Journal of Hematology & Oncology

REVIEW

Open Access

Recent progress on vascular endothelial growth factor receptor inhibitors with dual targeting capabilities for tumor therapy

Yun Liu[1,2†], Yang Li[1,2†], Yuxi Wang[2,3†], Congcong Lin[2], Dan Zhang[2], Juncheng Chen[2], Liang Ouyang[1,2], Fengbo Wu[4*], Jifa Zhang[1,2*] and Lei Chen[1*]

Abstract

Vascular endothelial growth factor receptors (VEGFRs) are a family of receptor protein tyrosine kinases that play an important role in the regulation of tumor-induced angiogenesis. Currently, VEGFR inhibitors have been widely used in the treatment of various tumors. However, current VEGFR inhibitors are limited to a certain extent due to limited clinical efficacy and potential toxicity, which hinder their clinical application. Thus, the development of new strategies to improve the clinical outcomes and minimize the toxic effects of VEGFR inhibitors is required. Given the synergistic effect of VEGFR and other therapies in tumor development and progression, VEGFR dual-target inhibitors are becoming an attractive approach due to their favorable pharmacodynamics, low toxicity, and anti-resistant effects. This perspective provides an overview of the development of VEGFR dual-target inhibitors from multiple aspects, including rational target combinations, drug discovery strategies, structure–activity relationships and future directions.

Keywords: VEGFR kinase, Antitumor drugs, Dual inhibitor, Antiangiogenesis treatment

Introduction

Abnormal angiogenesis can be considered an essential prerequisite for tumor progression and metastasis. Existing pieces of evidence have demonstrated that many extracellular, cell surface and intracellular molecules can directly or indirectly regulate angiogenesis [1, 2]. In particular, vascular endothelial growth factors (VEGFs) and their interaction with membrane receptors are of great significance during angiogenesis. In mammals, VEGF isoforms [VEGF-A, B, C, D and placental growth factor (PIGF)] are encoded by VEGF-related genes and interact specifically with the VEGF receptors (VEGFRs) family of VEGFR-1/Flt-1, VEGFR2/KDR and VEGFR-3/Flt-4 [3, 4]. These receptors share a high degree of structural similarity, but differ in activation mode, signal transduction and biological functions [5]. Table 1 summarizes the specific ligands, main functions and distinct domains of these receptors. Briefly, VEGFR1, VEGFR2 and VEGFR3 are essential for the development of hematopoietic cells, vascular endothelial cells and lymphatic endothelial cells, respectively. Nevertheless, VEGFR3 and its ligands play critical roles in lymphangiogenesis and the spread of tumor cells to regional lymph nodes [6, 7]. Structurally, VEGFRs consist of an extracellular part consisting of an extracellular ligand-binding domain (ECD) with seven immunoglobulin-like domains (IgD), a single transmembrane domain (TM), a juxtamembrane domain (JMD), a tyrosine kinase domain (TKD) with an insert of approximately 80 residues, and a carboxyl terminus (Fig. 1A, B)

†Yun Liu, Yang Li and Yuxi Wang contributed equally to this work

*Correspondence: Fbwu2013@scu.edu.cn; zjf298257@163.com; leilei_25@126.com

[1] Department of Neurology, Joint Research Institution of Altitude Health, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China
[4] Department of Pharmacy, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China
Full list of author information is available at the end of the article

Table 1 Unique characteristics of the VEGFR family

Receptor	VEGFR1	VEGFR2	VEGFR3
Protein size	180–185 kDa	210–230 kDa	195 kDa
Ligands	VEGF-A, VEGF-B and PIGF	VEGF-A, VEGF-C and VEGF-D	VEGF-C and VEGF-D
Functions	A negative regulator of angiogenesis, vasculogenesis and monocyte/macrophage motility	Vasculogenesis, angiogenesis, vascular permeability and endothelial cell motility and survival	Vascular and lymphatic development and maintenance
Full length	1338	1356	1363
Signal peptide	1–26	1–19	1–24
Receptor chain	27–1338	20–1356	25–1363
ECD	27–758	20–764	25–775
IgD1	32–123	46–110	30–127
IgD2	151–214	141–207	151–213
IgD3	230–327	224–320	219–326
IgD4	335–421	328–414	331–415
IgD5	428–553	421–548	422–552
IgD6	556–654	551–660	555–671
IgD7	661–747	667–753	678–764
TM	759–780	765–785	776–796
Cytoplasmic domain	781–1338	786–1356	797–1363

[8]. The activation of VEGFRs can be mediated by ligand binding. Subsequently, ligand-induced conformational changes in the VEGFRs intracellular domain promote receptor dimerization, leading to the autophosphorylation of specific tyrosine residues and the activation of several downstream enzymatic pathways, including p38/MAPK, RAS/RAF/MEK/ERK and PI3K/AKT/mTOR (Fig. 1C). At the same time, some receptors undergo internalization and form endosomes. In the early stages of internalization, receptors still exist as a membrane protein component of endosomes, and this receptor compartment, composed of microtubules and vacuoles, is widely distributed in the cytoplasm. During the transit of receptor across the endosomal membrane, the ligand–receptor complexes remain intact and the kinase function of the receptors remains activated. Finally, membrane fragments containing ligand–receptor complexes are squeezed into the lumen of the endosome as small vesicles, thus forming multi-vesicular endosomes. As a result, ligand–receptor complexes on the plasma membrane reach the lumen of the endosome and are widely distributed throughout the cytoplasm, along with other contents of the lumen. This process attenuates the continuous stimulation of growth factors at the cell surface and allows for a broad distribution of ligand–receptor complexes within the cytoplasm [9]. Importantly, it has been previously shown that VEGF could induce the internalization of VEGFR1 and VEGFR2 [10].

The dysfunctional VEGF-VEGFR signal axis is widely involved in human diseases, especially tumors. Inhibitors targeting VEGF signaling, including monoclonal antibodies targeting VEGF and small molecules targeting VEGFR, have shown treatment efficacy for different types of solid tumors. Specifically, bevacizumab, as a recombinant humanized monoclonal antibody targeting VEGF, exerts beneficial clinical effects. However, the main issues of anti-VEGF monoclonal antibodies are the high immunogenicity, high cost and low stability. Furthermore, the clinical application of anti-VEGF monoclonal antibodies is severely limited by considerable side effects associated with the inhibition of physiological angiogenesis, which is one of the most common side effects of antiangiogenic therapies. Currently, targeting tumor angiogenesis via inhibiting VEGFRs has become a successful strategy for oncotherapy [11]. To date, more than 340 clinical trials related to VEGFR inhibitors have been retrieved from the Web site of www.ClinicalTrials.gov. Specifically, several VEGFR inhibitors have been approved for clinical use, and their efficacy results are summarized in Table 2. Notably, VEGFR inhibitors could be divided into three classifications: type I inhibitors, type II inhibitors and type III inhibitors [12]. Type I inhibitors [*e.g.*, sunitinib (**2**), pazopanib (**3**), vandetanib (**4**), axitinib (**5**), ponatinib (**9**) and motesanib (**11**)], also known as ATP competitive inhibitors, could generate hydrophobic interactions with the adenine region and form one to three hydrogen bonds with the surrounding residues at the active site of the receptor, thereby competing for binding to the active "DFG-in" conformation in the ATP-binding pocket [13]. Type II inhibitors [e.g., sorafenib (**1**), carbozantinib (**6**),

