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Vascular Endothelial Growth Factor (VEGF) Receptors: Drugs and New Inhibitors

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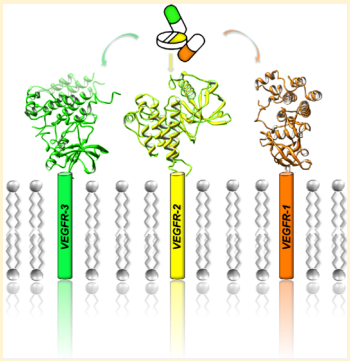
ABSTRACT: The recent launch onto the market of five VEGFR inhibitors indicates the therapeutic value of these agents and the importance of the research in the field of angiogenesis inhibitors for future oncologic therapy. In this Perspective we briefly report the inhibitors that are in clinical use, while we dedicate two wider sections to the compounds that are in clinical trials and to the new derivatives appearing in the literature. We especially consider the medicinal chemistry aspect of the topic and report the structure−activity relationship studies and the binding mode of some inhibitors as well as the biological data of the compounds discovered in the past 5 years.



INTRODUCTION

Angiogenesis is the process consisting of blood vessel formation sprouting or splitting from pre-existing vessels. This process begins with dilatation of pre-existing capillaries and venules, followed by an increase of vascular permeability. Endothelial cells, which constitute the vessels, become activated, detach, migrate, and proliferate to form new sprouts.[1,2] Normal angiogenesis occurs during ovulation, wound healing, and reproduction.[3] In these physiological conditions, angiogenesis is stimulated by a wide range of endogenous molecules, including growth factors, adhesion factors (integrins, cadherins), proteinases (matrix metalloproteinases, urokinase plasminogen activators), extracellular matrix proteins (fibronectins, collagens), transcription factors (hypoxia inducible factor, nuclear factor κB), and signaling molecules (mTOR, Akt, PKA, COX-2). A number of other molecules, including thrombospondin-1, TIMP-1 (tissue inhibitor of metalloproteinase-1), interleukin 10, angiostatins, and endostatins are antiangiogenic factors present in the organism.[4] Physiological angiogenesis is tightly controlled by a complex balance among endogenous proangiogenic and antiangiogenic factors.[5] Aberrant angiogenesis is present in a wide range of diseases including retinopathies, arthritis, endometriosis, atherosclerosis, and cancer.[6] Starting from Folkman's studies in the early 1970s,[7] it has been demonstrated that the growth of primary tumors and their subsequent metastasis are angiogenesis-dependent processes. In fact, in order to grow beyond a size of 1−2 mm, tumors need new blood capillaries to create their own nutrient supply to remove metabolic waste and to facilitate metastasis formation.[8] Tumor-derived factors promote angiogenesis, causing an imbalance between proangiogenic and antiangiogenic factors. Hypoxic conditions in the center of the tumor mass stimulate the release of proangiogenic factors from tumor cells that are located in the proximity of pre-existing blood vessels and lead to the formation of new capillaries around the tumor mass.[9] In these situations, endothelial progenitor cells are mobilized from the bone marrow, differentiate into endothelial cells, and participate in the angiogenic process, stimulating neovascularization of ischemic tissues.[10] Perivascular progenitor cells also differentiate into perivascular mural cells, which cover and stabilize vessels in tumor.[11]

In 1989 Ferrara and colleagues published the amino acid sequence of a peptide, VEGF, later identified as VEGF-A, which stimulates endothelial cell mitosis.[12] Subsequent studies demonstrated that this growth factor is a potent angiogenesis stimulator and is a member of a family consisting of five homodimeric glycoprotein members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF).[13]

VEGF RECEPTORS

VEGF family members bind in an overlapping pattern to three different but structurally correlated VEGF receptors (VEGFRs), which are transmembrane tyrosine kinases (TKs). VEGFR-1 (Flt-1) is critical for hematopoietic cell development, VEGFR-2 (human KDR or murine Flk-1) for vascular endothelial cell development, and VEGFR-3 (Flt-4) for lymphatic endothelial cell development. The expression of VEGFR-3 is associated with the dissemination of tumor cells to regional lymph nodes.[14] VEGFRs are type III transmembrane kinase receptors and share a common structure that has been extensively described elsewhere.[15] Briefly, these receptors consist of an extracellular part composed of an N-terminal signal sequence and seven immunoglobulin-like domains

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followed by a single transmembrane segment. The intracellular portion of the receptor is constituted by a juxtamembrane (JM) segment, a TK domain, which is divided into proximal and distal kinase domains by an insert domain of about 70 amino acid residues, and a carboxy-terminal tail (Figure 1).

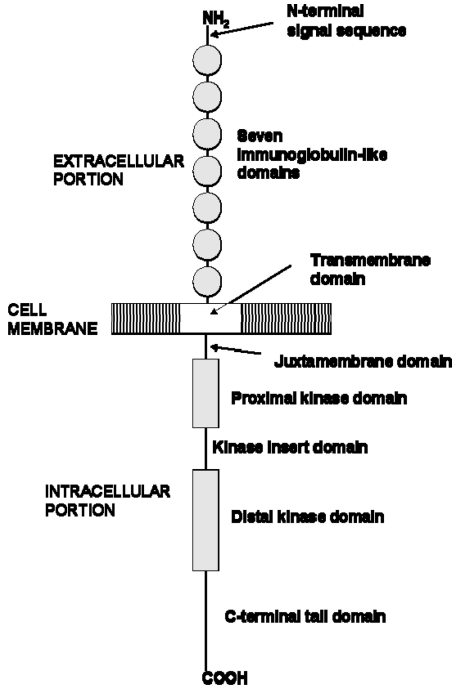

Figure 1. Schematic representation of VEGER structure.

The binding of VEGFs to VEGFRs induces receptor dimerization, causing modification in the intracellular domain conformation. These conformational changes lead to the exposure of the ATP-binding site, followed by ATP binding and auto- or transphosphorylation on specific tyrosine residues on the receptor dimers[13] and on downstream signal transducer proteins.[16] This mechanism leads to the initiation of a typical receptor signal transduction cascade, which activates several downstream enzymatic pathways, including p38MAPK, Raf/MEK/ERK, and PI3K/PKB pathways.[17] Tyrosine phosphorylation on VEGFRs is tightly regulated both by internalization and degradation and by dephosphorylation through different protein tyrosine phospatases.[18]

The biological and pathological roles of VEGFRs have been extensively studied and continue to be investigated.[19] VEGFR-1 is a 180−185 kDa glycoprotein activated by the binding of VEGF-A, VEGF-B, and PlGF. The crystal structure of the receptor binding domain of VEGF bound to the second Ig loop of VEGFR-1 indicates that hydrophobic interactions stabilize the ligand−receptor dimers.[20] VEGFR-1 is mainly expressed in vascular endothelial cells but is also present in nonendothelial cells, including monocytes, macrophages, renal mesangial cells, vascular smooth muscle cells, and dendritic cells. VEGFR-1 has been shown to mediate monocyte migration, recruitment of endothelial cell progenitors, hematopoietic stem cell survival, and release of growth factors from liver endothelial cells.[21]

The precise role of VEGFR-1 functions is still emerging, and it has been difficult to link the activation of VEGFR-1 to specific biological responses in cells that endogenously express this receptor. However, several studies in recent years have suggested that this receptor may play both negative and positive roles in angiogenesis. In fact, VEGFR-1 has negative functions, probably trapping VEGF-A in the embryo, and yet it shows a positive role in adulthood in a tyrosine-kinase-dependent manner.[22] Moreover, VEGFR-1 acts primarily as a decoy receptor, modulating the availability of VEGF-A and reducing its bioavailability to other receptors, in particular to VEGFR-2, which is the principal receptor in VEGF signaling.[23] Importantly, VEGFR-1-dependent signaling was shown to play a role in the angiogenesis of certain tumors as well as in the progression of rheumatoid arthritis and atherosclerosis.[24,25] VEGFR-1 is up-regulated in several tumor cell lines, including malignant prostate cells,[26] pancreatic cancer cells,[27] malignant melanoma cells,[28] and lung adenocarcinoma cells.[29] Importantly VEGFR-1 is involved in tumor metastasis.[30] Thus, blocking the activity of VEGFR-1 has emerged as an antimetastasis strategy to target angiogenesis, cancer cell survival, and migration. In addition, it has the potential to reduce the recruitment of tumor-growth-promoting bone-marrow-derived cells. However, it seems that selective inhibition of VEGFR-1 activity does not change the rate of spontaneous metastasis formation after surgical removal of primary tumors.[31] VEGFR-1 and VEGFR-2 also exist in soluble forms that are involved in angiogenesis and in different human diseases. High levels of soluble VEGFR-1 have been detected in several cancers, including breast, pancreatic, lung, and ovarian cancers and leukemias. On the other hand, administration of soluble VEGFR-1 has demonstrated antitumor effects, probably because the soluble receptor intercepts VEGF-A.[32]

VEGFR-2 is a 210−230 kDa glycoprotein that binds VEGF-A with a 10-fold lower affinity than VEGFR-1. It is expressed in vascular endothelial cells and also in hematopoietic stem cells.[16] VEGFR-2 is the major regulator of VEGF-driven responses in endothelial cells, including permeability, proliferation, invasion, and migration. Moreover, it is considered to be a crucial signal transducer in both physiologic and pathologic angiogenesis. Its signaling pathways are relatively well understood, with Y1175 and Y1214 in human VEGFR-2 being the main autophosphorylation sites following VEGF binding. Autophosphorylated VEGFR-2 is required for activation of several downstream pathways, including PI3K, p38MAPK, FAK, Src, Akt, which are usually hyperactivated in several tumors.[33] VEGFR-2 is overexpressed in several malignancies, including ovarian[34] and thyroid cancer,[35] melanoma,[36] and medulloblastoma.[37] VEGFR-2 and VEGFR-3 are primarily located and significantly up-regulated on the tumor vasculature (blood and/or lymphatic) that supports the majority of solid cancers. Moreover, the signal intensity of both receptors is significantly higher in vessels associated with malignant colorectal, lung, and breast tumor tissues than in adjacent nontumor tissues.[38]

VEGFR-3 is synthesized as a precursor protein of 195 kDa. The precursor is proteolytically cleaved in the fifth Ig-like domain, generating an N-terminal peptide that remains disulfide-bonded to the mother protein. VEGFR-3 is activated by the binding of VEGF-C and VEGF-D.[16] VEGFR-3 is deeply involved in the establishment and maintenance of the lymphatic system. Its expression in the adult seems to be largely restricted to lymphatic endothelial cells.[39] Furthermore, VEGFR-3 is strongly expressed in several human malignancies, including lung, cervical, breast, and colorectal cancers. Its higher levels are correlated with increased metastasis formation and shorter patient survival,[40] since the lymphatic vessels surrounding the tumor play important roles in metastasis formation.[41]

VEGFR INHIBITORS

Therapeutic strategies based on the inhibition of VEGF or its receptor signaling systems are an attractive approach for the treatment of different diseases, primarily tumors. TK inhibitors that target VEGFRs, reducing angiogenesis and/or lymphangiogenesis, have been shown to possess anticancer activity. Generally they are small synthetic molecules that act with an ATP-competitive mechanism by binding the ATP pocket of the protein kinase domain.[42,43] As a result of the similarity shared by VEGFR-1, -2, and -3, VEGFR inhibitors often target more than one member of the VEGFR family. However, this lack of selectivity among VEGFRs may be an advantage, since probably both VEGFR-1 and VEGFR-2 must be simultaneously inhibited to prevent tumor metastasis.[44] Furthermore the up-regulation of VEGFR-3 on tumor blood vessels indicates a potential additional antiangiogenic effect for dual VEGFR-2/VEGFR-3 inhibitors.[38] The lymphatic spread of malignancy may also be reduced by targeting VEGFR-3. A number of receptor TK families, such as PDGFRs, CSFR, c-Kit, and FLT3, share a substantial sequence homology with VEGFRs in their catalytic domains. For this reason, several compounds are not selective for VEGFRs. These often display high affinity for other TKs and are defined as multikinase inhibitors. Their poor selectivity among different TK families seems to offer different opportunities in cancer treatment. Several independent biological pathways that are vital for tumor proliferation and metastasis can be disrupted by these compounds.[4] Additionally, during VEGF inhibition, a hypoxic environment can drive a shift from VEGF dependent signaling to other proangiogenic pathways. For this reason, the use of multitargeted inhibitors should be of great therapeutic benefit.[19] Indeed, tumor angiogenesis is regulated by a number of proangiogenic factors other than VEGF, including FGFs (fibroblast growth factors), hepatocyte growth factor (HGF), and interleukin 8 (IL-8).[45] Even if the blockage of the VEGF/VEGFR signaling pathway alone has potent antitumor activity in animal models, this approach showed a reduced effect on some human tumors.[46] For example, a switch from VEGF-dependent angiogenesis to FGF-2-dependent angiogenesis has been clearly demonstrated in pancreatic islet tumors in response to chronic blockade of VEGF signaling.[47] Angiopoietin-Tie receptors, ephrin-Eph receptors, and Delta-Notch pathways have also been identified as other major angiogenesis regulators.[48]

These insights in angiogenesis regulation strongly support the rationale for the use of multitargeted TK inhibitors, which have been shown to be more useful from a therapeutic point of view than selective VEGFR inhibitors.

