$): *m/z* calcd for $C_{31}H_{36}BrN_5O_4$ 622 [$(M+H)^+$ for ^{79}Br], 624 [$(M+H)^+$ for ^{81}Br]. HRMS (ESI $(M+H)^+$ *m/z*) calcd for $C_{31}H_{36}BrN_5O_4$ 622.2023 found 622.1999.

*5.1.7.2. N-(3-Bromo-phenyl)-N′-{3-methyl-4-[(6-butoxy-7-diethylaminoethoxyquinazolin-4-yl)oxy]-phenyl}urea (**25**).* Compound **25** was synthesized from **19** and **22** using the general procedure described above to afford as a beige solid collected by crystallization from acetonitrile (52%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 169–171 °C. IR (cm^{-1}): 1699 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 0.98–1.05 (m, 9H, 3 CH_3), 1.5 (m, 2H, CH_2), 1.75 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 2.63 (m, 4H, $2CH_2$), 2.91 (m, 2H, CH_2), 4.10–4.15 (m, 4H, 2 CH_2), 6.99–7.15 (m, 3H, 3 ArH), 7.15–7.38 (m, 3H, 3 ArH), 7.51 (s, 1H, ArH), 7.82 (m, 1H, ArH), 7.91 (s, 1H, ArH), 8.20 (s, 1H, NH), 8.52 (s, 1H, ArH), 9.48 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.01, 14.19, 18.44, 19.22, 31.14, 47.75, 50.89, 68.67, 101.81, 107.78, 110.21, 117.16, 120.08, 120.56, 122.23, 123.04, 124.01, 124.59, 130.49, 131.20, 135.07, 142.14, 148.09, 149.08, 149.98, 152.69, 153.17, 155.42, 165.42. LC–MS (APCI$^+$): *m/z* calcd for $C_{32}H_{38}BrN_5O_4$ 636 [$(M+H)^+$ for ^{79}Br], 638 [$(M+H)^+$ for ^{81}Br]. HRMS (ESI $(M+H)^+$ *m/z*) calcd for $C_{32}H_{38}BrN_5O_4$ 636.2179 found 636.2157.

*5.1.7.3. N-(3-Bromo-phenyl)-N′-{4-[(6-methoxy-7-diethylaminoethoxyquinazolin-4-yl)oxy]-phenyl}urea (**26**).* Compound **26** was synthesized from **20** and **21** using the general procedure described above to afford as a white solid collected by filtration and washed with MeOH (15%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 144–146 °C. IR (cm^{-1}): 1699 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.02 (m, 6H, CH_3), 2.69 (m, 4H, CH_2), 2.98 (m, 2H, CH_2), 3.98 (s, 3H, OCH_3), 4.30 (m, 2H, CH_2), 7.11–7.62 (m, 9H, ArH), 7.88 (s, 1H, ArH), 8.51 (s, 1H, ArH), 8.92 (s, 1H, NH), 9.00 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.31, 47.48, 51.42, 56.48, 97.74, 101.18, 107.82, 110.07, 118.22, 121.85, 122.03, 122.28, 122.74, 123.27, 123.52, 125.18, 131.27, 133.92, 142.02, 148.13, 149.32, 151.33, 152.41, 152.49, 156.01, 165.32. LC–MS (APCI$^+$): *m/z* calcd for $C_{28}H_{30}BrN_5O_4$ 580 [$(M + H)^+$ for ^{79}Br], 582 [$(M+H)^+$ for ^{81}Br]. HRMS (ESI $(M+H)^+$ *m/z*) calcd for $C_{28}H_{30}BrN_5O_4$ 580.1553 found 580.1514.