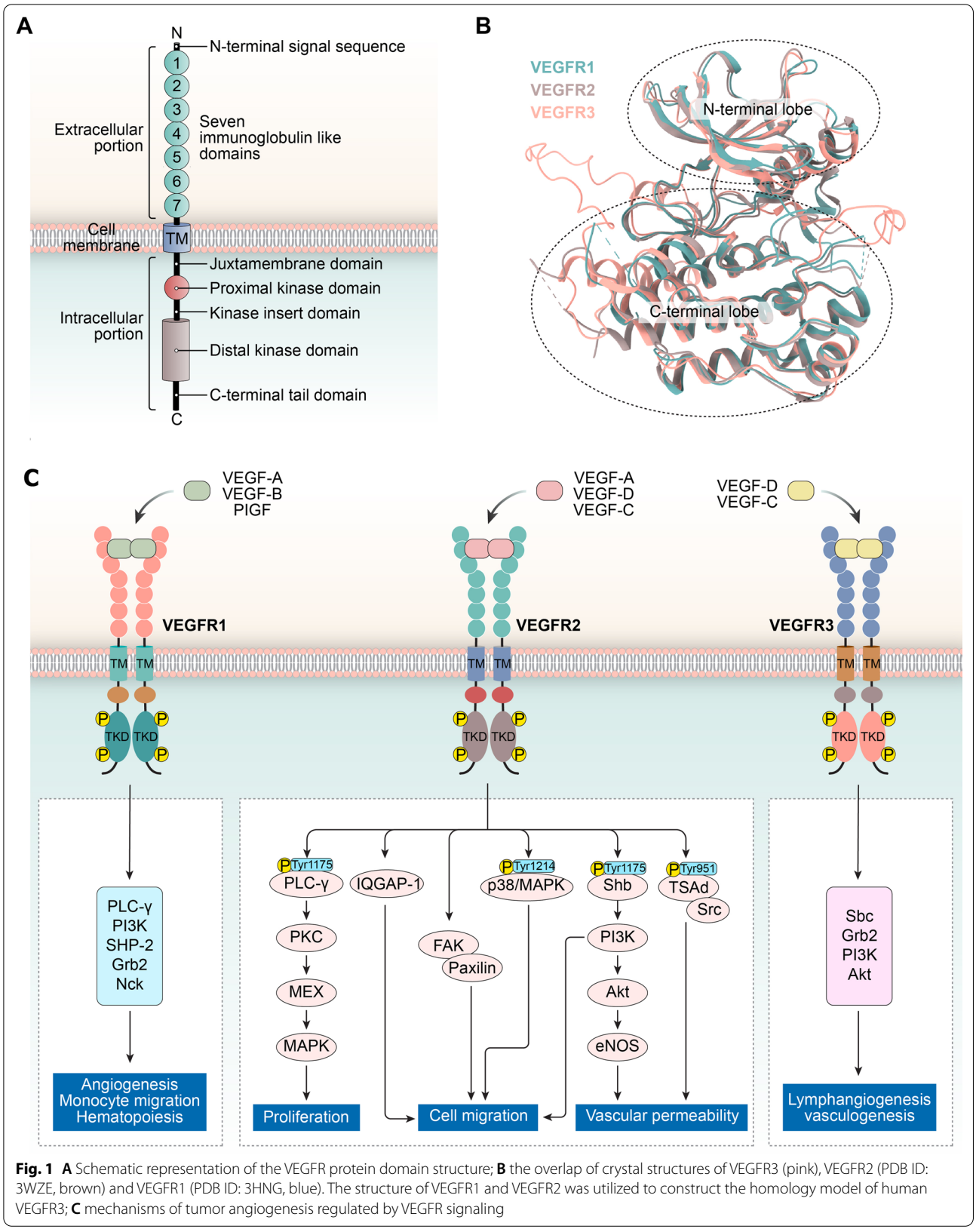


Fig. 1 **A** Schematic representation of the VEGFR protein domain structure; **B** the overlap of crystal structures of VEGFR3 (pink), VEGFR2 (PDB ID: 3WZE, brown) and VEGFR1 (PDB ID: 3HNG, blue). The structure of VEGFR1 and VEGFR2 was utilized to construct the homology model of human VEGFR3; **C** mechanisms of tumor angiogenesis regulated by VEGFR signaling

Table 2 Summary of clinically approved VEGFR inhibitors

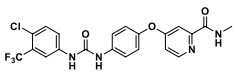
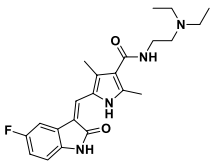
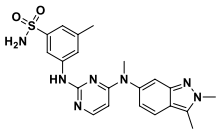
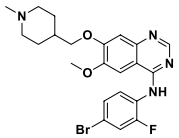
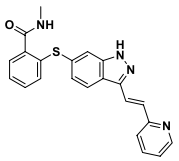
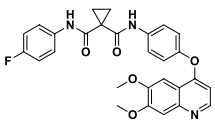
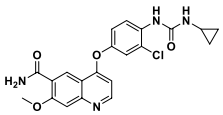
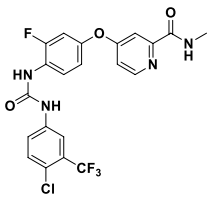
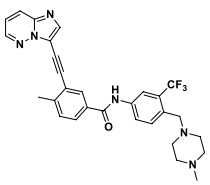
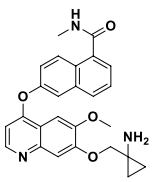
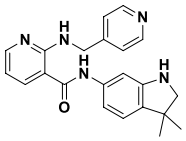
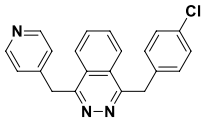
Drugs	Chemical structure	Target	Tumor types	Released date	Type	Ref
Sorafenib (1)		VEGFR2/3, PDGFRβ, c-Kit, BRAF	Advances renal cell carcinoma, hepatocellular carcinoma	2007	II	[25]
Sunitinib (2)		VEGFR1/2, PDGFRβ, FLT3	Renal cell carcinoma, gastrointestinal stromal tumors	2006	I	[26]
Pazopanib (3)		VEGFR2, PDGFRβ, c-Kit	Hepatocellular carcinoma	2012	I	[27]
Vandetanib (4)		VEGFR2/3, EGFR, RET	Late-stage metastatic medullary thyroid tumor	2011	I	[28]
Axitinib (5)		VEGFR1/2, c-Kit	Renal cell carcinoma	2012	I	[29]
Carbozantinib (6)		VEGFR2, c-Met, RET	Thyroid tumor	2012	II	[30]
Lenvatinib (7)		VEGFR1/2/3	Thyroid tumor	2015	II	[31]
Regorafenib (8)		VEGFR1/2/3, PDGFRβ, c-Kit, BRAF	Colorectal tumor, advanced GI stromal cancer, hepatocellular carcinoma	2015	II	[32]
Ponatinib (9)		Abl, PDGFRα, VEGFR2, FGFR1, Src	Chronic myeloid leukemia, acute lymphoblastic leukemia	2012	I	[33]
Lucitanib (10)		VEGFR1/2/3, FGFR-1, FGFR-2	Metastatic breast tumor	Phase II clinical trials	II	[34]

Table 2 (continued)

Drugs	Chemical structure	Target	Tumor types	Released date	Type	Ref
Motesanib (11)		VEGFR1/2/3	Non-small-cell lung tumor	Phase III clinical trials	I	[35]
Vatalanib (12)		VEGFR1/2/3, PDGFRβ, c-Kit	Colorectal tumor	Phase III clinical trials	III	[36]

lenvatinib (**7**), regorafenib (**8**) and lucitanib (**10**)] are characterized by binding to the inactive "DFG-out" conformation of the kinase and occupying a hydrophobic pocket adjacent to the ATP-binding site [14, 15]. Type III inhibitors [e.g., vatalanib (**12**)], or called covalent inhibitors, could exert their pharmacological functions through irreversibly binding to cysteines at specific sites on the kinases [16]. So far, numerous approved VEGFR inhibitors are type I inhibitors, which target the ATP-binding pocket. Based on the X-ray crystal structure of VEGFR2, several type I inhibitors have been reported to exert regulatory effects on tumor suppression. However, several studies have demonstrated that type II inhibitors possess certain advantages over type I inhibitors, including improved potency and selectivity [17, 18]. Structurally, the extension into the less conservative allosteric hydrophobic back pocket facilitates the affinity and selectivity of the type II inhibitors [19]. In addition, covalent enzyme inhibitors have been widely applied as therapeutic agents [20]. Generally speaking, most of these inhibitors can achieve continuous amelioration and even cure some tumor patients. However, their clinical application is limited by therapeutic resistance, limited efficacy and off-target toxicity [6]. Firstly, the mechanisms of resistance to VEGFR inhibitors are classified into the following sections: (i) activation of alternative pro-angiogenic signaling pathways; (ii) recruitment of local and distal stromal cells; and (iii) alternative modes of tumor vascularization (e.g., hypoxia). Secondly, due to similarities in the kinase domains of VEGFR and other receptors, these inhibitors showed cross-inhibitory activities against other targets such as PDGFR, c-KIT, and FLT3, leading to possible off-target effects. Several clinical toxic effects of VEGFR inhibitors have been investigated, such as hypertension, proteinuria, hypothyroidism, leukoencephalopathy syndrome and arterial thrombosis. Finally, accumulating pieces of evidence have confirmed that several VEGFR inhibitors have generally failed to reveal remarkable overall efficacy in the clinic [21, 22]. Therefore, clinical strategies to overcome these drawbacks, such as combination therapy, need to be well concerned [23, 24].

Combination therapy in human tumors not only increases the potency, but also reduces potential adverse events [37]. Since the therapeutic efficacy in tumors and relevant biological functions of these enzymes have been revealed, it is considered that a combination of them with VEGFR inhibitors (e.g., epigenetic agents, immunotherapeutic drugs and other RTK inhibitors) can be promising in antitumor treatment. However, it should be cautious that the complicated doses/schedule, dubious pharmacokinetic/pharmacodynamic profile and potential adverse events require in-depth explorations [38, 39]. As an alternative strategy to combination therapy, dual-target or multi-target drugs are characterized by reduced risk of adverse drug–drug interactions (DDIs), better pharmacokinetic (PK) profiles and guaranteed safety [40]. Based on these concepts, VEGFR dual-target inhibitors are emerging as an attractive approach.

Given the synergistic effect of VEGFR and other therapies in tumor development and progression, the identification of novel VEGFR dual-target inhibitors may provide an effective strategy for clinical practice. From this perspective, the research progress of dual-target VEGFR inhibitors is summarized, focusing on the rational targets selection, structure–activity relationships (SARs) and pharmacological activities of dual-target VEGFR inhibitors.

VEGFR2 as a therapeutic target

As mentioned above, each VEGFR family has unique characteristics. Among them, VEGFR2 has been identified as a promising tumor therapy target [41]. Accumulating pieces of evidence have confirmed that the abnormal expression of VEGFR2 in neovascular tumor endothelial cells is closely linked to the occurrence and development of multiple types of tumors [42, 43]. By blocking angiogenesis and lymphangiogenesis, all VEGFR2 inhibitors offer varying degrees of clinical benefit against different

types of tumors, although most of them lack specificity [44].