Therapies based on targeted antiangiogenic agents are supposed to be less toxic for chronic administration than conventional chemotherapy, since angiogenesis is a process usually restricted to the growing tumors.[49]

Beside its antiangiogenic effects, VEGFR inhibition may help to normalize tumor vasculature. In fact, because of elevated VEGF expression, the tumor vessels are abnormal and inefficient with many blind ends, and it is difficult for conventional drugs to gain access to the tumor tissue. Moreover, drug penetration is diminished by the high interstitial pressure of many tumors.[50] VEGFR inhibitors, producing vascular normalization, lead to a decrease of vascular volume and interstitial pressure, enhancing the delivery of chemotherapeutic agents.[51] A recent study is at odds with this hypothesis. Indeed, Van der Veldt and colleagues, using PET and radiolabeled docetaxel in NSCLC (non-small-cell lung cancer) patients receiving avastin (a monoclonal antibody inhibitor of VEGF-A) and docetaxel combination therapy, showed that there was no improvement in drug delivery to tumors.[52] On the other hand, it has been demonstrated that antiangiogenic drugs prevent a rapid tumor cell repopulation after cytotoxic chemotherapy,[53] strengthening the rationale for the combined use of conventional chemotherapeutic agents and VEGFR inhibitors.

VEGFR inhibitors ultimately target endothelial cells that are genetically stable in comparison with the genetically more labile tumor cells.[33] Consequently, this type of drug should be less prone to inducing enzymatic mutations that confer resistance to the drug itself.[54] This is in contrast to other TK inhibitors, such as the Bcr-Abl inhibitor imatinib used for chronic myeloid leukemia therapy,[55] which frequently leads to drug resistance. However, it has been observed that some tumors can also become resistant to antiangiogenic drugs.[56] Some possible reasons for this resistance include the overexpression of different angiogenic factors during tumor progression, mainly in response to antiangiogenic treatment or the development of tumor cell clones that may better survive in hypoxic tumors after angiogenesis inhibition.[11,57] Several studies have highlighted the possibility that VEGFR inhibitors can promote an invasive metastatic switch, in part by creating an increasingly hypoxic tumor microenvironment. Consequently, although the therapy based on VEGFR inhibitors has demonstrated delayed tumor progression, leading to progression-free survival and overall survival benefits compared with standard chemotherapy, a significant number of patients either do not respond to antiangiogenic agents or develop resistance to them. Moreover, some cancers, once they develop resistance, become more invasive or lead to metastatic disease.[58]

Another issue is the toxicity associated with the use of VEGFR inhibitors. Adverse event profiles are generally acceptable and can be classified in on-target adverse events caused by VEGFR inhibition and in off-target adverse events. The latter events are more frequent with multitargeted-kinase inhibitors[59] and are due to the simultaneous inhibition of kinases different from VEGFRs. Off-target effects are more dependent on the characteristics of patients, comorbidities, and the stage of the disease.[4] However, VEGFR inhibitors are generally less toxic than conventional chemotherapeutic agents, with the most common adverse effects being thromboembolic events, congestive heart failure, gastrointestinal perforation, and hypertension.[60] Particular attention must therefore be devoted to the toxicity evaluation of therapeutic protocols using associations of VEGFR inhibitors and conventional cytotoxic agents.

In this Perspective the inhibitors already in clinical use are briefly reported, since there are plenty of articles focused on clinical aspects of each single drug, while two wider sections are dedicated to the compounds that are in clinical trials and to the new derivatives appearing in the literature. We especially consider the medicinal chemistry aspect of the topic and report the structure−activity relationship (SAR) studies and the binding mode of some inhibitors as well as the biological (enzymatic, in vitro and in vivo) data of all the compounds.

Enzymatic data are given for each single kinase when available from the literature. For cell data and especially for in vivo data it is very difficult to determine the contribution of the inhibition of every single enzyme to the global activity of the drug (or inhibitor).

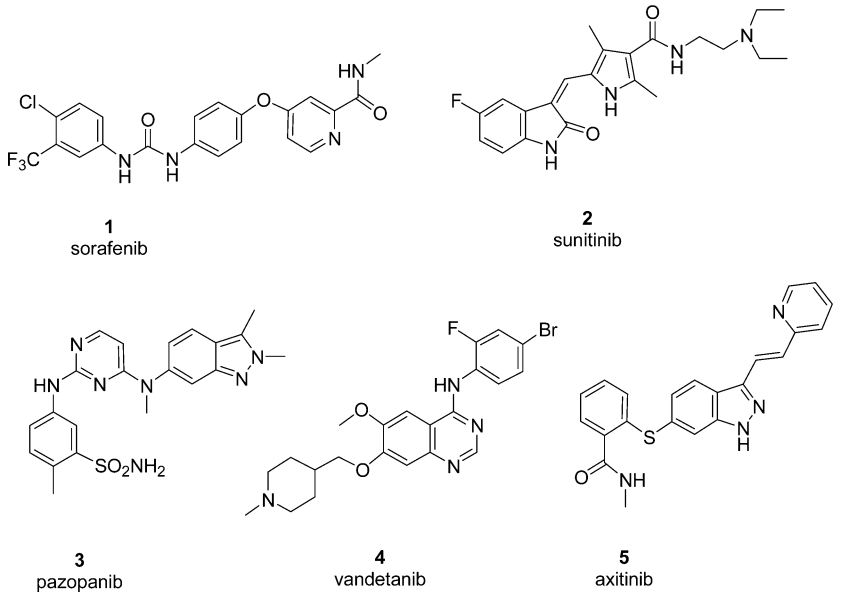

Figure 2. VEGFR/multitargeted inhibitors approved for clinical use.

VEGFR Inhibitors Approved for Clinical Use. Targeting VEGF receptors represents one approach that has enjoyed a great therapeutic success. At the time of writing, five drugs targeting VEGFRs and usually also other kinases have been approved for clinical use. The literature on these compounds is very extensive, and studies on their use for different tumors are ongoing. An overview of the compounds currently used in clinical settings is given below in order to provide a basis for comparison with other inhibitors that have appeared more recently in the literature.

Sorafenib, **1** (BAY-439006) (Figure 2), trade name Nexavar, codeveloped and comarketed by Bayer and Onyx Pharmaceuticals, is a biarylurea multitargeted kinase inhibitor. It inhibits, among others, VEGFR-2 and VEGFR-3, PDGFRβ, c-Kit, and Raf .[61] Importantly, it is the only approved drug shown to inhibit Raf, which is a critical component of the Ras pathway, a fundamental enzymatic cascade for cell division. Sorafenib was approved by U.S. FDA in 2005 for the treatment of advanced renal cell carcinoma (RCC) and in 2007 for the treatment of hepatocellular carcinoma (HCC).[62] All the drugs reported in this section have been approved for RCC but only sorafenib for HCC. Probably the positive clinical trial results that led to sorafenib's approval for HCC are due to the inhibitory activity of this drug on Raf. Indeed, overexpression of Raf and its pathway are frequently observed in HCC.[63,64] However, further efforts to understand the complete role of sorafenib in the treatment of HCC are still necessary.[62]

Sunitinib, **2** (SU11248) (Figure 2), trade name Sutent, by Sugen-Pfizer, is a multikinase indol-2-one inhibitor targeting VEGFR-1 and VEGFR-2, PDGFRβ, and other kinases, including FLT3, which has been shown to be involved, especially in mutated forms, in acute leukemia.[65] It also inhibits c-Kit, hyperactivated by mutations in gastrointestinal stromal tumor (GIST). Sunitinib was approved in 2006 by the FDA for the treatment of RCC and of GIST.[66]

Pazopanib, **3** (GW-786034) (Figure 2), trade name Votrient, by GlaxoSmithKline, is a potent pan-VEGFR inhibitor (indeed it inhibits VEGFR-1, -2, and -3). Its chemical structure is quite unusual among kinase inhibitors, bearing a 2,4-pyrimidinediamine core substituted by an indazole ring and a 2-methylbenzenesulfonamide moiety.[67] Pazopanib also inhibits PDGFRα/β and c-Kit. In 2009 it was approved by the FDA for RCC and in April 2012 for soft tissue sarcoma. Pazopanib also appears to be effective in the treatment of ovarian and in NSCLC.[68]

Vandetanib, **4** (ZD6474) (Figure 2), trade name Caprelsa, previously called Zactima, produced by AstraZeneca, inhibits VEGFR-2, VEGFR-3, EGFR, and RET.[69] It is an anilinoquinazoline derivative bearing a water-solubilizing basic side chain. In April 2011, vandetanib became the first drug to be approved by the FDA for treatment of late-stage (metastatic) medullary thyroid cancer in adult patients who are ineligible for surgery.[70] This therapeutic activity is likely caused by inhibition of RET, a TK hyperactivated by mutations in medullary thyroid cancer.[71]

Axitinib, **5** (AG013736) (Figure 2), trade name Inlyta, is an indazole derivative developed by Pfizer as a mutikinase inhibitor. It is active on VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, and c-Kit.[72] Pfizer researchers studied the effects of the juxtamembrane (JM) domain on VEGFR-2 enzymatic activity, autophosphorylation, and inhibition by axitinib, using kinetic, biophysical, and structural methods. In particular they obtained highly purified preparations of the VEGFR-2 catalytic domain without (VEGFR-2-CD) and with (VEGFR-2-CD/JM) the JM and showed that although the catalytic parameters for both constructs were similar, the autophosphorylation rate of VEGFR-2-CD/JM was faster than that of VEGFR-2-CD. Axitinib was found to have 40-fold enhanced biochemical potency toward VEGFR-2-CD/JM compared to VEGFR-2-CD, which correlates better with cellular potency. This study, identifying potential functions for the VEGFR-2 JM domain, emphasizes the importance of selecting the proper protein construct for mechanistic studies of receptor TKs and for the design of their inhibitors.[73]

On January 2012, the U.S. FDA approved axitinib for use in patients with RCC that had failed to respond to a previous treatment.

Among these drugs, sorafenib, sunitinib, and pazopanib are administered as salts tosylate, malate, and hydrochloride, respectively. Common doses, $T_{1/2}$, and T_{max} of the five inhibitors are reported in Table 1. Importantly, all these

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Table 1. Comparison of Some Pharmacokinetic Parameters for Approved Compounds[a]

compd	trade name	administration	salt	pharmacokinetics	
				$T_{1/2}$	T_{max} (h)
1, sorafenib	Nexavar	400 mg orally twice daily	tosylate	25−48 h	3
2, sunitinib	Sutent	50 mg orally once daily	malate	40−60 h	6−12
3, pazopanib	Votrient	800 mg orally once daily	hydrochloride	30.9 h	2−4
4, vandetanib	Caprelsa	300 mg orally once daily		19 days	4−10
5, axitinib	Inlyta	5 mg orally twice daily		2.5−6.1 h	2.5−4.1

[a]Data from http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm.

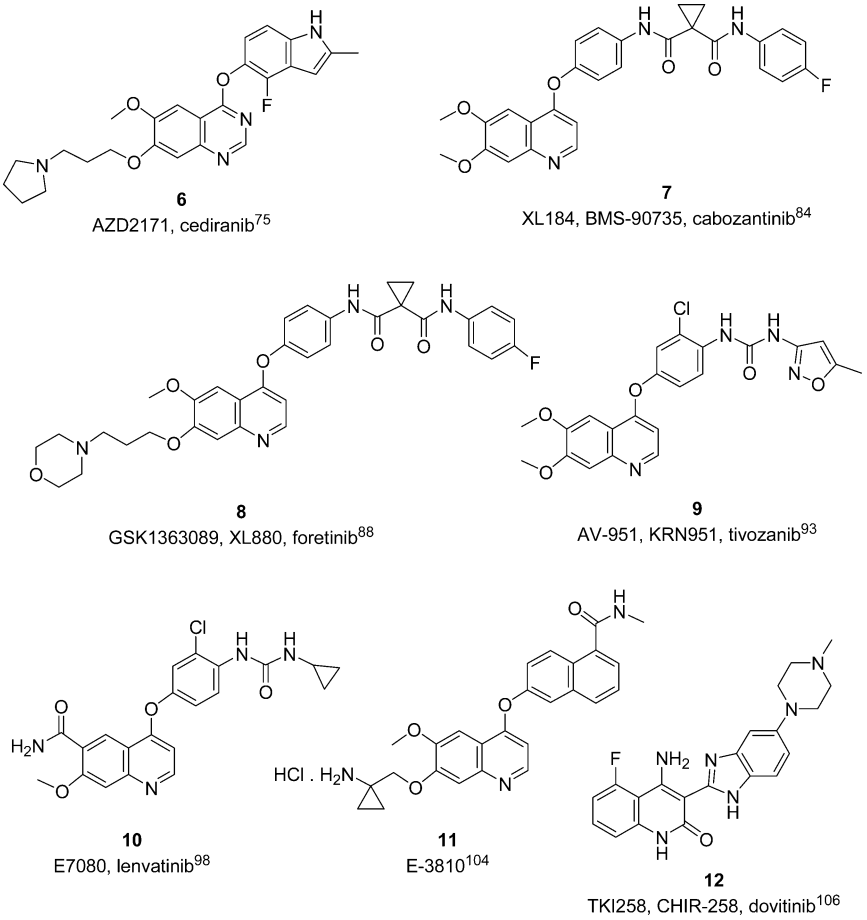

Figure 3. Quinazoline and quinoline derivatives in clinical trials.

compounds are administered per os, different from other chemotherapeutic agents that need iv administration.

Further insights on each drug can be found in several articles that have recently appeared in the literature, where detailed information on all that is known about these inhibitors, especially from the biological and medical perspectives, is reported.

VEGFR Inhibitors in Clinical Trials. A number of VEGFR and multitargeted inhibitors that have entered clinical trials are reported in this section. Emphasis has been placed on chemical structures and on the most important biological results obtained with their use. Data are from the past 5 years. In all the figures the superscript number refers to the main bibliografic reference for the compounds.

