Recent Advances in Antiangiogenic Agents with VEGFR as Target

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Abstract: Angiogenesis is required for invasive tumor growth and metastasis and constitutes an important point in the control of cancer progression. Its inhibition may be a valuable approach to cancer therapy. Antiangiogenic agents are designed to attack the tumor vasculature and cut off the tumor's supply of nutrients. Systemic blockade of angiogenesis has been recently approved for the treatment of several types of human cancers. Antiangiogenic therapy presents various advantages as compared to conventional treatment. Vascular endothelial growth factor (VEGF) is considered to be one of the most important regulators of angiogenesis and a key target in anticancer treatment. VEGF binding to its receptor (VEGFR) leads to cell proliferation and new vascular formation by tyrosine kinase (TK) pathway. VEGF/VEGFR pathway is becoming attractive target for anticancer drug design. It is believed to be important in the control of angiogenesis. Antiangiogenic therapy based on inhibition of VEGFR was reported to be powerful clinical strategies. In this review, the authors describe the existing literature regarding VEGFR inhibitors in the last few years. We attempt to cover all essential publications on the medicinal chemistry in terms of chemical structure, pharmacological profile and structure-activity relationships.

Keywords: Antiangiogenic agents, angiogenesis, VEGFR, tyrosine kinase, anticancer.

1. INTRODUCTION

Angiogenesis is the process of new capillary formation from pre-existing blood vessels [1]. It is believed that angiogenesis is regulated by two counter-balancing systems: angiogenic stimulators and angiogenic inhibitors. The balance between angiogenesis inducers and inhibitors in the microenvironment controls the rate of new blood vessel formation [2]. The disruption of the balance has an essential role in the development of a variety of diseases such as retinopathies, arthritis, endometriosis and cancer [3]. Angiogenesis plays an important role in the growth and spread of cancer. As early as the 1970s, Folkman provided strong evidence linking tumor growth and metastases with angiogenesis and suggested inhibiting new blood vessel formation as a way to fight cancer [4].

Angiogenesis is a key event of tumor progression and metastasis and hence a target for cancer chemotherapy. Antiangiogenic agents have been studied in the laboratory for many years. A great advantage of such drugs is that they are likely to be less toxic than the existing chemotherapy agents [5]. Antiangiogenic therapy offers a number of potential benefits including lack of drug resistance for some agents, synergistic interaction with other modalities, lack of significant toxicity compared with conventional agents, and a potent antitumor effect [6]. Angiogenesis inhibitors seem to help some chemotherapy drugs and radiation therapy work more effectively when given in combination [7].

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VEGFs and respective family of receptor tyrosine kinases (RTKs) are key proteins modulating angiogenesis. The binding of VEGF ligands to VEGFRs in the cell membrane induces dimerization and activation of the later, initiating intracellular signaling cascades that result in proliferation, survival, migration and increased permeability of vascular endothelial cells [8]. Although both VEGFR-1 and -2 are expressed in the vascular endothelium, the angiogenic activities of VEGFs are transduced mainly through VEGFR-2 *in vivo*.

Based on the importance of VEGF/VEGFR in cancer, many companies and institutes are now trying to generate appropriate small molecules as well as proteins that strongly antagonized the VEGF/VEGFR pathway. They are studying natural and synthetic angiogenesis inhibitors targeting VEGFR in the hope that these chemicals will prevent or slow down the growth of cancer by blocking the formation of new blood vessels. Hundreds of VEGFR inhibitors have been characterized, and a number of them are FDA-approved and even more are currently in clinical trials [9].

Several approaches to block VEGF/VEGFR activity are under evaluation, including biological agents (soluble receptors, VEGF splice variants, anti-VEGF or -VEGFR antibodies, VEGF transcription inhibitors), small-molecule ATP competitive VEGFR tyrosine kinase inhibitors (TKIs) and antisense oligonucleotides. This review describes current efforts in the field of small-molecule VEGFR inhibitors development.

2. THE BIOLOGICAL FUNCTIONS OF VEGFR

VEGF was initially purified as vascular permeability factor (VPF) from tumor cell ascites, and it is now known to be a multifunctional peptide capable of inducing receptor-

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mediated endothelial cell proliferation and angiogenesis [10]. VEGF is a substance made by cells that stimulates new blood vessel formation. It is a sub-family of growth factors, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor (PlGF) [11]. VEGF has been studied extensively for its angiogenic behavior in physiological and pathological conditions. VEGF-A, also called VEGF, is considered as the most important factor among VEGF family.

VEGF primarily binds to three transmembrane receptors with intracellular TK activity: VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR) and VEGFR-3 (Flt-4) [12]. The binding of VEGF to VEGFR causes receptor dimerization, kinase activation and autophosphorylation of specific tyrosine residues within the dimeric complex [13]. The VEGFRs have long been recognized as crucial components of regulatory systems that govern the formation and maintenance of blood vessels under physiological and pathological conditions, including cancer. The multiple effects of VEGF are mediated through several different receptors. Although both VEGFR-1 and VEGFR-2 are expressed in the vascular endothelium, the angiogenic activities of VEGFs are transduced mainly through VEGFR-2.

VEGFR-2 is the predominant receptor in angiogenic signaling. It regulates endothelial cell migration, proliferation, differentiation, and survival as well as vessel permeability and dilation. VEGFR-1 is able to mediate the growth and survival effects of VEGF, and it may also function as a negative regulator of angiogenesis by binding VEGF and preventing its binding to VEGFR-2. VEGFR-3 is predominantly expressed on lymphatic endothelial cells [14].

Recent research on VEGF/VEGFR pathway has led to the development of novel antiangiogenic agents. Clinical trials have shown inhibitors to this pathway are effective in reducing tumor size, metastasis and blood vessel formation [15]. Antiangiogenic therapies based on inhibition of VEGF/VEGFR pathway were reported to be powerful clinical strategies in oncology. In this review, the authors elucidated key aspects of antiangiogenic agents, with an emphasis on small-molecule VEGFR inhibitors.

3. VEGF/VEGFR INHIBITORS AS ANTIANGIOGENIC AGENTS

After several decades of theorizing, the use of antiangiogenic agents as anticancer therapy has finally moved from the realm of research to reality. Indeed, the effectiveness of VEGFR inhibitors as anti-tumor agents has been demonstrated in animal models and in human clinical trials. Several approaches to block VEGF/VEGFR signaling pathway are under evaluation, including monoclonal antibody (MAb) and small-molecule VEGFR TK inhibitors (TKIs).

3.1. Monoclonal Antibody

The VEGF/VEGFR receptor complex presents an attractive target for the specific delivery of drugs or other effectors to tumor endothelium. Neutralizing antibodies to VEGF/VEGFR prevent binding to and activation of VEGFRs and the subsequent activation of downstream signaling pathways. The MAbs that selectively bind to the VEGF/VEGFR have attracted considerable interests as clinical modalities of choice.

Clinical trials with anti-VEGF antibodies in a variety of malignancies are ongoing. Recently, a series of humanized anti-VEGF monoclonal antibodies have been approved by the FDA as first-line treatment for cancer in combination with chemotherapy [16].

Blockade of the respective VEGFRs provides alternative targets for antiangiogenic therapy. Antibodies against each of the VEGFRs can interfere with VEGF/VEGFR interactions in a highly receptor-specific manner. Anti-VEGFR MAbs prevent VEGF-induced signaling in VEGFR-positive cells, and result in impairment of essential functions of vessel endothelial cells. Anti-VEGFR MAbs are currently being investigated as cancer therapeutics in clinical trials [17].

The field of antiangiogenic cancer therapeutics is still in an early stage of development. Only a few antibodies are

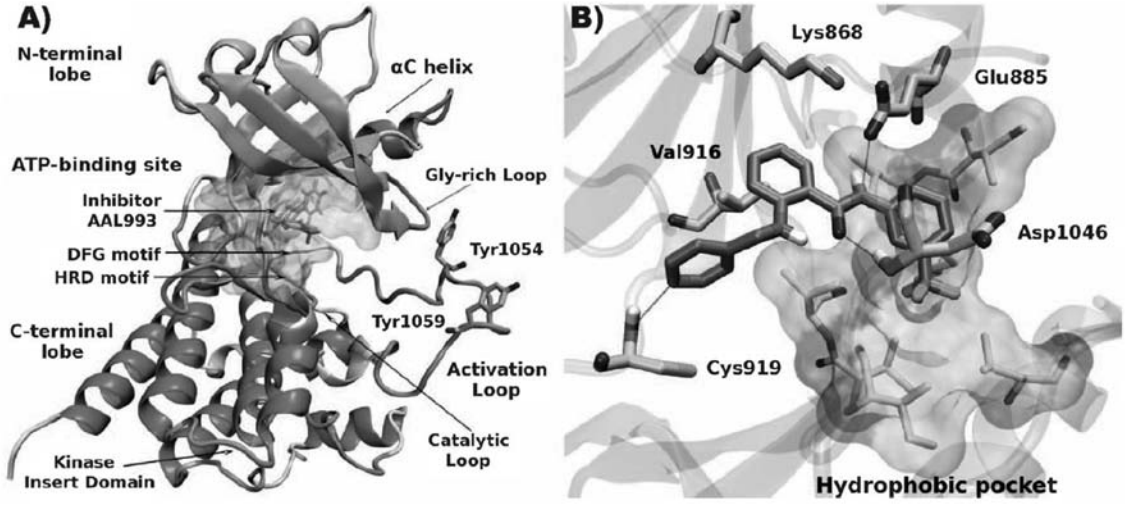
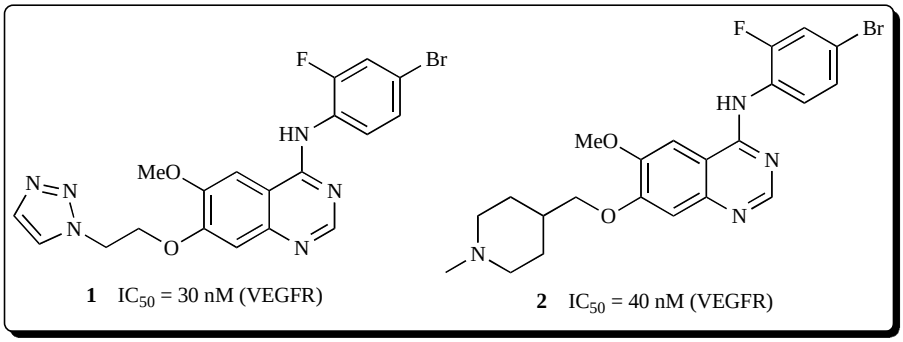


Fig. (1). A)Model of VEGFR-2 kinase domain based on the X-ray crystal structure (PDB ID: 1YWN); **B**) Close-up view of inhibitor docked into the ATP binding site of VEGFR-2.



1 IC_{50} = 30 nM (VEGFR) **2** IC_{50} = 40 nM (VEGFR)

approved for therapeutic use. Monoclonal antibodies directed against the VEGF and VEGFR have been studied in depth, and VEGF/VEGFR pathway inhibitory antibodies show broad therapeutic promise.

3.2. Small-Molecule VEGFR TK Inhibitors

Along with the development of therapies based on antibodies, extensive studies of small-molecule VEGFR TK inhibitors (TKIs) have been performed in the past two decades. Clinical trials have show inhibitors of VEGFR TK are effective in reducing tumor size, metastasis and blood vessel formation. We will now introduce the VEGFR TK inhibitors according to the structure.

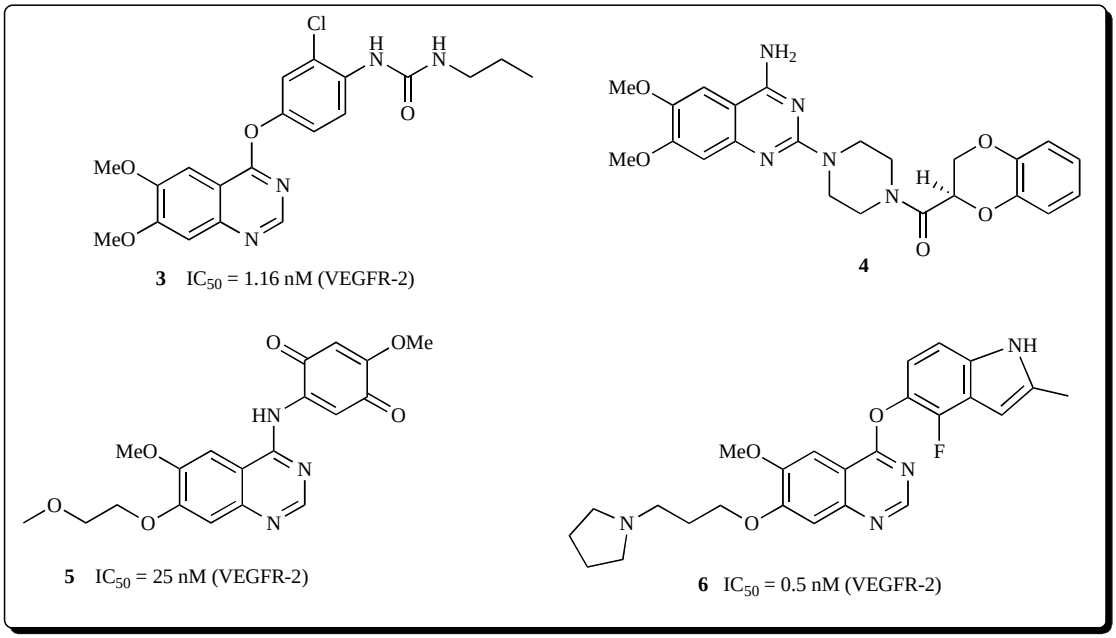
As reported for the inhibitor-VEGFR-2 complex, the inhibitor binds to the kinase through three hydrogen bonds between three residues of active site: the backbone NH of Cys 919 in the hinge region, the backbone NH of Asp 1046 of converved DFG (Asp-Phe-Gly) motif, and the side chain carboxylate of Glu 885. The hydrophobic group of inhibitors is accommodated within a lipophilic pocket defined by residues Ile 888, Leu 889, Ile 898, Val 899, Leu 1019, and Ile 1044, while the phenyl ring is sandwiched between the hydrophobic side chain components of Val 914 and Lys 868 (Fig. **1**) [18].

3.2.1. Antiangiogenic Agents of Quinazoline Type

The synthesis of most potent quinazoline inhibitors of VEGFR has been reported in the past two decade. Structure activity relationship for quinazoline as VEGFR inhibitors has also been established.

As early as in 1999, Hennequin described the synthesis and structure-activity relationship (SAR) of substituted 4-anilinoquinazolines as potential VEGFR inhibitors [19]. Most of these derivatives are potent submicromolar inhibitors of huaman endothelial cell proliferation stuimulated by VEGF. The anilinoquinazoline **1** (ZD4190) was identified as one of the most promising representatives of this new series of molecules. Compound **1** inhibited the growth of established Calu-6 lung carcinoma xenograft by 75% following daily oral administration of 100 mg/kg for 21 days.