Research status of VEGFR2 inhibitors

In the pharmaceutical field, the discovery and development of highly potent VEGFR2 inhibitors have always been a research hotspot. Co-crystal structures of VEGFR2 complexed with FDA-approved inhibitors reveal structural information for the structure-based design of VEGFR2 inhibitors. Structurally, the catalytic domain of VEGFR2, as a bi-lobed structure with a small N-lobe and a large C-lobe, plays a key role in the inhibitory potency of these inhibitors. Specifically, the active site of VEGFR2 consists of the following subregions: hydrophobic region I (encapsulated by the residues Leu840, Phe918 and Gly922), hydrophobic region II (encapsulated by the residues Leu889, Ile892, Val898 and Ile1044) and one linker region (encapsulated by the residues Ala866, Val914, Leu1035 and Cys1045) [45]. As shown in Fig. 2, the co-crystal structures of VEGFR2 in complex partial FDA-approved inhibitors revealed that these inhibitors, although highly structurally diverse, share conclusive pharmacophoric characteristics. Firstly, a flat heteroaromatic ring system of the primary skeleton that adopts the active site through the formation of a key hydrogen bond with Cys residue, and the essential residue in the catalytic ATP-binding pocket. Thus, at least one hydrogen bond acceptor should be included in this flat system (N atoms are preferred, followed by O atoms). Secondly, the linker region between the ATP-binding pocket and the DFG domain of the enzyme is occupied by a central aryl ring or spacer. Thirdly, functional groups such as amides or ureas, which are pharmacophores, form two hydrogen bonds with the side chain of Glu885 residue in the C-helix and the backbone NH of Asp1046 residue in the DFG motif, respectively. Fourthly, the terminal hydrophobic moiety of these inhibitors forms the hydrophobic interaction with the allosteric hydrophobic pocket [46, 47]. Based on these findings, several inhibitors of VEGFR2 containing different cores have been reported to suppress tumor growth.

Synergistic effects of VEGFR2 inhibitors and other antitumor agents

Through preclinical or clinical evaluation, the antitumor potency of VEGFR2 inhibitors combined with other antitumor drugs has been extensively identified. Existing pieces of evidence have shown that the combination of other RTK inhibitors and VEGFR inhibitors exerted a favorable clinical perspective [48, 49]. Firstly, the combination therapy of the dual epidermal growth factor receptor (EGFR) inhibitor cetuximab and VEGFR inhibitor

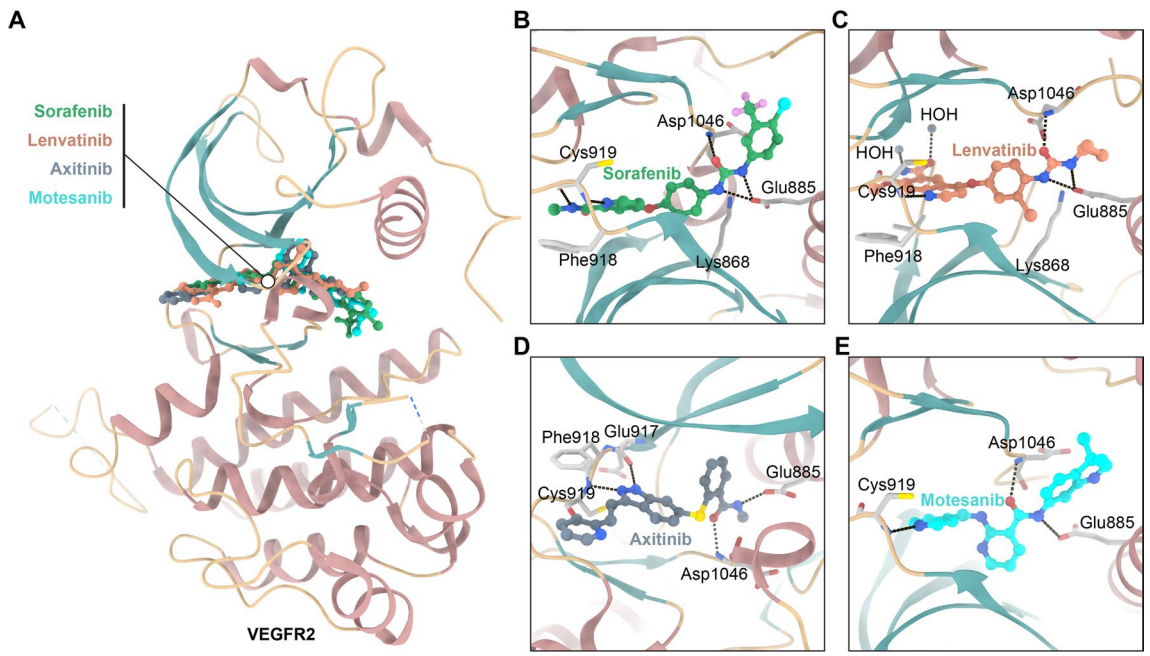


Fig. 2 Crystal structure of the FDA-approved VEGFR inhibitors—VEGFR2 complex. The corresponding PDB codes are 3WZE (sorafenib), 3WZD (lenvatinib), 4AGC (axitinib) and 3EFL (motesanib). Hydrogen binding (black) interactions are shown as dashed lines. **A** The overlap of co-crystal structures of VEGFR2 with sorafenib (green), lenvatinib (orange), axitinib (gray) and motesanib (blue); **B–E** Binding modes of VEGFR2 with sorafenib, lenvatinib, axitinib and motesanib

sorafenib prominently enhanced the clinical benefit of KRAS-mutated metastatic colorectal tumor in phase II clinical trial (NCT00326495). Secondly, aberrant expression of VEGFR and genetic alteration of fibroblast growth factor receptor (FGFR) have been reported to be involved in the development of solid tumors, synergistically promoting angiogenesis and fibrosis. Pieces of clinical evidence have suggested that lucitanib, as a dual VEGFR–FGFR inhibitor, exhibited significant inhibitory activities against solid tumors (NCT01283945). Finally, available pieces of evidence have demonstrated that the synergistic collaboration of VEGFR and c-Met promotes the process of angiogenesis and the development of multiple types of tumors [50]. Clinical evidence has also demonstrated the enhanced therapeutic efficacy of c-Met inhibitor tivantinib in combination with the VEGFR inhibitor pazopanib in advanced solid tumors (NCT01468922).

Rapidly accelerating fibrosarcoma (RAF) homologs, as serine threonine kinases, are of significance in regulating the RAS-RAF-MEK-ERK pathway, which have been highlighted as a potent antitumor target. Among mammalian genes, RAF homologs are encoded by three independent genes, including ARAF, BRAF and CRAF. Among them, BRAF presents the most remarkable reactivity of the others and can be activated by mutation in tumor cells. To date, mutations at valine 600 (V600D, V600E, V600K and V600R) have been detected in different types of tumors. Compared with wild-type BRAF, $BRAF^{V600E}$, the most common mutation, can significantly improve (approximately 600-folds) the kinase activity and ultimately promote the development of tumors [51, 52]. Recently, multiple studies have demonstrated that BRAF and VEGFR2 exert a synergistic effect on the development of tumors, and thus, combination therapy involving VEGFR2 inhibitors and BRAF inhibitors has been identified as a promising strategy for the treatment of tumors [53, 54]. Despite the multi-target inhibitors **1** and **8**, RAF265, a potent RAF/VEGFR2 dual inhibitor, has been identified and successfully applied to clinical treatment (NCT00304525) [55].

HDAC isozymes can be utilized as promising therapeutic targets for tumors. So far, accumulating pieces of preclinical and clinical evidence have shown that the combination of HDAC inhibitors with VEGFR inhibitors is promising in antitumor therapy. Particularly, in vitro and in vivo pieces of evidence proved the synergistic effects of compound **3** and diverse HDAC inhibitors for drug resistance reversal and enhanced antitumor efficacy [56, 57]. Moreover, a phase I trial evaluated the application of an HDAC inhibitor, SAHA, in combination with compound **3** in patients with mutant *TP53*, particularly in patients with metastatic sarcoma or metastatic colorectal tumor, and exerted considerable toxicities [58]. In another phase I trial, combination therapy with compound **3** and the HDAC inhibitor abexinostat demonstrated that HDAC inhibition could promote response and reverse resistance to compound **3** in patients with renal cell carcinoma and other solid tumor malignancies [59]. Severing as key components of cytoskeletons in eukaryotic cells, microtubules play an important role in a number of cellular functions. Due to their specific functions in crucial cellular processes, microtubules have been highlighted as potent antitumor targets [60]. In clinical trials, the combination of bevacizumab (anti-VEGF monoclonal antibody) and paclitaxel (microtubule-targeting agents) significantly increased the antitumor responses [61].

The estrogen receptor alpha (ERα) is utilized as a promising therapeutic target for breast tumor therapy, and VEGFRs play an important role in the development of breast tumors. In 2010, Roshani et al. reported the therapeutic effect of combining tamoxifen, a selective estrogen receptor modulator (SERM), and brivanib in human breast cancer cells. In vitro and in vivo pieces of evidence supported the role of combination therapy involving SERM and VEGFR2 inhibitors in improving therapeutic efficacy, as well as inhibiting the growth of SERM-resistant tumors [62].

Previous studies have proven the vital role of hypoxia-mediated abnormal expression of PIM1 in antiangiogenic drug resistance [63]. In 2018, Andrea L et al. demonstrated that a combination of PIM1 kinase inhibitors with antiangiogenic drugs can be promising in the treatment of solid tumors [64]. In vitro and in vivo studies showed that the synergy of PIM1 inhibitors and VEGF-targeting agents led to reduced proliferation, lessened tumor vasculature and decreased metastasis [65].