The quinazoline and the quinoline moieties bearing C4 anilino or C4 oxy substituents and C6, C7 oxy substitents are present in several TK inhibitors, including vandetanib **4**, already reported, and the EGFR (ErbB1) kinase inhibitor gefitinib, approved by FDA in 2003 for the treatment of NSCLC, and in many Src/Abl dual inhibitors.[74]

Cediranib, **6** (AZD2171) (Figure 3) (tentative trade name Recentin), from AstraZeneca, is a 4,6,7-trioxyquinazoline, a potent oral inhibitor of VEGFRs, showing IC_{50} values of 5, 1, and 3 nM toward VEGFR-1, -2, and -3, respectively. It is also active toward PDGFRβ and c-Kit with similar potencies and possesses good selectivity over a panel of other tyrosine and serine/threonine kinases.[75] This inhibitor reduces VEGF-induced angiogenesis in vivo and shows activity in mice implanted with human tumor xenografts. It is currently in several phase 2/3 trials, alone or in combination with other antineoplastic agents, for the treatment of various solid tumors and leukemias, particularly acute myeloid leukemia (AML).[76] Results from a phase 2 clinical trial using cediranib monotherapy at a dose of 45 mg/day for recurrent glioblastoma showed encouraging proportions of radiographic response and 6-month progression-free patient survival, with manageable

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toxicity.[77] Unfortunately, it has been successively reported that recurrent glioblastomas relapse after initial response to cediranib treatment. Probably the tumor escapes antiangiogenic therapy by switching to VEGFR-independent pathways and can grow using blood vessels with normal molecular expression and morphology without a second wave of angiogenesis.[78] However, it has been very recently determined that a judicious application of antiangiogenic therapy in glioblastomas may normalize the structure and function of the tumor vasculature, promoting an improved blood perfusion. These findings offer direct clinical evidence in support of the hypothesis that vascular normalization can increase tumor drug perfusion and help improve glioblastoma patient survival.[79] Results from a phase 2 clinical trial indicated that cediranib monotherapy demonstrates significant evidence of antitumor activity in patients with advanced RCC. The adverse event profile is consistent with previous studies of cediranib 45 mg/day and includes diarrhea, hypertension, and fatigue.[80] Cediranib monotherapy has modest single-agent activity in malignant pleural mesothelioma after platinum-based therapy. However, some tumors are highly sensitive to cediranib. This study provides a rationale for further testing of cediranib plus chemotherapy in mesothelioma patients,[81] whereas the compound is not an effective treatment in patients with unresectable or metastatic HCC, due to its high toxicity in this type of patients.[82] In a phase 1 study cediranib was generally well tolerated and shows preliminary evidence of activity as a monotherapy in patients with AML.[83]

Cabozantinib, **7** (XL184, BMS-907351) (Figure 3), invented at Exelixis, later developed by Bristol-Myers Squibb and then returned to Exelixis in 2010, is a quinoline derivative bearing a cyclopropane-1,1-dicarboxylic acid amide substituent. It is a multikinase inhibitor that targets VEGFR-2, MET, and RET. The drug also inhibits Kit, FLT3, and Tek with IC_{50} values in the low nanomolar range in enzymatic assays. Preclinical studies demonstrate that cabozantinib potently inhibits multiple receptor TKs in various cancer cell lines and animal xenograft models, with significant oral bioavailability and blood–brain barrier penetration. Cabozantinib is also a promising agent for inhibiting tumor angiogenesis and metastasis in cancers with deregulated VEGFR, MET, and RET signaling. For these reasons it is a potential candidate for the oral treatment of medullary thyroid cancer, glioblastoma, and NSCLC.[84] The compound is being tested in several phase 1/2 trials for different malignancies and in phase 3 trials in patients with medullary thyroid and prostate cancer.[85−87] Cabozantinib was granted orphan-drug status by the FDA in 2011. This status will support its development for treatment against various cancers in the U.S., particularly NSCLC, glioblastomas, and medullary thyroid cancer, which are listed as rare diseases by the Office of Rare Diseases of the National Institutes of Health.

Foretinib, **8** (GSK1363089, XL880) (Figure 3), was synthesized at Exelixis and then licensed by GSK in 2007. It is a cabozantinib derivative in which the 7-methoxy group of the quinoline ring has been substituted by the 3-morpholin-4-yl-propoxy group as a solubilizing basic side chain. It is a multitargeted inhibitor that is especially active on VEGFR and MET and shows IC_{50} values of 0.8, 6.8, and 2.8 nM against VEGFR-1, VEGFR-2, and VEGFR-3, respectively, and of 0.5 nM on MET. It also inhibits PDGFRs and the angiopoietin-1 receptor Tie-2, shows modest activity on FGFR and EGFR, and is inactive against 50 serine/threonine kinases. In human umbilical vein endothelial cells (HUVEC), it reduces VEGFR-2 phosphorylation with an IC_{50} of 16 nM.[88] Foretinib blocks tumorigenesis and reduces invasive tumor growth in different models of ovarian cancer by affecting several critical tumor functions. This provides the rationale for its further clinical development for the treatment of ovarian cancer.[89] The significant antitumor activities in patient-derived HCC xenograft models, which it demonstrates, justifies its clinical investigation in patients with advanced HCC.[90] Foretinib is being tested in phase 1−2 clinical trials on solid malignancies. In a phase 1 clinical trial, the recommended dose of foretinib is 240 mg, given on the first 5 days of a 14-day cycle.[91]

Tivozanib, **9** (KRN951, AV-951) (Figure 3), was originally synthesized at Kirin Brewery, now Kyowa Hakko Kirin, then licensed to AVEO in 2007, which in turn licensed the compound to Astellas in 2011. It is a quinoline derivative bearing a urea moiety in turn substituted with an isoxazolyl group. The urea function is often present in kinase inhibitors, since it is a suitable group for hydrogen binding interactions with amino acids of the kinase catalytic cleft.[92] Tivozanib is a potent inhibitor of VEGFR-1, VEGFR-2, and VEGFR-3, showing IC_{50} values of 0.21, 0.16, and 0.24 nM, respectively, and nanomolar activity values on PDGFRβ and c-Kit. It displays antitumor activity in a variety of human tumor xenografts, such as lung, breast, colon, ovarian, pancreas, and prostate cancer.[93] Tivozanib is undergoing clinical trial investigation for the treatment of solid tumors[94,95] at a recommended daily dose of 1.5 mg in a 4-week-on and 2-week-off dosing regimen.[96] Phase 2 results suggest that tivozanib is active and well tolerated in patients with advanced RCC. These data support additional development of tivozanib in advanced RCC.[97] Phase 3 results on advanced RCC suggest a 30% or 3 months improvement in progression free survival compared to sorafenib.

Lenvatinib, **10** (E7080) (Figure 3), also possesses a urea moiety, whereas the C6 methoxy group present in the previously reported quinolines has been replaced with an amide group. The compound produced by Eisai Co. is a potent dual inhibitor of VEGFR-2 (IC_{50} = 4.0 nM) and of VEGFR-3 (IC_{50} = 5.2 nM). In preclinical assays lenvatinib suppresses lymph node and lung metastases of human breast tumor MDA-MB-231 via inhibition of angiogenesis and lymphangiogenesis.[98] Treatment with lenvatinib potently inhibits the proliferation of malignant pleural mesothelioma cell lines, markedly prolongs mouse survival, and is associated with fewer tumor vessels and proliferating cells in the tumor.[99] The compound also shows activity on human sarcoma xenografs.[100] It is being tested in phase 1−2 clinical trials against solid tumors, including HCC, melanoma, and thyroid cancer of medullary, papillary, and follicular subtypes.[101] Results from phase 1 studies indicate that lenvatinib has manageable toxicity up to 13 mg b.i.d. when administered in a 2-week-on/1-week-off cycle. It also shows preliminary activity for durable disease control. Biomarker analysis suggests that its antiangiogenic activity is correlated with antitumor activity in patients with a wide range of solid tumors.[102] Results have been promising in patients with melanoma and RCC.[103]

11 (E-3810) (Figure 3) is a dual inhibitor of VEGFR and FGFR. It shows IC_{50} values of 7, 25, and 10 nM on VEGFR-1, -2, and -3, respectively, and of 17.5 and 82.5 nM on FGFR-1 and -2, respectively, whereas it possesses low activity on PDGFRs and on c-Kit. The compound suppresses phosphorylation of VEGFR-2 and FGFR-1 in HUVEC growth at nanomolar concentrations. In a variety of tumor xenograft

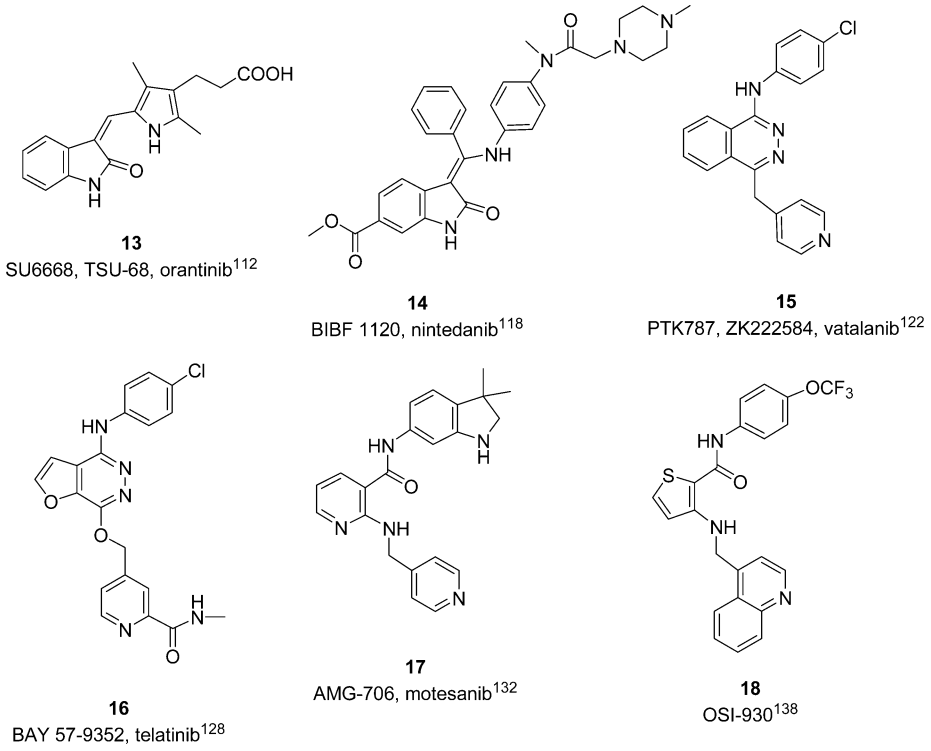

Figure 4. Other derivatives in clinical trials.

models, **11** exhibits antitumor properties at well-tolerated oral doses administered daily, making it a potent antiangiogenic agent with a favorable pharmacokinetic profile and broad spectrum antitumor activity.[104] The compound is being tested in a phase 1 clinical trial. A high-performance liquid chromatography−tandem mass spectrometry method has been developed and validated to evaluate **11** pharmacokinetics in cancer patients with solid tumors who are receiving daily oral doses of the drug during the phase 1 trial.[105]

Dovitinib, **12** (CHIR-258, TKI258) (Figure 3), developed by Novartis/Chiron,[106] is a benzymidazolhydroquinolinone multi-targeted inhibitor active on VEGFR-1, -2, -3, FGFR, PDGFRβ, c-Kit, and FLT3 with IC_{50} values in the low nanomolar range. Renhowe and colleagues developed a homology model to study the interaction of dovitinib with the catalytic site of VEGFR-2. Briefly, the compound binds to the active and so-called "in" conformation of the enzyme in an ATP-competitive fashion and forms three hydrogen bonds to the hinge region (to Glu917-CO, Cys919-NH, and Cys919-CO). Moreover, it makes a pattern of van der Waals interactions with other amino acids, including Val916, which is the gatekeeper residue of the catalytic site, and with some residues in the purine pocket. The good pharmacokinetic profile and the pharmacological activity in preclinical models have prompted further investigation on this compound.[107] Dovitinib shows antiangiogenic activity in vivo and, when orally administered, inhibits tumor growth in colon cancer animal models in a dose dependent manner.[108] Because of its action against FLT3, a TK mutated in AML, it is also active in experimental tumor xenograft models of this disease[109] and in orthotopic multiple myeloma models in mice.[110] Despite its preclinical activity on hematologic malignancies, at the moment dovitinib is in clinical trials for solid tumor treatment only. In a phase 3 trial dovitinib activity is compared with that of sorafenib in patients with metastatic RCC, whereas in other phase 2 trials it is being tested on HCC, breast cancer, GIST, endometrial cancer, and melanoma. In the latter tumor, treatment with 400 mg/die of dovitinib showed an acceptable safety profile but limited clinical benefit.[111]

Orantinib, **13** (SU6668, TSU-68) (Figure 4), synthesized by Sugen, is an indolin-2-one structurally similar to sunitinib[112] but bearing an acid group on the pyrrole moiety. It is a soluble and orally bioavailable nanomolar inhibitor of VEGFR-2, PDGFRβ, and FGFR-1. In animal models it produces tumor vascular normalization because of its antiangiogenic effect.[113] It also reduces chondrosarcoma growth in severe combined immunodeficient (SCID) mice[114] and prevents liver metastasis of colon cancer xenografts.[115] The compound is currently being tested in phase 1−2 clinical trials for the treatment of solid tumors, especially HCC. At a dose of 200 mg b.i.d. it shows promising preliminary efficacy with a high safety profile in patients with HCC who had been heavily pretreated[116] and satisfactory activity against breast cancer in combination with other antineoplastic agents.[117]

Nintedanib (or intenanib), **14** (BIBF 1120) (Figure 4), Vargatef, is a 6-methoxycarbonyl-substituted indolinone reported by Boehringer Ingelheim Pharma GmbH & Co. and defined as a triple angiokinase inhibitor targeting VEGFR, PDGFR, and FGFR. It shows IC_{50} values of 104, 5, and 5 nM for VRGFR-1, -2, and -3, respectively, and of 38 and 18 nM for FGFR-1 and PDGFRα, respectively, in enzymatic assays. The compound is an ATP-competitive inhibitor, as indicated by the homology model of a similar derivative in the VEGFR-2 kinase domain. The typical canonical hydrogen bonds are formed between the lactam moiety and the hinge region (Glu917 and Cys919). In addition, the carbonyl oxygen of the C6 group can form an additional hydrogen bond with Lys868, probably accounting for the high potency of this family of compounds. Nintedanib shows antiproliferative activity on VEGF-related endothelial cell with additional efficacy on pericyctes and

