Recent Progress of Small Molecular VEGFR Inhibitors as Anticancer Agents

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Abstract: Vascular endothelial growth factor receptor (VEGFR) is an important receptor tyrosine kinase (RTK) in the induction of angiogenesis. Abnormal activation of VEGFR leads to several disorders including cancer. Nowadays, inhibition of VEGFR kinase has been one of the most powerful clinical strategies in cancer treatment and great efforts to design and synthesize small molecular VEGFR inhibitors for cancer research have been made in recent years. This review highlights the major progress and development of them, including their structure and pharmacophore features, biological activities and structure-activity relationships (SAR). Special attentions are paid to the compounds available in market or in advanced clinical stages.

Keywords: Vascular endothelial growth factor receptor, anticancer, inhibitor, angiogenesis, small molecular.

1. INTRODUCTION

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is a normal and vital process in growth and development [1], as well as in wound healing and female reproductive cycling [2]. However, Abnormal regulation of angiogenesis has been reported in the pathogenesis of several disorders including coronary artery disease [3, 4], inflammation [5, 6] and age-related macular degeneration [7]. Especially, it is also a fundamental step in the transition of tumors from a dormant state to a malignant one [2, 8, 9].

The concept of angiogenesis inhibition is a promising therapeutic strategy against cancer. In 1971, Dr. Judah Folkman first proposed the hypothesis that tumor growth and their subsequent metastasis are angiogenesis dependent [10] because neovascularization permits further growth of the primary tumor while also providing a potential pathway for migrating tumor cells to gain access to the systemic circulation and establish distant metastases. Based on further study, he proposed that tumors cannot grow beyond 2mm in diameter without developing a vascular supply [11]. In the absence of a functional vascular supply tumors remain dormant and unable to metastatize [10, 12]. Since studies have shown that angiogenesis is a key pathway for tumor growth and invasion, targeting angiogenesis represents a new and hot strategy for the development of anticancer therapies.

In a pathological condition such as cancer, angiogenesis is tightly regulated and balanced by endogenous proangiogenic and antiangiogenic factors. These two endogenous factors are also called, respectively, angiogenesis activators and angiogenesis inhibitors [9, 13]. The balance of activators and inhibitors governs the angiogenic switch. In some tissues, the absence of angiogenesis activators, or inhibitors outweighing the activators would keep the switch "off", while in others if the level of activators is higher than that of inhibitors, the angiogenic switch may be turned ''on'' for cancer progression. Therefore, either reducing the inhibitor concentration or increasing the activator levels can each change the balance and activate the switch, leading to tumor angiogenesis [14].

Vascular endothelial growth factor (VEGF) is one of the most important activitors of angiogenesis. VEGF activation of its receptor VEGFR has a fundamental role in inducing vascular endothelial cell proliferation, migration, and angiogenesis, which is closely linked to the development of cancer [2, 14-16]. So inhibition of the VEGF/VEGFR signaling system is an attractive approach for the treatment of cancer.

In order to cure cancer by inhibiting abmormal angiogenesis, VEGF/VEGFR inhibitors were proposed and have made great progress in the recent years. The most studied and developed inhibitors are monoclonal antibodies that neutralize VEGF, ribozymes, and small molecule VEGFR inhibitors. Some of them have been approved by U.S. Food and Drug Administration (USFDA) for cancer treatment.

2. VEGF AND ITS RECEPTOR VEGFR

VEGFs are potent mitogens produced by cells that stimulate the growth of new blood vessels. They are approximately 34–46-kDa homodimeric glycoproteins and have been identified as principal factors in the induction of angiogenesis [17]. As a potent and specific proangiogenic factor, the VEGF gene family consists of six groups including VEGF-A, -B, -C, -D, -E and the placental growth factor (PlGF) [18, 19].

VEGFRs are indentified as members of the Receptor Tyrosine Kinase (RTK) superfamily. There are three main subtypes: VEGFR-1 (also known as Flt-1), VEGFR-2 (also

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known as KDR/Flk-1) and VEGFR-3 (also known as Flt-4). These unliganded receptors are clearly monomeric while VEGFs have distinctive binding specificities for the three transmembrane tyrosine kinase receptors, leading to the dimerisation of VEGFRs and activating them [20, 21]. In response to ligands binding, VEGFRs lead to the autophosphorylation of intracellular tyrosine kinases and subsequent activate a network of distinct downstream signaling pathways that elicit various angiogenic programs [22]. In adults, VEGFR-2 is located on the surface of vascular endothelial cells and is reported as the key mediator of VEGF-induced angiogenesis. VEGFR-1 is expressed on haematopoietic stem cells, macrophages and monocytes as well as on the vascular endothelium. VEGFR-3 is restricted largely to the lymphatic endothelium, its expression is associated with the dissemination of tumor cells to regional lymph nodes [20, 22].

VEGF/VEGFR signaling system fulfills numerous functions in the regulation of the tumor vasculature, including endothelial cell proliferation, migration, invasion, vascular permeability and vasodilation. Considerable evidence from clinical observations and laboratory investigations indicate that VEGF/VEGFR-induced abnormal angiogenesis is implicated in many tumor entities, including stomach [23], lung [24], bladder [25], kidney [25], ovarian [26], prostate [27] and gastrointestinal tract cancer [28] as well as melanoma [29], Leukaemia, lymphoma and multiple myeloma [30].

3. MECHANISM OF VEGF/VEGFR SIGNALING SYSTEM INDUCING TUMOR ANGIOGENESIS

Among the three VEGFR subtypes, VEGFR-2 is critical for VEGF-induced mitogenic and chemotactic signaling in vascular endothelial cells and plays a critical role in tumor angiogenesis development and hematopoiesis, while VEGFR-1-dependent signaling plays a role in angiogenesis of certain tumors, the progression of rheumatoid arthritis, and atherosclerosis and VEGFR-3 is related to the process of lymphangiogenesis. VEGFR-2 is composed of seven extracellular immunoglobulin homology domains containing the ligand-binding region, a single short transmembrane domain, and an intracellular tyrosine kinase signaling domain, split by a 70-amino-acid insert [20, 21]. The binding domain is in the extracellular region and binds all VEGF-A isoforms, VEGF-C, -D and –E.

Under normal conditions, unliganded VEGFR-2 is clearly monomeric, and its extracellular domains appear to adopt essentially random conformations. Binding of VEGF to immunoglobulin homology domains of one VEGFR-2 increases the probability that another VEGFR-2 monomer can bind the already tethered ligand. The immunoglobulin homology domains 2 and 3 have been shown to mediate ligand binding [31]. Once two receptors are bridged by VEGF ligand to be cross-linked to each other, their seven extracellular immunoglobulin homology domains are held in close proximity. The low-affinity homotypic interaction be-