*5.1.7.4. N-(3-Bromo-phenyl)-N′-{4-3-methyl-[(6-methoxy-7-diethylaminoethoxyquinazolin-4-yl)oxy]-phenyl}urea (**27**).* Compound **27** was synthesized from **20** and **22** using the general procedure described above to afford as a white solid collected by filtration and washed with CH_2Cl_2 (28%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 128–130 °C. IR (cm^{-1}): 1640 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.00 (m, 6H, 2 CH_3), 2.31 (s, 3H, CH_3), 2.62 (m, 4H, CH_2), 2.92 (m, 2H, CH_2), 3.98 (s, 3H, OCH_3), 4.26 (m, 2H, CH_2), 7.05–7.42 (m, 6H, ArH), 7.52 (s, 1H, ArH), 7.80–7.90 (m, 2H, ArH), 8.53 (s, 1H, ArH), 8.59 (s, 1H, NH), 10.05 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 10.19, 18.81, 47.61, 50.11, 56.61, 66.08, 101.43, 108.13, 110.45, 116.88, 119.92, 120.25, 122.14, 122.70, 123.92, 124.31, 130.32, 131,12, 135.30, 142.41, 147.79, 149.01, 150.44, 152.85, 153.41, 154.68, 165.50. LC–MS (APCI$^+$): *m/z* calcd for $C_{29}H_{34}BrN_5O_4$ 594 [$(M+H)^+$ for ^{79}Br], 596 [$(M+H)^+$ for ^{81}Br]. HRMS (ESI $(M+H)^+$ *m/z*) calcd for $C_{29}H_{34}BrN_5O_4$ 594.1710 found 594.1686.

*5.1.7.5. N-(3-Bromo-phenyl)-N′-{4-3-chloro-[(6-methoxy-7-diethylaminoethoxyquinazolin-4-yl)oxy]-phenyl}urea (**28**).* Compound **28** was synthesized from **20** and **23** using the general procedure described above to afford as a white solid collected by filtration and washed with MeOH (13%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 157–159 °C. IR (cm^{-1}): 1648 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.00 (t, 6H, J = 6.8 Hz, 2 CH_3), 2.59 (m, 4H, CH_2), 2.88 (t, 2H, J = 5.9 Hz, CH_2), 3.95 (s, 3H, OCH_3), 4.13 (t, 2H, J = 5.9 Hz, CH_2), 7.28–7.58 (m, 7H, ArH), 7.90 (m, 1H, ArH), 8.19 (m, 1H, ArH), 8.47 (s, 1H, NH), 8.59 (s, 1H, ArH), 9.61 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.32, 47.45, 51.36, 56.51, 68.08, 101.17, 107.80, 109.97, 117.43, 120.84, 122.00, 122.26, 122.80, 123.24, 123.58, 125.10, 131.26, 133.80, 141.58, 147.75, 149.33, 150.65, 152.55, 152.57, 155.53, 165.10. LC–MS (APCI$^+$): *m/z* calcd for $C_{28}H_{29}BrClN_5O_4$ 614 [$(M + H)^+$ for $^{35}Cl/^{79}Br$], 616 [$(M+H)^+$ for $^{35}Cl/^{81}Br$], 616 [$(M+H)^+$ for $^{37}Cl/^{79}Br$], 618 [$(M+H)^+$ for $^{37}Cl/^{81}Br$]. HRMS (ESI $(MH)^+$ *m/z*) calcd for $C_{28}H_{29}BrClN_5O_4$ 616.1134 found 616.1090.

*5.1.7.6. N-(3-Bromo-phenyl)-N′-{4-[(6-methoxy-7-piperidinoethoxyquinazolin-4-yl)oxy]-phenyl}urea hydrochloride (**29**).* Compound **29** was synthesized from **16** and **21** using the general procedure described above to afford as a pink solid collected by precipitation in a mixture of diethyl ether/hydrochloric acid 6 N in isopropanol (15%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 222–224 °C. IR (cm^{-1}): 2380 (NH^+); 1707 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 0.92–1.06 (m, 2H, CH_2), 1.28–1.41 (m, 4H, 2 CH_2), 1.69–1.75 (m, 4H, $2CH_2$), 3.59–3.62 (m, 4H, 2 CH_2), 4.02 (s, 3H, OCH_3), 7.13–7.37 (m, 5H, 5 ArH), 7.50–758 (m, 3H, 3ArH), 7.66 (s, 1H, ArH), 7.86 (s, 1H, ArH), 8.67 (s, 1H, ArH), 9.44 (s, 1H, NH), 9.53 (s, 1H, NH), 10.51 (s, 1H, NH^+). ^{13}C NMR (DMSO-d_6): δ (ppm) 24.32, 26.17, 54.62, 56.47, 57.58, 68.12, 102.17, 107.92, 111.10, 116.18, 120.12, 120.72, 122.34, 123.22, 124.12, 124.67, 130.72, 131.20, 135.10, 142.10, 148.18, 149.22, 151.62, 152.73, 153.32, 155.08, 165.50. LC–MS (APCI$^+$): *m/z* calcd for $C_{29}H_{30}BrN_5O_4$ 592 [$(M+H)^+$ for ^{79}Br], 594 [$(M+2+H)^+$ for ^{81}Br]. HRMS (ESI $(M+H)^+$ *m/z*) calcd for $C_{29}H_{30}BrN_5O_4$ 592.1553 found 592.1532.