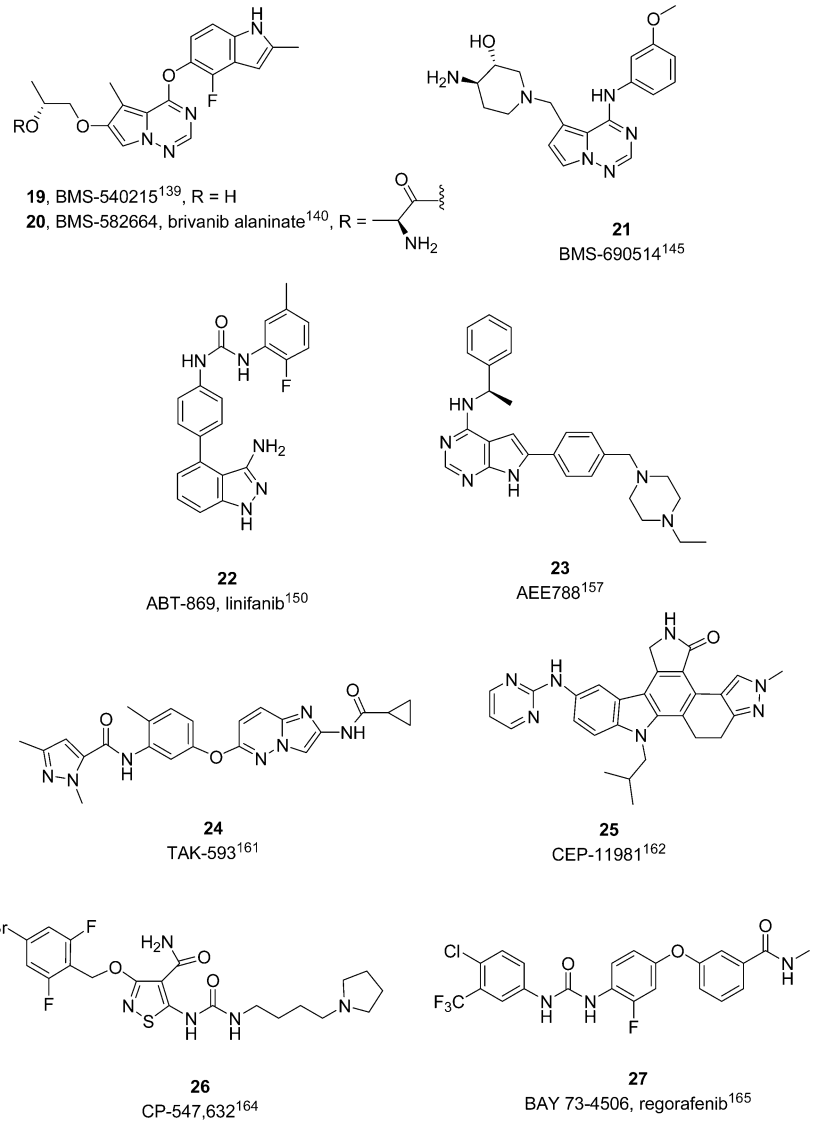

Figure 5. Other derivatives in clinical trials.

smooth muscle cells. However, no direct inhibition of tumor cell proliferation is observed. It is orally available and displays encouraging efficacy in in vivo tumor models while being well tolerated. It is currently in phase 3 clinical trials for the treatment of NSCLC.[118,119] Current phase 2 trials are investigating the effect of the compound in ovarian cancer[120] and on other solid malignancies.[121]

Vatalanib, **15** (PTK787, ZK222584) (Figure 4), a phthalazino derivative disclosed by Novartis and Schering AG in 1998, is one of the most potent and selective first-generation VEGFR kinase inhibitors, with IC_{50} values of 110, 43, 195 nM against VEGFR-1, -2, and -3, respectively. It also inhibits other kinases, including PDGFRβ and c-Kit, but at higher concentrations. Vatalanib is an ATP competitive inhibitor. Docking studies indicate that it does not form direct hydrogen bonds with the backbone of the hinge region as many reported TK inhibitors do. In detail, the aniline moiety is located in a hydrophobic pocket, and the phthalazine bicycle also makes hydrophobic contacts with other amino acids. Although no direct hydrogen bond with the hinge region is established, the aniline NH group forms water-mediated hydrogen bonds with Glu915 and Cys917 of the hinge region, and the pyridyl nitrogen is assumed to form a hydrogen bond with Lys1060, a residue of the kinase activation loop.[122] The compound has been extensively investigated in phase 1 and 2 clinical trials for the treatment of solid tumors and hematological malignancies. Results from a phase 2 trial on mesothelioma patients show partial responses in 6% of patients and stable disease in 72% of patients, with mild toxicity.[123] It has been shown to be quite active in metastatic GIST,[124] NSCLC,[125] and melanoma patients, although in the last cancer overall survival is disappointing.[126] The compound is being tested in a number of phase 3 trials in advanced colorectal cancer. Results from two phase 3 studies (CONFIRM 1 and 2) evaluating vatalanib in the first- and second-line treatment of advanced colorectal cancer indicated that the addition of vatalanib to standard treatment did not result in improved outcomes.[127]

Telatinib, **16** (BAY 57-9352) (Figure 4), is characterized by a furo[2,3-*d*]pyridazine scaffold, isoster to the phthalazinic one present in vatalanib. It has been patented by Bayer as a potent inhibitor of VEGFR, PDGFR, and c-Kit, with IC_{50} values of 6 and 4 nM in enzymatic assays for the inhibition of VEGFR-2 and VEGFR-3, respectively. It inhibits HUVEC growth in proliferation assays, although it is not directly active against

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tumor cells, possessing a purely antiangiogenic mechanism of action. In in vivo assays, telatinib is orally active at a dose of 2.5−60 mg/kg in different xenograft models[128] and has been tested in a number of phase 1 trials for the treatment of advanced solid tumors. Telatinib is safe and well tolerated up to 1500 mg twice daily. On the basis of pharmacodynamic and pharmacokinetic end points, telatinib 900 mg twice daily is the recommended dose for subsequent phase 2 studies.[129,130] A phase 2 study on telatinib used in combination with chemotherapy as first-line therapy in subjects with advanced gastric cancer has been just completed, but the results are not yet available.

Motesanib, **17** (AMG 706) (Figure 4), produced by Amgen, is derived from the anthranilamide class of VEGFR inhibitors. The anthranilamide scaffold, where an intramolecular H-bond between NH and CO forms a "pseudo ring", mimics the phthalazine binding mode, giving the same type of interactions with the enzyme.[131] Motesanib is a multikinase inhibitor that possesses IC_{50} values of 2, 3, 6 nM for VEGFR-1, -2, -3 and is also active on PDGFR, c-Kit, and RET. Oral administration of the compound potently inhibits VEGF-induced angiogenesis in the rat corneal model and induces regression of established A431 xenografts.[132] The compound also shows antitumor activity in breast cancer,[133] medullary thyroid cancer xenografts,[134] and GIST,[135] and it is currently being studied in clinical trials for the treatment of thyroid cancer and other advanced solid tumors.[100] Unfortunately, a 2011 report indicated that a phase 3 trial did not show benefit for advanced NSCLC.[136]

18 (OSI-930) (Figure 4), patented by OSI Pharmaceuticals in 2004,[137] is a thiophene isoster of anthranilamide derivatives and bears a quinoline group, which constitutes the hinge-region binding element. It potently inhibits VEGFR-2 with an IC_{50} of 9 nM, together with c-Kit, c-Raf, and Lck (IC_{50} values of 10, 41, 22 nM, respectively) in different enzymatic assays and in intact cells, including HUVECs. More importantly, it has been found to be active in different tumor xenograft models when administered daily at the maximally efficacious dose of 200 mg/kg.[138] Phase 1 dose escalation studies of **18** in healthy volunteer patients have been completed, and a phase 1 dose escalation study in cancer patients is ongoing.

Bristol-Myers Squibb reported a series of 4-(4-fluoro-1*H*-indol-5-yloxy)pyrrolotriazines as VEGFR-1, VEGFR-2, and FGFR-1 inhibitors, including **19** (BMS-540215) (Figure 5), endowed with good kinase selectivity and potent in vivo efficacy versus H3396 and L2987 human lung carcinoma xenografts implanted in athymic mice.[139] On the other hand, the compound showed suboptimal physicochemical and pharmacokinetic properties and particularly low aqueous solubility that presumably contributed to dissolution rate-limited absorption. To overcome this potential development issue, a prodrug approach has been investigated and led to the preparation of brivanib alaninate, **20** (BMS-582664) (Figure 5), in which the secondary alcohol of **19** was linked to alanine through a metabolically labile ester linkage. Brivanib alaninate is converted in vivo to the active compound **19** and demonstrates excellent pharmaceutical properties and significant antitumor activity against L2987 human lung carcinoma xenografts.[140] Brivanib alaninate is also very active in other preclinical models,[141] including in mouse models of human HCC,[142] and has entered clinical trials for the treatment of solid malignancies.[143] Unfortunately, the failure of brivanib in a phase 3 head to head study with nexavar has been very recently reported. Brivanib demonstrates promising antitumor activity and a manageable safety profile as first-line therapy in patients with advanced, unresectable HCC. In a phase 2 open-label study, brivanib was administered orally at a dose of 800 mg once daily as second-line therapy in patients with advanced HCC who had failed prior antiangiogenic treatment. Results of this trial showed that brivanib has a manageable safety profile and is one of the first agents to possess promising antitumor activity in advanced HCC patients treated with prior sorafenib.[144] At the time of writing, the compound is being tested in a phase 3 study with the same objective as the phase 2 study just reported.

21 (BMS-690514) (Figure 5) is another pyrrolotriazine by Bristol-Myers Squibb. The compound is a pan HER/VEGFR orally active inhibitor that also targets other kinases, including FLT3 and Lck. It exerts antiproliferative and proapoptotic effects on NSCLC cell lines, with prominent efficacy on cells expressing the T790M mutation that confers resistance to the EGFR inhibitor erlotinib.[145] Moreover, it shows antitumor activity on NSCLC xenografts and induces synergistic effect with radiation.[146] The potency and selectivity of **21** have been successively evaluated by using an extensive array of enzymatic assays, as well as cellular assays, whereas the antitumor activity was evaluated by using multiple xenograft models. The antiangiogenic properties of the compound were assessed in a matrigel plug assay, and the effect on tumor blood flow was measured by dynamic contrast-enhanced magnetic resonance imaging. Overall, the results demonstrated that **21** is a novel targeted agent that disrupts signaling in the tumor and its vasculature.[147] The preclinical ADME properties of **21** suggest good oral bioavailability in humans and metabolism by multiple pathways including oxidation and glucuronidation. On the basis of the preliminary results on compound pharmacokinetics, the efficacious dose for humans is predicted to be in the range of 100−200 mg.[148] Considering the submicromolar plasma concentration at the anticipated clinical dose of 200 mg, **21** is unlikely to cause clinically relevant drug−drug interactions when coadministered with other medications. In addition, because multiple enzymatic clearance pathways are available for the compound, inhibition of an individual metabolic pathway either via coadministered drugs or gene polymorphisms is not expected to cause pronounced increases in **21** exposure.[149] The compound is currently being tested in different phase 1 clinical trials on solid tumors and in a phase 2 trial on NSCLC.

Linifanib, **22** (ABT-869) (Figure 5), is a multitargeted indazolyldiphenylurea inhibitor, developed by Abbott, which potently inhibits VEGFR-2 and PDGFRβ with IC_{50} values of 4 and 2 nM, respectively, and shows a similar behavior against FLT3 and CSF1-R in both enzymatic and cell assays.[150] It possesses antiproliferative and apoptotic effects on kinase-dependent cancer cell lines and favorable pharmacokinetic profiles across different species and displays significant tumor growth inhibition in multiple xenograft models, including human fibrosarcoma, breast, colon, and lung carcinomas.[151] Similar to other urea-based kinase inhibitors, the compound forms three hydrogen bonds with the catalytic site of VEGFR-2 (Figure 6), more specifically between the urea external NH and Glu885 carboxylate, between the 3-amino group of the indazole and Glu917 backbone carbonyl, and between the ring nitrogen (N2) and Cys919 NH.[149]

The antiangiogenic activity of the compound has been recently confirmed in HT1080 fibrosarcoma and SW620 colon carcinoma cells[152] and in an orthotopic rat glioma model.[153]

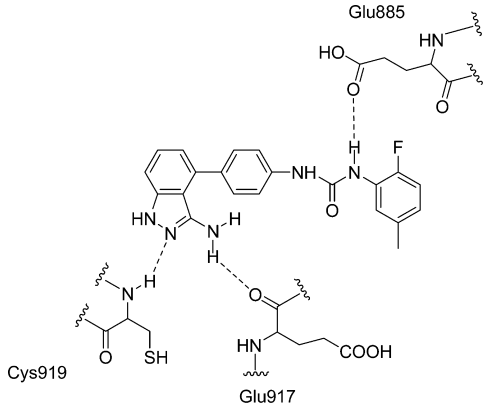

Figure 6. Schematic representation of hydrogen bonds (dotted lines) between **22** and VEGFR-2 catalytic site.