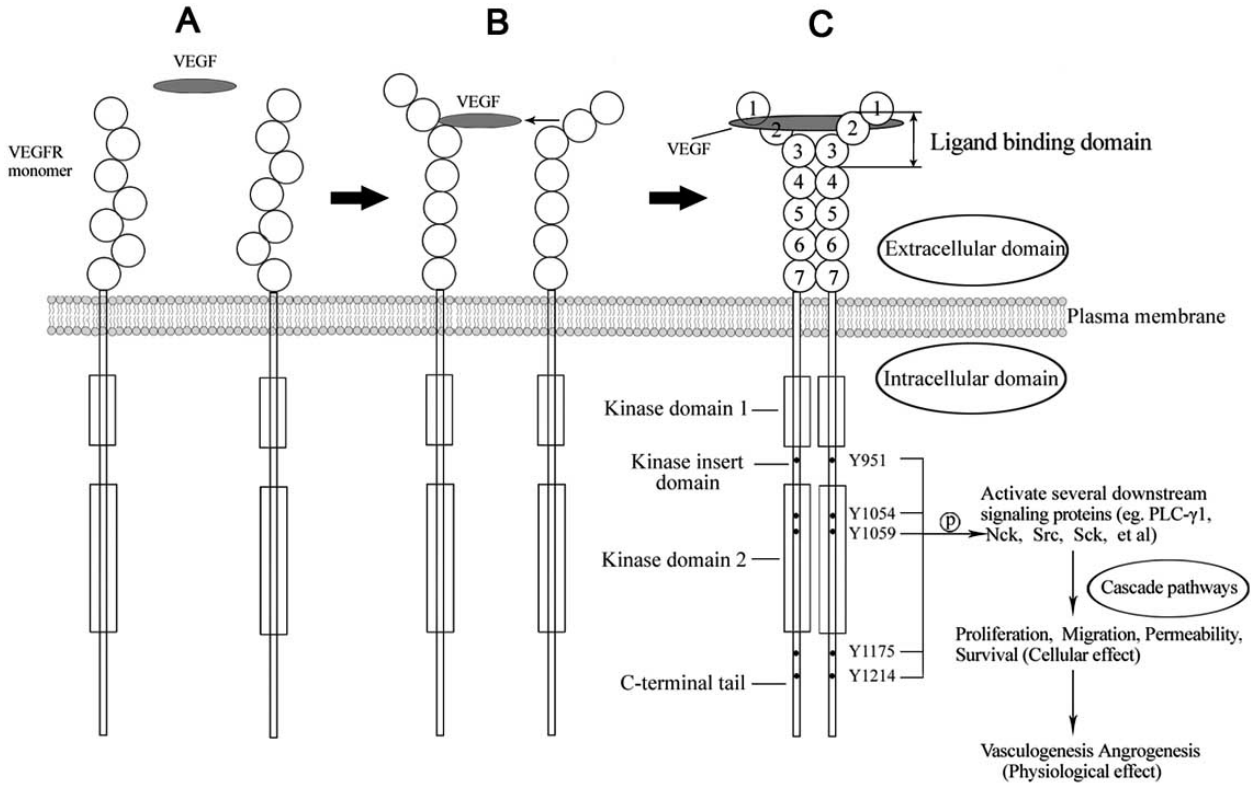


Fig. (1). The binding mode of VEGF and VEGFR-2. **Part A.** Unliganded VEGFR-2 is monomeric**.** Its extracellular domain is composed of seven immunoglobulin homology domains, which appear to adopt essentially random conformations. **Part B.** Binding of VEGF to one VEGFR-2 monomer increases the probability that another monomer tends to bind the already tethered ligand. The immunoglobulin homology domains 2 and 3 mediate the VEGF binding. **Part C.** Binding of VEGF induces the dimerization of VEGFR-2, leading to autophosphorylation of intracellular tyrosine residues and subsequent initiating the signal transduction cascade, resulting in angiogenesis.

tween these domains can promote structural rearrangements of the receptor dimmer [32]. The ligand-induced dimerization of VEGFR-2 can be communicated across the membrane, initiating signaling through intracellular tyrosine kinase domains and resulting in autophosphorylation. Five tyrosine residues have been identified as the major phosphorylation sites, such as Y951 in the tyrosine kinase insert domain, Y1054 and Y1059 in the activation loop of the tyrosine kinase domain 2, Y1175 and Y1214 in the carboxyl-terminal tail (Fig. **1**) [33]. Phosphorylation of these sites, together with the adjacent amino-acid sequence subsequently leads to phosphorylation of several intracellular signaling proteins including phospholipase C-γ1 (PLC-γ1), Src family tyrosine kinases, phosphatidylinositol 3-kinase (PI3K), oncogenic adaptor protein Nck, the adaptor protein Shb and Sck. These signaling proteins activate the cascade downstream pathways that have close relationships with tumor angiogenesis [34, 35].

4. RECENT PROGRESS OF DIFFERENT TYPES OF VEGFR INHIBITORS

Since VEGF/VEGFR signaling pathway is closely related to tumor angiogenesis, tremendous progress has been made in the discovery of blocking the VEGF/VEGFR signaling system. Five agents have been approved by the USFDA for the treatment of various cancers (Table **1**). Numerous potent, specific and well-tolerated inhibitors are in the clinical trials. All the inhibitors are divided into four types: 1) neutralizing monoclonal antibodies against VEGF and VEGFR; 2) small molecule VEGFR tyrosine kinase inhibitors; 3) soluble VEGFR and 4) ribozymes [36].

Recent studies have shown that most of small molecular VEGFR inhibitors are ATP-binding site inhibitors. They inhibit the VEGFR activity by directly occupying the ATP-binding site of the respective intracellular kinase domain, leading to the inhibition of VEGFR phosphorylation and, ultimately, to the apoptotic death of the aberrant endothelial cells. This review focuses on these small molecular VEGFR inhibitors, with an emphasis on the compounds in the market or in advanced clinical stages (Table **2**).

4.1. Quinazolines and Quinolines

The quinazoline and quinoline skeletons were present in many tyrosine kinase (TK) inhibitors. It was reported that the first synthesized potent and selective small molecule TK inhibitor was PD-153035 (**1**) (Fig. **2**) [37], targeted at the epidermal growth factor receptor (EGFR). Its structure was based on a quinazoline skeleton. Many compounds containing quinazoline or quinoline skeleton also played important roles as VEGFR inhibitors.

4.1.1. Vandetanib

Vandetanib (ZD6474, developed by AstraZeneca) (**2**) (Fig. **2**) was a potent, orally active inhibitor of VEGFR-2 kinase (IC_{50} = 40 nM) and also exhibited activity against VEGFR-1, -3, EGFR-1, RET and platelet derived growth factor receptor-β (PDGFR-β), and moreover, it was shown potent inhibition of VEGF-stimulated proliferation of human umbilical vein endothelial cells (HUVEC; IC_{50} = 60 nM), which naturally expressed the VEGFR-2 receptor [38]. On account of its inhibition of VEGFR-2, Vandetanib was reported to inhibit the growth of experimental lung metastasis and to reduce the weight and the size of lung nodules in a non-small cell lung cancer (NSCLC) model [39]. Besides, *in vitro* and *in vivo* studies proved that Vandetanib targeted not only tumors that use VEGF signaling for the promotion of angiogenesis, but also inhibited the growth of several EGFR-expressing human cancer cell lines that lack VEGFR-2 expression, thus potentially expanding possible future indications for the drug [40].