A novel subseries of 4-anilinoquinazolines that possess basic side chains at the C-7 position of the quinazoline nucleus was reported by the same group [20]. Anilinoquinazolines possessing C-7 basic side chains are potent nanomolar inhibitors of VEGFR, and their enzyme inhibition profiles translate very well into endothelial cells. The presence of a basic side chain confers excellent physicochemical properties including good aqueous solubility, improved



3 IC_{50} = 1.16 nM (VEGFR-2) **4**

5 IC_{50} = 25 nM (VEGFR-2) **6** IC_{50} = 0.5 nM (VEGFR-2)

bioavailability and reduced protein binding. Compound **2** (ZD6474) exhibited excellent selectivity for the inhibition of VEGFR TK (IC_{50} = 40 nM) vs the other kinase (IC_{50} > 1100 nM). ZD 6474 (**2**), or vandetanib (Zactima^{TM}, AstraZeneca) is a new oral anti-cancer drug being investigated in clinical trials. The vandetanib clinical trials are now on Phase III development in a broad range of patients.

A novel quinazoline urea derivative, KRN633 (**3**), inhibited tyrosine phosphorylation of VEGFR-2 (IC_{50} = 1.16 nM) in human umbilical vein endothelial cells (HUVEC) was discovered by Nakamura [21]. KRN633 could strongly and selectively inhibit VEGFR TK and intracellular VEGF signaling. KRN633 was well tolerated and had no significant effects on body weight or the general health of the animals.

The quinazoline-derived doxazosin (**4**), could affect the growth of human vascular endothelial cells and modulate VEGF-mediated angiogenesis [22]. Their findings may have potential therapeutic significance in using this quinazoline-based compound as an antiangiogenic agent for the treatment of advanced prostate cancer. A series of quinazoline derivatives with different substituents at the 4-position of quinazoline core was reported as potent covalent-binding, irreversible inhibitors of VEGFR-2 [23]. Several of the compounds inhibited VEGF-stimulated autophosphorylation in intact cells. One compound of them (**5**) displayed antitumor activity in an *in vivo* model.

The novel indole-ether quinazoline inhibitor (AZD2171, **6**) is a highly potent inhibitor of VEGFR-2 [24]. It is a highly potent (IC_{50} < 1 nM), orally bioavailable, ATP-competitive inhibitor of recombinant VEGFR TK. AZD2171 inhibited VEGF-stimulated proliferation and VEGFR phosphorylation with IC_{50} values of 0.4 and 0.5 nM, respectively. AZD2171, as known as Cediranib (Recentin), is being developed by AstraZeneca as a possible anti-cancer chemotherapeutic agent for oral administration.

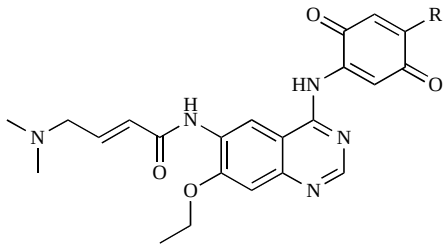
On the basis of covalent binding inhibitors of EGFR and HER2 kinase, Wissner designed dual irreversible inhibitors (**7-16**) [25]. The results of the biological assays are shown in Table **1**. It is clear from the data in Table **1** that all the compounds inhibit both EGFR and VEGFR-2. The relative dependence of the IC_{50} values on the concentration of ATP used in the assays suggests that these compounds appear to function as irreversible inhibitors of TK.

Last year, Hu E reported the discovery of several aminoquinazoline pyridones (**17-21**) with VEGFR inhibitory activity [26]. However, SAR studies resulted in the identification of potent inhibitors of c-Kit with greater than 200-fold selectivity against VEGFR.

3.2.2. Antiangiogenic Agents of Quinoline Type

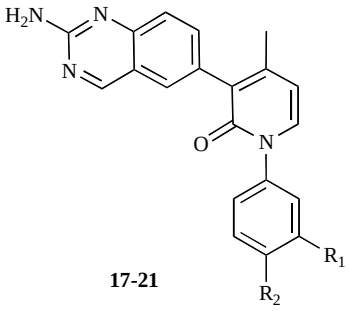
Quinoline is another important group of small-molecule VEGFR inhibitors. Kubo found a novel class of quinoline derivatives as VEGFR TKIs on the basis of PDGFR TKIs [27]. A representative compound (**22**), termed Ki8751, inhibited VEGFR-2 phosphorylation with an IC_{50} value of 0.90

Table 1.

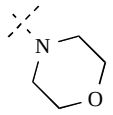
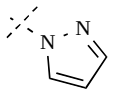


7-16 IC_{50} = 25 nM (VEGFR-2)

Compound	R	EGFR IC_{50} (nM)	VEGFR IC_{50} (nM)
7	OCH_3	799.3	378.7
8	$OCH(CH_2F)_2$	283.3	109.3
9	OCH_2Ph-3-F	4.1	113.4
10	OCH_2Ph-3,4-di-F	166.7	111.6
11	OCH_2Ph	18.7	102.3
12	OCH_2-2-pyridyl	90.8	135.0
13	OCH_2Ph-3-Cl	12.2	80.2
14	OCH_2-2-thiophene	47.5	76.2
15	OCH_2Ph-3-OCH_3	107.3	371.5
16	OCH_2Ph-3-CH_3	24.9	188.7



17-21

Compound	R_1	R_2	VEGFR IC_{50} (μM)
17	H	^{t}Bu	0.011
18	H	CF_3	1.8
19	CH_3	F	4.3
20	H		4.4
21	H		3.4

nM. Ki8751 showed an excellent antitumor activity against some human tumor xenografts in nude mice and rats.

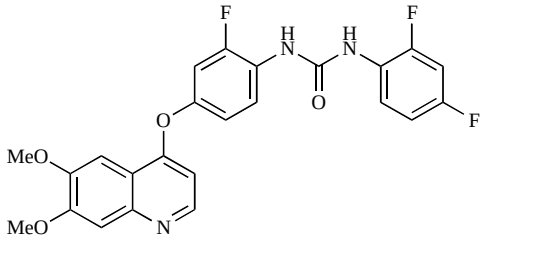
Another quinoline-urea derivative, KRN951 (**23**), is a novel VEGFR inhibitor with antitumor angiogenesis activities [28]. KRN951 potently inhibited VEGF-induced VEGFR-2 phosphorylation in endothelial cells at *in vitro* subnanomolar IC_{50} values (IC_{50} = 0.16 nM). KRN951 is currently under evaluation in a phase I clinical trial.

The efficacy of a novel VEGFR-2 TKI (YM-359445, **24**) was analyzed both *in vitro* and *in vivo* by Amino [29]. Their studies suggested that YM-359445 would be a novel, orally available antiangiogenesis agent that inhibited VEGFR-2 in low IC_{50} values (IC_{50} = 8.5 nM). The results showed that YM-359445 was more potent than orally bioavailable VEGFR-2 TKIs, which led to great expectations for clinical applicability.

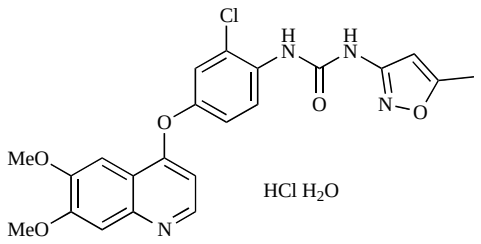
SAR studies of naphthyl-based VEGFR inhibitors led to the identification of a novel series of quinoline derivatives as potential inhibitors [30]. Numerous analogues demonstrated low nanomolar inhibition of VEGF-dependent HUVEC proliferation. Compound (**25**) demonstrated good pharmacokinetics and significant inhibition of VEGF-induced proliferation of HUVEC with IC_{50} values of 8 nM.

Weiss and coworkers prepared another series of naphthamides as potent and selective inhibitors of VEGFR-2 [31]. Three of them (**26, 27, 28**) exhibited good pharmacokinetics following oral dosing and showed potent inhibition of VEGF-induced angiogenesis in the rat cornea. In the same year, La DS reported several 2,3-dihydro-1,4-benzoxazines as inhibitors of intrinsic VEGFR activity and HUVEC proliferation with IC_{50} < 0.1 μM [32]. Compound (**29**) was identified as a potent and selective inhibitor that exhibited efficacy in angiogenic *in vivo* models.

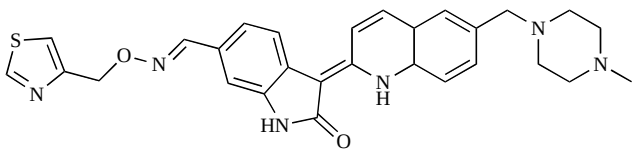
The discovery of 1,2,3,4-tetrahydroisoquinolines and 3,4-dihydroisoquinoline-1(2*H*)-ones as potent inhibitors of VEGFR-2 was reported soon later [33]. Compound (**30**) was identified as a potent and selective inhibitor of VEGFR-2



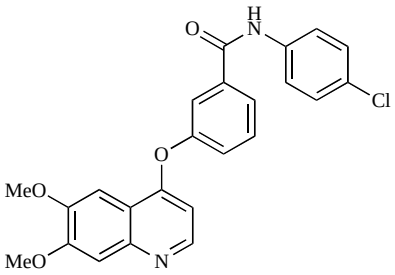
22 IC_{50} = 0.9 nM (VEGFR-2)



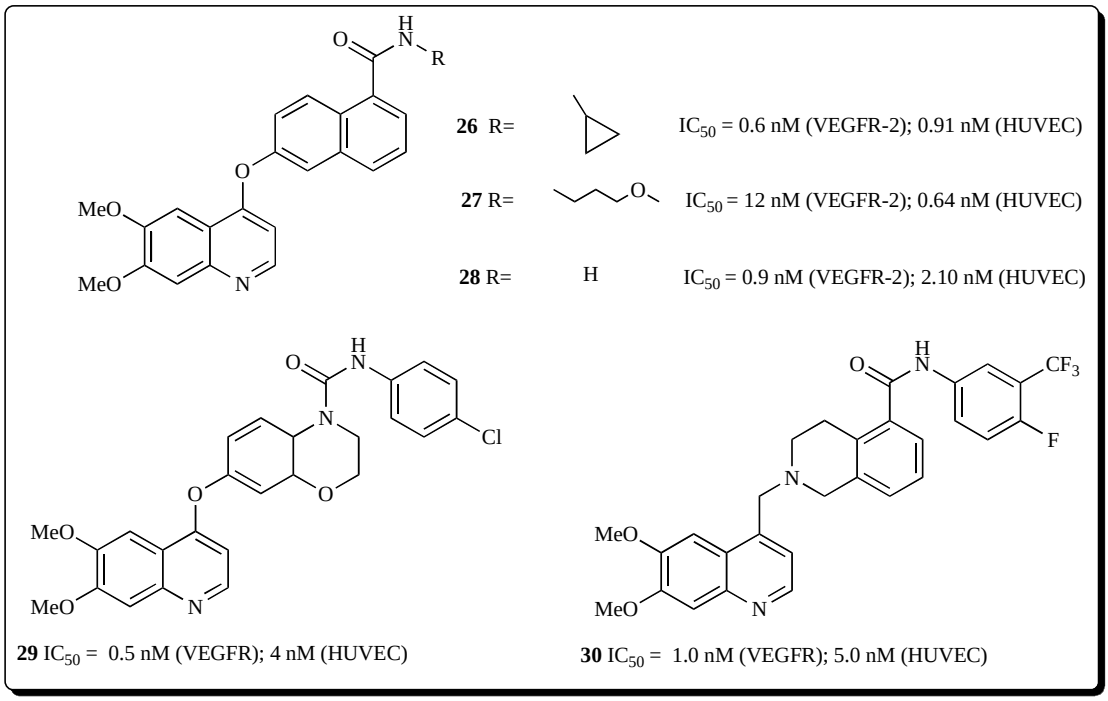
23 IC_{50} = 0.16 nM (VEGFR-2)

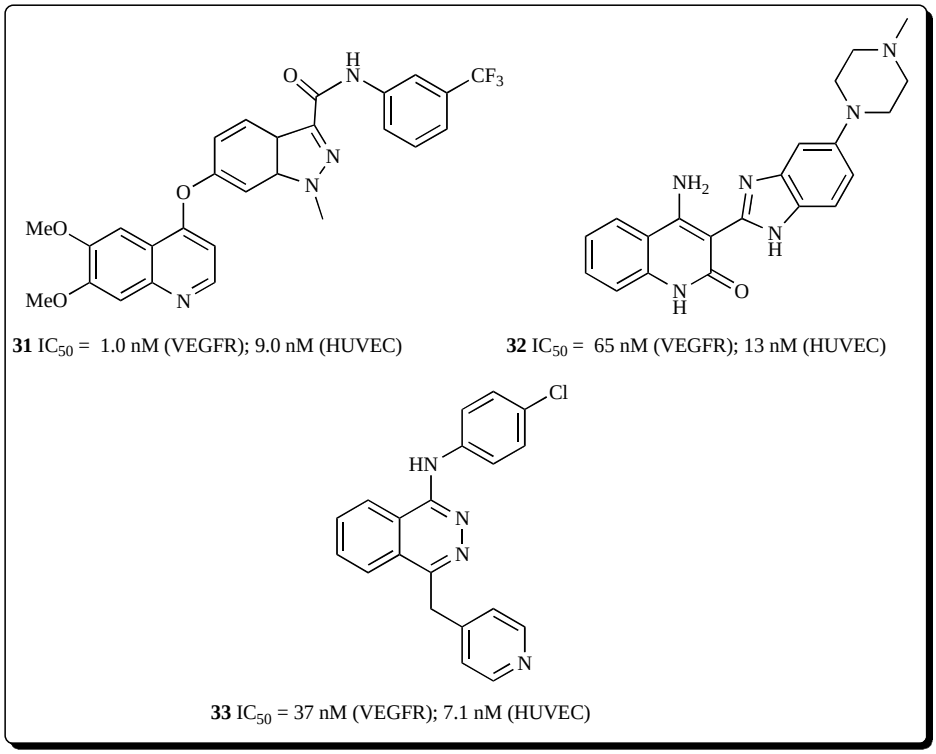


24 IC_{50} = 8.5 nM (VEGFR-2)



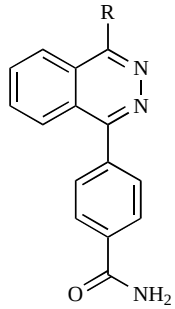
25 IC_{50} = 0.5 nM (VEGFR-2)





with improved solubility. On the basis of (**29**), Bauer and coworkers prepared a novel series of potent and selective inhibitors of VEGFR-2 incorporating an indazole moiety [34]. They successfully replaced the 1-aminonaphthyl core by a 3-aminoindazolyl core as illustrated by the selectivity, rat pharmacokinetic properties, and pharmacological activity of (**31**). Compound (**31**) could inhibit VEGF-induced vascular permeability in a dose-dependent fashion.

Last year, Renhowe synthesized a novel scaffold with multi-kinase affinity [35]. These compounds inhibited receptor tyrosine kinases (RTKs) such as VEGFR-2, FGFR-1, PDGFR, and Flt-3, possessed good pharmacokinetic and pharmacological characteristics. Compound (**32**) (TKI258) was reversible inhibitor of VEGFR-2 with IC_{50} values of 65 nM. On the basis of its favorable *in vitro* and *in vivo* properties, compound (**32**) was currently in phase I clinical trials.