Linifanib is currently being tested in a number of phase 1−2 clinical trials for the treatment of solid tumors, including colorectal cancer and NSCLC and hematological malignancies. Some results have been published indicating that the compound is well tolerated and shows promising preliminary clinical activity in Japanese patients with solid tumors,[154] in patients with advanced RCC after sunitinib failure,[155] and in advanced NSCLC patients as second- or third-line therapy. Increased adverse event rates were observed at a dose of 0.25 mg/kg once daily, whereas lower doses are quite well tolerated.[156]

23 (AEE788) (Figure 5) is a 7*H*-pyrrolo[2,3-*d*]pyrimidine synthesized by Novartis as a dual inhibitor of EGFR and VEGFR. It shows IC_{50} values of 2, 77, 59 nM toward EGFR-2, VEGFR-2, and VEGFR-1, respectively, in enzymatic assays. Orally administered, **23** shows favorable pharmacokinetic properties and potent antitumor and antiangiogenic activity in different animal models of cancer.[157] As an example, **23** reduces tumor growth by inhibiting proliferation and vascularization in human HCC xenografts in nude mice[158] and shows growth-suppressive activities in chemosensitive and chemoresistant medulloblastoma cells in vitro and in xenografts.[159] The compound is in phase 1/2 clinical trials on patients with advanced cancer or recurrent glioblastoma. Unfortunately, it has been very recently reported that once-daily **23** was associated with unacceptable toxicity and minimal activity for the treatment of recurrent glioblastoma. This finding led to the premature discontinuation of the trial.[160]

Table 2. Compounds in Clinical Trials

			clinical trial	
compd	common name	chemical class	phase 1/phase 2	phase 3
AZD2171 (**6**, Figure 3)	cediranib	quinazoline	alone or in combination for hematological and solid tumors	in combination for solid tumors (glioblastoma, colorectal, ovarian, and lung cancers)
XL184, BMS-907351 (**7**, Figure 3)	cabozantinib	quinoline	alone or in combination for hematological and solid tumors	alone for prostate and medullary thyroid cancers
XL880, GSK1363089 (**8**, Figure 3)	foretinib	quinoline	alone or in combination for solid tumors	
KRN951, AV-951 (**9**, Figure 3)	tivozanib	quinoline	alone or in combination for solid tumors	alone for RCC
E7080 (**10**, Figure 3)	lenvatinib	quinoline	alone or in combination for solid tumors	alone for refractory differentiated thyroid cancer
E-3810 (**11**, Figure 3)		quinoline	alone for solid tumors	
CHIR-258, TKI258 (**12**, Figure 3)	dovitinib	quinolin-2-one	alone or in combination for solid tumors	alone for metastatic RCC
SU6668, TSU-68 (**13**, Figure 4)	orantinib	indolinone	alone for solid tumors	in combination for HCC
BIBF 1120 (**14**, Figure 4)	nintedanib, intenanib	indolinone	alone or in combination for hematological and solid tumors	alone or in combination for solid tumors (ovarian, lung and liver cancers)
PTK787, ZK222584 (**15**, Figure 4)	vatalanib	phtalazine	alone or in combination for hematological and solid tumors	in combination for metastatic colorectal cancer
BAY 57-9352 (**16**, Figure 4)	telatinib	furopyridazine	in combination for gastric cancer	
AMG 706 (**17**, Figure 4)	motesanib	2-aminonicotinamide	alone or in combination for solid tumors or lymphoma	in combination for NSCLC
OSI-930 (**18**, Figure 4)		3-aminothiophene-2-carboxamide	alone or in combination for solid tumors	
BMS-540215 (**19**, Figure 5)		pyrrolotriazine	alone or in combination for solid tumors	alone or in combination for solid tumors (liver and colorectal cancers)
BMS-582664 (**20**, Figure 5)	brivanib alaninate	pyrrolotriazine	alone or in combination for solid tumors	alone or in combination for solid tumors (liver and colorectal cancers)
BMS-690514 (**21**, Figure 5)		pyrrolotriazine	alone or in combination for solid tumors	
ABT-869 (**22**, Figure 5)	linifanib	indazole	alone or in combination for solid tumors	alone for advanced HCC
AEE788 (**23**, Figure 5)		pyrrolopyrimidine	alone or in combination for solid tumors	
TAK-593 (**24**, Figure 5)		imidazopyridazine	alone for solid tumors	
CEP-11981 (**25**, Figure 5)		indazolopyrrolocarbazolone	alone for solid tumors	
CP-547,632 (**26**, Figure 5)		isothiazole	alone or in combination for solid tumors	
BAY 73-4506 (**27**, Figure 5)	regorafenib	diphenylurea	alone or in combination for solid tumors (e.g., colorectal cancer)	

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24 (TAK-593) (Figure 5), an imidazo[1,2-*b*]pyridazine derivative by Takeda Pharmaceuticals, potently inhibits TKs from the VEGFR and PDGFR families, specifically VEGFR-1, -2, and -3 with IC_{50} values of 3.2, 0.95, and 1.1 nM, respectively. **24** is selective for these families, with $IC_{50} > 1$ μM when tested against more than 200 protein and lipid kinases. It displays competitive inhibition for ATP and binds to the inactive conformation of the enzyme, behaving as a type II kinase inhibitor.[161] The authors, studying its kinetic and interaction with VEGFR-2, concluded that the compound binds the enzyme in a way similar to that recently reported for axitinib, interacting also with the juxtamembrane region.[73] This inhibitor targets the enzyme with a two-step slow binding mechanism, and this accounts for the longest residence times of any VEGFR inhibitor yet reported, probably ensuring a prominent and durable effect at very low doses in vivo. It has been tested in a phase 1 clinical and pharmacokinetic study in subjects with nonhematologic advanced cancer, but no result is available.[161]

25 (CEP-11981) (Figure 5) is an indazolo[5,4-*a*]pyrrolo[3,4-*c*]carbazolone, very recently reported by Cephalon as a potent multitargeted inhibitor, being especially active on Tie-2, VEGFR-1, -2, and -3 and FGFR-1. In detail, it shows IC_{50} values of 4 and 22 nM on VEGFR-2 and Tie-2, respectively, and less than 10 nM in VEGFR-2 cells. The compound shows antiangiogenic activity on ex vivo rat aortic ring explant cultures and in vitro HUVEC capillary-tube formation bioassays, as well as reduced tumor growth of glioblastoma and melanoma xenografts in nude mice. Its inhibitory activity on Tie-2 seems to play important roles in the antiangiogenic activity.[162] Indeed, Tie-1 and Tie-2, together with their ligands, the angiopoietins, have been implicated in vessel stabilization, maturation, remodeling, and organization of the rudimentary vasculature. The inhibition of tumor angiogenesis and vascular remodeling by modulating the angiopoietin/Tie-2 axis and the VEGF/VEGFR-2 axis has been demonstrated using different approaches against a variety of human tumors in preclinical studies.[163] **25** possesses favorable pharmacokinetic properties; a clinical phase 1 study assessing its safety and pharmacokinetics has been recently completed. Phase 2 proof-of-concept studies are planned in select genotyped cancer patient populations to fully assess the therapeutic potential of this compound.[162]

26 (CP-547,632) (Figure 5), produced by Pfizer, is a substituted isothiazoleurea identified as a potent, ATP-competitive, reversible inhibitor of VEGFR-2 and FGFRβ, with IC_{50} values in enzymatic assays of 11 and 9 nM, respectively, and of 6 nM in VEGFR-2 cells. It possesses antiangiogenic activity, assessed in vivo by functional assays. The compound, orally administered to athymic mice bearing human xenografts, inhibits tumor growth by 85% and is well tolerated. It is currently in phase 2 clinical trials for the treatment of refractory solid tumors.[164]

Regorafenib, **27** (BAY 73-4506) (Figure 5), is a fluoro derivative of sorafenib, developed by Bayer.[165] It inhibits angiogenic kinases (VEGFR-1/3, PDGFRβ, FGFR1, and Tie-2) and the mutant oncogenic kinases Kit, RET, and B-Raf with IC_{50} values in the low nanomolar range. The compound shows antiproliferative effects on different cancer cell lines and is active in various preclinical human xenograft models in mice, where it also demonstrated antiangiogenic activity.[166] Regorafenib is currently being tested in several phase 1/2 clinical trials. The first results indicate that it is active in patients with advanced solid tumors[167] and in particular with metastatic colorectal cancer.[168]

VEGFR inhibitors currently in clinical trials for the treatment of different cancers are summarized in Table 2 (data from clinicaltrials.gov).

VEGFR Inhibitors Recently Published. A high number of VEGFR inhibitors have recently been reported, highlighting the interest of medicinal chemists from both academia and pharmaceutical companies in this class of therapeutic agents. This final section of the article deals with synthetic VEGFR inhibitors that have appeared in the literature over the past few years, with an emphasis on medicinal chemistry in terms of chemical structure, mechanisms of action, and SAR. Even if experience frequently shows a decrease of inhibitor activity when passing from in vitro to in vivo assays, preliminary data on the biological profile of some of these compounds are extremely interesting. Some of the compounds described below are derived from minor chemical modifications of lead compounds already approved or in clinical trials. It is likely that the majority of the new inhibitors will prove to be less active than their parent drugs as the biological evaluation continues. On the other hand, the introduction of a small substituent on a suitable chemical scaffold can lead to surprising results. The most famous example is imatinib, the first kinase inhibitor approved for clinical use and the first drug of choice for chronic myeloid leukemia (CML) treatment. It is derived from an optimization process on phenylaminopyrimidine derivatives active as protein kinase C (PKC) and other kinase inhibitors. The introduction of a simple methyl group (often referred to as the "magic methyl") at position 6 of the diaminophenyl ring abolished PKC inhibitory activity and retained, or even enhanced, activity against protein tyrosine kinases, thus giving rise to imatinib that is active on Abl, the TK etiologic agent of CML.[169] It is impossibile to rule out a similar advance in the field of VEGFR/multitargeted inhibitors. Regorafenib, a close analogue of sorafenib, which bears only one fluorine atom more than its parent, appears to be doing surprisingly well in clinical trials, especially for metastatic colorectal cancer.

The complex nature of the interactions between a small molecule drug and a protein target is very difficult to fully determinate a priori, even using advanced techniques such as X-ray crystallography or molecular modeling studies. So it is impossible to predict whether or not a very small change in a molecule will give rise to a large variation in its biological activity. It is important to point out that, besides the possible structural drug−target collaboration just mentioned, a small variation of chemical structure can lead to a different solubility or permeability and consequently to a different biological behavior, especially when the compound in question is an apolar molecule, as several TKs inhibitors are.

In our opinion, the search for new TK inhibitors endowed with antiangiogenic activity is still an open road. Systematic and sophisticated procedures for designing the inhibitor provide opportunity for further promising results, as does serendipity.

Pyrimidine Derivatives. Quinazolines. The quinazoline scaffold is present in potent VEGFR inhibitors, including vandetanib, **4**, and cediranib, **6**. Garofalo and colleagues reported a series of quinazolineurea derivatives as VEGFR-2 inhibitors. The molecules are derived from a previously synthesized series of quinolines active as dual EGFR/VEGFR-2 inhibitors, modified following the suggestion of computational studies performed by the same authors. Compound **28**

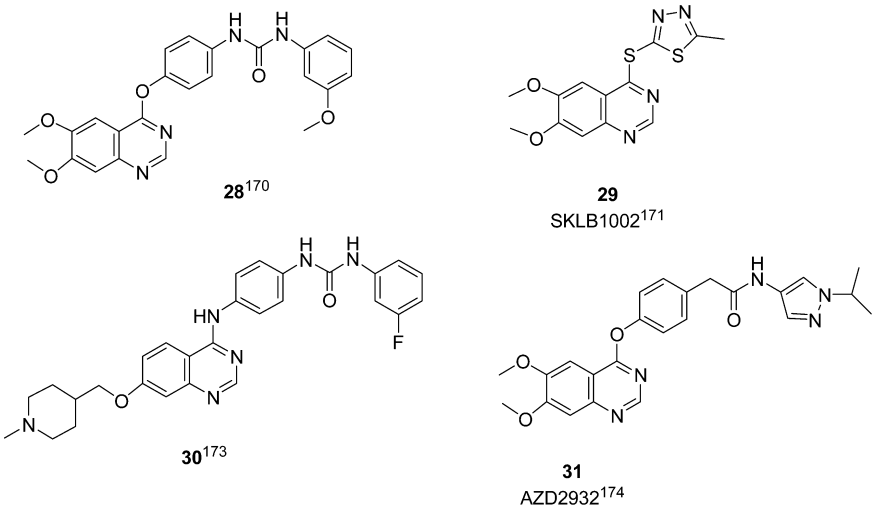

Figure 7. Quinazolines.

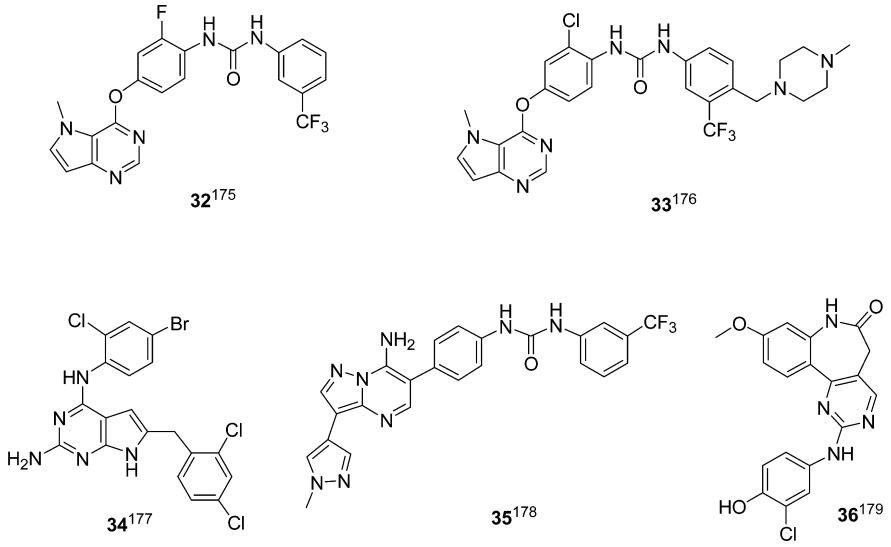

Figure 8. Pyrrolopyrimidines and other fused pyrimidines.