*5.1.7.7. N-(3-Bromo-phenyl)-N′-{3-methyl-4-[(6-methoxy-7-piperidinoethoxyquinazolin-4-yl)oxy]-phenyl}urea (**30**).* Compound **30** was synthesized from **16** and **22** using the general procedure described above to afford as a white solid collected by filtration and washed with MeOH (12%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 206–208 °C. IR (cm^{-1}): 2935 (piperidine), 1643 (C=O). ^1H NMR (DMSO-d_6): δ (ppm) 1.34–1.42 (m, 2H, CH_2), 1.45–1.56 (m, 4H, 2 CH_2), 2.28 (s, 3H, CH_3), 2.46–2.50 (m, 4H, 2 CH_2), 2.75 (t, 2H, J = 6.6 Hz, CH_2), 3.97 (s, 3H, OCH_3), 4.29 (t, 2H, J = 6.6 Hz, CH_2), 7.01–7.16 (m, 3H, 3ArH), 7.21–7.32 (m, 2H,

2ArH), 7.41 (s, 1H, ArH), 7.54 (s, 1H, ArH), 7.81–7.91 (m, 2H, 2 ArH), 8.10 (s, 1H, NH), 8.53 (s, 1H, ArH), 9.24 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 18.29, 24.37, 26.02, 54.88, 56.46, 57.50, 67.36, 101.23, 107.97, 110.12, 117,26, 120.08, 120.67, 122.22, 123.24, 124.01, 124.65, 130.66, 131.18, 135.00, 142.06, 148.20, 149.21, 150.55, 152.72, 153.10, 155.39, 165.42. LC–MS (APCI$^+$): *m/z* calcd for $C_{30}H_{32}BrN_5O_4$ 606 [(M+H)$^+$ for ^{79}Br], 608 [(M+2+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ *m/z*) calcd for $C_{30}H_{32}BrN_5O_4$ 606.1710 found 606.1686.

5.1.7.8. N-(3-Bromo-phenyl)-N′-{3-chloro-4-[(6-methoxy-7-piperidinoethoxyquinazolin-4-yl)oxy]-phenyl}urea (***31***).
Compound **31** was synthesized from **16** and **23** using the general procedure described above to afford as a white solid collected by crystallization from acetonitrile (13%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 204–206 °C. IR (cm^{-1}): 2935 (piperidine), 1649 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.32–1.42 (m, 2H, CH_2), 1.44–1.58 (m, 4H, $2CH_2$), 2.49 (m, 4H, $2CH_2$), 2.74 (t, 2H, J = 6.0 Hz, CH_2), 3.97 (s, 3H, OCH_3), 4.29 (t, 2H, J = 6.0 Hz, CH_2), 7.29–7.37 (m, 3H, 3 ArH), 7.41 (m, 1H, ArH), 7.53–7.57 (m, 2H, 2ArH), 7.84 (dd, 1H, J = 9.6, 2.4 Hz, ArH), 8.16 (d, 1H, J = 9.6 Hz, ArH), 8.43 (s, 1H, ArH), 8.51 (s, 1H, ArH), 8.56 (s, 1H, NH), 9.58 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 24.30, 26.11, 54.58, 56.52, 57.65, 68.07, 101.92, 106.99, 11.12, 116.20, 120.32, 120.41, 122.40, 123.18, 124.10, 124.70, 131.81, 132.21, 135.12, 142.15, 148.27, 149.21, 151.70, 152.71, 153.08, 155.68, 165.13. LC–MS (APCI$^+$): *m/z* calcd for $C_{29}H_{29}BrClN_5O_4$ 626 [(M+H)$^+$ for ^{35}Cl/^{79}Br], 628 [(M+H)$^+$ for ^{35}Cl/^{81}Br], 628 [(M+H)$^+$ for ^{37}Cl/^{79}Br], 630 [(M+H)$^+$ for ^{37}Cl/^{81}Br]. HRMS (ESI (M+H)$^+$ *m/z*) calcd for $C_{29}H_{29}BrClN_5O_4$ 628.2714 found 628.2783.