	Compound	R	VEGFR-2, IC_{50} (nM)
 **34-38**	**34**	NH(4-OCF_2Cl)Ph	190
	35	NH(3-*t*-Bu)Ph	470
	36	NH(4-*t*-Bu)Ph	78
	37	NH(4-Br)Ph	100
	38	NH(3,4-Cl_2)Ph	840

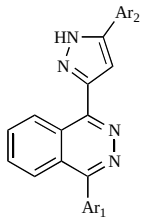
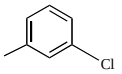
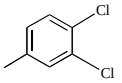
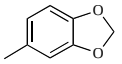
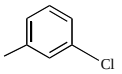
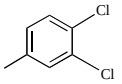
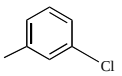
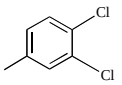
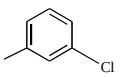
3.2.3. Antiangiogenic Agents of Phthalazine Type

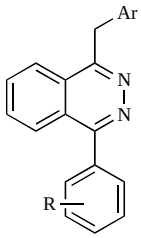
This section illustrates chemical diversity of VEGFR inhibitors of phthalazine type and their bioisosteric analogues. PTK787/ZK 222584 (**33**) is a potent inhibitor of VEGFR, active in the submicromolar range [36]. PTK787, also named Vatalanib, inhibited VEGF-induced autophosphorylation of VEGFR-2, endothelial cell proliferation, migration. Modification of the 1-anilino moiety afforded derivatives with higher selectivity for the VEGFR TKs. This novel compound had therapeutic potential for the treatment of solid tumors.

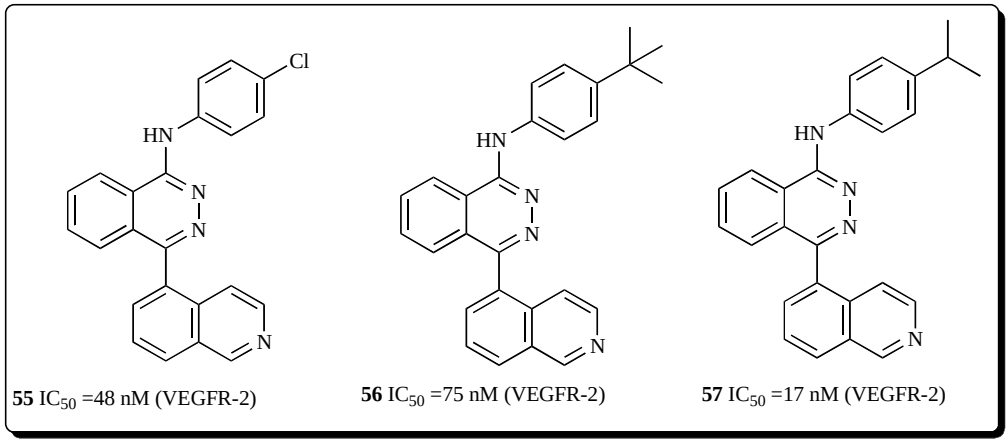
A novel series of phthalazine chemotype was then identified as inhibitors of VEGFR-2 [37]. Several compouinds displayed potent VEGFR-2 inhibitory activity with an IC_{50} as low as 78 nM. These phthalazine derivatives incorporating a 4-carbamoylphenyl group were effective at inhibiting VEGFR-2. For example, compounds (**34-38**) exhibited submicromolar activity against VEGFR-2.

Kiselvov's group described a novel class of derivatives of phthalazine as inhibitors of VEGFRs [38]. Majority of new phthalazine derivatives exhibited robust inhibitory activity against VEGFR-2. The biological assays indicated that eight VEGFR-2 active phthalazine derivatives (**39-46**, IC_{50} = 38-110 nM) displayed good activity against VEGFR-1 with the IC_{50} values in the 170-650 nM range. This outcome should be of benefit in the clinical setting as both receptors are reported to mediate VEGF signaling in the angiogenesis.

In the same year, they prepared another series of phthalazine derivatives as potent VEGFR inhibitors [39]. The results indicated that all the six VEGFR-2 active phthalazine derivatives (**47-52**, IC_{50} = 48-120 nM) also displayed good activity against VEGFR-1 with the IC_{50} values in the 130-

	Comp	Ar_1	Ar_2	VEGFR-2, IC_{50} (nM)	HUVEC, IC_{50} (nM)
 **39-46**	**39**	4-$CONH_2$-(C_6H_4)		44	280
	40	4-$CONH_2$-(C_6H_4)		95	650
	41	4-$CONH_2$-(C_6H_4)		110	540
	42	Isoquinolin-5-yl		38	180
	43	Isoquinolin-5-yl		55	240
	44	4-(4-1H-tetrazol-1-yl)-Ph		49	170
	45	4-(4-1H-tetrazol-1-yl)-Ph		66	240
	46	4-(4-1H-tetrazol-1-yl)-Ph		63	210

	Comp	Ar_1	Ar_2	VEGFR-2, IC_{50} (nM)	HUVEC, IC_{50} (nM)
 **47-54**	**47**	4-Cl(C_6H_4)	4-*N*-tetrazolo	68	130
	48	4-*t*-Bu(C_6H_4)	4-*N*-tetrazolo	92	220
	49	4-*i*-Pr(C_6H_4)	4-*N*-tetrazolo	48	170
	50	4-Cl(C_6H_4)	4-*N*-(1,2,4-triazolo)	79	250
	51	4-Cl(C_6H_4)	4-*N*-tetrazolo	72	130
	52	4-*i*-Pr(C_6H_4)	4-*N*-tetrazolo	120	330
	53	4-ClF_2CO(C_6H_4)	4-*N*-tetrazolo	80	1190
	54	4-ClF_2CO(C_6H_4)	4-*N*-tetrazolo	87	1230



55 IC_{50} =48 nM (VEGFR-2) **56** IC_{50} =75 nM (VEGFR-2) **57** IC_{50} =17 nM (VEGFR-2)

330 nM range. Another two compounds (**53, 54**) showed good potential for the development as VEGFR-2 specific inhibitors (15-20 fold) against VEGFR-1. Duntton disclosed a series of novel 1-(isoquinolin-5-yl)-4-arylamino pathalazines as highly potent VEGFR-2 inhibitors based on their previous findings [40]. Many compounds exhibited VEGFR-2 inhibitory activity with an IC_{50} as low as 17 nM. The results indicated that isoquinoline-substituted phthalazines exhibited excellent inhibitory activity. For example, the *para*-chloro-, *tert*-butyl-, and *iso*-propyl derivatives (**55-57**) wee highly active with IC_{50} values of 48, 75 and 17 nM, respectively.

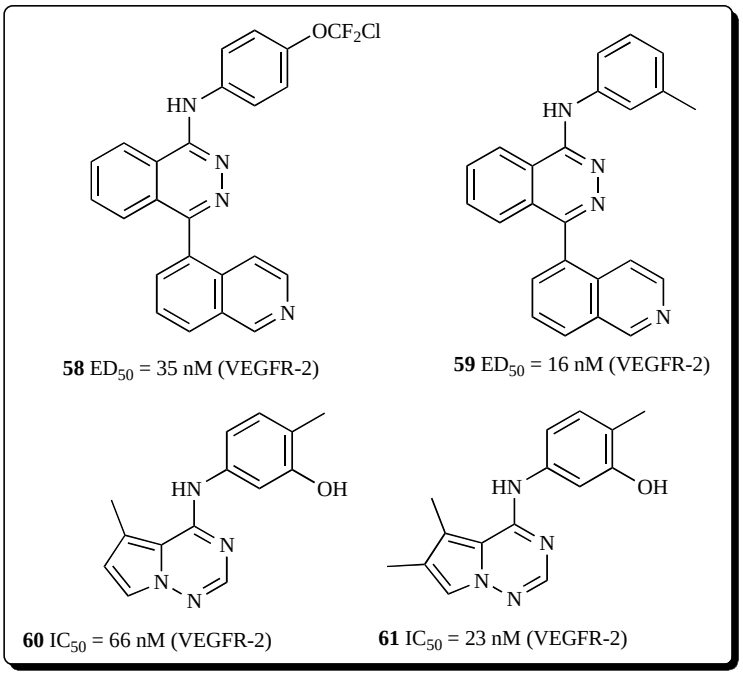
Last year, the same group reported a novel series of arylphthalazine derivatives [41]. The biological test identified two compounds (**58, 59**) which exhibited excellent inhibition against VEGFR-2 chimeric receptor expressed in HEK-293 cells with with ED_{50} of 35 and 16 nM, respectively. The further results indicated that this series of compounds possessed favorable cellular potency and pharmacokinetic profiles which would be suitable for their further development as drug candidates.

3.2.4. Antiangiogenic Agents of Triazine Type

Pyrrolo[1,2,4]triazine nucleus was identified as a novel TKIs template which effectively mimics the well-known quinazoline template [42]. Attachment of substituent at position 4 to the template provided several novel pyrrolotriazine-based inhibitors of VEGFR-2. The 5-methyl analague (**60**) and 5,6-dimethyl analogue (**61**) are the most potent inhibitors identified of the TK activity of VEGFR-2, with IC_{50} values of 66 and 23 nM, respectively. Preliminary inhibition studies with varying ATP concentrations indicated that, like the quinazoline-based inhibitors, the pyrrolotriazine-based inhibitors bound in the ATP pocket.

A novel series of 2-hydroxy-4,6-diamino-[1,3,5]triazines was then identified as a novel class of potent VEGFR-2 TKIs [43]. Four compounds (**62-65**) of them showed excellent enzyme inhibitory activities against VEGFR-2. SAR studies on the effects of substitution at 5, 6, and 7-positions yielded a novel class of pyrrolo[1,2,4]triazines as potent and selective inhibitors of VEGFR-2 and FGFR-1 [44]. Several derivatives demonstrated low nanomolar inhibitory activity against VEGF-dependent HUVEC proliferation. The favorable *in vitro* pharmacology and pharmacokinetic properties exhibited by oxadiazole analogues **66, 67**, and **68** translated into excellent antitumor activity.

In the same year, another series of TKIs based on pyrrolo[1,2,4]triazine template containing a 3-hydroxyphenylamino of the pyrrolotriazine was identified [45]. Replacement of the C6-ester substituent of the core with heterocyclic bioisosteres afforded compounds with excellent oral bioavailability. Five of the compounds (**69-73**) exhibited excellent VEGFR-2 TK inhibitory activity and VEGF-stimulated HUVEC proliferation.

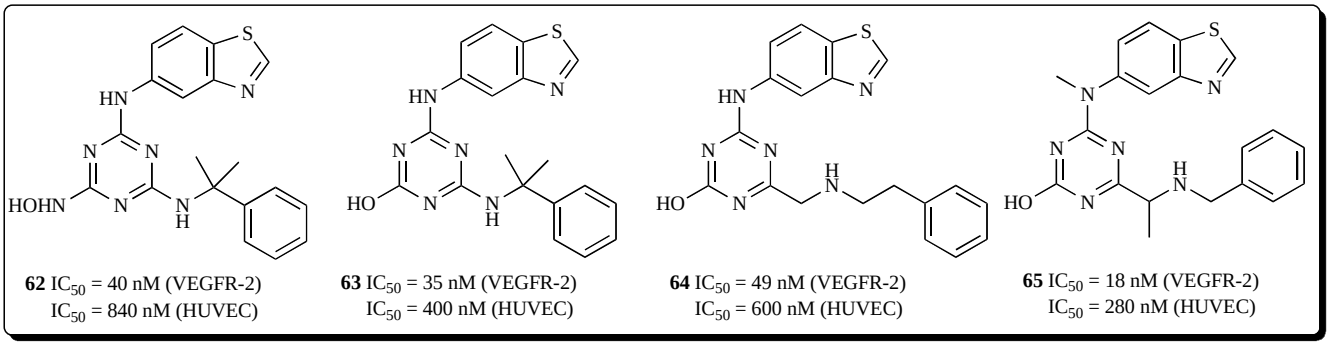


58 ED_{50} = 35 nM (VEGFR-2)

59 ED_{50} = 16 nM (VEGFR-2)

60 IC_{50} = 66 nM (VEGFR-2)

61 IC_{50} = 23 nM (VEGFR-2)

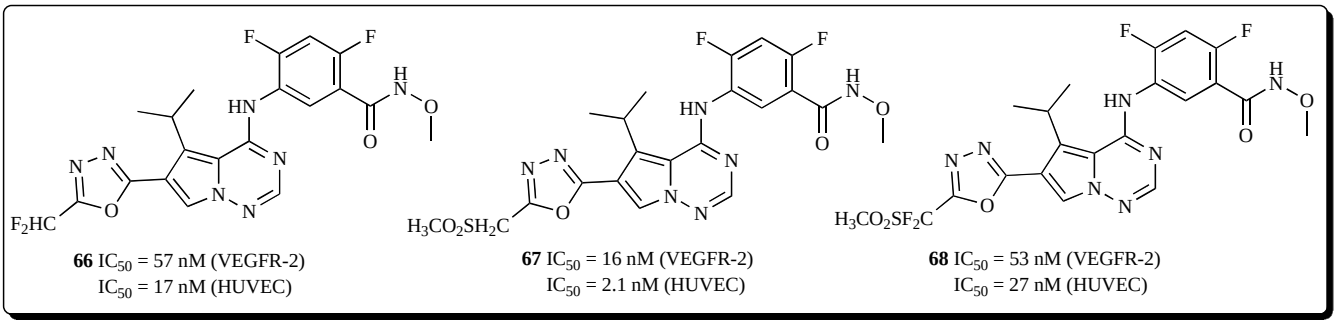


62 IC_{50} = 40 nM (VEGFR-2)
IC_{50} = 840 nM (HUVEC)

63 IC_{50} = 35 nM (VEGFR-2)
IC_{50} = 400 nM (HUVEC)

64 IC_{50} = 49 nM (VEGFR-2)
IC_{50} = 600 nM (HUVEC)

65 IC_{50} = 18 nM (VEGFR-2)
IC_{50} = 280 nM (HUVEC)



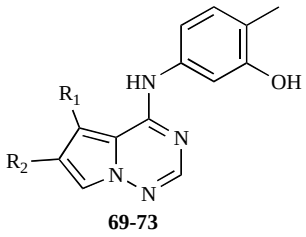
66 IC_{50} = 57 nM (VEGFR-2)
IC_{50} = 17 nM (HUVEC)

67 IC_{50} = 16 nM (VEGFR-2)
IC_{50} = 2.1 nM (HUVEC)

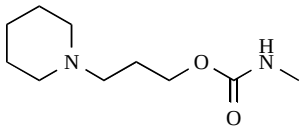
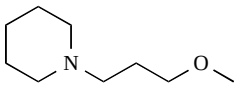
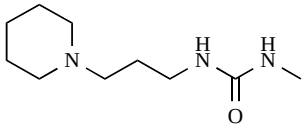
68 IC_{50} = 53 nM (VEGFR-2)
IC_{50} = 27 nM (HUVEC)

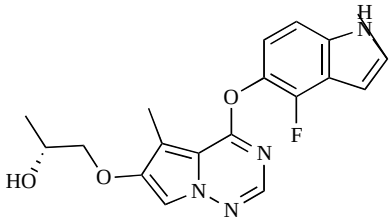
Indole-based pyrrolo[1,2,4]triazines were prepared to improved pharmacokinetic properties of phenol-substituted triazines [46]. Biochemical potency, kinase selectivity, and pharmacokinetics of the series were optimized and *in vitro* safety liabilities were minimized to afford BMS-540215 (**74**). A series of amino acid ester prodrugs of (**74**) was identified in an effort to improve the aqueous solubility and oral bioavailability [47]. *In vitro* and *in vivo* evaluation led to identification of compounds **75, 76**, and **77** as potential lead prodrugs. The *L*-alanine prodrug of BMS-540215, BMS-582664 (**75**), was currently under evaluation in clinical trials for the treatment of solid tumors. A series of 7-azaindoles were also investigated as inhibitors of VEGFR-2 TK [48]. Biochemical potency and kinase selectivity optimization afford BMS-645737 (**78**) as antiangiogenic agent with good preclinical *in vivo* activity against human tumor xenograft models.