Collectively, these studies demonstrated a significant therapeutic advantage for VEGFR inhibitor-based combination therapies. Specifically, they not only present favorable potency, but may also reverse drug resistance. However, drug combination therapies are limited by the complicated doses/schedule, dubious pharmacokinetic/pharmacodynamic profile and potential adverse events. Encouragingly, the cognition of synergetic efficacy of these drug combinations by clinical investigations and phenotype screenings facilitated the rational combinations of numerous targets to identify dual-target VEGFR inhibitors.

Design approaches for dual-target VEGFR inhibitors

Dual-target strategies possess several advantages over single-target drugs and drug combination therapy. Firstly, dual-target drugs not only retain most of the advantages of combination therapy, but also partially overcome the shortcomings of combination therapy. Specifically, due

to one integrated molecule, dual-target drugs possess no or lower risks of drug–drug interactions, lower adverse reactions, more effortlessly predictable PK profiles, lower incidence of target-based resistance and higher patient compliance [66].

Rational target combinations have been found to play a key role in the clinical successes of dual-target drugs, ultimately facilitating the development of dual-target VEGFR inhibitors [67]. To date, great efforts have been made to identify dual-target drugs. In general, design strategies such as drug repurposing, pharmacophore-based combination and computational approaches are frequently used for dual-target drug discovery [68]. Specifically, drug repurposing is the application of conventional agents to novel therapeutic fields and is characterized by exerting a shorter development process [69]. Additionally, most dual-target VEGFR inhibitors are identified via the pharmacophore-based approach. This approach is characterized by integrating the potency of two selective inhibitors into a single molecule and is carried out by linking or merging the pivotal pharmacophores of selected maternal inhibitors. A pharmacophore-linked method is a simple approach through directly connecting pharmacophores or via a conjugate linker. However, the pharmacophore-linked molecules also possibly suffer from high molecular weight, low absorption and poor PK properties. In addition, an inappropriate linker would hamper the interaction of the ligand moiety with the target protein [70]. Similar to a hybrid design, the pharmacophore-merged strategy is an approach to obtaining new chemical structures by maximizing the overlapping level of pharmacophores, resulting in smaller molecular weight, simple skeleton and better physicochemical properties than those of the parent drugs [71]. However, any alterations in the structure of the parent drug may result in vital changes in biological activities [72]. Thus, it is important to determine the mutual pharmacophores of both VEGFR and other targets before designing dual-target VEGFR inhibitors. Undoubtedly, drug repurposing and pharmacophore-based approaches are essential for the discovery of dual-target drugs. However, the application of these strategies is based on known small molecules, thus leading to the poor structural diversity of dual-target VEGFR inhibitors. Nowadays, computational approaches have been successfully applied to identify dual-target drugs with desired activity profiles, including ligand/structure-based drug design, in silico screening and data mining [73]. Specifically, these approaches are capable of predicting novel targets of reported ligands and are also of significance for the identification or optimization of novel ligands for desired targets. In particular, several dual-target VEGFR inhibitors, containing novel scaffolds, are identified via molecule docking, pharmacophore studies and binding pocket similarity search, showing better therapeutic efficacy for tumors.

Dual inhibitors of VEGFR2 and other tumor-associated targets

Dual VEGFR2–EGFR inhibitors

Accumulating pieces of evidence have confirmed that VEGFR2 is closely related to EGFR and is involved in the development of multiple types of tumors [74, 75]. Thus, the combination of VEGFR2 inhibitors and EGFR inhibitors can be promising in antitumor treatment. Accordingly, several VEGFR2–EGFR dual inhibitors have been reported to exert promising effects on tumor suppression. Chemical structure, in vitro potency and optimization of dual VEGFR–EGFR inhibitors are illustrated in Fig. 3.

Due to their suitable physicochemical properties, diaryl urea and amide groups have been widely used in VEGFR2 inhibitors design. In 2017, the analogue **14** (Fig. 3) was obtained by optimizing the side chain and introducing chlorine within the central core of compound **13** (vandetanib). It shows a remarkable potency against VEGFR2 and EGFR with IC_{50} values of 1 nM and 79 nM, respectively. Compared with parent compound **13**, **14** exerts superior inhibitory activities against HT-29 and MCF-7 cells (IC_{50} = 1.76 μM and 7.28 μM, respectively). Preliminary SAR studies showed that (i) compounds containing the 4-methylpiperazine group in the region I position exerted higher potency against VEGFR2 and EGFR; (ii) the introduction of chlorine in the region II position could facilitate the kinases inhibition of both VEGFR2 and EGFR; (iii) the introduction of the diaryl urea group at region III is beneficial to improve the potency; and (iv) benzene ring with a methyl group at C-3 and C-4 positions in region IV could improve potency.

More and more pieces of evidence have confirmed that hypoxia is closely linked to the occurrence and development of multiple types of tumors. Additionally, hypoxia is the main cause of therapeutic resistance, especially in radiotherapy. Presently, owing to the significance indicated in tumor progression and drug resistance, molecules in hypoxia-driven pathways are considered as potential therapeutic targets for tumors [76]. Based on these studies, a series of hypoxia-targeted EGFR/VEGFR2 dual inhibitors containing 3-nitro-1,2,4-triazole core is prepared by Wei et al. [77]. Compared with **13**, most of these compounds exert superior potency against EGFR, with IC_{50} values in the low nanomolar range. Moreover, they also show good-to-moderate inhibitory activities against VEGFR2 with IC_{50} values in the concentration range between 36.8 nM and 4.09 μM. Among these compounds, compounds **16** and **17** (Fig. 3) showed

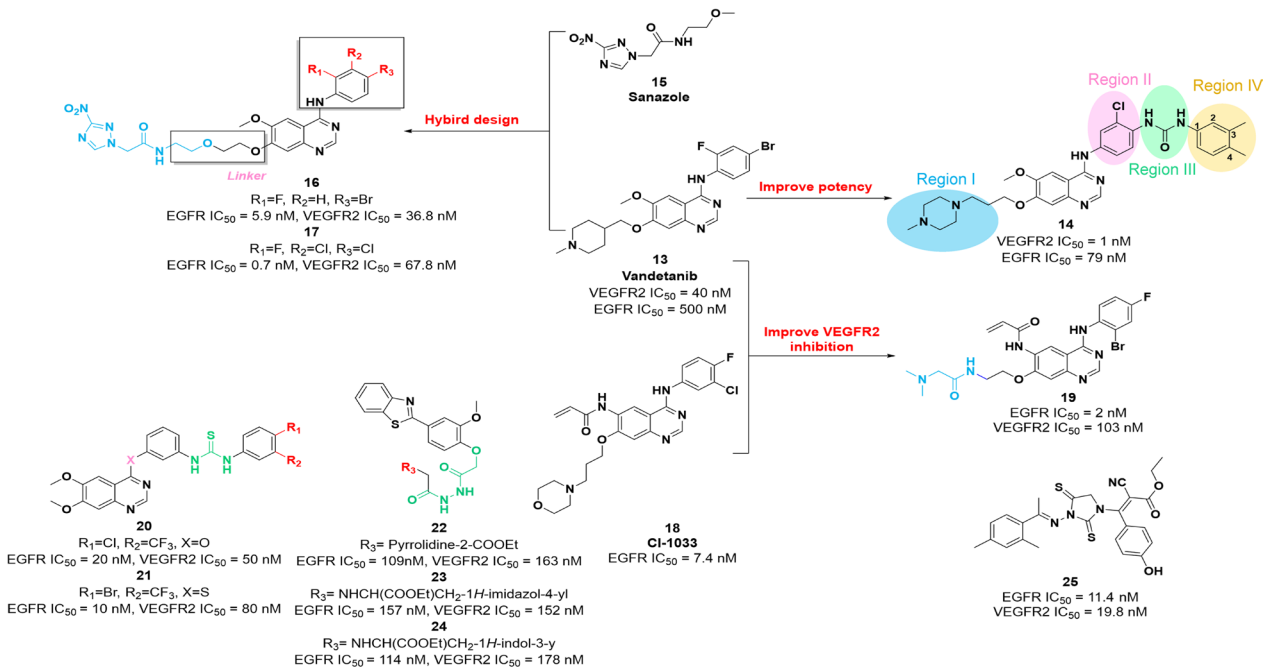


Fig. 3 Chemical structures of dual VEGFR2–EGFR inhibitors **14**, **16**, **17** and **19–25** and their inhibitory activities against VEGFR2 and EGFR

the most remarkable inhibitory activity against VEGFR2 and EGFR. Furthermore, in vitro and in vivo evidence proved that compound **16** has superior therapeutic efficacy, target selectivity and acceptable tolerance. Further SAR studies showed that the length of the linker could dramatically influence the potency of target compounds against EGFR, and bulky and heavy halogen-substituted benzene contributed to the improvement in inhibitory activities against VEGFR2.