5.1.7.9. N-(3-Bromo-phenyl)-N′-{4-[(6-methoxy-7-pyrrolidinoethoxyquinazolin-4-yl)oxy]-phenyl}urea (***32***).
Compound **32** was synthesized from **17** and **21** using the general procedure described above to afford as a gray solid collected by filtration and washed with MeOH (24%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 179–181 °C. IR (cm^{-1}): 2950 (pyrrolidine), 1503 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.64–1.82 (m, 4H, $2CH_2$), 2.54–2.62 (m, 4H, $2CH_2$), 2.93 (m, 2H, CH_2), 3.98 (s, 3H, OCH_3), 4.30 (m, 2H, CH_2), 7.12–7.15 (m, 2H, 2ArH), 7.20–7.23 (m, 2H, 2ArH), 7.35 (m, 1H, ArH), 7.40 (m, 1H, ArH), 7.56 (m, 3H, 3ArH), 7.87 (s, 1H, ArH), 8.54 (s, 1H, ArH), 8.94 (s, 1H, NH), 8.99 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 23.58, 54.35, 54.49, 56.46, 68.18, 101.24, 107.81, 110.14, 117.48, 119.91, 119.92, 120.85, 122.15, 122.81, 122.82, 124.70, 131.09, 137.44, 141.98, 147.35, 149.16, 150.51, 152.68, 152.94, 155.27, 165.40. LC–MS (APCI$^+$): *m/z* calcd for $C_{28}H_{28}BrN_5O_4$ 578 [(M+H)$^+$ for ^{79}Br], 580 [(M+2+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ *m/z*) calcd for $C_{28}H_{28}BrN_5O_4$ 578.1397 found 578.1372.

5.1.7.10. N-(3-Bromo-phenyl)-N′-{3-methyl-4-[(6-methoxy-7-pyrrolidinoethoxyquinazolin-4-yl)oxy]-phenyl}urea (***33***).
Compound **33** was synthesized from **17** and **22** using the general procedure described above to afford as a white solid collected by filtration and washed with MeOH (25%). TLC: R_f 0.6 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 193–197 °C. IR (cm^{-1}): 2950 (pyrrolidine), 1651 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.70–1.72 (m, 4H, $2CH_2$), 2.28 (s, 3H, CH_3), 2.51–2.55 (m, 4H, 2 CH_2), 2.90 (t, 2H, J = 6.1 Hz, CH_2), 3.99 (s, 3H, OCH_3), 4.30 (t, 2H, J = 6.1 Hz, CH_2), 7.08–7.35 (m, 6H, 6ArH), 7.41 (s, 1H, ArH), 7.56 (s, 1H, ArH), 7.81–7.84 (m, 1H, ArH), 8.11 (s, 1H, NH), 8.58 (s, 1H, ArH), 9.30 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 18.30, 23.62, 54.43, 54.49, 56.44, 68.37, 101.19, 107.77, 110.11, 117.26, 120.08, 120.68, 122.22, 123.25, 124.00, 124.63, 130.65, 131.15, 135.00, 142.07, 148.20, 149.19, 150.52, 152.70, 153.11, 155.34, 165.41. LC–MS (APCI$^+$): *m/z* calcd for $C_{29}H_{30}BrN_5O_4$ 592 [(M+H)$^+$ for ^{79}Br], 594 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ *m/z*) calcd for $C_{29}H_{30}BrN_5O_4$ 592.1553 found 592.1534.