Incorporation of the 2,4-difluoro-5-(cyclopropylcarbamoyl)phenylamino group at the C-4 position of the

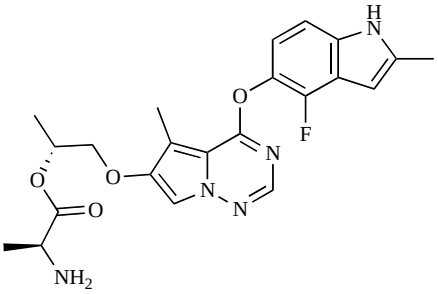


69-73

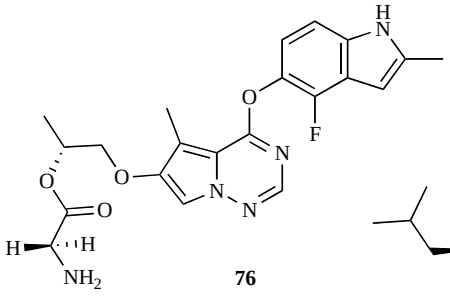
Compound	R_1	R_2	VEGFR-2, IC_{50} (nM)	HUVEC, IC_{50} (nM)
69	*n*-Pr	-COOEt	3	6
70	*i*-Pr	-COOEt	14	4
71	*i*-Pr		5	1
72	*i*-Pr		4	1
73	*i*-Pr		5	5



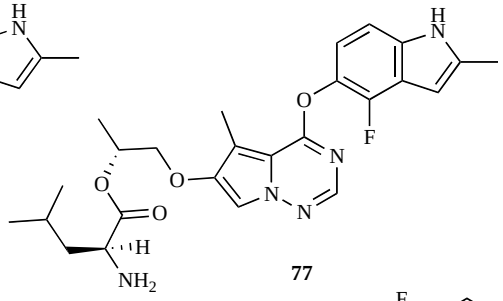
74 IC_{50} = 25 nM (VEGFR-2)



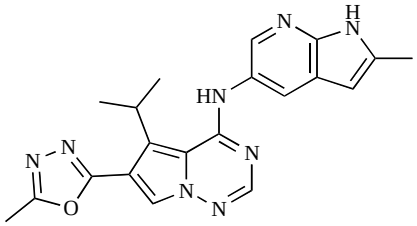
75 BMS-582664



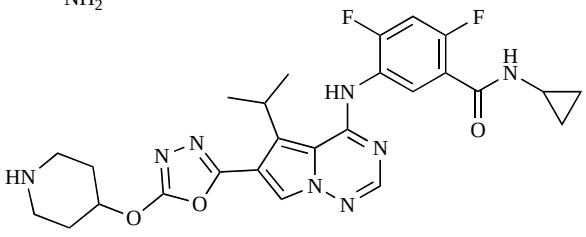
76



77

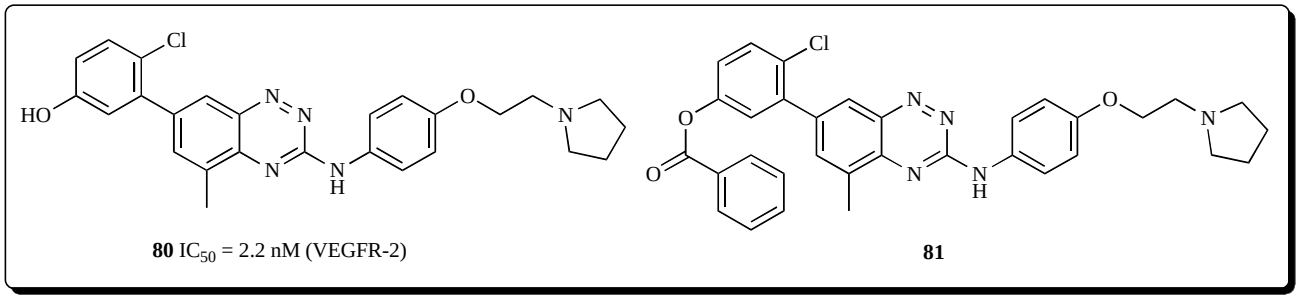


78 IC_{50} = 25 nM (VEGFR-2)
IC_{50} = 13 nM (HUVEC)



79 IC_{50} = 11 nM (VEGFR-2)
IC_{50} = 23 nM (HUVEC)

pyrrolo[2,1-f][1,2,4] triazine template led to the discovery of a novel sub-series of VEGFR-2 inhibitors [49]. SAR studies of substituents on 1,3,4-oxadiazole provided a series of compounds with potent enzymatic and VEGF-stimulated HUVEC cellular inhibitory activity against VEGFR-2. The *in vivo* anticancer activity of (**79**) was identified in a L2987



80 IC_{50} = 2.2 nM (VEGFR-2)

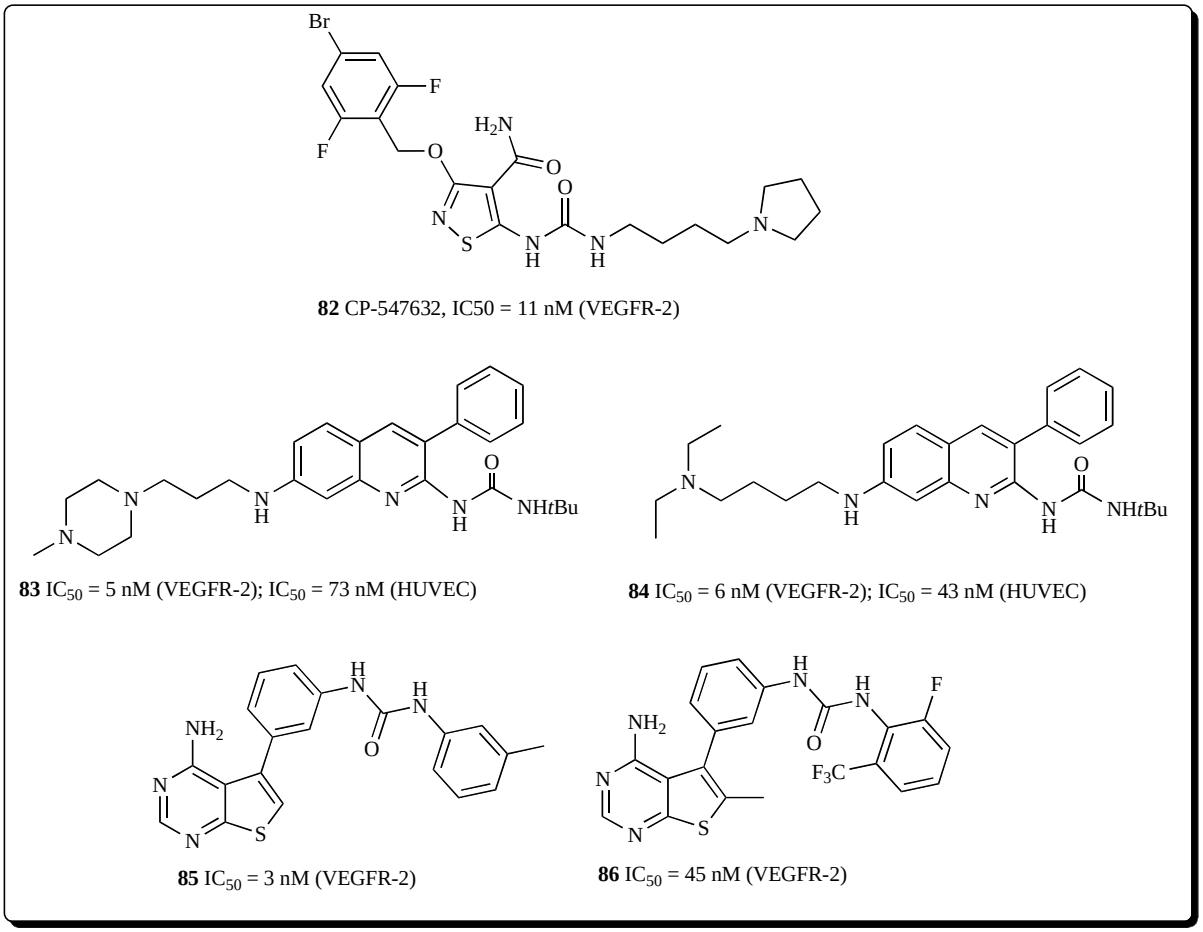
81

human lung carcinoma xenografts in athymic mice. A series of substituted benzotriazines was disclosed as potent inhibitors of VEGFR by Palanki in 2008 [50]. One of the most potent compounds was (**80**), a dual inhibitor of both VEGFR-2 and Src family kinases. They subsequently prepared several prodrugs of (**80**) and evaluated their thermal stability. One of the prodrugs (**81**) showed much better pharmacokinetic properties compared to (**80**).

3.2.5. Antiangiogenic Agents of Urea Type

Urea subunits are common components of lead pharmaceuticals. A novel isothiazole, CP-547632 (**82**), was identified as a potent inhibitor of the VEGFR-2 and bFGF kinase (IC_{50} = 11 and 9 nM, respectively) [51]. CP-547632 potently inhibited bothe bFGF and VEGF-induced angiogenesis and inhibited tumor growth as much as 85%. CP-547632 represented an exciting new chemotype for clinical development in the area of VEGFR inhibitors.

Thompson *et al* identified a series of urea derivatives as potent inhibitors of both FGFR-1 and VEGFR-2 [52]. Several compounds were also low nanomolar inhibitors of the growth of HUVECs stimulated by VEGF. Two compounds of them, (**83**) and (**84**), were worthy of further evaluation as antiangiogenesis agents. Investigation of structure-activity relationships at the 5- and 6-positions of the thienopyrimidine nucleus led to a series of *N,N'*-diaryl ureas that potently inhibit VEGFR and PDGFR [53]. A number of compounds had been identified to possess superior *in vivo* activity. In particular, compounds (**85**) and (**86**) had demonstrated significant oral efficacy in tumor growth inhibition and displayed favorable pharmacokinetic profiles.



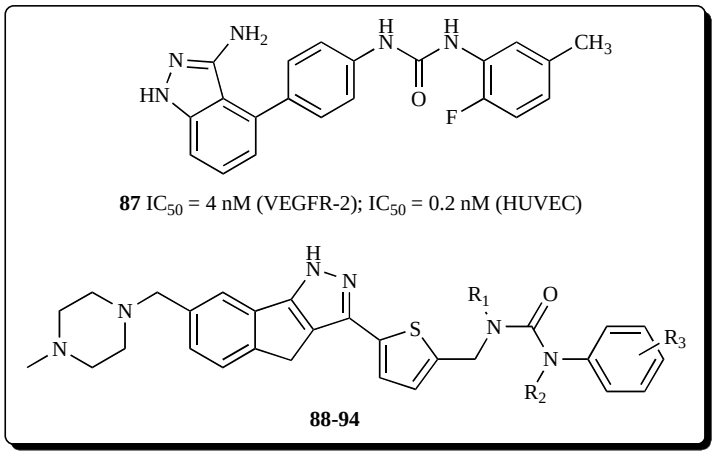
82 CP-547632, IC50 = 11 nM (VEGFR-2)

83 IC_{50} = 5 nM (VEGFR-2); IC_{50} = 73 nM (HUVEC)

84 IC_{50} = 6 nM (VEGFR-2); IC_{50} = 43 nM (HUVEC)

85 IC_{50} = 3 nM (VEGFR-2)

86 IC_{50} = 45 nM (VEGFR-2)



87 IC_{50} = 4 nM (VEGFR-2); IC_{50} = 0.2 nM (HUVEC)

88-94

Compound	R_1	R_2	R_3	VEGFR-2, IC_{50} (nM)
88	CH_3	H	3-CF_3	11
89	CH_3	H	3-Cl	12
90	CH_3	H	4-F	16
91	CH_3	H	4-OCF_3	17
92	CH_3	H	3,4-di-Cl	18
93	CH_3	H	4-Br	19
94	CH_3	H	4-Cl	21

ABT-869 (**87**) was a structurally novel, potent inhibitor of members of the VEGF and PDGF receptor families (VEGFR-2, IC_{50} = 4 nM) [54]. The inhibition profile of ABT-869 was evident in cellular assays of RTK phosphorylation and VEGF-stimulated proliferation (IC_{50} = 0.2 nM for human endothelial cells). The preclinical characteristics of ABT-869 suggested that this molecule may offer distinct advantages in the realm of kinase inhibitors in cancer therapy.

Dinges and coworkers prepared a series of 1,4-dihydroindenol[1,2-*c*]pyrazoles with 3-thiophene substituent carrying a urea-type side chain as potent multiargeted RTKIs in 2006 [55]. Further optimization of the urea-type side chain and the position of a basic subtituent on the core led to a series of potent VEGFR-2 TKIs (**88-94**).

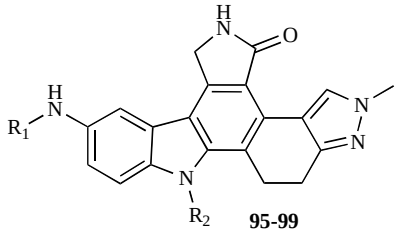
A novel class of C-3 urea fused dihydroindazolocarbazole analogs was identified as highly potent dual inhibitors of Tie-2 and VEGFR-2 [56]. Five of them (**95-99**) exhibited potent activity against VEGFR-2 with IC_{50} < 10 nM. Good cell activity was also observed for compound (**98**) and (**99**). E7080 (**100**) was an orally active inhibitor of multiple RTK including VEGFR, FGFR and SCFR [57]. E7080 caused regression of H146 tumors as a result of antiangiogenic activity *via* inhibition of both Kit and VEGFR-2 signaling.

Ji Z and colleagues synthesized and evaluated a series of *N,N'*-diphenyl urea derivatives as potent VEGFR-2 TKIs [58]. Optimization of the urea moiety led to several compounds that displayed high oral bioavailability and excellent *in vivo* efficacy. In particular, compound (**101**) exhibited high oral bioavailability and improved antitumor efficacy. Usui T reported a novel class of indenopyrazoles as EGFR TKIs by the highthroughput screening in 2008 [59]. Unexpectedly, significant inhibition of VEGFR-2 TK was observed in compounds (**102**), (**103**), and (**104**) at 1 μM concentration.