In 2018, Bang et al. identified compound **19** based on the structures of molecule **13** and second-generation EGFR inhibitor **18** (CI-1033), which is a powerful VEGFR2/EGFR dual inhibitor [78]. In vitro enzymatic inhibition assay showed that **19** exerts potent inhibitory potency against both VEGFR2 and EGFR with the IC_{50} values of 103 nM and 2 nM, respectively. Furthermore, **19** showed remarkable inhibitory activities against EGFRT790M and EGFRT790M/L858R mutants with IC_{50} values of 11 nM and 3 nM, respectively. In 2017, compounds **20** and **21** were developed based on the structure of **1**, which present selective, cell active and potent potency against VEGFR2 (IC_{50} = 50 nM and 80 nM, respectively) and EGFR (IC_{50} = 20 nM and 10 nM, respectively) in vitro. In in vivo models, **20** and **21** present a competitive tumor suppression role than molecule **1** [79]. Similarly, compounds **22**, **23** and **24**, as derivatives of **1**, were also identified by Eman et al. These molecules exert potential inhibitory activities against VEGFR2 (IC_{50} = 163 nM, 152 nM and 178 nM, respectively) and EGFR (IC_{50} = 109 nM, 157 nM and 114 nM, respectively). Furthermore, these compounds showed low micromolar potency against different types of tumor cells in vitro [80]. In 2021, Mourad et al. identified a series of novel VEGFR2–EGFR dual inhibitors containing 2-thioxoimidazolidin-4-one scaffold [81]. Among these compounds, compound **25** has a promising potency for VEGFR2 and EGFR (IC_{50} = 19.8 nM and 11.4 nM, respectively), and stronger antitumor effects on human breast cancer cell lines MCF-7 compared with that of molecule **1** and EGFR inhibitor erlotinib. Furthermore, **25** promotes cell apoptosis and the prolongation of cell cycle progression in the G2/M-phase against MCF-7 cells.

Dual VEGFR2–FGFR inhibitors

Accumulating pieces of evidence have confirmed that the binding of VEGF ad VEGFR and the binding of FGF2 and FGFR are synergistically involved in angiogenesis and fiber formation, thereby mediating the development of tumors [82]. Up to now, several dual inhibitors of VEGFR and FGFR containing different cores have been reported to suppress tumor growth [83]. Their chemical structure, in vitro and in vivo potency, and optimization are illustrated in Fig. 4.

In 2006, compound **26** (brivanib) was identified as a promising inhibitor of VEGFR2 and FGFR by Bhide et al. [84]. **26** exerts remarkable inhibitory potency

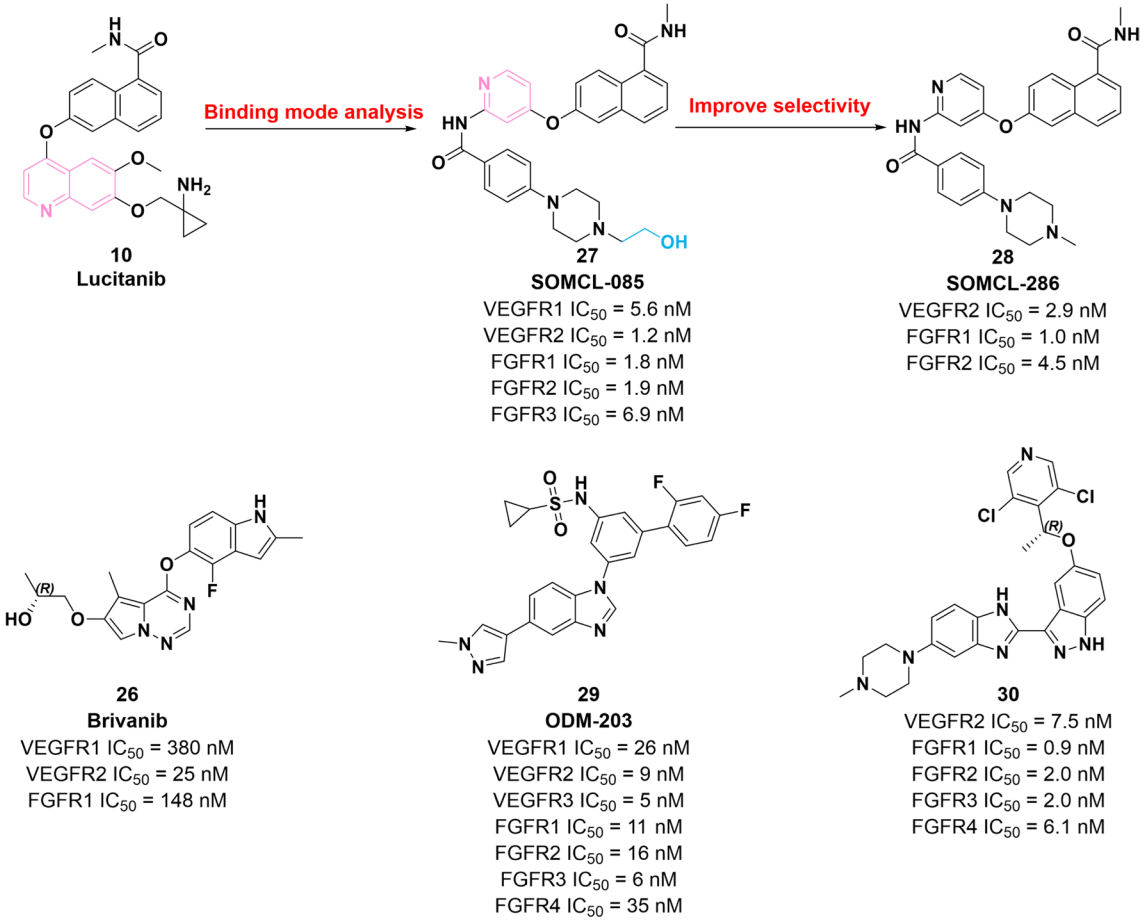


Fig. 4 Chemical structures of dual VEGFR2–FGFR inhibitors **26**–**30** and their inhibitory activities against VEGFR2 and FGFR

against VEGFR1, VEGFR2 and FGFR1 with the IC_{50} values of 380 nM, 25 nM and 148 nM, respectively. Preliminary SAR studies showed that (i) the introduction of methyl group at the 5-position of the pyrrole[2,1-*f*] [1,2,4]triazine ring improves the inhibitory activity against VEGFR2; (ii) the substitution of indole ring at the 4-position of fluorine atom is beneficial to improve the potency against VEGFR2; (iii) the superior enzyme potency is attributed to the replacement of ester group at 6-position with an ether group; and (iv) CYP3A4 can be strongly suppressed through introducing the amino side chain. However, **26** is limited by the poor oral bioavailability and low absorption. Thus, an ester pro-drug of **26** is prepared by Cai et al. by introducing L-alanine in the side chain of molecule **26** [85]. Preclinical studies have demonstrated that **26** exerts a significant antiangiogenic efficacy through simultaneously blocking FGF and VEGF pathways [86]. Until now, there have been several clinical trials of **26** in the treatment of different types of tumors (NCT04395612, NCT03895788, NCT03516071 and NCT04212221).

Based on the study of binding mode of **10** and FGFR, compound **27** (SOMCL-085) is further discovered to present powerful potency against FGFR1, FGFR2, FGFR3, VEGFR1, VEGFR2, PDGFRα and PDGFRβ (IC_{50} = 1.8 nM, 1.9 nM, 6.9 nM, 5.6 nM, 1.2 nM, 22.6 nM and 7.8 nM, respectively). Specifically, **27** is obtained through opening the quinoline fragment of 10 and introducing the amide as a hydrogen bond acceptor and donor. In the following in vitro and in vivo assays, compound **27** is determined to present a considerable selectivity profile and antiproliferative activities [87]. In 2018, Wei et al. identified compound **28** (SOMCL-286) based on the structure of molecule **27**, which is a potent VEGFR2/FGFR dual inhibitor [88]. In vitro enzymatic inhibition assay showed that **28** exerts potent inhibitory potency against VEGFR2, FGFR1 and FGFR2 with the IC_{50} value of 2.9 nM, 1.0 nM and 4.5 nM, respectively. Nevertheless, **28** presents superior selectivity for VEGFR2 and FGFR compared with that of molecule **27**. Therefore, **28** theoretically exerts superior curative effect and low toxicity. However, compound **28** is

limited by poor oral bioavailability with a low *F*% of 14.9.

Compound **29** (ODM-203), as a potent VEGFR2/FGFR inhibitor, exerts remarkable inhibitory activities against the VEGFR and FGFR family with IC_{50} values in the low nanomolar range. Moreover, it is selective for VEGFR and FGFR over other kinases. In vitro and in vivo pieces of evidence have proven the significant tumor suppression role (TGI = 92%) of **29** in FGFR-dependent cell lines RT4 [89]. Notably, it has been evaluated in clinical trials for solid tumor therapy (NCT02264418) [90].