5.1.7.11. N-(3-Bromo-phenyl)-N′-{3-chloro-4-[(6-methoxy-7-pyrrolidinoethoxyquinazolin-4-yl)oxy]-phenyl}urea (***34***).
Compound **34** was synthesized from **17** and **23** using the general procedure described above to afford as a beige solid collected by filtration and washed with MeOH (9%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 187–189 °C. IR (cm^{-1}): 2950 (pyrrolidine), 1645 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.70 (m, 4H, 2 CH_2), 2.60–2.65 (m, 4H, $2CH_2$), 2.90 (t, 2H, J = 6.7 Hz, CH_2), 4.00 (s, 3H, OCH_3), 4.32 (t, 2H, J = 6.7 Hz, CH_2), 7.12–7.48 (m, 5H, 5ArH), 7.55–7.62 (m, 2H, 2 ArH), 7.91 (s, 1H, ArH), 8.12–8.20 (m, 1H, ArH), 8.48 (s, 1H, NH), 8.60 (s, 1H, ArH), 9.61 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 23.58, 54.37, 54.42, 56.36, 68.37, 101.12, 107.84, 111.02, 117.52, 120.05, 120.08, 120.92, 122.33, 122.80, 122.86, 124.72, 131.13, 137.54, 141.99, 147.33, 148.98, 150.37, 152.71, 152.91, 155.31, 165.37. LC–MS (APCI$^+$): *m/z* calcd for $C_{28}H_{27}BrClN_5O_4$ 612 [(M+H)$^+$ for ^{35}Cl/^{79}Br], 614 [(M+H)$^+$ for ^{35}Cl/^{81}Br], 614 [(M+H)$^+$ for ^{37}Cl/^{79}Br], 616 [(M+H)$^+$ for ^{37}Cl/^{81}Br]. HRMS (ESI (M+H)$^+$ *m/z*) calcd for $C_{28}H_{27}BrClN_5O_4$ 614.0978 found 614.0955.

5.1.7.12. N-(3-Bromo-phenyl)-N′-{4-[(6-methoxy-7-piperidinopropoxyquinazolin-4-yl)oxy]-phenyl}urea (***35***).
Compound **35** was synthesized from **18** and **21** using the general procedure described above to afford as a white solid collected by crystallization from acetonitrile (26%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 179–181 °C. IR (cm^{-1}): 2935 (piperidine), 1712 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.68–1.73 (m, 4H, $2CH_2$), 2.15–2.25 (m, 4H, $2CH_2$), 2.75–2.78 (m, 2H, CH_2), 2.93–2.99 (m, 2H, CH_2), 3.05–3.17 (m, 2H, CH_2), 4.00 (s, 3H, OCH_3), 4.15–4.21 (m, 2H, CH_2), 7.13–7.32 (m, 6H, 6ArH), 7.62 (m, 2H, 2ArH), 7.68 (s, 1H, ArH), 7.75 (s, 1H, ArH), 8.27 (s, 1H, NH), 8.37 (s, 1H, ArH), 9.30 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 24.62, 26.07, 26.42, 54.77, 56.02, 56.50, 67.48, 100.89, 107.52, 110.81, 116.38, 120.87, 121.03, 122.91, 122.99, 123.20, 123.66, 125.13, 125.18, 132.03, 133.95, 142.64, 147.71, 149.30, 151.69, 153.03, 157.60, 165.37. LC–MS (APCI$^+$): *m/z* calcd for $C_{30}H_{32}BrN_5O_4$ 606 [(M+H)$^+$ for ^{79}Br], 608 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ *m/z*) calcd for $C_{30}H_{32}BrN_5O_4$ 606.1701 found 606.17017.