In order to identify potent and novel small-molecule VEGFR inhibitors, Dai Y *et al* discovered that 3-aminoindazole could serve as a novel kinase hinge-binding template for TKIs [60]. By incorporating a diaryl urea unit at C4-position, a number of potent and orally bioavailable VEGFR/PDGFR inhibitors were identified. Three of them, (**105-107**), possessed reasonable pharmacokinetic profiles and were promising as oral agents. The binding mode of (**106**) with VEGFR-2 was constructed to interpret the difference in potency (Fig. **2**). The results indicated that the 3-amino pyrazolopyridine core was anchored to the KDR hinge region *via* two hydrogen bouns. The urea portion occupied the back of hydrophobic pocket with addition hydrogen bonds.

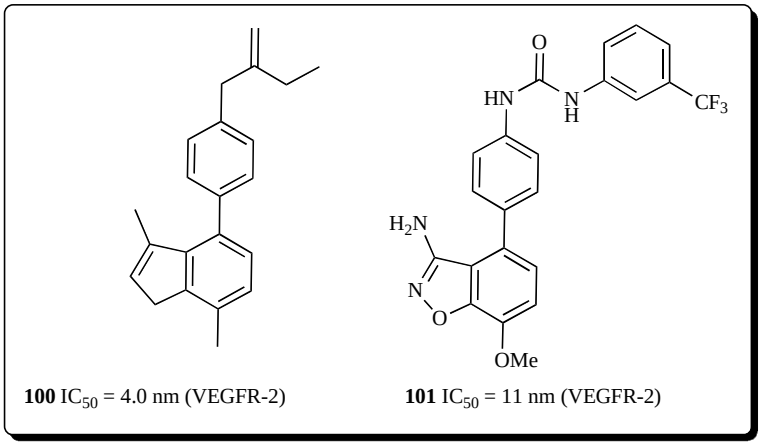
3.2.6. Antiangiogenic Agents of Indole Type

In order to improve the anticancer activity and optimize the pharmaceutical properties of indolin-2-ones, Sun L *et al* prepared a number of basic analogues of indolin-2-ones [61]. One of them (**108**) (SU 11248) possessed the best overall profile in terms of inhibitory activity against VEGFR-2. Furthermore, (**108**) also was highly efficacious in a number of preclinical tumor models. SU 11248 was currently in phase I clinical trials for the treatment of cancers.



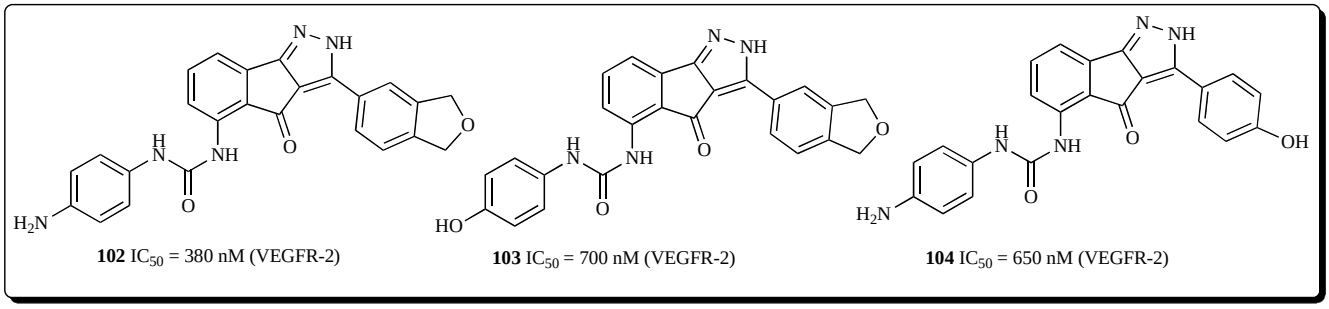
95-99

Compound	R_1	R_2	VEGFR-2, IC_{50} (nM)
95	4-Me-phenylNHCO	$CH_2CH_2CH_3$	8
96	Phenyl(Me)NCO	$CH_2CH_2CH_3$	7
97	2-F-phenylNHCO	$CH_2CH_2CH_3$	9
98	2-Cl-phenylNHCO	$CH_2CH_2CH_3$	9
99	2-Br-phenylNHCO	$CH_2CH_2CH_3$	10



100 IC_{50} = 4.0 nm (VEGFR-2)

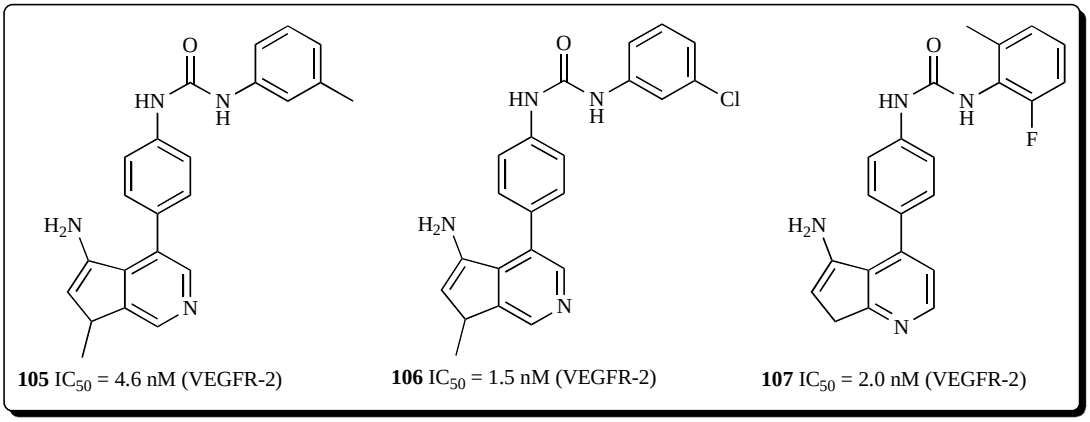
101 IC_{50} = 11 nm (VEGFR-2)



102 IC_{50} = 380 nM (VEGFR-2)

103 IC_{50} = 700 nM (VEGFR-2)

104 IC_{50} = 650 nM (VEGFR-2)



105 IC_{50} = 4.6 nM (VEGFR-2)

106 IC_{50} = 1.5 nM (VEGFR-2)

107 IC_{50} = 2.0 nM (VEGFR-2)

Modification to the basic side-chain of lead structures of indolyl quinolinones resulted in a novel class of VEGFR-2 TKIs with improved pharmacokinetic [62]. Potency was further optimized by substitution of the benzimidazole moiety with an indoyl nucleus. Introduction of a basic side chain at the 5-position of indolyl core provided compounds with im-

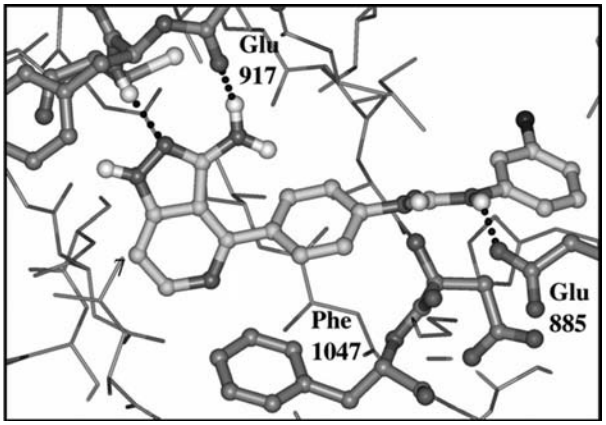


Fig. (2). Binding model of (**106**) in an inactive form of VEGFR-2 (DFG-out).

proved physical properties and excellent cellular activity. However, only (**109**) exhibited favorable pharmacokinetic properties. The selectivity profiling of compound (**110**) in a panel of 20 protein kinases and molecular modeling indicated (**110**) to be highly active and selective for VEGFR-2/3 [63]. It may have potential for development as a potent and selective VEGFR-2/3 inhibitor in angiogenic-relevant diseases.

SU14813 (**111**) was identified from the same chemical library used to isolate sunitinib [64]. SU14813 had broad-spectrum RTK inhibitory activity. In cellular assays, SU14813 inhibited ligand-dependent and ligand-independent proliferation, migration and survival of endothelial cells. The results supported the ongoing phase I clinical evaluation of SU14813 in advance malignancies.

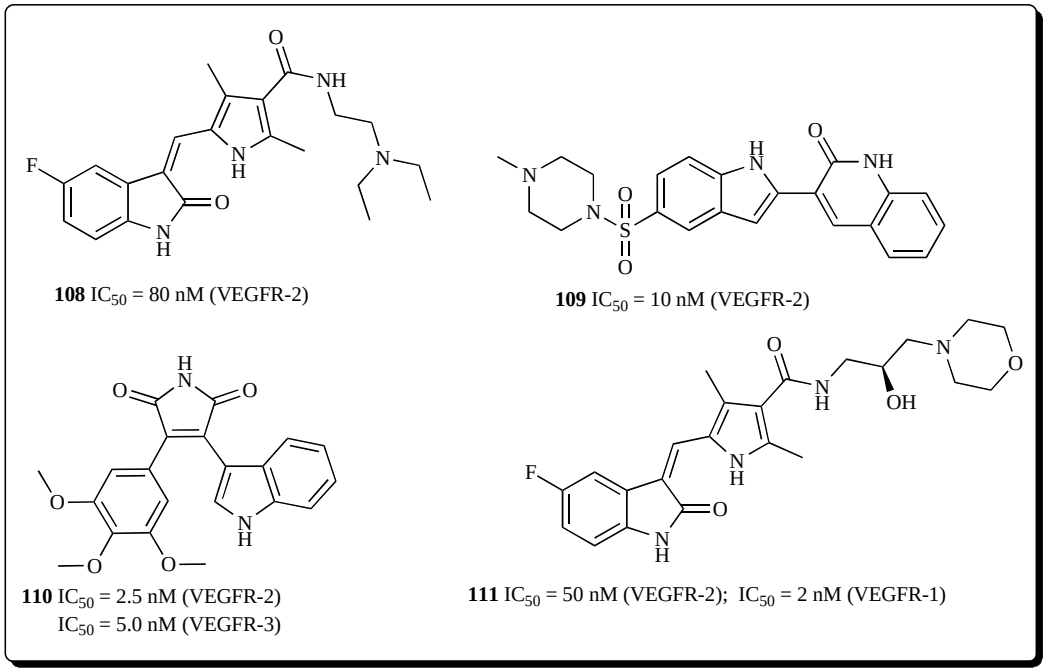
A novel class of VEGFR-2 inhibitors bearing heterocyclic substituted pyrazolones as template was identified by structure-based design strategy [65]. SAR studies on pyrazolones resulted in inhibitors which were extremely potent against the isolated enzyme as well as in cells. One of them (**112**) demonstrated excellent anti-angiogenic activity and oral anticancer efficacy in nude mice. Optimization and modifications of these compounds led to the identification of two series of 1,2,3-thiadiazole substituted pyrazolones as potent and cell-permeable VEGFR-2 TKIs [66]. One methylthiadiazole compound (**113**) showed modest enzymatic inhibitory activity, but exhibited 29% oral bioavailability in rats. Orally bioavaiable, dual inhibitors of TIE-2/VEGFR-2 were prepared by optimization of a pyrrolodihydroindazolocarbazole scaffold [67]. Two thiophencarbonyl analogs (**114, 115**) were active in functional cellular assays and were orally bioavailable when assessed in rats.

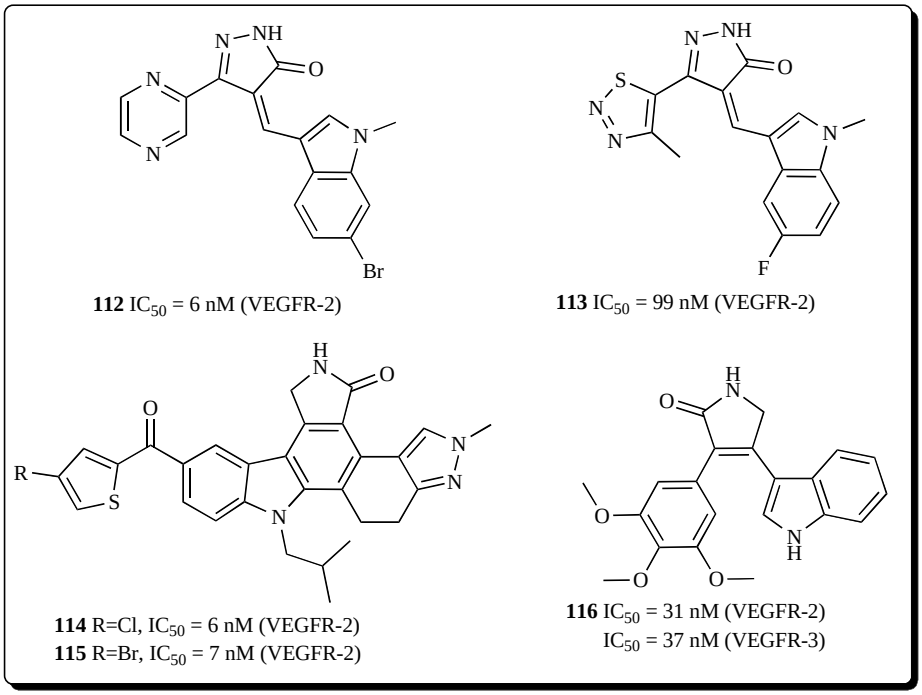
On the basis of the binding mode of compound (**110**) in the ATP pocket, Peifer's gruop designed a series of novel regioselective substituted 3,4-diaryl-2*H*-pyrrole-2-ones as VEGFR-2/3 TKIs [68]. (**116**) was identified as a highly active and selective VEGFR-2/3 inhibitor with IC_{50} values of 31 and 37 nM, respectively. This compound may have potential for clinical development as an angiangiogenic agent.

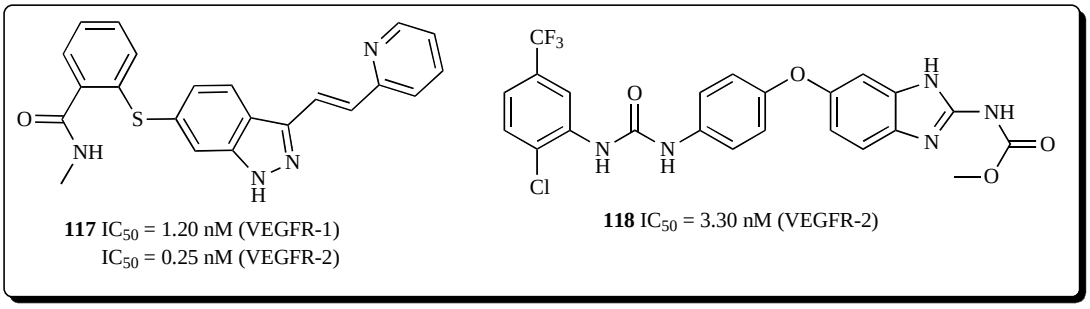
3.2.7. Antiangiogenic Agents of Indazole Type

In 2006, Giles and coworkers reported an oral antiangiogenesis agent with activity against a variety of RTKs, AG-013736 (**117**) [69]. AG-013736 (also known as Axitinib) is a small molecule TKI under development by Pfizer. It has been shown to significantly inhibit growth of breast cancer in xenograft models and has been successful in trials in several tumor types.