Table 1. Marketed Compounds and Representative Inhibitors in Clinical Trials Targeted at VEGF/VEGFR-Induced Tumor Angiogenesis

Agent	Originators	Highest development Phase	Molecular target
Bevacizumab	Genentech	Launched in 2004	Recombinant humanized monoclonal antihuman VEGF antibody
Pegaptanib	Eyetech Inc	Launched in 2004	Anti-VEGF synthetic RNA oligomer
Ranibizumab	Genentech	Launched in 2006	Recombinant humanized monoclonal antihuman VEGF antibody
Sorafenib	Bayer, Onyx	Launched in 2005	PDGFR-β, Raf and VEGFR-2/3
Sunitinib	Pfizer, Sugen	Launched in 2006	Flt-3, c-Kit, PDGFR-β, VEGFR-1/2/3, CSF-1R and RET
Pazopanib	GlaxoSmithKline	Launched in 2009	c-Kit, PDGFR and VEGFR-1/2/3
Vandetanib	AstraZeneca	Phase III	EGFR, RET, PDGFR-β and VEGFR-1/2/3
Vatalanib	Novartis, Shering AG	Phase III	c-Kit, PDGFR and VEGFR-1/2/3
Axitinib	Pfizer	Phase III	c-Kit, PDGFR and VEGFR-1/2/3
Cediranib	AstraZeneca	Phase III	c-Kit, PDGFR and VEGFR-1/2/3

4.1.2. Other Quinazoline and Quinoline Agents

Further optimization of Vandetanib was replacing the anilino -NH- group with an oxygen atom that bonded to various aromatic rings.

Cediranib (AZD2171, developed by AstraZeneca) (**3**) (Fig. **2**) was a highly potent and selective oral inhibitor that against VEGFR-2 (IC_{50} < 1 nM), with activity also observed against VEGFR-1 (IC_{50} = 5 nM) and VEGFR-3 (IC_{50} = 3 nM). It also inhibited PDGFR protein kinase family, including c-Kit, PDGFR-β at the nanomolar concentration, and with good selectivity over a panel of other tyrosine and serine-threonine kinases [41, 42]. This agent is currently undergoing Phase III trials for the treatment of NSCLC, colorectal cancer, breast cancer, liver and ovary cancers [43].

KRN633 (**4**), KRN951 (**5**), Ki8751 (**6**) and compound (**7**) (Fig. **2**) were quinazoline and quinoline derivatives containing the urea fragments, an important class of TK inhibitors. All the four agents exhibited potent activities against VEGFR kinase and the VEGF-induced proliferation of HUVECs at very low concentrations [44-47]. Furthermore, Ki8751 (**6**) was a selective kinase inhibitor that showed a potent inhibitory activity for VEGFR-2 and a high selectivity for many other kinases over 1000-fold [46].

Merck researchers prepared a library of the indolyl quinolinone class. Compound (**8**) (Fig. **2**) showed high activity of VEGFR-2 (IC_{50} = 5 nM). In addition, it exhibited favourable pharmacokinetic properties and potentially safe ancillary profiles [48].

4.2. Phthalazines, Anthranilamides and their Derivatives

4.2.1. Valatanib

Valatanib (PTK787/ZK222584) (**9**) (Fig. **3**), disclosed by Novartis and Schering AG, was a potent, relatively selective, orally administered first generation VEGFR inhibitor. It has been shown previously to inhibit VEGFR-1, -2 and -3, with IC_{50} values of 77, 37 and 66 nM, respectively. It also inhibited other TKs, such as PDGFR and c-Kit, but at least 10 fold higher concentrations [49-51]. Besides, this compound did not have any cytotoxic or antiproliferative activity against cells not expressing VEGF receptors. Vatalanib is in Phase III development for the first- and second-line treatment of metastatic colorectal cancer [52].

A binding mode by docking Valatanib into a model of the human VEGFR-2 (KDR) ATP-binding site demonstrated that Valatanib did not form direct hydrogen bond interactions with the backbone of the hinge region, but rather occupied the hydrophobic regions of the binding site. The aniline

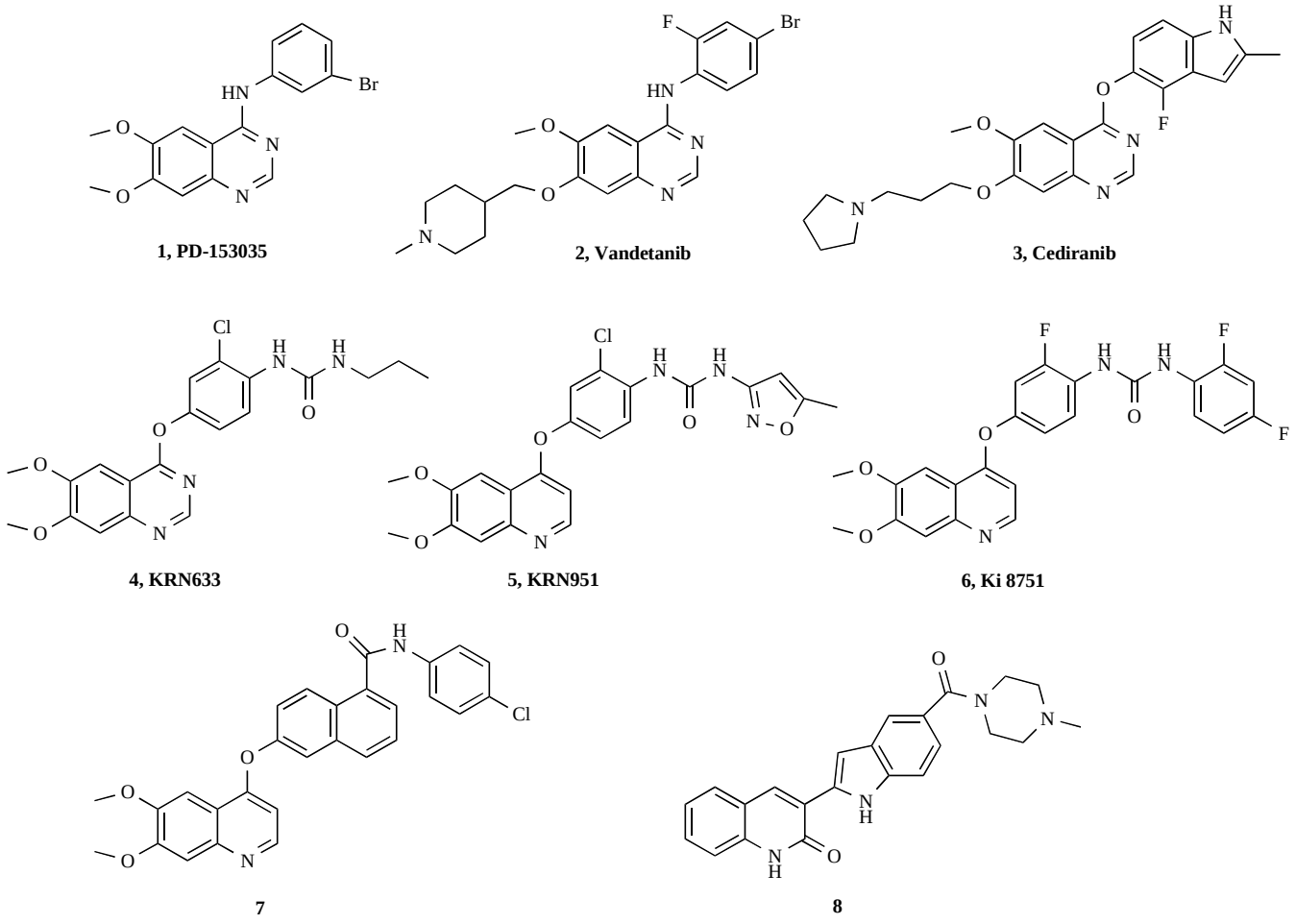


Fig. (2). PD-153035, quinazolines and quinolines.