(Figure 7) shows an IC_{50} of 40 nM on VEGFR-2, whereas it is almost inactive on EGFR (IC_{50} > 10 μM) and exerts antiproliferative activity on PC3 prostate cancer cells, HT29 colon cancer cells, and MCF-7 breast cancer cells with IC_{50} values of about 9 μM.[170]

29 (SKLB1002) (Figure 7), a quinoline substituted with a (5-methyl-1,3,4-thiadiazol-2-yl)thio group in C4, is a VEGFR-2-selective inhibitor (IC_{50} = 32 nM) while exhibiting reduced or little activity against other kinases. It is an effective antiangiogenic agent, showing 98% inhibition of HUVEC tube formation at 10 μM and 80% inhibition of zebrafish embryos intersegmental vessel growth at 2.5 μM. The compound suppresses SW620- and HepG2-derived tumor growth in mice (by 72% and 63%, respectively, in 18 days at 100 mg kg^{-1} day^{-1}).[171] Moreover, **29** induces vascular normalization and enhances anticancer drug delivery, which is associated with a synergistic effect in vivo.[172]

Yu and colleagues prepared a series of 4-aminoquinazoline VEGFR inhibitors based on the vandetanib, **4**, structure. The newer compounds, however, lack the C6 methoxy group and bear a urea group at the para position of the C4 anilino moiety. Compound **30** (Figure 7) was found to be 6-fold more potent than the positive control **4**, showing IC_{50} values of 5.5 and 9.6 nM for VEGFR-2 and VEGFR-3, respectively, and a good selectivity on a panel of other kinases. It is also efficacious in HepG2 human tumor xenograft model in BALB/c-nu mice.[173]

AstraZeneca researchers further expanded the quinazoline family of kinase inhibitors and very recently reported **31** (AZD2932) (Figure 7) as a high affinity inhibitor of VEFGR-2 and PDGFR. The N1-alkylated 4-aminopyrazole in the amide portion leads to compounds with a balanced ∼1:1 ratio of activity vs both VEGFR-2 and PDGFRβ, including **31**, which shows IC_{50} values of 8 and 4 nM against the two enzymes, respectively. It is also active on c-Kit and FLT3 with good selectivity on a panel of kinases. The pharmacokinetic behavior and the preclinical antitumor activity in nude mice bearing C6 rat glial tumors indicate that **31** has the potential to become an antiangiogenic agent in the clinic.[174]

Other Fused Pyrimidines. Two families of pyrrolo[3,2-*d*]pyrimidine derivatives have been published by Oguro and colleagues at Takeda Pharmaceuticals, as VEGFR-2 kinase inhibitors. Incorporation of a diphenylurea moiety at the C4-position of the pyrrolopyrimidine core via an oxygen linker

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resulted in compounds that are type II inhibitors, binding to the inactive conformation of VEGFR-2 kinase.

Compound **32** (Figure 8) shows IC_{50} values of 6.2, 15, 35, and 96 nM against VEGFR-2, VEFGR-1, PDGFRα, and PDGFRβ, respectively. It also inhibits Tie-2 with good selectivity on a panel of kinases. In order to confirm the binding mode of such compound, the crystal structure of the complex between **32** and VEGFR-2 was solved (PDB code 3VHE). The kinase adopts an inactive conformation (DFG-out) so that the urea portion occupies the back hydrophobic pocket with additional hydrogen-bonding interactions. The N1 of the pyrrolo[3,2-*d*]pyrimidine core interacts with the main chain NH group of Cys919 in the hinge region. The urea moiety binds to the protein through two hydrogen-bonding interactions: both urea NH groups interact with Glu885, and the carbonyl interacts with Asp1046. The external 3-(trifluoromethyl)phenyl moiety occupies the hydrophobic pocket created by the conformational rearrangement of Phe1047 (DFG-out) (Figure 9). The compound inhibits

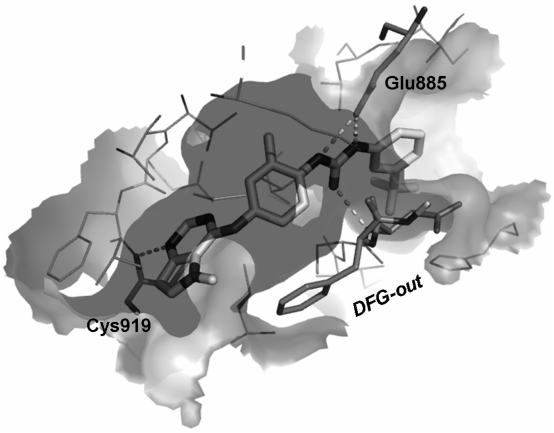


Figure 9. Graphical representation of the binding mode of compound **32** (sticks) into the ATP binding site of VEGFR-2. For sake of clarity, only a few residues are labeled, nonpolar hydrogen atoms are omitted, and hydrogen bond interactions are represented by dashed lines.

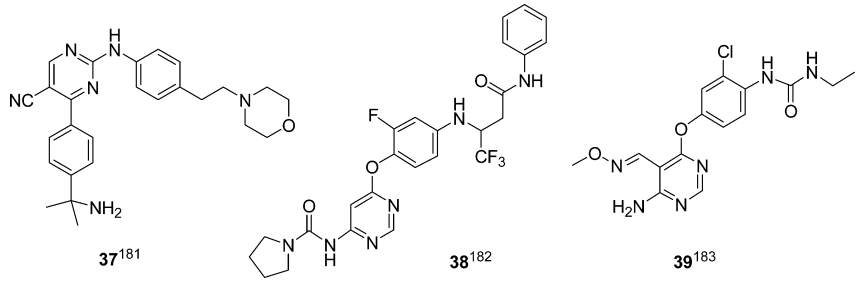
HUVEC cell proliferation with an IC_{50} of 4.4 nM, possessing good pharmacokinetic parameters. Oral administration of its hydrochloride salt inhibits tumor growth in a DU145 human prostate cancer cell xenograft nude mouse model.[175]

Further modifications, performed using the cocrystal structure analysis of VEGFR-2 and **32**, led to the identification of potent dual VEGFR-2/FGFR-1 inhibitors. Among these compounds the urea derivative **33** (Figure 8), having a piperazine moiety on the terminal benzene ring, shows IC_{50} values of 9.3 and 14 nM, respectively, for the two enzymes. A binding model of **33** complexed with VEGFR-2 suggested that the piperazine moiety forms additional interactions with Ile1025 and His1026. Compound **33** shows strong inhibitory activities against both VEGF- and FGF-stimulated HUVEC proliferation and improved solubility. Since FGF-FGFR signaling has been shown not only to influence tumor angiogenesis but also to directly contribute to tumor growth and survival, simultaneous inhibition of these receptor TKs may be useful for the antitumor activity.[176]

The isoster heterocyclic scaffold, i.e., the pyrrolo[2,3-*d*]pyrimidine, also provided interesting VEGFR-2 inhibitors. Gangjiee and colleagues prepared a series of N^4-phenyl-substituted-6-(2,4-dichlorophenylmethyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine-2,4-diamines, among which compound **34** (Figure 8) inhibits VEGFR-2 with an IC_{50} of 100 nM and shows an excellent selectivity on EGFR, VEGFR-1, and PDGFβ, which are inhibited only at doses of >300 μM. The compound was further evaluated in a mouse orthotopic model of melanoma and showed significant inhibition of tumor growth, angiogenesis, and metastasis formation.[177]

Among fused pyrimidine derivatives, other potent TK inhibitors are present. 7-Aminopyrazolo[1,5-*a*]pyrimidines, again substituted with a urea function, gave different VEGFR and PDGFR inhibitors. Several of these compounds were selective for VEGFR-2. For example, **35** (Figure 8) shows IC_{50} values of 3 and 0.7 nM in enzymatic and in cell assays, respectively. Compound **35** possesses a favorable pharmacokinetic and oral efficacy in the estradiol-induced murine uterine edema assay, an in vivo model of VEGF-induced vascular permeability. Modeling and SAR studies suggest that this compound binds in the ATP pocket of the enzyme with the 7-aminopyrazolo[1,5-*a*]pyrimidine core forming a bidentate hydrogen bonding interaction with the hinge region, in particular between the exocyclic amine and Glu917 backbone carbonyl and between the N1 of the pyrazolopyrimidine and Cys919 NH. The the *N*,*N*′-diarylurea occupies the hydrophobic pocket. Asp1046 and Phe1047 of the "DFG" motif are in the inactive conformation ("DFG-out").[178]

Egert-Schmidt and colleagues developed a family of dual polo-like kinase 1 (PLK1)/VEGFR-2 inhibitors.[179] The serine/threonine kinase PLK1 is an important regulator of the cell cycle, involved in the formation of the mitotic spindle and in the activation of CDK/cyclin complexes during M-phase of the cycle. Experimental evidence demonstrated that PLK1 is a valid biological target for potential anticancer agents.[180] Accordingly, dual PLK1/VEGFR-2 inhibitors should display an improved antitumor activity compared with selective VEGFR inhibitors. 2-Anilino-9-methoxy-5,7-dihydro-6*H*-pyrimido[5,4-*d*][1]-benzazepin-6-ones consist of the seven-membered lactam structure present in the paullone basic scaffold, which might



37[181] **38**[182] **39**[183]

Figure 10. Nonfused pyrimidines.

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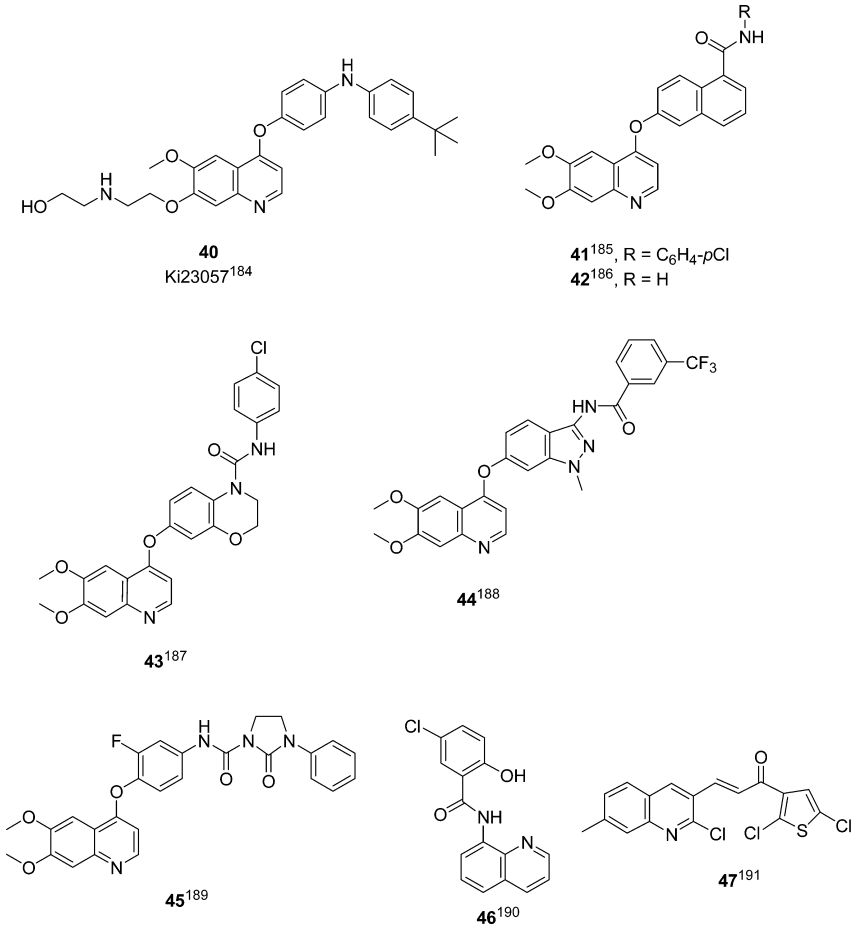

Figure 11. Quinolines.

serve as a suitable hinge-binder template for the design of dual VEGFR-2/PLK1 inhibitors. Among these, compound **36** (Figure 8) shows IC_{50} values of 11 and 360 nM against VEGFR-2 and PKL1, respectively, inhibitory activity in a cellular angiogenesis model and antiproliferative activity on cancer cell lines in vitro.[179]

Nonfused Pyrimidines. The isolated pyrimidine ring is present in a number of VEGFR-2 inhibitors. A series of 4-aryl-5-cyano-2-aminopyrimidines shows potent VEGFR-2 kinase inhibitory activity. The phenylethylmorpholino derivative **37** (Figure 10) has an IC_{50} of 27 nM on the isolated enzyme and is orally active in the corneal micropocket assay, suggesting the inhibition of in vivo neovascularisation.[181]

Optimization of a series of pyrimidine derivatives bearing a urea function and a malonamide type group, where one amido group is substituted with the amide isostere triflouoroethylamine unit, led to potent and selective VEGFR-2 inhibitors. These include **38** (Figure 10) (IC_{50} = 40 nM), which reduces VEGF-dependent HUVEC proliferation. In an in vitro angiogenesis assay it significantly affects tubule growth and branch formation and inhibits tubule growth and junction formation.[182]

4-Aminopyrimidine-5-carbaldehydeoximes bearing a phenylurea function on C4 were good and selective VEGFR-2 inhibitors when tested on a panel of 13 kinases. Derivative **39** (Figure 10) shows an IC_{50} of 11 nM in the enzymatic assay and is active against a variety of xenografts in mice. It demonstrates good bioavailability and no evident toxicity in animals.[183]