A novel chemical series of benzimidazole-ureas was disclosed as VEGFR-2 and Tie-2 inhibitors [70]. SAR studies indicated the important role of the N1 nitrogen and the urea moiety when these compounds were bond to VEGFR-2. Majority of the compounds exhibited excellent TK inhibitory







activity (IC_{50} < 200 nM). A representative compound (**118**) was administered orally to indicate that this urea-benzimidazole series could be employed as a systemically active drug.

3.2.8. Antiangiogenic Agents of Anthranilamide Type

Based on the binding model and the key pharmacophore elements of PTK787, it was postulated that an anthranilamide scaffold in which an intramolecular H-bond between the aniline-NH and the benzanilide-C=O would favour a conformation having a high degree of limilarity to that of PTK787 (Fig. **3**).

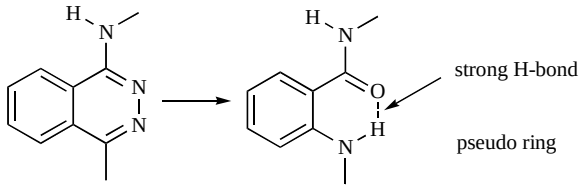
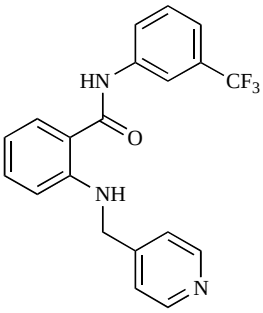


Fig. (3). The conformation of the phthalazine and anthranilamide scaffold.

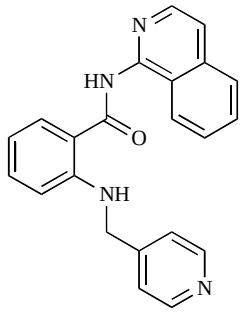
Based on the anthranilic acid amide scaffold, Manley prepared and evaluated two anthranilamides (**119,** AAL993) and (**120**) as angiogenesis inhibitors [71]. They identified that (**119**) and (**120**) were highly potent and selective inhibitors of recombinant VEGFR kinase. The antiangiogenic activity of the two compounds translated into potent inhibition of primary tumor growth. Consequently, these anthranilamides represent a new structural class of VEGFR TKIs possessing potent antiangiogenic and antitumor properties [72].

The development of a hypothesis concerning the bioactive conformation of PTK 787 (**33**) led to the discovery of a novel class of potent inhibitors of VEGFR-2 and Flt-1 [73]. Conformational analysis of these compounds by computational methods led to identification of an exact anthranilic amide mimetic (**121**) of PTK787. When tested, compound (**121**) exhibited the same level of potency and selectivity as PTK787.

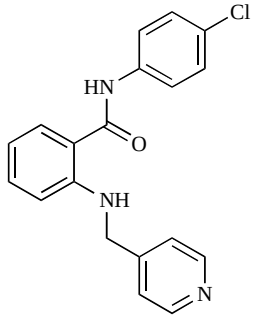
Another series of anthranilic amides, salicylic amides and anthranilic amidines was prepared as selective inhibitors of VEGFR [74]. Biological assay for inhibitory activity toward autophosphorylation led to identification of salicylic amide (**122**) and anthranilic amidine (**123**). The 4,5-dimethoxy-salicylic group of (**123**) occupied the binding site of $CF_3C_6H_4$ groups of (**122**).



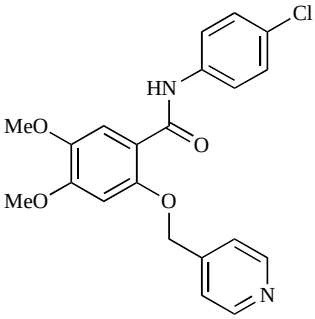
119 IC_{50} = 1.24 nM (VEGFR-2)



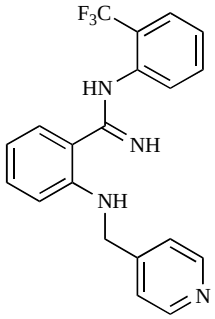
120 IC_{50} = 1.16 nM (VEGFR-2)



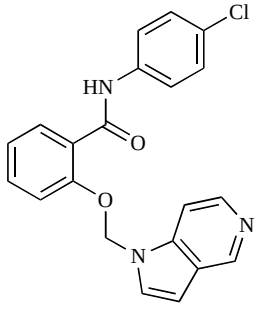
121 IC_{50} = 20 nM (VEGFR-2)



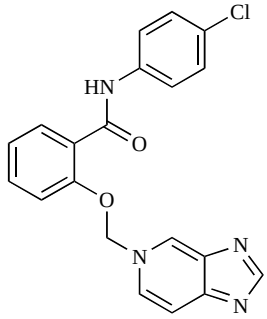
122 55% of inhibition(VEGFR-2)



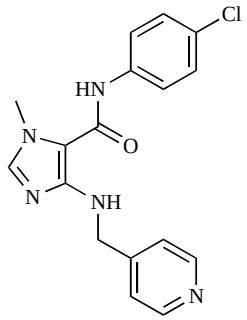
123 44% of inhibition (VEGFR-2)



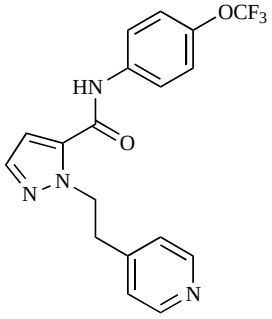
124 IC_{50} = 92nM (VEGFR-2)



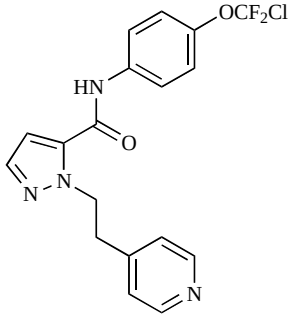
125 IC_{50} =47 nM(VEGFR-2)



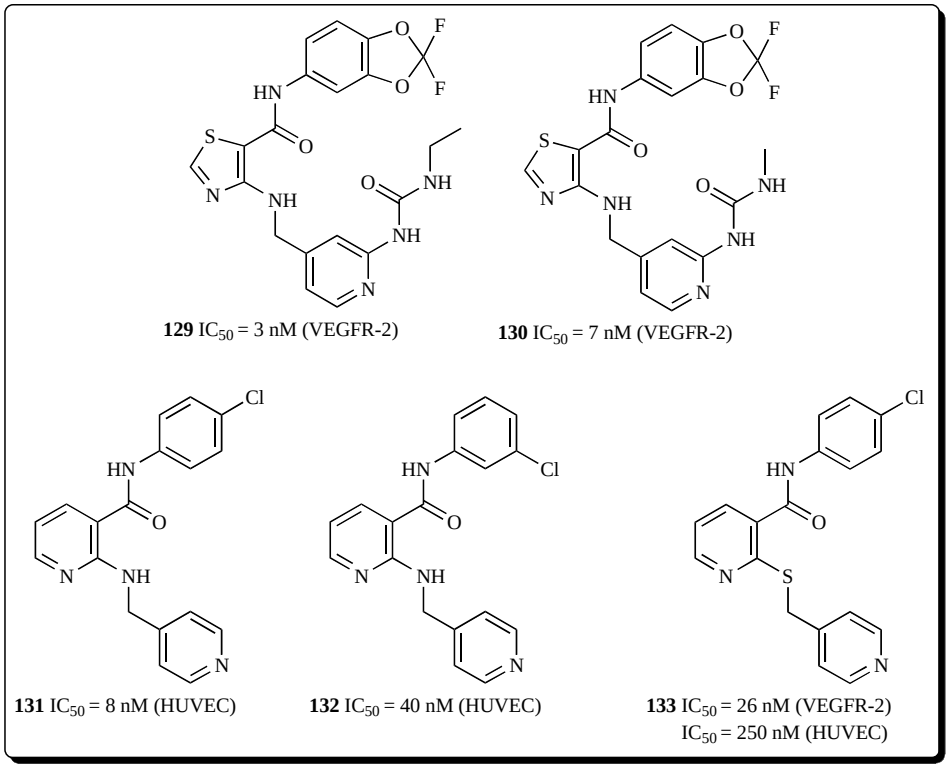
126 IC_{50} = 250 nM (VEGFR-2)



127 IC_{50} = 28 nM (VEGFR-2)



128 IC_{50} = 17 nM (VEGFR-2)



Kiselyov and coworkers identified a series of *N*-arylbenzamide derivatives as potent ATP-competitive inhibitors of the VEGFR-2 [75]. All potent compounds were stable towards hydrolysis and displayed good solubility (>3 nM) in the screening buffer. Two of them, (**124**) and (**125**), showed robust inhibitory activity against VEGFR-2 which was similar in potency to PTK787 (**124, 125**; IC_{50} = 92 and 47 nM, respectively). One year later, the same group developed another series of novel potent *ortho*-substituted azole derivatives as specific and dual inhibitors of VEGFR-1/2 [76]. They reasoned that these non-phthalazine templates could provide for a proper pharmacophore arrangement consistent with the model proposed for PTK787. The most specific molecule (**126**) displayed > 10-fold selectivity for VEGFR-2 over VEGFR-1. In the same year, they also reported a series of novel potent azole-5-carboxamide derivatives active against VEGFR-1/2 [77]. The results indicated that two of the title compounds, (**127**) and (**128**), exhibited the best activity in this series, and could be further developed for *in vivo* studies as both VEGFR-2 specific and VEGFR-1/-2 dual inhibitors.

Based on a continued interest in VEGFR-2 inhibitors and their activity profiles, a chemical library of anthranilamides was synthesized, and broadly screened against a panel of tumor associated kinases [78]. The most promising compounds resulting from these efforts are (**129**) and (**130**). Both of the two compounds showed strong VEGFR-2 inhibitory activity, with IC_{50} values of 3 and 7 nM, respectively.

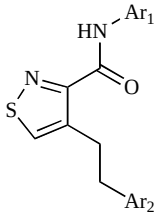
Dominguez and colleagues reported several potent and efficacious 2-aminonicotinamide VEGFR-2 inhibitors on the basis of PTK 787 [79]. These compounds bound to VEGFR in a DFG-out conformation. Among them, compounds (**131**) and (**132**) were potent in VEGF-induced HUVEC proliferation assays (8 nM and 40 nM, respectively), displayed good selectivity in bFGF-induced HUVEC proliferation.

In 2008, Honda developed novel 4-pyridylmethyl derivatives and evaluated their VEGFR-2 inhibitory activities [80]. The conformation-activity relationships indicated that a non-bond intramolecular interaction was a critical structural property for inhibitory activity. 4-pyridylmethylthio derivative (**133**) was identified to be very effective in antiangiogenesis activity. It was also effective in hihibition of VEGFR-2 TK (IC_{50} = 26 nM). Novel derivatives of isothiazoles were identified as potent inhibitors of VEGFR-1/2 in 2009 [81]. The biological assays indicated that all VEGFR-2 active compounds (**134-138**) consistently exhibited good activity against VEGFR-1 with the IC_{50} values in the 31-580 nM range. Several derivatives featuring bulky meta-substituents in the amide portion of the molecule displayed 4- to 8-fold specificity for VEGFR-2 versus VEGFR-1. Active molecules also showed high intrinsic permeability (>30 $\times 10^{-5}$ cm/min) across Caco-2 cell monolayer.

In a communication, the same group expanded upon their initial findings and disclosed potent VEGFR-2 inhibitors based on (1,2,3-triazol-4-yl)benzenamines [82]. Three of the title compounds, (**139-141**), showed sound potency against VEGFR-2 with the IC_{50} values of 51-87 nM. This outcome could be of benefit in the clinical setting as both receptors were reported to mediate VEGF-signaling in the angiogenesis.

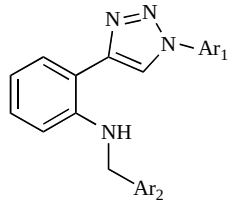
3.2.9. Antiangiogenic Agents of Pyrimidine Type

Many derivatives bearing variously substituted pyrimidine core have been developed as VEGFR TKIs.



134-138

Compound	Ar_1	Ar_2	VEGFR-2, IC_{50} (nM)	VEGFR-2, IC_{50} (nM)
134	3-$F_3C(C_6H_4)$	4-Pyridine	41	170
135	3-$F_3CO(C_6H_4)$	4-Pyridine	33	260
136	3-*t*Bu-(C_6H_4)	4-Pyridine	35	310
137	3-Br-(C_6H_4)	4-Pyridine	27	55
138	4-Me-3-$F_3C(C_6H_4)$	4-Pyridine	43	190



139-141

Compound	Ar_1	Ar_2	VEGFR-2, IC_{50} (nM)	VEGFR-2, IC_{50} (nM)
139	3-Cl-(C_6H_4)	4-Pyridine	87	130
140	3-F_3C-(C_6H_4)	4-Pyridine	51	66
141	3-$F_3CO(C_6H_4)$	4-Pyridine	66	72

Pyrimidine derivatives, which are useful as VEGFR-2 inhibitors are reviewed herein.

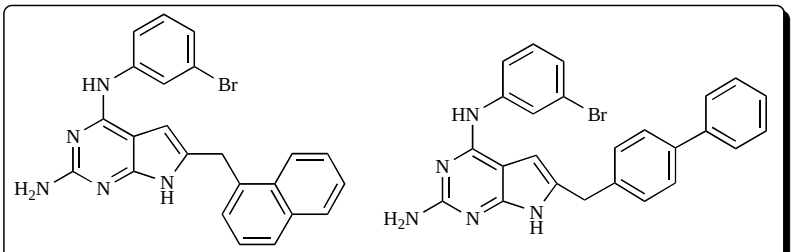
On the basis of pharmacophore model, Gangjee and colleagues designed RTKIs using the pyrrolo[2,3-*d*]pyrimidine scaffold with a 2-NH_2 moiety [83]. These compounds were synthesized and evaluated as inhibitors of VEGFR-1/2, EGFR, and PDGFR-β. Two of them, (**142**) and (**143**), exhibited high antiangiogenic activity in the CAM assay.

Thienopyrimidine was then identified as VEGFR inhibitors from high-throughput screening. The template was structurally attractive in that it contained three motifs that could be easily modified. Munchhof developed a series of novel thienopyrimidines as potent inhibitors of VEGFR-2 [84]. Four of the title compounds, (**144-147**), exhibited excellent VEGFR-2 inhibitory activity with the IC_{50} values < 10 nM.

4-NH_2-furo[2,3-d]pyrimidines bearing a diarylurea substituent at 5-position was reported as potent dual inhibitors of Tie-2 and VEGFR-2 [85]. (**148**) was the most active analogue with the IC_{50} values of 3 nM against VEGFR-2. It also showed potent cellular inhibitory activity versus proliferation of HUVEC stimulated by VEGF.

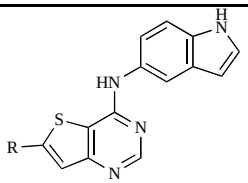
RO4383596 (**149**) is a pyrimidopyrimidone inhibitor of key pro-angiogenic receptor tyrosine kinases involved in tumor angiogenesis [86]. In cellular assays, RO4383596 potently and selectively inhibited the proliferation of VEGF-induced HUVEC. This agent has an excellent pharmacokinetic profile and is highly efficacious in rodent models of angiogenesis upon oral administration.