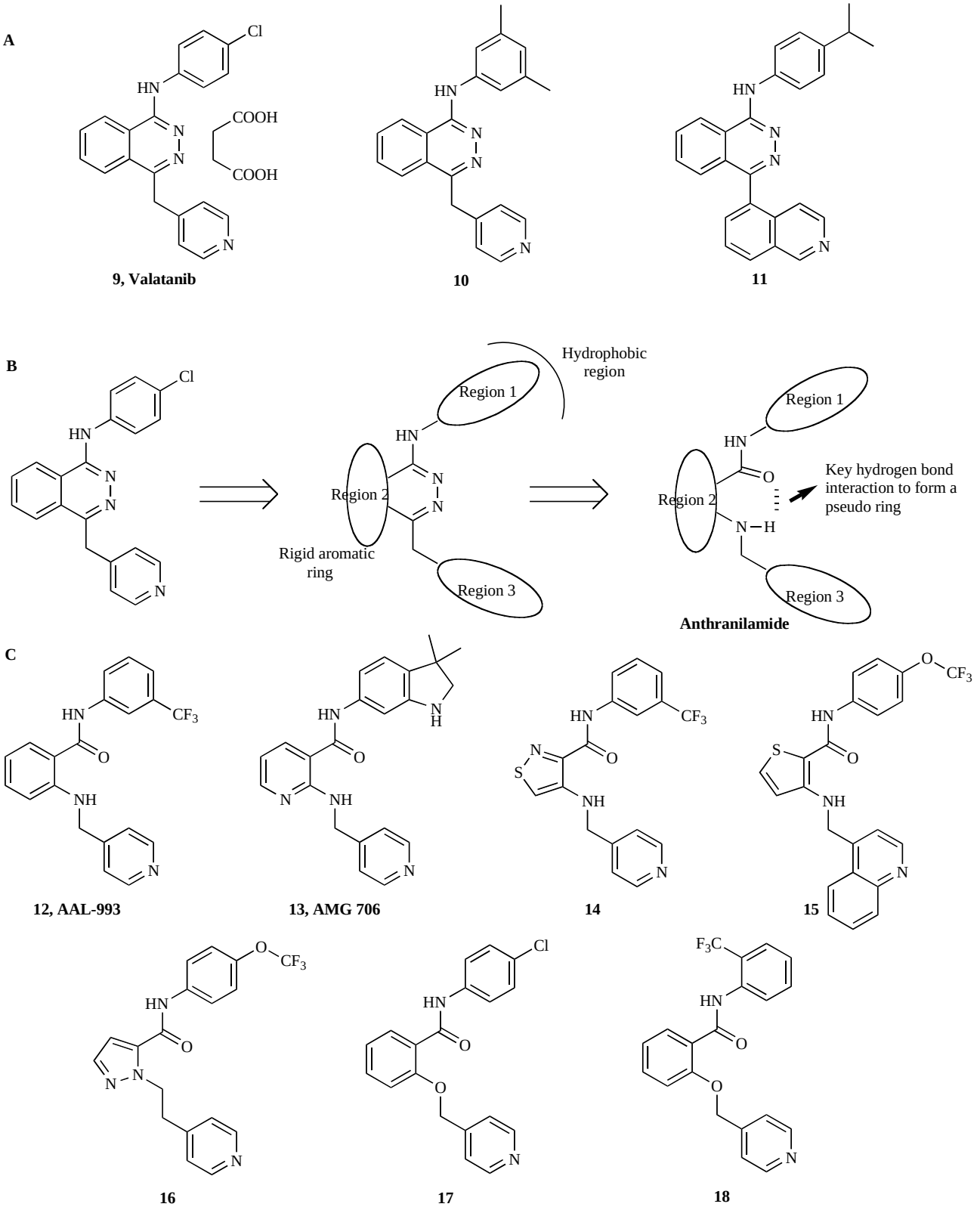


Fig. (3). Phthalazines, Anthranilamides and their derivatives.

Part A. Phthalazines: Valatanib and its analogues.

Part B. Discovery of anthranilamides.

Part C. Anthranilamides derivatives.

moiety was located in a hydrophobic pocket, while the phthalazine core made hydrophobic contacts with amino-acid residues Leu 1033, Gly 920, and Leu 838. The pyridil nitrogen was assumed to form a hydrogen bond with Lys1060 in the kinase activation loop [49]. This binding mode, accompanied with KDR inhibitory data from *in vitro* studies suggested a structure feature that phthalazine derivatives including Valatanib had: 1) one substituted aniline function at position 1 of the phthalazine ring; 2) one hydrogen bond acceptor such as 4-pyridylmethyl and isoquinolin-5-yl connected to the position 4 *via* an appropriate linker. Some representative phthalazine VEGFR inhibitors including compound (**10**) and (**11**) all possess the structure feature (Fig. **3**) [49, 53].

4.2.2. Anthranilamides and their Derivatives

According to the structure feature and space conformations of Valatanib and other phthalazine inhibitors as lead compounds, scientists from Novartis reported a novel series with excellent potency and selectivity [54]. The optimization was based on an anthranilamide scaffold, where an intramolecular hydrogen bond between –NH- and carbonyl group would hold the shape of the molecule into a pseudo ring system, mimicing the phthalazine binding mode (Fig. **3**). A number of literatures and patents about anthranilamide VEGFR inhibitors have been published since then.

AAL-993 (**12**) (Fig. **3**) was the potent one of this series. It was reported to selectively inhibit VEGFR-2 (IC_{50} = 23 nM) and VEGFR-3 (IC_{50} = 18 nM), and readily penetrate cells to inhibit VEGFR-2 autophosphorylation. Upon chronic oral dosing in rodents, AAL-993 was well tolerated with no overt signs of toxicity [54]. Its derivative the nicotinamide AMG 706 (**13**) (Fig. **3**) was also effective at inhibiting VEGFR-1, -2, -3 and reducing VEGF-stimulated vascular permeability in animal models [55].

Later, studies focused on the modifications of the phenyl ring and the amine –NH- group.

Compare to the previous anthranilamides, the phenyl ring were substituted by isothiazole ring, thiophene ring and pyrazole ring to obtain compound (**14**), (**15**) and (**16**) (Fig. **3**) with potent activities against VEGFR [56-58]. With the mechanism of intramolecular nonbonded O-O interaction, researchers also tried to replace the amine –NH- group by an oxygen atom to obtain compound (**17**) and (**18**) (Fig. **3**). However, neither of them displayed potent activity [59, 60].