Pyridine Derivatives. Quinolines. The quinoline ring, often bearing C6 and C7 methoxy or oxy groups, is the most represented scaffold among different kinase inhibitors. A C4 oxy substituent is commonly present in VEGFR inhibitor structures. **40** (Ki23057) (Figure 11) is a multitargeted receptor-TK, active on VEGFR-2, PDGFRβ, FGFR-2, and c-Kit (IC_{50} values in the range 69−100 nM) but not on EGFR, IGF1R, or MET. The compound inhibits VEGF-induced proliferation of HUVECs, whereas no inhibitory effect on the proliferation of three colon cancer cell lines (LM-H3, LoVo, and LS174T) was observed. On the other hand, **40** inhibits the growth of the xenografted LM-H3 tumors and the spreading of cancer cells to the liver. Fewer microvessels are present in the xenograft tumors treated with **40** compared with controls, indicating the antiangiogenic activity of the compound.[184]

Amgen Inc. researchers synthesized different series of quinolines, two of them bearing C4 naphthamide substituents. Among the first series, one of the most active compounds is the 4-chlorophenyl derivative **41** (Figure 11) which shows IC_{50} values of 0.5 and 8 nM in enzymatic test and in HUVEC cells, respectively, and significant antitumor efficacy against established HT29 human colon adenocarcinoma xenografts implanted in athymic mice. Authors obtained a X-ray cocrystal structure of VEGFR-2 with naphthamide **41**. As illustrated in Figure 12, the compound binds to the ATP binding site of VEGFR-2 and forces the protein to adopt a "DFG-out" conformation, enabling the *p*-chlorophenyl ring to penetrate into the extended hydrophobic pocket (PDB accession code 3B8Q). A conserved hydrogen-bond interaction is formed

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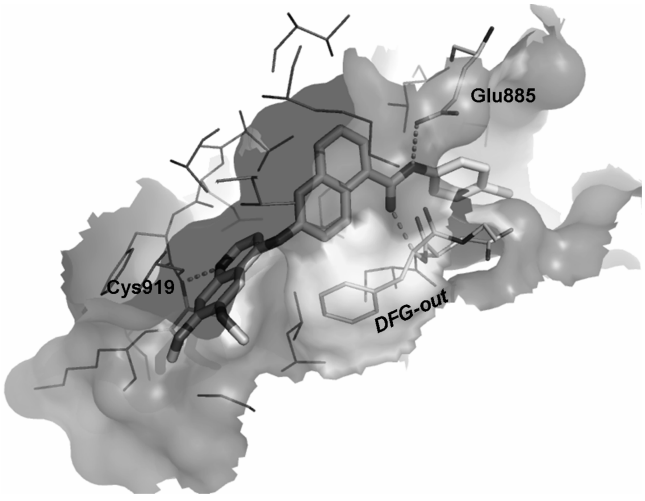

Figure 12. Graphical representation of the binding mode of compound **41** (sticks) into the ATP binding site of VEGFR-2. For sake of clarity, only a few residues are labeled, nonpolar hydrogen atoms are omitted, and hydrogen bond interactions are represented by dashed lines.

between the backbone amide NH of Cys919 and the nitrogen of the quinoline ring; the carbonyl and NH groups of the amide form critical hydrogen bonds with the backbone NH of Asp1046 and the side chain of Glu885, respectively.[185]

The same group synthesized a series of *N*-alkyl and N-unsubstituted naphthamides, with the aim of obtaining more selective compounds, lacking the Aurora B and Lck activity characteristic of **41**. The new compounds target the hydrophobic pocket of the enzyme and behave as type I inhibitors. Indeed, the enzyme is maintained in a "DFG-in" conformation after inhibitor binding, typical of the active kinase. One of the most active compounds of the new series is the unsubstituted naphthamide **42** (Figure 11), which shows IC_{50} values of 0.9 and 2.1 nM in enzymatic and cell assays, respectively, and exhibits good pharmacokinetics. Once-daily oral administration of **42** for 14 days led to 85% inhibition of established HT29 colon cancer and Calu-6 lung cancer xenografts.[186]

More soluble compounds have been obtained by the substitution of the naphthalene moiety with a 2,3-dihydro-1,4-benzoxazine group, which retains the shape and contact elements with the protein while improving the solubility. Compound **43** (Figure 11) shows IC_{50} values of 0.5 and 4 nM for VEGFR-2 and HUVEC cells, respectively, and is also quite selective, inhibiting MET, Tie-2, and Lck with IC_{50} values in the range 20−100 nM.[187]

6,7-Dimethoxyquinolines bearing an indazole moiety proved to be potent VEGFR-2 inhibitors. Among these, the *m*-trifluorophenyl derivative **44** (Figure 11) has IC_{50} values of 1 and 9 nM in enzymatic and HUVEC cell assays, respectively, and inhibits VEGFR-induced vascular permeability in a dose-dependent fashion. Tested on a small panel of kinases, the compound is quite selective for VEGFR-2. Aurora-1 is the only other kinase significantly inhibited by **44**.[188]

A series of *N*-(4-(6,7-disubstituted-quinolin-4-yloxy)-3-fluorophenyl)-2-oxo-3 phenylimidazolidine-1-carboxamides was found to target MET and VEGFR-2 with IC_{50} values in the low nanomolar range. Since MET cooperates synergistically with the VEGFRs in tumor angiogenesis and its deregulation is associated with tumorigenesis, such dual inhibitors represent a promising approach to cancer treatment. Compound **45** (Figure 11), which also demonstrates good bioavailability within this series, was evaluated for its ability to inhibit the proliferation of several human cancer cell lines and showed potent antiproliferative effects. This compound was active in a mouse xenograft model bearing tumors derived from the MET-driven MNNG-Hos cells.[189]

Quinoline amides, e.g., **46** (Figure 11),[190] and quinolylthienyl chalcones, e.g., **47** (Figure 11),[191] made up of a differently decorated quinoline ring, are active as VEGFR-2 inhibitors in enzymatic and in cell assays.

Other Fused Pyridines. The pyridine ring fused with a number of eterocycle moieties has provided VEGFR/multi-targeted inhibitors. A family of dual MET/VEGFR-2 targeted molecules, including compound **48** (Figure 13), is structurally and biologically similar to **44** but is based on a substituted thieno[3,2-*b*]pyridine scaffold instead of the quinolinic one.[192] A further development of such derivatives is a series of arylmalonamides, in particular **49** (Figure 13), characterized by the introduction of a cyclopropyl moiety at the methylene unit

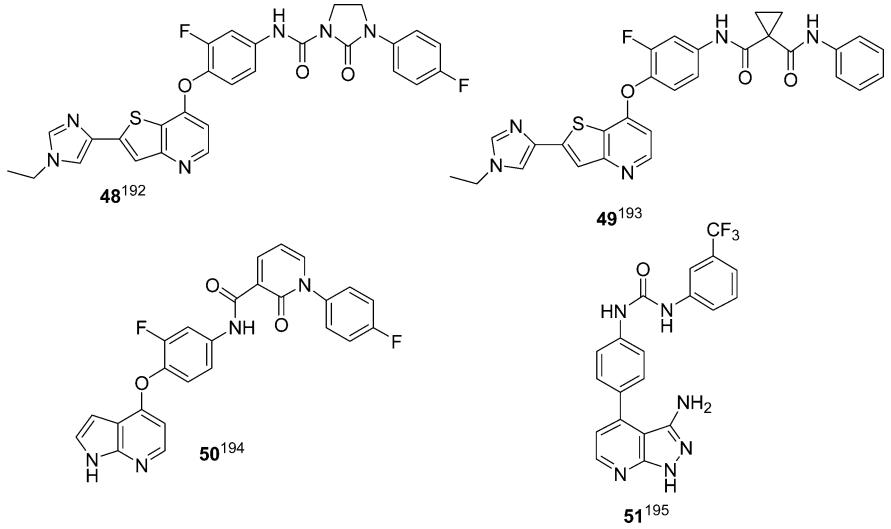

Figure 13. Fused pyridines.

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of the malonamide fragment. This side chain is present in cabozantinib and foretinib, which have been discussed elsewhere in this paper. Compound **49** shows IC_{50} values of 8 and 24 nM for VEGFR-2 and MET, respectively, and is efficacious in different human tumor xenograft models.[193]

Pyrrolopyridinepyridone derivatives such as **50** (Figure 13) are multitargeted inhibitors active in the low nanomolar range on VEGFR-2, FLT3, and MET, metabolically stable, and endowed with good efficacy in a GTL-16 human gastric carcinoma xenograft model.[194]

Aminopyrazolo[3,4-*b*]pyridineureas, aza analogues of **22**, were shown to be VEGFR/PDGFR inhibitors, but were not active on other structurally nonrelated kinases, such as Fyn and Src. Compound **51** (Figure 13) inhibits VEGFR-2 with IC_{50} values of 1.7 and 2.6 nM in enzymatic and cell assays, respectively, and is orally active in an estradiol-induced mouse uterine edema model.[195]

Nonfused Pyridines. A number of VEGFR inhibitors with structures miming the anthranilamide moiety and containing a nonfused pyridine ring have been synthesized in the past few years, including 3,4-disubstituted isothiazoles such as **52** (Figure 14)[196] and (1,2,3-triazol-4-yl)benzenamines such as

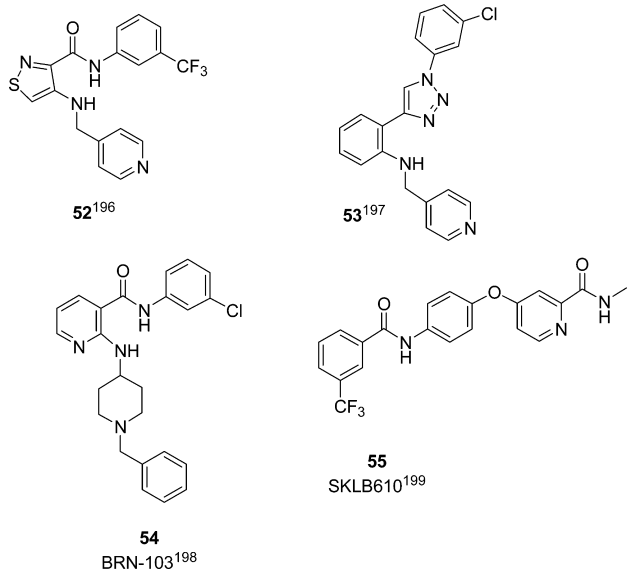

Figure 14. Nonfused pyridines.

53 (Figure 14),[197] both reported by Kiselyov and colleagues. These compounds are potent VEGFR-1 and -2 inhibitors with activity comparable to that of vatalanib in enzymatic and cell assays.

A more recent example of this type of VEGFR inhibitors is represented by **54** (BRN-103) (Figure 14), a nicotinamide derivative that inhibits VEGF-induced migration, proliferation, capillary-like tube formation of HUVECs, and vessel sprouting from mouse aortic rings, in a dose dependent fashion. **54** was found to suppress the VEGF-induced phosphorylation of VEGFR-2 and the activation of AKT and eNOS.[198]

A differently structured compound containing the isolated pyridine ring is the *N*-methyl-4-(4-(3-(trifluoromethyl)-benzamido)phenoxy)picolinamide **55** (SKLB610) (Figure 14), a multitargeted inhibitor active on VEGFR-2 and FGFR-2. The derivative has antiangiogenic effects, inhibiting HUVEC capillary-tube formation and the subintestinal vein formation of zebrafish in vivo. It has antiproliferative effects, especially on human NSCLC cell line A549 and human colorectal cancer cell line HCT116, and is also active on tumor xenografts in nude mice without evident toxicity.[199]

Pyrrolocarbazoles, Indolopyrrolones, and Indolinones. Cephalon Inc. synthesized different families of dihydroindazolo[5,4-*a*]pyrrolo[3,4-*c*]carbazole derivatives active as dual Tie-2/VEGFR-2 inhibitors, including **56**, **57**, and **58** (Figure 15),[200−202] bearing different C3 groups. These studies led very recently to the identification of **25**,[162] currently in clinical trials and discussed elsewhere in this paper. All these compounds are derived from modifications/simplifications of staurosporine, one of the first pan-kinase inhibitors.