5.1.7.13. N-(3-Bromo-phenyl)-N′-{3-methyl-4-[(6-methoxy-7-piperidinopropoxyquinazolin-4-yl)oxy]-phenyl}urea hydrochloride (***36***). Compound **36** was synthesized from **18** and **22** using the general procedure described above to afford as a beige solid collected by precipitation in a mixture of diethyl ether/hydrochloric acid 6 N in isopropanol (18%). TLC: R_f 0.5 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp > 250 °C. IR (cm^{-1}): 2510 (NH^+); 1705 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.70–1.80 (m, 4H, $2CH_2$), 2.22–2.31 (m, 4H, 2 CH_2), 2.94–2.98 (m, 2H, CH_2), 3.10–3.16 (m, 2H, CH_2), 3.21–3.29 (m, 2H, CH_2), 4.01 (s, 3H, OCH_3), 4.27–4.30 (m, 2H, CH_2), 4,33 (s, 3H, CH_3), 7.03–7.51 (m, 5H, 5ArH), 7.47 (m, 2H, 2ArH), 7.61 (s, 1H, ArH), 7.88 (s, 1H, ArH), 8.54 (s, 1H, NH), 8.69 (s, 1H, ArH), 9.99 (s, 1H, NH), 10.17 (s, 1H, NH^+). ^{13}C NMR (DMSO-d_6): δ (ppm) 24.54, 27.01, 27.05, 53.98, 56.32, 56.59, 68.50, 101.32, 107.57, 110.83, 114.03, 120.02, 121.38, 122.47, 122.91, 123.05, 123.47, 125.32, 125.87, 132.04, 133.48, 143.05, 149.15, 149.73, 152.99, 153.05, 158.02, 165.48. LC–MS (APCI$^+$): *m/z* calcd for $C_{31}H_{34}BrN_5O_4$ 620 [(M + H)$^+$ for ^{79}Br], 622 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ *m/z*) calcd for $C_{31}H_{34}BrN_5O_4$ 620.1866 found 620.1842.

5.1.7.14. N-(3-Bromo-phenyl)-N′-{3-chloro-4-[(6-methoxy-7-piperidinopropoxyquinazolin-4-yl)oxy]-phenyl}urea (***37***).
Compound **37** was synthesized from **18** and **23** using the general procedure described above to afford as a white solid collected by

filtration and washed with MeOH (21%). TLC: R_f 0.7 (CH_2Cl_2:MeOH(NH_3) 9:1 v/v). Mp: 198–200 °C. IR (cm^{-1}): 2935 (piperidine), 1708 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.69–1.78 (m, 4H, $2CH_2$), 2.15–2.28 (m, 4H, $2CH_2$), 2.68–2.72 (m, 2H, CH_2), 3.01–3.12 (m, 2H, CH_2), 3.15–3.23 (m, 2H, CH_2), 3.99 (s, 3H, OCH_3), 4.20–4.25 (m, 2H, CH_2), 7.10–7.15 (m, 5H, 5 ArH), 7.36–7.42 (m, 2H, 2 ArH), 7.56 (s, 1H, ArH), 7.78 (s, 1H, ArH), 8.34 (s, 1H, NH), 8.43 (s, 1H, ArH), 9.27 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 24.59, 26.06, 26.46, 54.56, 55.44, 56.47, 67.69, 101.15, 107.67, 109.93, 117.43, 120.85, 121.98, 122.27, 122.77, 123.21, 123.56, 125.05, 125.10, 131.25, 133.80, 141.57, 147.73, 149.33, 150.62, 152.55, 155.59, 165.09. LC–MS (APCI$^+$): *m/z* calcd for $C_{30}H_{31}BrClN_5O_4$ 640 [(M+H)$^+$ for ^{35}Cl/^{79}Br], 642 [(M+H)$^+$ for ^{35}Cl/^{81}Br], 642 [(M+H)$^+$ for ^{37}Cl/^{79}Br], 644 [(M+H)$^+$ for ^{37}Cl/^{81}Br]. HRMS (ESI (M+H)$^+$ *m/z*) calcd for $C_{30}H_{31}BrClN_5O_4$ 640.1299 found 640.1304.