A series of pyrimidines with RTK and dihydrofolate reductase inhibitory activity in single molecules were prepared by Gangjee [87]. These analogs were designed with the po-



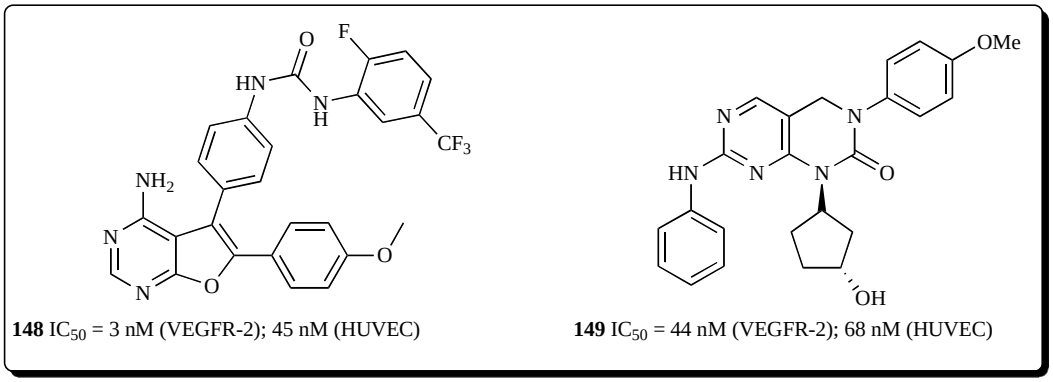
142 IC_{50} = 53 nM (CAM assay)

143 IC_{50} = 52 nM (CAM assay)



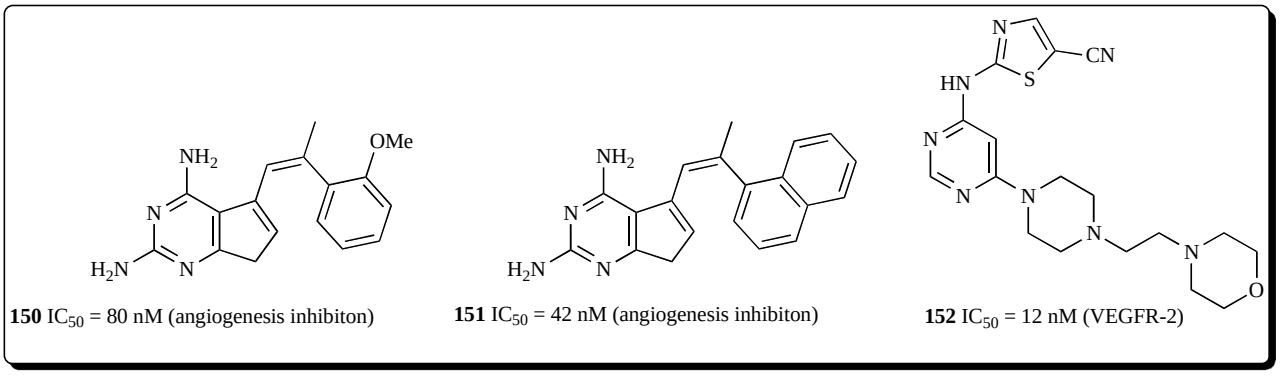
144-147

Compound	R	VEGFR-2, IC_{50} (nM)
144	4-$PhCH_2NH(CH_2)_2NHAc$	4
145	4-$PhCH_2N$-pipiperdine-4-$CONH_2$	2
146	4-$PhCH_2NH(CH_2)_3N$-1-imidazole	4
147	4-$PhCH_2NHC(CH_3)_2CH_2OH$	3



148 IC_{50} = 3 nM (VEGFR-2); 45 nM (HUVEC)

149 IC_{50} = 44 nM (VEGFR-2); 68 nM (HUVEC)



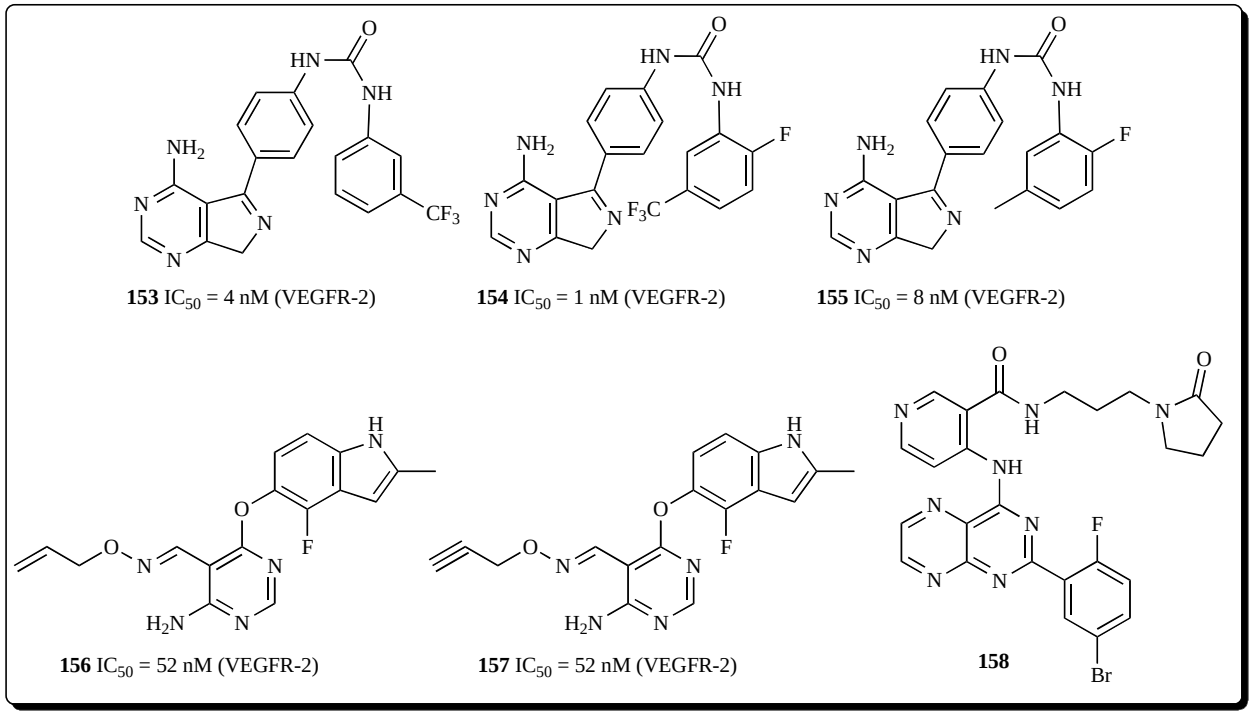
150 IC_{50} = 80 nM (angiogenesis inhibiton)

151 IC_{50} = 42 nM (angiogenesis inhibiton)

152 IC_{50} = 12 nM (VEGFR-2)

tential to possess both cytostatic activity and cytotoxic activity in a single molecule. The *in vivo* antitumor evaluation indicated that compound (**150**) and (**151**) were active as anticancer agents *in vivo*, both against primary tumors and metastases. Pyrimidino-thiazolyl carbonitriles had been identified as VEGFR-2 inhibitors [88]. The modification of lead compounds resulted in a representative compound (**152**) which possessed the best overall profile of VEGFR-2 inhibitory activity.

Ji Z and colleagues developed a series of novel isothiazolopyrimidines as potent VEGFR-2 inhibitors [89]. SAR studies led to isothiazolopyrimidines urea analogs (**153, 154**,



153 IC_{50} = 4 nM (VEGFR-2)

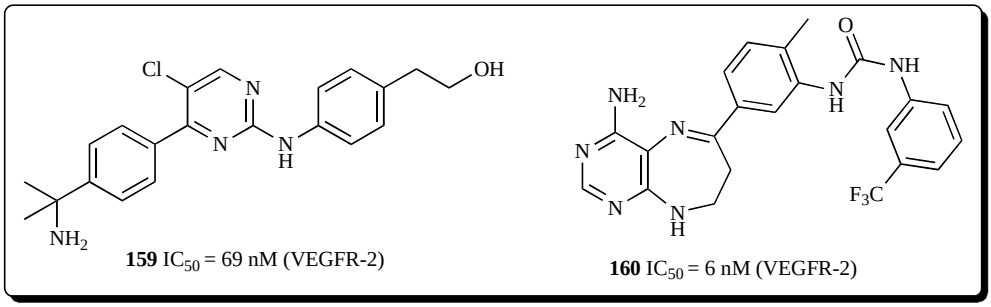
154 IC_{50} = 1 nM (VEGFR-2)

155 IC_{50} = 8 nM (VEGFR-2)

156 IC_{50} = 52 nM (VEGFR-2)

157 IC_{50} = 52 nM (VEGFR-2)

158



159 IC_{50} = 69 nM (VEGFR-2)

160 IC_{50} = 6 nM (VEGFR-2)

and **155**) which exhibited excellent VEGFR-2 TK inhibitory activity (VEGFR-2 enzymatic and cellular IC_{50} values below 10 nM). A novel 4-aminopyrimidine-5-carboxaldehyde oxime scaffold with VEGFR-2 inhibitory activity was identified by Huang [90]. This scaffold exhibited high selectivity for VEGFR-2 and antiproliferative activity against several cancer cell lines. When the oxime side chain was an allyl or propargyl group (**156**, or **157**), high potency for both VEGFR-2 and cell proliferation was achieved.

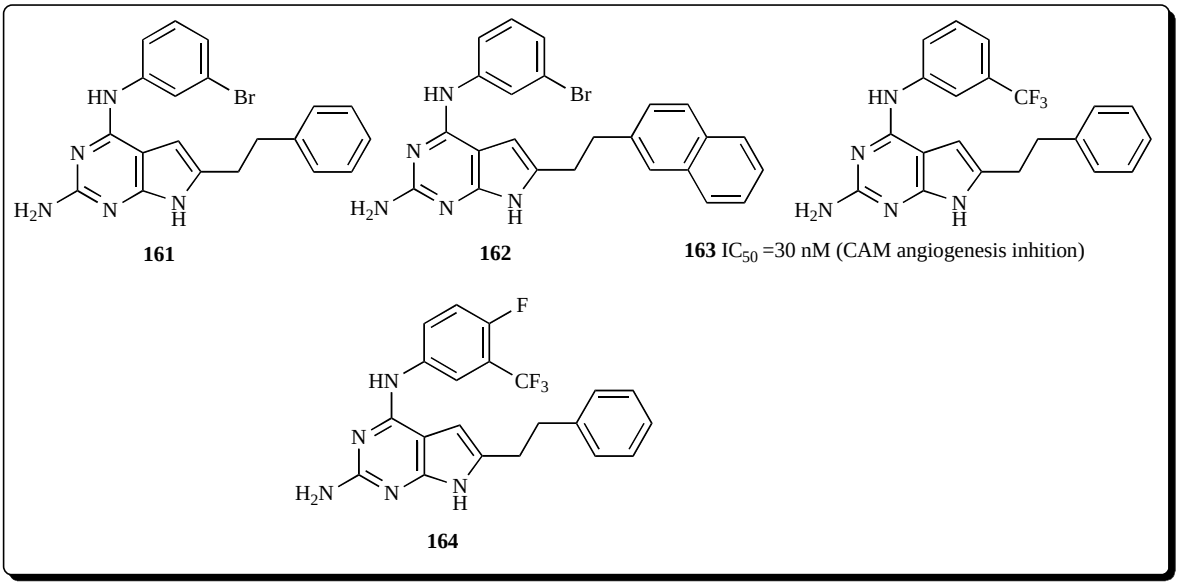
In order to develop potent and non-cytotoxic HCV inhibitors, a series of pteridines were prepared [91]. Fortunately, some of these compounds exhibited potent VEGFR inhibitory activity. (**158**) exhibited a selective inhibition profile against VEGFR kinases 1-3 (VEGFR-1, IC_{50} = 47 nM; VEGFR-2, IC_{50} = 140 nM; and VEGFR-3, IC_{50} = 123 nM). On the basis of initial research, Huang disclosed a related series of novel chloropyrimidines as potent VEGFR-2 inhibitors [92]. Their work was surprising in light of the potent VEGFR-2 inhibition shown by all compounds and the robust antiproliferative activity exhibited by compound (**159**).

Structure-based design led to the discovery of compound (**160**) with potent inhibitory activity against VEGFR-2 RTKs [93]. It showed low nanomolar VEGFR-2 enzymatic and cellular potencies (IC_{50} = 6 and 52 nM, respectively). Gangjee developed a novel class of substituted pyrrolo[2,3-*d*]pyrimidines as multiple RTK inhibitors and antiangiogenic agents [94]. Novel multi-RTK inhibitors (**161, 162**) and potent inhibitors of angiogenesis (**163, 164**) were identified. The best compound (**163**) exhibited excellent potency with an IC_{50} value of 30 nM in the CAM angiogenesis inhibition assay.

Novel 7-aminopyrazolo[1,5-*a*]pyrimidine inhibitors of VEGFR and PDGFR were reported in 2008 [95]. Several of them, such as (**165**), were potent inhibitor of VEGFR-2 both enzymatically (<10 nM) and cellularly (<10 nM). Dependent on the region-substitution of the purine scaffold, Peifer developed a series of novel N_9 or N_7 -arylethanone-substituted 6-aminopurines as VEGFR inhibitors [96]. Two of them (**166, 167**) were interesting lead structures for the development of potent and selective VEGFR-2 inhibitors.

3.2.10. Antiangiogenic Agents of Miscellaneous Type

Sridhar developed a novel pharmacophore based on the binding of ATP to the hinge region of the kinase domain of VEGFR and conducted a database search of 18,000 compounds [97]. Novel cores had been identified containing pthalimide. The research afforded (**168, 169**) with good antiangiogenic activity by optimization of the lead compound. (**169**) exhibited 60% inhibition against sprouting and formation of the tubular networks at 50 nM. Harris PA developed 2-anilino-5-aryloxazole derivatives as a novel class of potent VEGFR-2 TKIs in the same year [98]. Optimization of both aryl rings led to two potent inhibitors (**170, 171**) at both the enzymatic and cellular levels.