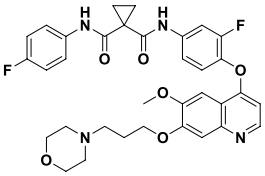
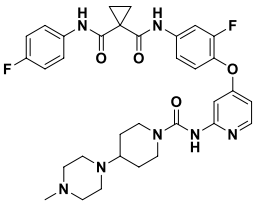
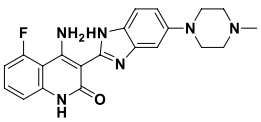
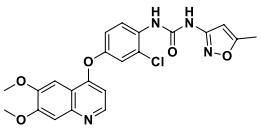
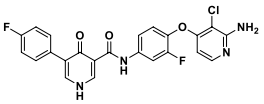
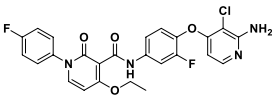
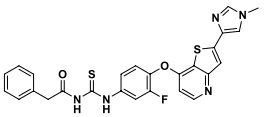
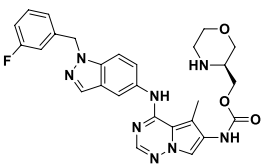
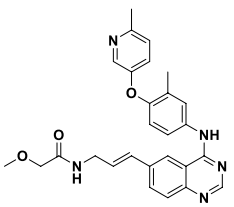
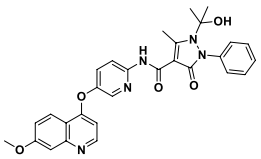
In 2016, Yan et al. designed and synthesized dual inhibitors of FGFR and VEGFR2 through knowledge- and structure-based methods [91]. Among them, molecule **30**, containing a 3-benzimidazol-5-pyridine alkoxy-1H-indole scaffold, shows significant inhibitory activities against VEGFR2 and FGFR1-4 with IC_{50} values of 7.5 nM, 0.9 nM, 2.0 nM, 2.0 nM and 6.1 nM, respectively. Furthermore, compound **30** not only potently inhibits a panel of FGFR-amplified cell lines in vitro, but also presents considerable bioavailability (33% F) and tumor growth suppression (TGI = 96.9%) in vivo.

Dual VEGFR2–c-Met inhibitors

Although the combination of VEGFR inhibitors and c-Met inhibitors inhibits both VEGFR and c-Met signaling pathways, it significantly suppresses the development of different types of tumors [92]. Therefore, the identification of dual-target VEGFR/c-Met inhibitors has been boosted. As shown in Table 3, several pyridine- or pyrimidine-based inhibitors of VEGFR2/c-Met have been identified and are being used in clinical trials, including **31** (foretinib)**, 32** (golvatinib), **33** (dovitinib), **34** (tivozanib), **35** (BMS-794833), **36** (BMS-777607), **37** (MGCD-265), **38** (AC480), **39** (CP-724714) and **40** (AMG-458). These inhibitors are characterized by acting on multiple targets and exerting remarkable potency against tumor cells. Their active scaffolds warrant further investigation, thereby promoting the development of VEGFR/c-Met dual inhibitors. As shown in Fig. 5, most VEGFR2/c-Met dual inhibitors shared the following characteristics: (i) in the region I, different nitrogen-containing aromatic heterocycles, including pyridine, pyrrolidine and quinazoline, can be introduced to form a hydrogen bond with the amino acid residues of VEGFR (Cys919) and c-Met (Met1160). Additionally, the side chains of aromatic heterocycles have a significant effect on the affinity of molecule and target; (ii) region II is composed of a pyridine ring or benzene ring, which can be either unsubstituted or mono-substituted; (iii) in the region III, the introduction of a flexible chain or rigid ring structure (5-atom linker group), containing one or more hydrogen bond donors or receptors, promotes the efficiency of the molecule. Hydrogen bonds formed between this region with the amino acid residues of VEGFR (Lys868, Asp1046, etc.) and c-Met (Asp1220, Lys1110, Leu1245, etc.); (iv) region IV is made up of a six-membered aromatic heterocyclic ring, which can be either unsubstituted, mono- or di-substituted [93]. Here, we summarized the major achievements of dual VEGFR/c-Met inhibitors, and their chemical structure, potency and development are illustrated in Figs. 6 and 7.

Pyridine/pyrimidine scaffolds have been widely applied in RTK inhibitors including VEGFR. Particularly, the pyridine motif stretches into the ATP-binding pocket of the target protein and interacts with the adjacent residues in the hinge region [102]. In 2016, a series of aminopyrimidine derivatives were designed and synthesized to evaluate their inhibitory activities against VEGFR2 and c-Met [103]. Among these compounds, molecule **41** has considerable potency against VEGFR2 and c-Met with IC_{50} values of 170 nM and 210 nM, respectively. In the following year, Zhao et al. identified compound **42** based on the structure of molecule **41**, which is a potent VEGFR2/c-Met dual inhibitor [104]. Moreover, **42** presents a cell active and potent potency against VEGFR2 (IC_{50} = 55 nM) and c-Met (IC_{50} = 17 nM) in vitro. Preliminary SAR for these compounds demonstrated that the introduction of a chlorine atom can positively regulate the kinase inhibitory activities against VEGFR2 and c-Met. In the same year, Gu et al. designed and synthesized a series of novel VEGFR2/c-Met dual inhibitors. Among them, compound **43** has a promising potency against targets [IC_{50} = 160 nM (VEGFR2) and 110 nM (c-Met)], and inferior antiproliferative effects on human vascular endothelial cells HUVEC and BaF3-TPR-Met cells compared with that of positive control **6** [105]. Docking studies further confirmed that molecule **43** occupies the ATP-binding pocket of VEGFR2 and c-Met, and its pyridine and triazole moiety forms hydrogen bonds with the amino acid residues of VEGFR2 (Cys919) and c-Met (Tyr1159 and Met1160). Moreover, the 5-atom linker group (pyrazolone moiety) of compound **43** generates at least one hydrogen bond with the amino acid residues of VEGFR2 (Val898 and Lys868) and c-Met (Asp1222). Similarly, using the scaffold hopping strategy, compound **44** containing the 1,6-naphthyridine scaffold is identified as a potent dual inhibitor of VEGFR2 and c-Met with IC_{50} values of 68 nM and 9.8 nM, respectively [106]. In addition, **44** exerts an unfavorable pharmacokinetic profile (***F***% = 12, CL = 5.0 L/h/kg). Further optimization has been performed based on the structure of molecule **44**, and as a result, compound **45** was identified as a potent inhibitor of c-Met (IC_{50} = 7.1 nM) with selectivity over VEGFR2. PK studies revealed moderate clearance (CL = 0.02 L/h/kg) and suitable oral bioavailability

Table 3 Summary of clinically approved dual VEGFR–c-Met inhibitors

Drugs	Chemical structure	Target	Tumor types	Phase	Ref.
Foretinib (31)		VEGFR2/3, c-Met, Tie-2	Gastric tumor and head/neck tumor	II	[94]
Golvatinib (32)		VEGFR2, c-Met	Head and neck tumor, liver tumor	II	[94]
Dovitinib (33)		FLT3, FGFR1/3, VEGFR1,2,3, EGFR, c-Met	Solid tumor	IV	[95]
Tivozanib (34)		VEGFR1/2/3, c-Met, PDGFR, c-Kit	Advanced renal cell carcinoma	III	[96]
BMS-794833 (35)		VEGFR2, c-Met, Ron, Axl, FLT3	Gastric tumor	I	[93]
BMS-777607 (36)		VEGFR, c-Met, Ron, Axl,	Advanced solid tumor	II	[97]
MGCD265 (37)		VEGFR1/2, c-Met, Ron	Non-small cell lung tumor	II	[98]
AC480 (38)		VEGFR2, HER1/2/4, c-Kit, Met, Lck	Advanced solid tumor	I	[99]
CP-724714 (39)		HER2, EGFR, VEGFR2, c-Met	Advanced solid tumor	II	[100]
AMG-458 (40)		VEGFR2, c-Met	Solid tumor	Non-medicinal	[101]

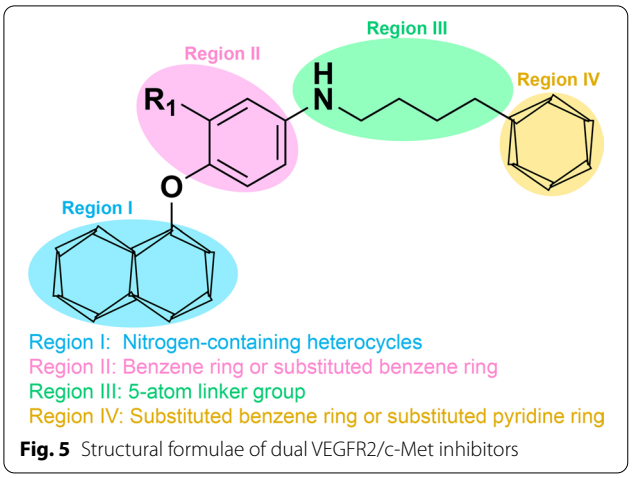


Fig. 5 Structural formulae of dual VEGFR2/c-Met inhibitors

(F = 57%) of **44**. SAR studies showed that substitutions on the 2-aminopyrimidine skeleton were more resistant to c-Met efficacy than VEGFR2.