4.3. 2-Oxindoles

4.3.1. Sunitinib

Sunitinib (SU-11248/Sutent, developed by Sugen, Pfizer) (**19**) (Fig. **4**) was an oral small molecular tyrosine kinase inhibitor that exhibited potent antiangiogenic and antitumor activity. It not only displayed significant activity against VEGFR-1, -2, -3, with IC_{50} values of 15, 38, 30 nM, respectively, but also inhibited Flt-3, c-Kit, PDGFR-β and colony-stimulating factor-1 receptor (CSF-1R) at the nanomolar concentration [61]. Distinguished from other 2-Oxindole analogues, Sunitinib possessed a diethylaminoethyl group that ensured good pharmacologic property and bioavailability [62]. Preclinical studies data reported that Sunitinib had effective antitumor activity, which not only inhibited tumor growth, but also induced tumor regression in models of colon cancer, NSCLC, melanoma, renal carcinoma, and squamous cell carcinoma. The activity and function were associated with inhibition of VEGFR and PDGFR phosphorylation [63]. Sunitinib was approved by the USFDA in 2006 for treatment of gastrointestinal stromal tumor (GIST) and advanced renal cell carcinoma (RCC). Pfizer has been evaluating Sunitinib further for the treatment of various cancer diseases, including liver cancer, breast cancer, prostate cancer and NSCLC.

4.3.2. Other 2-Oxindole Agents

Using a random screening approach with 2-oxindoles, researchers found several candidate compounds, including Semaxanib (SU-5416) (**20**) (Fig. **4**) and SU-6668 (**21**) (Fig. **4**). Semaxanib was one of the first small molecule tyrosine kinase inhibitors to reach clinical trials. It was a multi-kinase inhibitor targeted VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, CSF-1R, and Flt-3 [64]. However, due to its poor pharmacologic property and limited efficacy *in vivo*, and even the severe side effects, such as thromboembolic events observed in phase II clinical trials, the drug was suspended in clinical trials [65]. SU-6668 had a protein kinase inhibitory profile similar to but even more potent than that of Semaxanib. However, its toxicity led to the discontinuation of clinical trials, also [66, 67].

BIBF 1000 (**22**) (Fig. **4**) and BIBF 1120 (**23**) (Fig. **4**) were novel indolinone multi-kinase inhibitors containing methoxycarbonyl group substituted on the position 6. They displayed potent and selective activities against to VEGFR, PDGFR and fibroblast growth factor receptor (FGFR), and moreover, they were shown encouraging efficacy in various *in vivo* tumor models while being well tolerated. BIBF 1120 is currently in phase III clinical trials for the treatment of NSCLC [68-70].

4.4. Pyrimidine and Pyridine Derivatives

Pyrimidine and pyridine rings have been characterized as kinase-privileged structures with specific binding affinity to various kinases. And data analysis of a large number of kinase inhibitors suggested that most of the bisarylaniline moiety with a heteroaromatic nitrogen *ortho*- to the –NH- linker, was a fragment to increased activity within kinases [71]. Many derivatives containing variously substituted pyrimidine or pyridine ring have been reported as VEGFR inhibitors. Most of them possess the bisarylaniline moiety (Fig. **5**).

4.4.1. Pyrimidine Derivatives

AEE788 (**24**) (Fig. **5**), obtained by optimization of the 7H-pyrrolo[2,3-d]pyrimidine lead scaffold, was a dual potent inhibitor of EGFR and VEGFR both at the isolated enzyme level and in cellular systems. At the enzyme level, AEE788 inhibited EGFR-2, VEGFR-1 and VEGFR-2 at nanomolar concentration, with IC_{50} values of 2, 59 and 77 nM, respectively. In addition, AEE788 also demonstrated antiproliferative activity against EGF-stimulated and VEGF-stimulated HUVECs. These antitumor properties, combined with a favorable pharmacokinetic profile in oral administration indi-

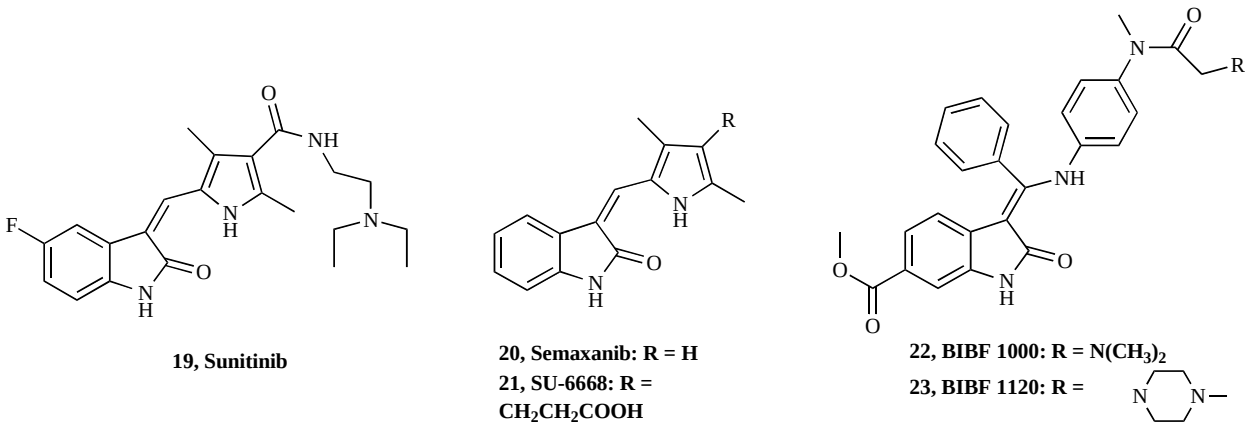


Fig. (4). 2-Oxindole: Sunitinib and its analogues.

cated that AEE788 had potential to be an anticancer agent targeting deregulated tumor cell proliferation as well as angiogenic parameters. The drug is currently in Phase II clinical trials in oncology by Novartis [72].

Pfizer researchers reported thieno-pyrimidines as potent dual inhibitors of VEGFR-2 and EGFR. Such as compound (**25**) (Fig. **5**), it exhibited encouraging activity against these two TKs, with IC_{50} values of 2 and 7 nM, respectively [73].

Some compounds bearing isolated pyrimidine core have also been reported as effective VEGFR inhibitors, especially 2, 4-disubstituted and 2, 6-disubstituted pyrimidines. Pazopanib (GW786034) (**26**) (Fig. **5**) was a multi-targeted TK inhibitor of VEGFR, PDGFR and c-Kit. It demonstrated potent IC_{50} values of 10, 30 and 47 nM against VEGFR-1, -2, -3, respectively. The agent also showed strong selectivity for VEGF-induced HUVEC proliferation compared to several tumor cell lines and fibroblasts, respectively [74, 75]. In the year 2009, USFDA granted approval to pazopanib for the treatment of patients with advanced renal cell carcinoma (RCC).

Scientists from Johnson and Johnson Pharmaceuticals reported a new series of inhibitors towards VEGFR with the 2, 6-disubstituted cyano-pyrimidine. By designing, synthesizing and optimizing many kinds of derivatives, JNJ-17029259 (**27**) and compound (**28**) (Fig. **5**) were identified as the most potent compounds aganist VEGFR-2 with the IC_{50} values of 21 nM and 7 nM respectively, and demonstrated good activities in different human tumor cell lines. Furthermore, JNJ-17029259 was reported to inhibit VEGF-induced HUVEC proliferation and migration, while compound (**28**) had potent activity in inhibiting cyclin dependent kinase 1 (CDK1), which plays an important function in the cell proliferation cycle and is a potential target for cancer therapy [76, 77].

4.4.2. Pyridine Derivatives

Several VEGFR inhibitors have been reported to possess the pyridine moieties, which are either isolated or fused to form heterocyclic rings in the molecules.