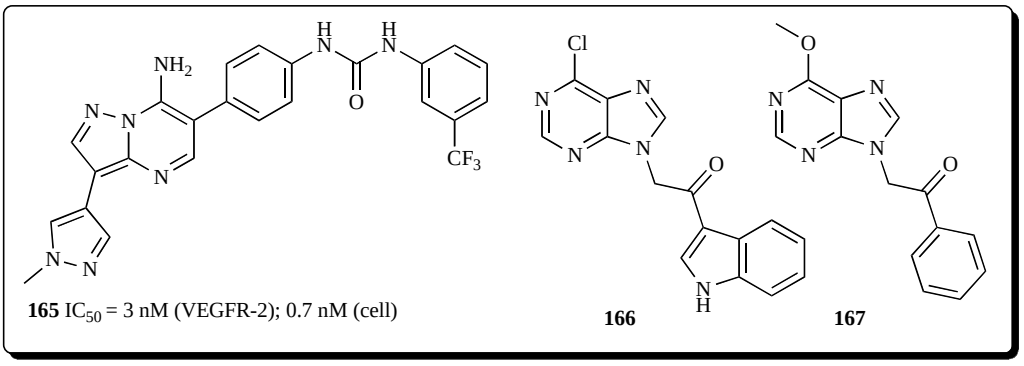


161

162

163 IC_{50} =30 nM (CAM angiogenesis inhition)

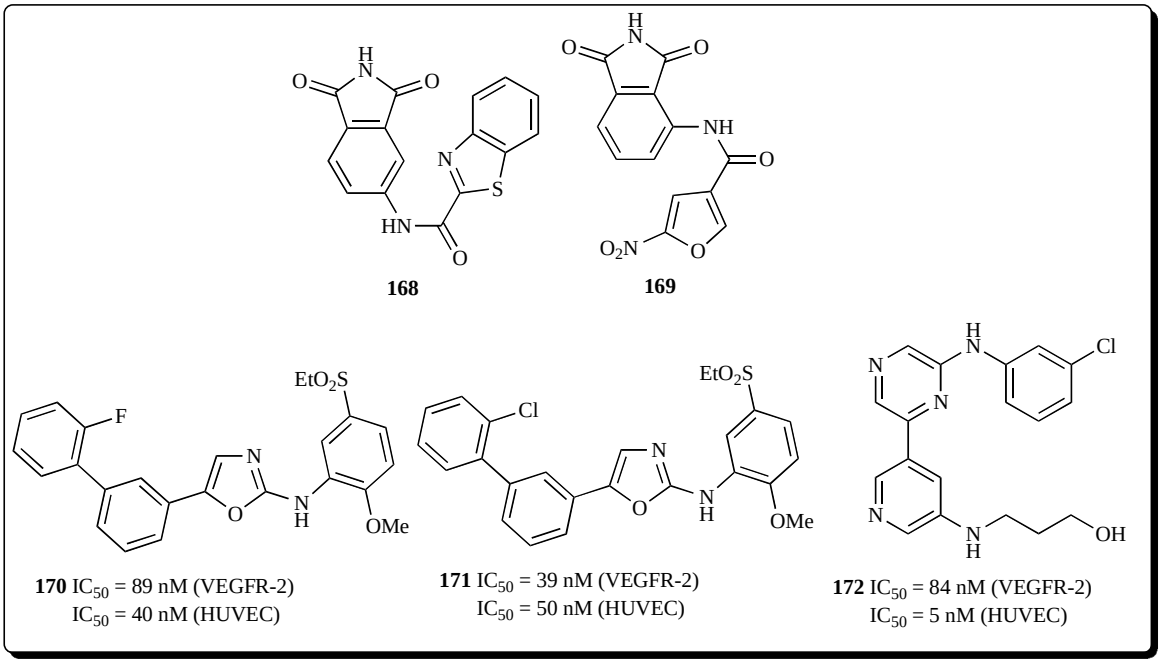
164



165 IC_{50} = 3 nM (VEGFR-2); 0.7 nM (cell)

166

167



168

169

170 IC_{50} = 89 nM (VEGFR-2)
IC_{50} = 40 nM (HUVEC)

171 IC_{50} = 39 nM (VEGFR-2)
IC_{50} = 50 nM (HUVEC)

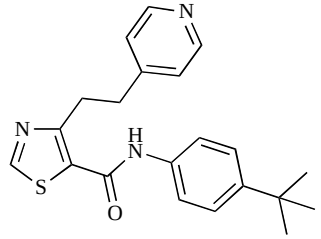
172 IC_{50} = 84 nM (VEGFR-2)
IC_{50} = 5 nM (HUVEC)

Before long, Kuo reported the unexpected discovery of a pyrazine-pyridine biheteroaryl as a novel series of potent VEGFR-2 TKIs [99]. Among them, (**172**) which had IC_{50} = 84 nM inhibitory activity against VEGFR-2 exhibited high inhibitory selectivity against VEGF-induced HUVEC proliferation (IC_{50} = 5 nM).

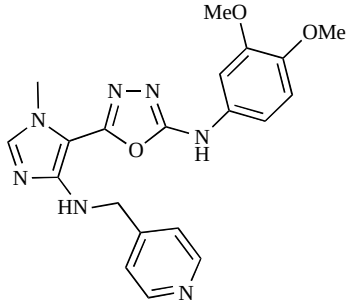
A novel series of ((pyridine-4-yl)ethyl)pyridines were reported as potent VEGFR-2 inhibitors [100]. One compound (**173**) of them possessed good VEGFR-2 inhibitory activity in both enzymatic (IC_{50} = 110 nM) and cellular assays (IC_{50} = 73 nM). They also identified a novel class of imidazole derivatives as potent inhibitors of VEGFR-2 in both enzymatic and cellular assays (IC_{50} < 100 nM) [101]. (**174**) exhibited excellent inhibitory activity and could be further developed for *in vivo* studies. They reported another series of novel potent ((pyridine-4-yl)ethyl)pyridine derivatives active against kinases VEGFR-1/2 [102]. Notably, (**175**) with potent activity yielded over 20-fold selectivity for the VEGFR-2 versus VEGFR-1 kinase.

Borzilleri discovered a ATP-competitive VEGFR-2 inhibitor, BMS-605541 (**176**), using compouter-assisted drug design techniques [103]. It exhibited excellent inhibitory activity, kinase selectivity, favorable pharmacokinetic properties, and robust *in vivo* efficacy. In 2007, Akritopoulou-Zanze developed a series of 5-substituted 1,4-dihydroindeno [1,2-*c*]pyrazoles as novel potent multitargeted kinase inhibitors [104]. Two of them (**177, 178**) exhibited good VEGFR-2 enzymatic and cellular activity (IC_{50} = 19 and 62 nM, respectively). Novel derivatives of 1,2,4-trizaoles were then developed as potent inhibitors of both VEGFR-1/2 [105]. Both enzymatic and cellular activities of (**179**) were comparable to the clinical candidates.

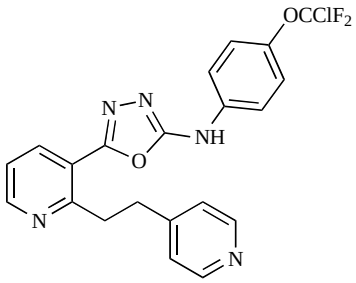
On the basis of (**178**), Dinges discovered two novel VEGFR-2 TKIs (**180, 181**) by optimization of the acetylene-type side chain [106]. The selectivity profile for the two compounds revealed that both compounds were multitargeted RTKIs with low nanomolar potencies against VEGFR kinase subfamilies. Guided by X-ray crystallography and molecular modeling, a series of 2-aminobenzimidazoles and 2-aminobenzoxazoles were identified as potent inhibitors of VEGFR-2 in both enzymatic and HUVEC cellular proliferation assays [107]. Among them, (**182**) exhibited good potencies and pharmacokinetic profile.



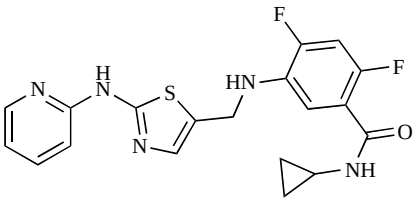
173 IC_{50} = 110 nM (VEGFR-2)



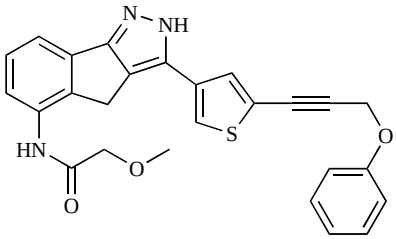
174 IC_{50} = 52 nM (VEGFR-2)



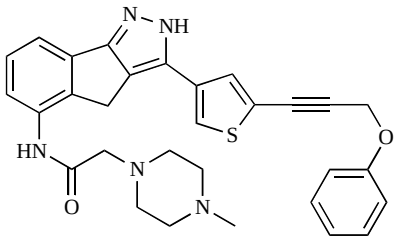
175 IC_{50} = 31 nM (VEGFR-2)
IC_{50} = 730 nM (VEGFR-1)



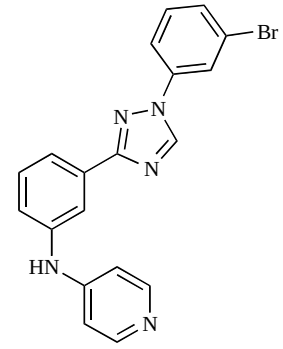
176 IC_{50} = 23 nM (VEGFR-2)
IC_{50} = 25 nM (HUVEC)



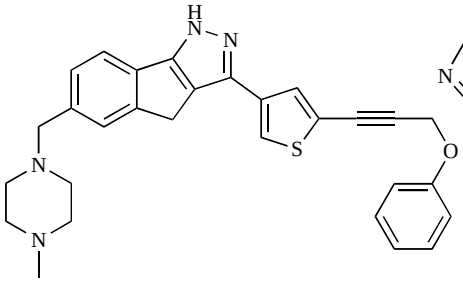
177 IC_{50} = 53 nM (VEGFR-2)



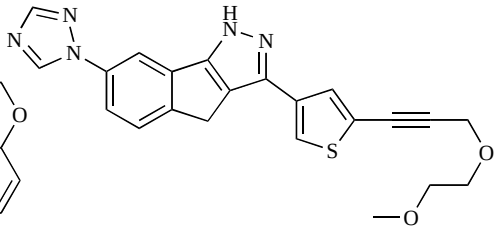
178 IC_{50} = 53 nM (VEGFR-2)



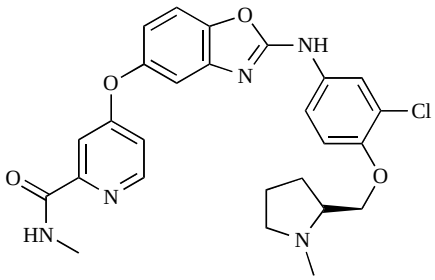
179 IC_{50} = 46 nM (VEGFR-2)
IC_{50} = 68 nM (VEGFR-1)



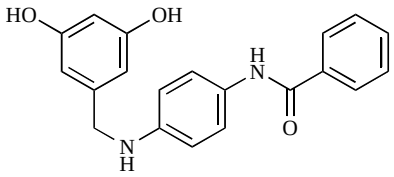
180 IC_{50} = 6 nM (VEGFR-2)



181 IC_{50} = 5 nM (VEGFR-2)



182 IC_{50} = 3 nM (VEGFR-2)
IC_{50} = 15 nM (HUVEC)



183 IC_{50} = 570 nM (VEGFR-2)

A novel class of *N*-benzylbenzene-1,4-diamine derivatives were described as new candidates for VEGFR-2 inhibitors using *de nono* drug design systems based on the X-ray structures of VEGFR-2 kinase domain [108]. Among the title compounds, (**183**) exhibited significant inhibitory activity of VEGFR-2 TK with the IC_{50} value of 570 nM. Furthermore, it could suppress the VEGF-stimulated phosphorylation of VEGFR-2 in HUVEC cells.

4. SIDE-EFFECTS OF ANGIOGENESIS INHIBITORS

The inhibition of VEGFR TKs that are implicated in tumor vasculature formation and maintenance, as well as tumor progression and metastasis, has been a major focus in oncology research. Clinical experience with VEGF/VEGFR pathway targeting angiogenesis inhibitors is rapidly increasing, and several compounds have already been approved for anticancer treatment. Apart from their activity, much attention should be focused on the clinical toxicity profile of these compounds. Angiogenesis inhibitors therapy may not necessarily kill tumors, but instead may keep tumors stable. Therefore, this type of therapy may need to be administered over a long period. Because angiogenesis is important in wound healing and in reproduction, long-term treatment with angiogenic agents could cause problems with bleeding, blood clotting, heart function, the immune system, and the reproductive system [109]. Besides the haematological side effects of most of TKIs like anemia, thrombopenia and neutropenia, the most common extra-heamatologic adverse effects are edema, nausea, hypothyroidism, vomiting and diarrhea [110]. According to several clinical studies, targeting VEGF/VEGFR signaling alone is sometimes insufficient to stop tumor growth. The combined administration of antiangiogenic and cytotoxic therapies can yield additional therapeutic benefits as compared with monotherapy [111].

Clinical trials of VEGFR inhibitors in metastatic medullary thyroid cancer suggested that caution should be taken because of the rare but fatal toxicity potentially associated with VEGFR inhibitors [112]. Another research indicated that treatment with VEGFR inhibitors was usually associated with a significant increase in the risk of arterial thromboembolic events [113].

Recent evidence suggested that combination therapy of cancer with RTK inhibitors, which are usually cytostatic, with conventional chemotherapeutic agents, which are usually cytotoxic, provide an improved treatment option.

5. CONCLUSIONS

The VEGF/VEGFR pathway plays a central role in tumor vasculature. Because angiogenesis in healthy adults is generally absent, interruption of VEGF/VEGFR signaling is an attractive strategy to selectively inhibit angiogenesis in solid tumors. Antiangiogenic therapies based on inhibition of VEGFR TK were reported to be powerful clinical strategies in oncology.

Identification of novel VEGFR inhibitors continues to be an intense area of investigation in anticancer research. Since all VEGFR inhibitors show anticancer efficacy attributable to antiangiogenic activity, it is very probable that the efficacy is a result of VEGFR inhibition mechanism. In addition to the VEGF/VEGFR signaling pathway, other targets are also being explored, including angiopoietin/Tie receptors, EGF/EGFR, ephrins/Eph receptors, PlGF, bFGF, TNF, and others. The potential advantages of agents targeting at VEGF/VEGFR include easy access to targets because of intravascular receptor-ligand interactions, broad activity against many tumor types. Because angiogenesis is infrequent in the adult, there is the potential to development antiangiogenic agents with less toxic.

The success of these SBDD studies with VEGFR TK improves current knowledge on cancer, and facilitates the development of novel approaches of treatment of cancer. The crystallographic studies, molecular modeling and computational chemistry analysis, understanding of the enzyme mechanism and synthetic chemistry may provide encouragement for future studies of VEGFR TKIs as anticancer agents.

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ABBREVIATIONS

DFG	=	Asp-Phe-Gly
EGF	=	Epidermal Growth Factor
EGFR	=	Epidermal Growth Factor Receptor
FDA	=	Food and Drug Administration
FGF	=	Fibroblast Growth Factor
FGFR	=	Fibroblast Growth Factor Receptor
bFGF	=	basic Fibroblast Growth Factor
HUVEC	=	Human Umbilical Vein Endothelial Cell
KDR	=	Kinase Insert Domain Receptor
MAb	=	Monoclonal Antibody
PlGF	=	Placenta Growth Factor
RTK	=	Receptor Tyrosine Kinase
SAR	=	Structure-Activity Relationship
SBDD	=	Structure-Based Drug Design
Tie-2	=	Tyrosine Kinase with Immunoglobulin and Epidermal Growth Factor Homology Domains-2
TK	=	Tyrosine Kinase
TKIs	=	Tyrosine Kinase Inhibitors
TKR	=	Tyrosine Kinase Receptor
VEGF	=	Vascular Endothelial Growth Factor
VEGFR	=	Vascular Endothelial Growth Factor
VPF	=	Vascular Permeability Factor

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