The quinoline core is widely used to design several active molecules with different biological properties. In 2016, based on the SAR studies of compound **31**, molecule **46** was identified as an effective inhibitor of c-Met (IC_{50} = 1.57 nM) by Liu et al. [107]. The selectivity of **46** for c-Met is 306 times higher than that of VEGFR2 and 100 times higher than that of PDGFRα and Ron. Additionally, **46** exerts significant inhibitory potency against different types of tumor cells (HT-29, H460, A549 and MKN-45) with the IC_{50} values of 80 nM, 140 nM, 110 nM and 30 nM, respectively. Further SAR studies revealed that the replacement of the 5-atom linker group (the cyclopropane-1,1-dicarboxamide moiety) of **31** with the 5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-3-carboxamide fragment and structural modification (methyl, ethyl or cyclopropyl group) on the 4-position of the 1,2,4-triazole skeleton can be tolerable. Interestingly, the introduction of a fluorine atom at 2-position of benzene ring at the end would be beneficial to the potent cytotoxicity. Similarly, the 3-oxo-3,4-dihydroquinoxaline-2-carboxamide moiety displayed similar properties to the cyclopropane-1,1-dicarboxamide moiety, in particular containing hydrogen bond donor and acceptor. These studies accelerated the identification of compound **47**, which contains the 3-oxo-3,4-dihydroquinoxaline-2-carboxamide moiety at the 5-atom linker group [108]. **47** exerts superior inhibitory activities against c-Met (IC_{50} = 0.9 nM) compared with that of molecule **31** (IC_{50} = 1.41 nM). Furthermore, **47** exhibits high inhibitory potency against c-Kit (IC_{50} = 2.45 nM) and exerts considerable efficacy against Ron, VEGFR2 and FLT3 with IC_{50} values of 82.56 nM, 151.47 nM and 268.81 nM, respectively. Further in vitro studies showed that **47** displayed remarkable cytotoxicity (IC_{50} values in the nanomolar concentration range) against different types of tumor cells. Regrettably, studies in vivo are lacking.

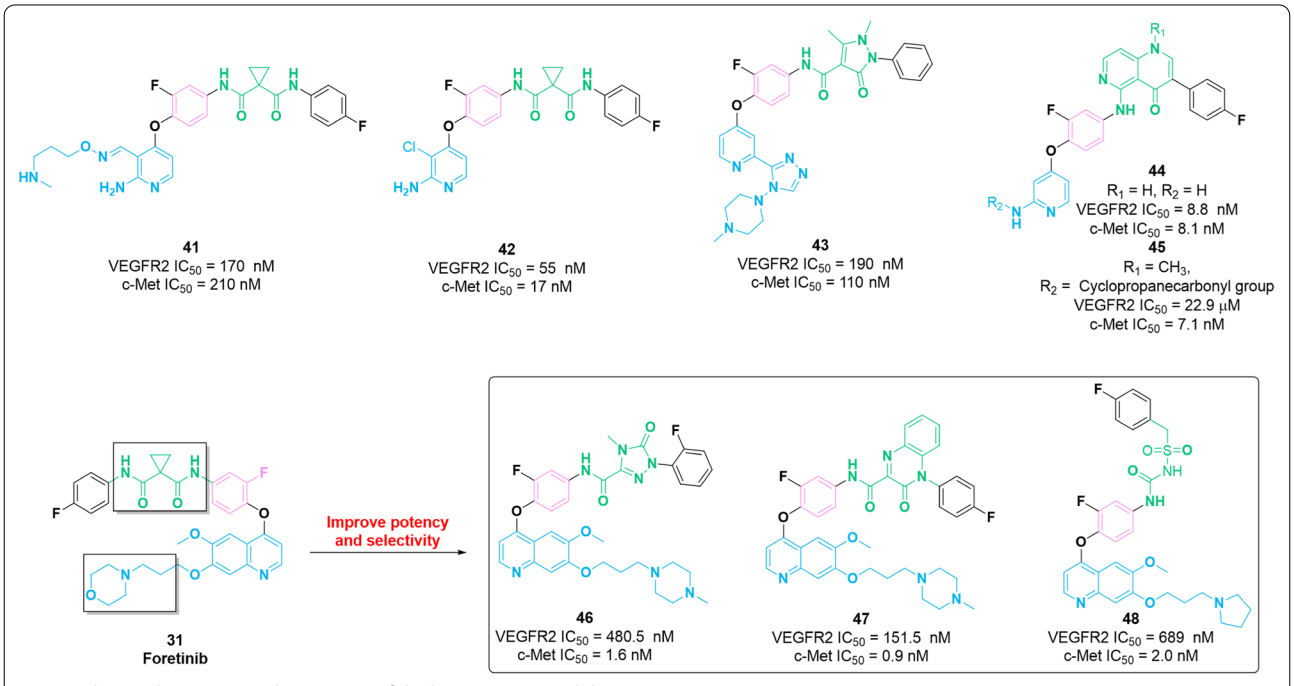


Fig. 6 Chemical structure and properties of dual VEGFR–c-Met inhibitors **41**–**48**

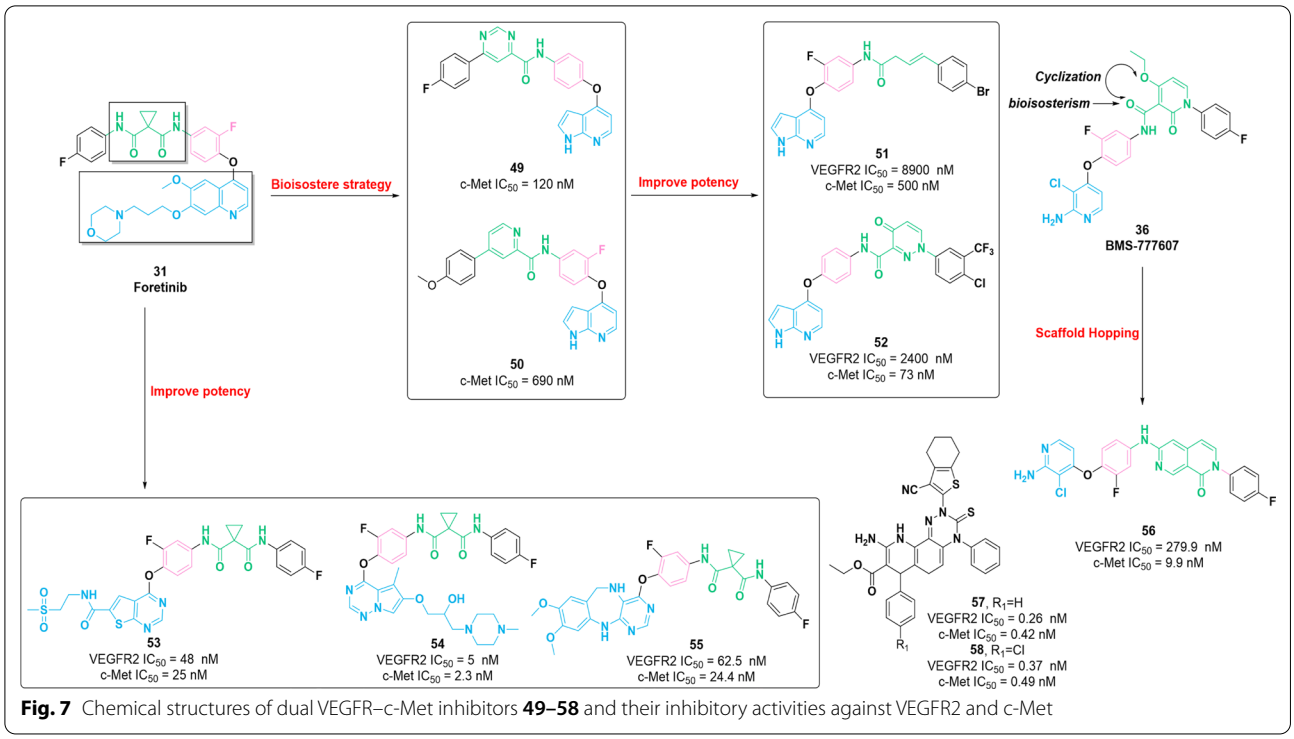


Fig. 7 Chemical structures of dual VEGFR–c-Met inhibitors **49–58** and their inhibitory activities against VEGFR2 and c-Met

In 2019, a c-Met inhibitor **48** containing 4-phenoxyquinoline skeleton and sulfonylurea moiety is discovered by the structural optimization of compound **31** [109]. It presents an excellent inhibitory effect against c-Met with an IC_{50} value of 1.98 nM. Moreover, **48** is highly selective for c-Met over 347 times higher than that of VEGFR. **48** has strong antiproliferative activity against different types of tumor cell lines with nanomolar potency in vitro. The SAR studies demonstrated that the introduction of sulfonylurea fragment as the 5-atom linker group could maintain remarkable potency. In recent years, pyrrolopyridine derivatives, as biologically active molecules, occupy a unique place in medicinal chemistry [110]. Zhu et al. identified compounds **49** and **50** as potent inhibitor of c-Met (IC_{50} = 120 nM and 670 nM, respectively) using the bioisostere strategy, which possesses excellent cytotoxicity activities against different types of tumor cells [111, 112]. Based on the structures of **49** and **50**, compound **51**, containing an N-acylhydrazones group, was identified as a potential c-Met inhibitor (IC_{50} = 0.5 μM) by Wang et al. [113]. In addition, **51** shows a considerable selectivity profile for c-Met over other kinases (FLT3, VEGFR2 and EGFR) and exerts significant inhibitory activities against diverse types of tumor cells through arresting the cell cycle in the G2/M-phase inducing cell apoptosis. Similarly, compound **52** was identified by the same team by introducing 4-oxo-pyridazinone fragment into 5-atom linker group of molecule **49**. It displays remarkable inhibitory activities against c-Met with an IC_{50} value of 73 nM. The selectivity of **52** for c-Met is approximately 15 times higher than VEGFR2 and c-Kit and, specifically, 7 times higher than that of FLT3. In vitro assay showed that molecule **52** exerts favorable inhibitory activities against different types of tumors [114].