A series of pyrazine-pyridine derivatives were identified by Johnson and Johnson Pharmaceuticals as potent VEGFR-2 inhibitors. One of the thoroughly evaluated members, compound (**29**) (Fig. **5**), with IC_{50} value of 63 nM against VEGFR-2, showed good selectivity towards other kinases [78].

A series of compounds based on thieno[3, 2-b]pyridine were discovered as dual inhibitors of VEGFR-2 and c-Met. Compound (**30**) (Fig. **5**) not only inhibited VEGFR-2 (IC_{50} = 10 nM), but also performed well *in vivo* against a panel of different human tumor types [79].

The scientists from Merck reported imidazopyridine compounds as a novel class of potent and selective VEGFR-2 inhibitors. Such as compound (**31**) (Fig. **5**), displayed strong inhibitory activity of VEGFR-2 (IC_{50} = 28 nM). In addition, its piperidine terminal group led the compound to possess good aqueous solubility. In this class of compounds, it was found that the introduction of pyridone functionality at position C-7 could significantly improve potency and physical properties [80].

4.5. Ureas

Kinase inhibitors are enriched in NH-containing linkers, especially the urea (-NHCONH-), being found with higher frequency in the kinase collection [71]. Several compounds containing disubstituted urea moieties were reported as VEGFR inhibitors with potent activity.

4.5.1. Sorafenib

Using a combination of medicinal and combinatorial chemistry approaches, Sorafenib (BAY 43-9006, developed by Bayer) (**32**) (Fig. **6**) was initially developed as a specific inhibitor of Raf kinases, which are members of serine/threonine kinases and may lead to a sustained proliferative signal resulting in tumor growth and progression. Subsequent studies have shown that Sorafenib was a multi-kinase inhibitor that also targeted several tyrosine kinases involved in tumor progression and angiogenesis, including VEGFR-2 (IC_{50} = 90 nM for KDR) and PDGFR-β (IC_{50} = 57 nM) [81]. Thus, Sorafenib inhibited tumor growth by a dual mechanism, acting both directly on tumor progression (inhibiting Raf signaling) and on tumor angiogenesis (inhibiting VEGFR and PDGFR signaling). Sorafenib inhibited various cancer cell lines and tumor xenografts and exhibited effec-

tive oral antitumor activity in a broad spectrum of human tumor xenograft models. Furthermore, this compound showed limited side effects and disease stabilization in clinical trails. Based on these results with both robust antitumor effects and limited toxicity, Sorafenib was approved by the USFDA in December 2005 for patients with advanced renal cancer [67, 81-83].

4.5.2. Other Urea Agents

ABT-869 (**33**) (Fig. **6**), bearing indazolyl-diphenyl urea core structure, was discovered by Abbot. It was a multi-targeted inhibitor that was mainly focused on the VEGFR and PDGFR families (IC_{50} = 3, 8 and 25 nM for VEGFR-1, VEGFR-2 and PDGFR-β, respectively) and lacked activity against off-target kinases. The phase I trial for ABT-869 was recently completed and it demonstrated respectable efficacy in solid tumors including lung and hepatocellular carcinoma with acceptable side effects. The compound is currently tested in phase II trials [84, 85].

Researchers from Abbot also reported a series of N, N'-diaryl ureas that had similar structures with ABT-869. These compounds showed significant activity against the VEGFR and PDGFR families. Typical compounds were compound (**34**), (**35**) and (**36**) (Fig. **6**). They all possessed high potency against VEGFR-2 kinase, with IC_{50} values of 3, 45 and 11 nM, respectively [86-88]. Their binding modes with KDR demonstrated that these compounds bound to the inactive conformation of the enzyme with the urea portion extending into the back hydrophobic pocket adjacent to the ATP binding site [87].

KRN633 (**4**), KRN951 (**5**), Ki8751 (**6**) (Fig. **2**) in quinazoline and quinoline family cited above also possess the disubstituted urea moieties.

4.6. Others

Besides the potent inhibitors stated above, there have been some other compounds containing novel skeletons that were used as VEGFR inhibitor scaffolds.

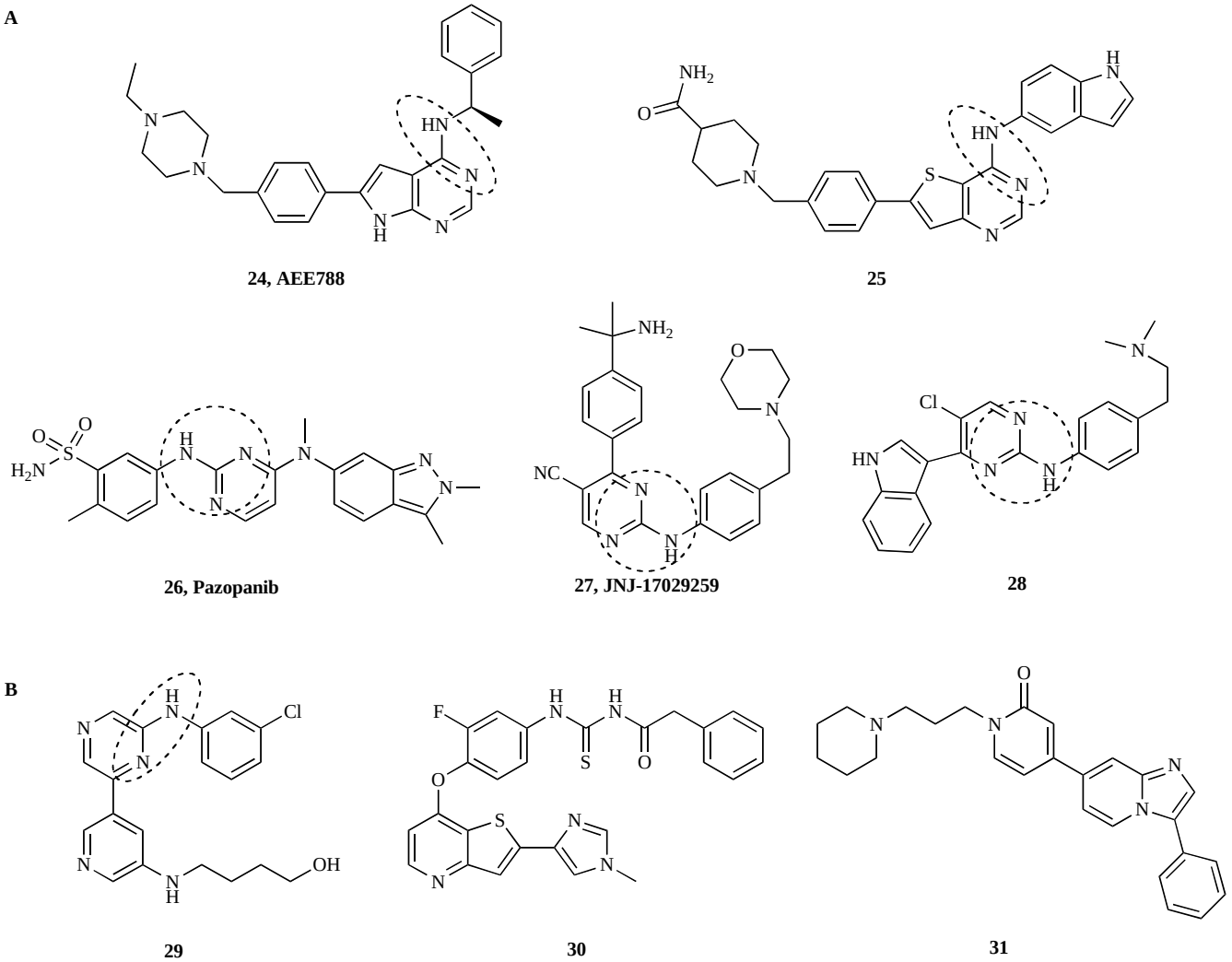
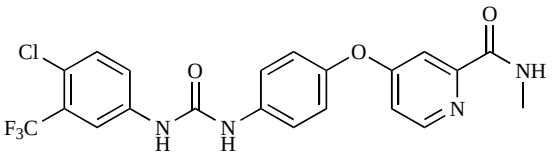