5.2. Biological assays and methods

5.2.1. In vitro kinase assays

Kinase assays were performed in 96-well plates (Multiscreen Durapore. Millipore) using [γ-^{32}P]ATP (Perkin Elmer) and the synthetic polymer poly(Glu4/Tyr) (Sigma Chemicals) as a phosphoacceptor substrate. Tested compounds were dissolved in DMSO. The final concentration of DMSO in assay solutions was 0.1%, which was shown to have no effect on kinase activity.

5.2.2. EGFR tyrosine kinase activity

20 ng of EGFR (purified from human carcinoma A431 cells, Sigma Chemicals) were incubated for 1 h at 28 °C using various concentrations of tested compounds in kinase buffer (HEPES 50 mM pH 7.5, BSA 0.1 mg/mL, $MnCl_2$ 10 mM $MgCl_2$ 5 mM, Na_3VO_4 100 μM, DTT 0.5 mM, poly(Glu4/Tyr) 250 μg/mL, ATP 5 μM [γ-^{32}P] ATP 0.5 μCi).

5.2.3. VEGFR-2 tyrosine kinase activity

10 ng of VEGFR-2 (Recombinant Human Protein, Invitrogen) were incubated for 1 h at 28 °C using various concentrations of tested compounds in kinase buffer (Tris 50 mM pH 7.5, BSA 25 μg/mL, $MnCl_2$ 1.5 mM, $MgCl_2$ 10 mM, DTT 2.5 mM, Na_3VO_4 100 μM, ß-glycerophosphate 5 mM, poly(Glu4/Tyr) 250 μg/mL, ATP 5 μM [γ-^{32}P]ATP 0.5 μCi).

The reaction was stopped by adding 20 μL of trichloroacetic acid, 100%. Wells were washed 10 times with trichloroacetic acid, 10%. Plates were counted in a Top Count (Perkin Elmer) for 1 min per well.

5.2.4. Cells and materials

Human prostate cancer cells PC3, breast cancer cells MCF7, colon cancer cell line HT29 and human fetal lung fibroblast cells MRC5 were obtained from European Collection of Cell Cultures. There were cultured, at 37 °C in a CO_2 incubator, respectively in RPMI-1640, MEM and DMEM + Glutamax-I medium supplemented with 10% fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 μg/mL). FBS, medium, penicillin-streptomycin, and other agents used in cell culture studies were purchased from Invitrogen. Human umbilical vein endothelial cells (HUVEC) and ECGM medium + SupplementMix were purchased from Promocell.

5.2.6. Cell proliferation

Briefly, cells were plated at a density of 3×10^3 cells/well in 96-well plates for 24 h. Then the medium was removed, and cells were treated with either DMSO as a control or various concentrations of compounds. The final concentration of DMSO in the medium was <0.1% (v/v). After the cells were incubated for 72 h, cell growth was estimated by colorimetric MTS test.

5.2.7. In vitro endothelial tube formation assay

Matrigel® was thawed overnight at 4 °C in an ice bath, and then 50 μL of solution was used to coat 96-well plates. The plates were then incubated at 37 °C for 60 min to ensure complete gelation of the matrix. HUVEC cells were then seeded into 96-well plates at a cell density of 20 000 cells/well and allowed to incubate for 10 h at 37 °C. Cells were treated with different concentrations of compound (0.1, 1, 5 and 10 μM). The morphological changes of the cells and tubes formed were observed under inverted microscope and photographed with Moticam®.

5.2.8. Cell invasion assay

In vitro invasion assay was carried out using invasion chamber, coated with Matrigel® matrix, with 6.4 mm diameter PET membrane (8 micron pore size, BD Biocat). The bottom chambers were filled with ECGM medium with 20 ng/mL VEGF and the top chambers were seeded with ECGM medium (without growth factors) and HUVEC (1.10^5 cells per well). The top chamber contained vehicle or compound at various concentrations (0.01, 0.1 or 1 μM). Cells were allowed to migrate for 24 h. Non migrated cells were scraped with a cotton swab, and migrated cells were fixed with 100% methanol and stained with 0.05% crystal violet. The number of HUVEC that penetrated the membrane was quantified by manual counting and photographed with Moticam®. The percentage of migrated cells was expressed on the basis of vehicle control wells.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.007.

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