The pyrrol-2-one **59** (Figure 15), derived from a further simplification on dihydroindazolo[5,4-*a*]pyrrolo[3,4-*c*]-carbazole structure of **56**−**58**, is a potent VEGFR-2/3 inhibitor showing IC_{50} values of 31 and 37 nM, respectively, and specificity over a panel of other 22 proteine kinases. The inhibitor forms the two canonical hydrogen bonds to the hinge region, whereas the indole ring is located in the hydrophobic region I with $\pi-\pi$ stacking interactions to Phe1047 (Figure 16). It demonstrates a strong in vivo activity in the human lung derived microvascular endothelial cells sprouting assay at 1.3 μM and induction of apoptosis in the human dermal microvascular endothelial cells assay at 2.6 μM. Thus, in light of the high in vitro activity, selectivity, and good bioavailability in the cellular models, **59** may have potential for clinical development as an antiangiogenic drug.[203]

A number of 2-indolinone derivatives synthesized in the past few years are multikinase inhibitors that are also active on VEGFR-2. Among them, some compounds substituted with pyrrolo-fused six- and seven-membered heterocycles, including **60** (Figure 15), are potent inhibitors of VEGFR-2, PDGFR, and c-Kit both enzymatically (<50 nM) and cellularly (<50 nM). Moreover, compound **60** possesses a favorable pharmacokinetic profile and demonstrates good efficacy against human HT-29 cell colon tumor engrafts in nude mice.[204]

Derivative **61** (SIM010603) (Figure 15) inhibits VEGFR-2, PDGFR, c-Kit, RET, and FLT3 with IC_{50} values in the range from 5 to 68 nM. It also inhibits endothelial cell proliferation and chemotaxis, corneal angiogenesis, and tumor growth in animal models, in particular reducing microvascular density in a T241-VEGF-A tumor engraft model.[205]

Miscellaneous Compounds. Two series of pyrrolotriazine derivatives, similar to the Bristol-Myers Squibb clinical candidate brivanib alaninate **20**, have been reported by the same company. Compound **62** (Figure 17), bearing the 2,4-difluoro-5-(cyclopropylcarbamoyl)phenylamino group at the C4 position and a substituted 1,3,5-oxadiazole ring appended to the C6 position of the pyrrolo[2,1-*f*][1,2,4]triazine scaffold, is a low nanomolar inhibitor of VEGFR-2, showing IC_{50} values of 11 and 25 nM in enzymatic and HUVEC assays, respectively. Furthermore, antitumor efficacy was observed with this derivative against L2987 human lung carcinoma xenografts in athymic mice.[206] Compound **63** (BMS-645737) (Figure 17) and its congeners, substituted in C4 with a pyrrolo[2,3-*b*]pyridin-5-yl moiety, are nanomolar VEGFR-2 inhibitors. Derivative **63** possesses good preclinical in vivo activity against human tumor xenograft models.[207]

Abbott synthesized a series of benzo[*d*]isoxazoles incorporating a *N*,*N*′-diphenylurea moiety at the 4-position as receptor TK inhibitors. In particular, compound **64** (Figure 17) potently inhibits VEGFR-2 with IC_{50} values of 11 and 31 nM in enzymatic and cell assays, respectively, and FLT3 and Kit in the

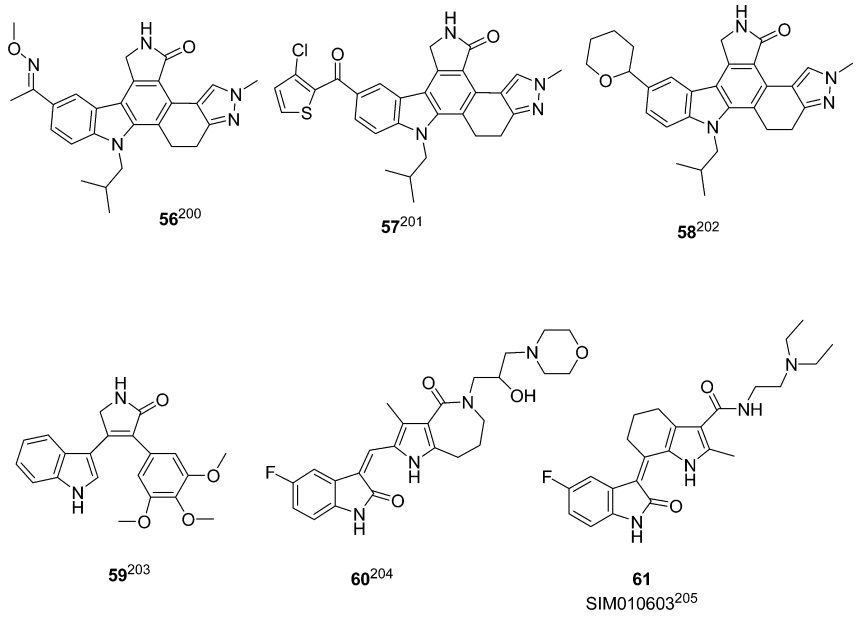

Figure 15. Pyrrolocarbazoles, indolopyrrolones, and indolinones.

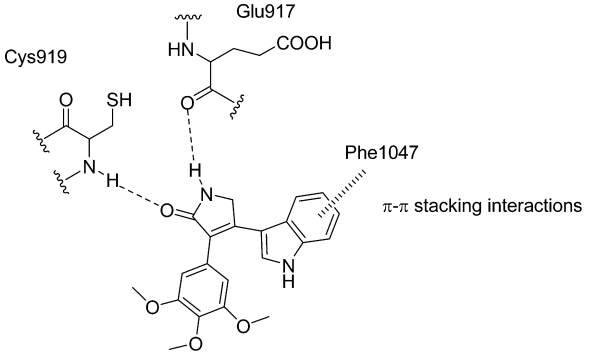

Figure 16. Schematic representation of hydrogen bonds (dotted lines) between **59** and VEGFR-2 catalytic site.

nanomolar range. This inhibitor is orally bioavailable and has a promising pharmacokinetic profile. It demonstrates in vivo efficacy in models of VEGF-stimulated vascular permeability, i.e., the uterine edema model. Moreover, it inhibits tumor growth in the human fibrosarcoma (HT1080) model by 81% at a dose of 10 mg kg^{-1} day^{-1}.[208]

The tetrasubstituted pyrazole **65** (Figure 17) and its analogues, derived from a family of p38MAPK inhibitors, are potent multikinase inhibitors active in the nanomolar range against Src, B-Raf wt, B-Raf V600E, EGFRs, and VEGFR-2. The last is inhibited by **65** with an IC_{50} of 34 nM.[209]

CONCLUSIONS

The admission into clinical use of five VEGFR inhibitors in 8 years, the last drug being pazopanib, approved in April 2012, has strongly indicated the therapeutic value of these agents and

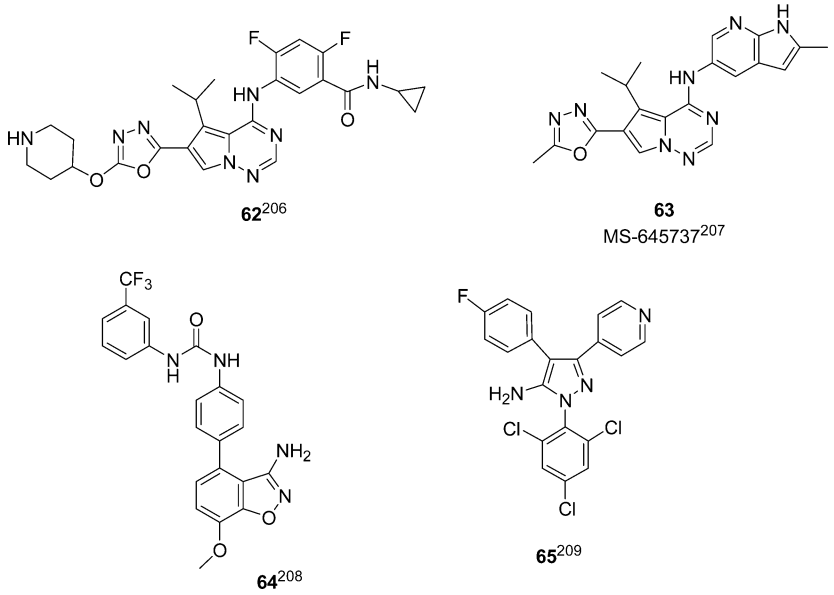

Figure 17. Miscellaneous compounds.

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the importance of research in the field of angiogenesis inhibitors for future oncology therapy.

Even if their initial uses in cancer treatment are currently restricted to advanced and metastatic renal-cell carcinoma, gastrointestinal stromal tumors, and hepatocellular carcinoma, ongoing investigations into different malignancies are likely to lead to the use of such compounds in other tumors as well.

However, there are undoubtedly many issues that are not yet fully explored, from both the clinical and biochemical perspectives. As to the clinical aspect, it has been reported in several reviews that the toxicity profile of VEGFR inhibitors is usually acceptable and manageable, considering the severity of the diseases in which such drugs are used. Some effects are, however, sufficiently severe or life-threatening to require discontinuation of therapy. VEGFR inhibitors are being tested in several combinations with conventional chemotherapy, which should improve patient outcomes and permit reduced doses of cytotoxic chemotherapy, although particular attention should be paid to the use of combination regimens in order to avoid risks of severe effects. Future studies will help to fine-tune the delicate balance between efficacy and tolerability.

From the biochemical point of view, much evidence indicates that VEGFR inhibition alone is not usually sufficient to block and eradicate the tumor. This aspect of the problem is partially mitigated by the fact that the most active compounds that have been mentioned in this article are multitargeted inhibitors and inhibit other kinases involved in angiogenesis or in cell growth. As an example, a number of potential drugs block both the VEGF/VEGFRs and angiopietin/Tie-2 pathways, which are both involved in angiogenesis. Manipulating these interacting pathways may make possible even more potent and targeted inhibition.

While in the recent past the research was focused on the discovery of selective agents that inhibited only one tyrosine or serine/threonine kinase, the more recent trend is to address the rational design of compounds active on multiple protein targets. Especially in the field of cancer treatment, nonselective drugs acting on more than one kinase and inhibiting different cell pathways can sometimes be more effective than a drug specific for one kinase, as indicated by the principles of multitargeted pharmacology.[210,211] The continuous development of new technologies in the chemical, computational, and biological fields, all applied to systems biology, will offer new means for discovering other molecules as potential new drugs. On the other hand, efforts to obtain inhibitors selective for VEGFRs or, preferably, for each member of the VEGFR family are very challenging and worthwhile, since the identification of VEGFR specific signaling pathways and gene expression remains to be fully understood.

In conclusion, the large number of studies and publications in the field of angiogenesis inhibitors shows a significant interest in VEGFR/multikinase inhibitors on the part of academic and pharmaceutical researchers. Several compounds are in advanced clinical trials, and very recently synthesized molecules, which have just entered preclinical study, could offer further opportunities to develop other drugs that are active on hematological and solid malignancies.

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Notes

The authors declare no competing financial interest.

Biographies

Francesca Musumeci graduated from the University of Genoa, Italy, in 2008 (marks, 110/110 cum laude), specializing in Medicinal Chemistry. In the same year, she received a fellowship for "new synthetic approaches toward interesting therapeutic molecules", working at the Department of Chemistry and Industrial Chemistry at University of Genoa. In 2012, she obtained a Ph.D. in Medicinal Chemistry at University of Genoa, working at synthesis of heterocycles compounds as protein kinases inhibitors. During her Ph.D. studies, she spent a period at National University of Ireland in Galway, working on "deuteration and functionalization of resorcylic acid lactones (RALs)". Since January 2012 she has a postdoctoral position at University of Genoa. She is coauthor of nine publications on international journals, a patent, and three oral communications to Congress.

Marco Radi graduated cum laude (November 2000) and was later awarded a Ph.D. in Medicinal Chemistry from the University of Siena, Italy (2004). He spent 1 year as Postdoctoral Research Associate at the University of Georgia (U.S.) under the supervision of Prof. David C. K. Chu. From January 2006 to January 2010 he worked as Postdoctoral Research Associate at the University of Siena, Italy, in collaboration with Prof. Maurizio Botta. From July 2010 to December 2011, he worked at the University of Siena. Since December 2011, he has been Assistant Professor at the Faculty of Pharmacy of the University of Parma, Italy. His research is in the field of synthesis of anti-infective and anticancer agents. He is author of 47 papers, 2 book chapters, and 6 patents.

Chiara Brullo received her Bachelors degree in Pharmaceutical Chemistry at the University of Genoa, Italy, in 1999 (marks, 110/110 cum laude). In 2003 she received a Ph.D. in Pharmaceutical Science, and from 2003 to 2009 she held five research fellowships at the University of Genoa, working on the synthesis of adenosine receptor ligands and on the synthesis of neutrophil chemotaxis. Since 2010 she has had a contract from the Department of Pharmaceutical Sciences, University of Genoa. Since November 2010 she has been a researcher at the Department of Pharmaceutical Sciences at the University of Genoa. She is the author of 39 scientific publications on international journals, 30 posters, 2 patents, and a book chapter.

Silvia Schenone obtained a degree in Medicinal Chemistry in 1987 and in Pharmacy in 1988, both with laude at the University of Genoa, Italy. In 1992 she received her Ph.D. in Medicinal Chemistry. Since 2001 she has been Associate Professor of Medicinal Chemistry at the same university. She is the author of 115 publications and 4 patents. Currently she is involved in the synthesis of pyrazolopyridine derivatives as adenosine antagonists and of heterocyclic derivatives as tyrosine kinases inhibitors. Prof. Schenone's group is also involved in structural studies on biological macromolecules (enzymes, receptors, growth factors) and molecular modeling application on medicinal subjects.

ABBREVIATIONS USED

VEGF, vascular endothelial growth factor; mTOR, mammalian target of rapamycin; TIMP-1, tissue inhibitor of metalloproteinase 1; PlGF, placenta growth factor; VEGFR, vascular endothelial growth factor receptor; TK, tyrosine kinase; Flt, fms-like tyrosine kinase; KDR, kinase insert domain; Flk, fetal liver kinase; JM, juxtamembrane; MAPK, mitogen activated protein kinase; Raf, rapidly accelerated fibrosarcoma; MEK, mitogen activated protein kinase/extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase; FAK,

focal adhesion kinase; PDGFR, platelet-derived growth factor receptor; CSFR, colony stimulating factor receptor; FLT3, fms-like tyrosine kinase receptor 3; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IL-8, interleukin 8; Tie-2, tyrosine kinase with immunoglobulin and epidermal growth factor homology domains 2; Eph, erythropoietin-producing human hepatocellular carcinoma receptor; PET, positron emission tomography; NSCLC, non-small-cell lung cancer; Bcr-Abl, breakpoint cluster region Abelson tyrosine kinase; RCC, renal cell carcinoma; HCC, hepatocellular carcinoma; GIST, gastrointestinal stromal tumor; EGFR, epidermal growth factor receptor; RET, rearranged during transfection; CD, catalytic domain; AML, acute myeloid leukemia; FGFR, fibroblast growth factor receptor; HUVEC, human umbilical vein endothelial cell; HER, human epidermal growth factor receptor; Lck, lymphoid cell kinase; ADME, adsorption, distribution, metabolism, and excretion; CML, chronic myeloid leukemia; PLK1, polo-like kinase 1; CDK, cyclin dependent kinase; eNOS, endothelial nitric oxide synthase

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