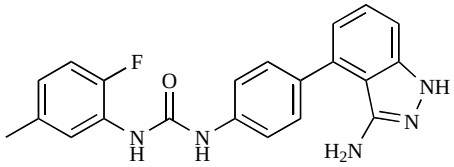
Fig. (5). Pyrimidine and pyridine derivatives. The bisaryl –NH- linkers are shown in the circle.

Part A. Pyrimidine derivatives.

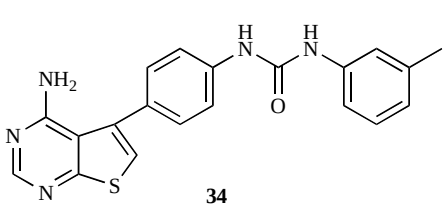
Part B. Pyridine derivatives.



32, Sorafenib



33, ABT-869



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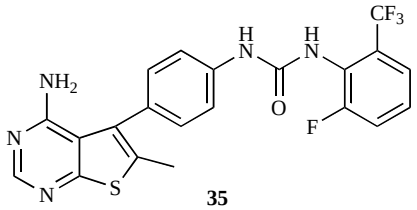

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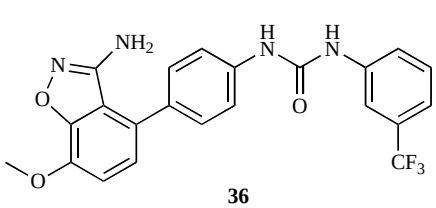

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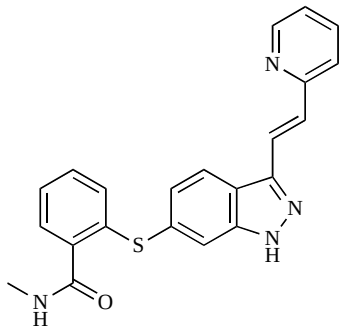
Fig. (6). Sorafenib and other urea agents.

Axitinib (AG-013736) (**37**) (Fig. **7**), was a substituted indazole derivative discovered by Pfizer. The agent not only displayed significant and selective activity against VEGFR-1, -2 and -3 kinases with respective IC_{50} values of 1.2, 0.25 and 0.29 nM, but also demonstrated broad-spectrum activity towards several types of tumors in clinical trails, including NSCLC, metastatic renal cell carcinoma (mRCC), metastatic breast cancer, pancreatic cancer and thyroid cancer. These results supported clinical assessment of Axitinib as a therapeutic agent for cancer [89].

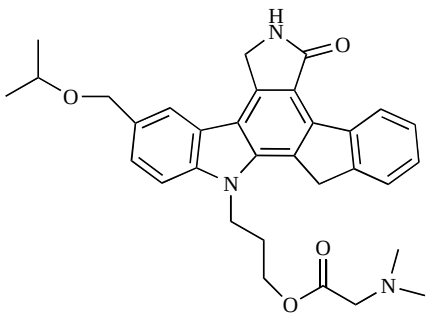
Indenopyrrolocarbazolone compounds, derived from modification and simplification of the staurosporine scaffold, showed multi-targeted TK inhibitory activity, including VEGFR kinases. One of the thoroughly evaluated members, CEP-7055 (**38**) (Fig. **7**), with IC_{50} values of 18 and 8 nM towards VEGFR-2 and VEGFR-3, demonstrated strong and durable antiangiogenic and antitumor efficiency, with no apparent toxicity [90].

Scientists from Bristol-Myers Squibb reported the discovery of Substituted 3-((2-(pyridin-2-ylamino) thiazol-5-ylmethyl)-amino) benzamides as a new series of VEGFR-2 kinase inhibitors. BMS-605541(**39**) (Fig. **7**) was identified as the most potent compound with the IC_{50} value of 23 nM towards VEGFR-2, and demonstrated excellent kinase selectivity, favorable pharmacokinetic properties in multiple species, and robust *in vivo* efficacy in human lung and colon carcinoma xenograft models [91].

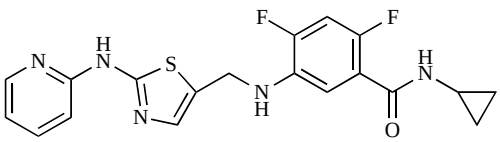
Amgen laboratories have employed the X-ray crystallography and molecular modeling assay to identify a series of



37, Axitinib



38, CEP-7065



39, BMS-605541

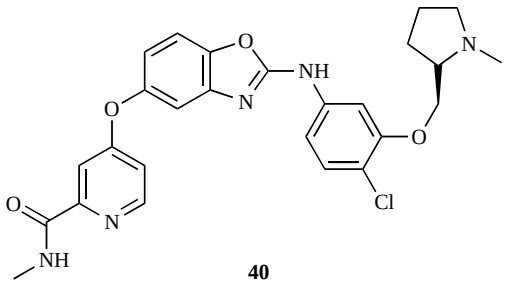


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Fig. (7). Some other VEGFR inhibitors.

Table 2. Representative Drugs of Different Kinds About VEGFR Inhibitors

Class	Representative drug	Highest development Phase	Inhibition (IC_{50}, nM)		Major targeted therapy in solid tumors
			VEGFR-2	VEGF-HUVEC	
Quinazolines and quinolines	Vandetanib (Fig. **2**)	Phase III	40	60	Non-small cell lung cancer
Phthalazines and anthranilamides	Valatanib (Fig. **3**)	Phase III	37	17	Metastatic colorectal carcinoma
2-Oxindoles	Sunitinib (Fig. **4**)	Launched in 2006	38	40	Gastrointestinal stromal tumor and advanced renal cell carcinoma
Pyrimidines and pyridines	Pazopanib (Fig. **5**)	Launched in 2009	30	21	Advanced renal cell carcinoma
Ureas	Sorafenib (Fig. **6**)	Launched in 2005	90	60	Advanced renal cell carcinoma
Others	Axitinib (Fig. **7**)	Phase III	0.25	60	Pancreatic carcinoma

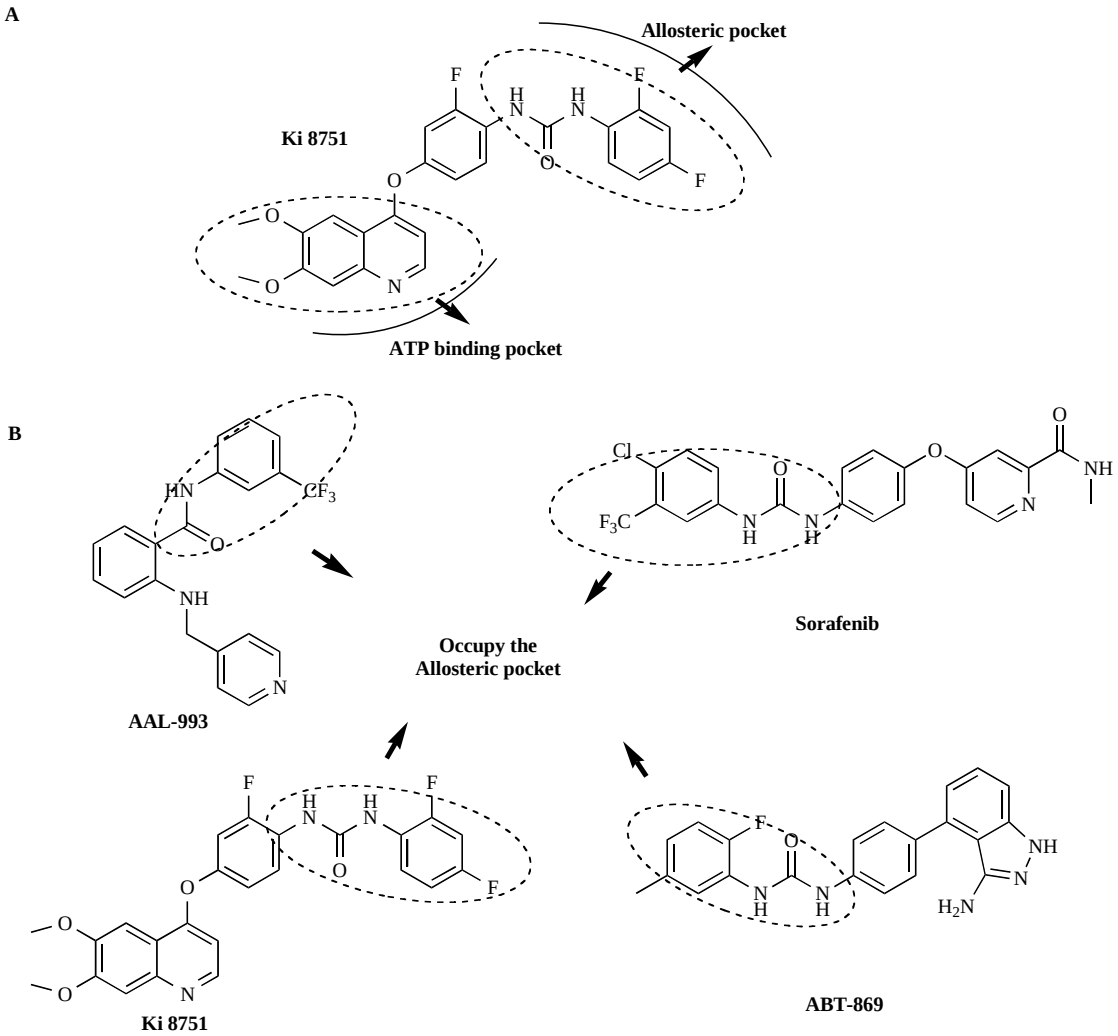

Fig. (8). Type II VEGFR inhibitors.

Part A. Ki8751: the quinoline moiety interacts with ATP binding pocket and the phenylurea moiety interacts with allosteric site.

Part B. Four type II VEGFR inhibitors. The fragments in the circle are used to interact with allosteric site.

2-aminobenzimidazoles and 2-aminobenzoxazoles as potent inhibitors of VEGFR-2 in both enzymatic and HUVEC cellular proliferation assays. Among them, compound (**40**) (Fig. **7**) emerged as the best candidate. The agent exhibited high potency against VEGFR-2 and good pharmacokinetic properties. In addition, it showed potent *in vivo* activity upon oral dosing in both the mouse matrigel and the rat corneal models [92].

4.7. Type II VEGFR Inhibitors

Recently, much more attention has been paid to type II kinase inhibitors which could bind to an inactive conformation of the kinase, thereby preventing activation. These special kinase inhibitors not only interact with the ATP binding site, but also occupy a new hydrophobic pocket that is adjacent to the ATP binding pocket created by a DFG-out conformation of the activation loop. This new hydrophobic pocket, which is only accessible in the inactive form of a protein kinase, is often defined as the allosteric site. Because the amino acids surrounding the allosteric site differ from each kinase, it has been proposed that type II inhibitors may achieve kinase selectivity easier than their type I counterparts, which could bind to the highly conserved ATP binding site only [93]. For VEGFR inhibitors cited above, several compounds such as compound (**6**) (Ki8751), compound (**7**), compound (**12**) (AAL-993), compound (**32**) (Sorafenib), compound (**33**) (ABT-869), compound (**34**), compound (**35**) and compound (**36**) can be defined as type II inhibitors (Fig. **8**). Besides interacting with the ATP binding pocket, they all posses an additional benzamido or phenylurea fragment to occupy the allosteric site of VEGFR. The –NH- and carboxyl oxygen groups provide hydrogen bonding interactions with the residues of the DFG-motif. The phenyl group can penetrate into the allosteric pocket and form the hydrophobic interaction with it. The capability of targeting both the ATP binding site and the allosteric binding site make these compounds posses greater cellular potency and altered selectivity relative to many other VEGFR inhibitors [94].

5. CONCLUSIONS

Antiangiogenic therapies based on the inhibition of VEGFR kinase have been reported to be one of the most powerful clinical strategies in cancer treatment. Great efforts to design and synthesize small molecular VEGFR inhibitor drugs for cancer research have been made in recent years. Sorafenib, Sunitinib and Pazopanib have been approved by the USFDA for the treatment of various cancers and many other promising compounds with high potency are in the clinical trials. In the near future, research of VEGFR kinase inhibitors may pay more attentions on type II inhibitors to increase the selectivity and affinity. In addition, as a novel method in the design of kinase inhibitor, study on type II inhibitors may provide plenty of lead compounds with various molecular structures and help fully explore the signaling pathways. With the increased understanding of the VEGF/VEGFR signaling pathways and mechanism of action between type II inhibitors and VEGFR kinase, more and more promising small molecular VEGFR inhibitors with higher selectivity and potency will emerge.

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ABBREVIATIONS

CDK	=	Cyclin dependent kinase
CSF-1R	=	Colony stimulating factor-1 receptor
EGFR	=	Epidermal growth factor receptor
FGFR	=	Fibroblast growth factor receptor
Flk	=	Fetal liver kinase
Flt	=	Fms-like tyrosine kinase
GIST	=	Gastrointestinal stromal tumor
HUVEC	=	Human umbilical vein endothelial cell
NSCLC	=	Non-small cell lung cancer
PDGFR	=	Platelet-derived growth factor receptor
PLC-γ1	=	phospholipase C-γ1
PlGF	=	Placental growth factor
Raf	=	Rapidly accelerated fibrosarcoma
RCC	=	Renal cell carcinoma
RET	=	Rearranged during transfection
RTK	=	Receptor tyrosine kinase
VEGF	=	Vascular endothelial growth factor
VEGFR	=	VEGF receptor

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