Similarly, based on the structure of molecule **31**, a series of thieno[2,3-*d*]pyrimidine derivatives were prepared as potent dual inhibitors of VEGFR2 and c-Met [115]. Among these compounds, molecule **53** exerts remarkable inhibitory activities against VEGFR2 and c-Met with IC_{50} values of 48 nM and 25 nM, respectively. Docking studies demonstrated that the thieno[2,3-*d*] pyrimidine scaffold of molecule **53** generates hydrogen bonds with the amino acid residues of VEGFR2 (Cys919) and c-Met (Met1160). Additionally, hydrogen bonds formed between 4-fluoro-phenyl-cyclopropane-1,1-dicarboxamide fragment with the amino acid residues of VEGFR (Asp1046 and Lys868) and c-Met (Phe1223). In 2018, compound **54** containing pyrrolo[1,2-*f*][1,2,4] triazine core is identified as a remarkable dual inhibitor of VEGFR2 (IC_{50} = 5.0 nM) and c-Met (IC_{50} = 2.3 nM) [116]. **54** showed remarkable inhibitory activities against BaF3-TPR-Met, HUVEC and different types of tumor cells. Besides, compound **54** possesses favorable physiochemical properties and an excellent pharmacokinetic profile (***F***% = 98.1). Further docking studies revealed that molecule **54** can completely occupy the ATP-binding

pocket of c-Met and VEGFR2, thereby generating important ligand interactions. In the same year, Huang et al. identified compound **55** as a c-Met inhibitor, which bears 6,11-dihydro-5*H*-benzo[e]pyrimido[5,4-*b*] [1, 4] diazepine scaffold [117]. Enzymatic inhibition assay showed that **55** exerts notable inhibitory potency against c-Met and VEGFR2 with IC_{50} values of 24.4 nM and 62.5 nM, respectively. Compound **55** has a selectivity profile for VEGFR2 and c-Met over other kinases. Furthermore, molecule **55** possesses favorable in vitro potency and moderate oral bioavailability (***F***% = 39). Further in vivo pieces of evidence demonstrated that compound **55** had a considerable therapeutic effect (TGI = 64.5%).

In 2019, Zhuo et al. designed and synthesized a series of 2,7-naphthyridinone-based c-Met inhibitors through knowledge- and structure-based methods [118]. In vitro assay showed that molecule **56**, as the most promising inhibitor, possesses a potent potency for c-Met (IC_{50} = 9.9 nM), and the selectiveness for c-Met is 28 times higher over VEGFR2. Additionally, compound **56** possesses favorable pharmacokinetic profile (***F***% = 54) and excellent in vivo efficacy (TGI = 95%) in mouse xenograft tumor models. Based on these studies, 2,7-naphthyridinone may be a promising skeleton for future drug development. In 2020, compounds **57** and **58** containing tetrahydrobenzo[*b*]thiophene scaffold are identified as multi-target RTK inhibitors [119]. Specifically, **57** and **58** possess remarkable potency against multi-kinase, including c-Met and VGEFR2, with IC_{50} values in the low nanomolar to picomolar concentration range. Moreover, they also exert antiproliferative activities in vitro against the six typical tumor cells, including A549, H460, HT-29, MKN-45, U87MG and SMMC-7721. However, in vivo studies are still lacking.

Dual VEGFR2–BRAF inhibitors

As we mentioned earlier, combination therapy involving VEGFR inhibitors and BRAF inhibitors has been identified as an effective therapeutic strategy [120]. Notably, RAF-265, a VEGFR2–BRAF dual inhibitor, has demonstrated its efficacy and safety profile in a I/II clinical phase trial (NCT00304525). Currently, several dual VEGFR2–BRAF inhibitors have been identified to suppress tumor growth. Their chemical structure, in vitro and in vivo potencies, and optimizations are illustrated in Fig. 8.

In 2017, Fu et al. identified compound **59** by using a structure-based drug design as an encouraging RAF inhibitor [121]. It presents remarkable inhibitory potency against c-RAF, wild-type BRAF and $BRAF^{V600E}$ with IC_{50} values of 8.5 nM, 51.5 nM and 23.6 nM, respectively. Additionally, the strong antiproliferative activity of **59** against four types of tumor cell lines with micromolar potency in vitro, and superior selectivity for RAF compared to other kinases have been confirmed. Particularly, **59** presents moderate inhibitory activity against VEGFR2 kinase. (The inhibition rate of VEGFR2 at 1 μM was 56%.) Mechanistic

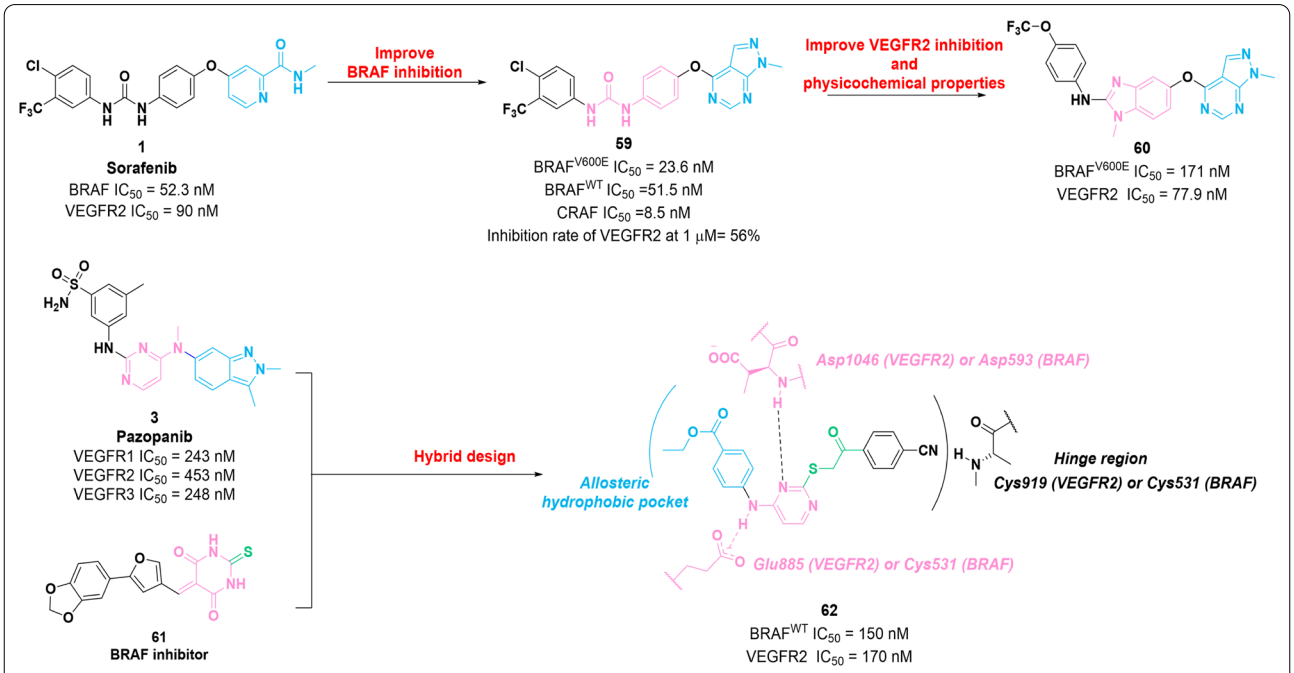


Fig. 8 Chemical structures of dual VEGFR–BRAF inhibitors **59**, **60** and **62** and their inhibitory activities against VEGFR2 and BRAF

studies revealed that **59** exerts antiproliferative activities on A375 and HT-29 cells by arresting the cell cycle progression in G0/G1 stage and significantly suppressing RAS/RAF/MEK/MAPK signaling pathways. The following year, compound **60** was developed based on the structural optimization of **59**, which has excellent selectivity for VEGFR2 (IC_{50} = 77.9 nM) and $BRAF^{V600E}$ (IC_{50} = 171 nM) over wild-type BRAF and other protein kinases [122]. Docking studies and molecular dynamics simulations demonstrated that **60** adopts a similar binding mode to that of compound **1** at the ATP-binding sites of $BRAF^{V600E}$ and VEGFR2. These studies indicated that **60** can sever as a lead compound for the identification of potent $BRAF^{V600E}$/VEGFR2 dual inhibitors.

In 2019, compound **62** was identified as an effective wild-type BRAF/VEGFR2 dual inhibitor through structural hybridization between VEGFR inhibitor **3** and BRAF inhibitor **61** [123]. **62** exerts potent inhibitory activities against wild-type BRAF and VEGFR2 with IC_{50} values of 150 nM and 170 nM, respectively. Moreover, **62** possesses antiproliferative activity against MCF-7 and T-47D cells with IC_{50} values in the micromolar concentration range. Docking studies revealed that **62** can bind to the active sites of VEGFR2 and BRAF, thereby accomplishing the key binding interactions.

Dual VEGFR2–HDAC inhibitors

Recent studies have shown that the combination of VEGFR inhibitors and HDAC inhibitors exerts promising potency in vitro and in vivo. Structurally, HDAC inhibitors generally consist of three parts: a zinc-binding group (ZBG), an appropriate linker and a capping group (CAP group). Notably, SAR studies showed that modification of the CAP group of HDAC inhibitor is tolerable. Hence, the CAP group could hybridize with VEGFR2 inhibitors for the identification of dual-target inhibitors [124]. In 2016, a series of VEGFR2/HDAC dual inhibitors containing *N*-phenylquinazolin-4-amine and hydroxamic acid moieties were obtained based on the parent compounds **4** and **63** (SAHA) [125]. Among them, molecule **64** (Fig. 9) possesses remarkable potency against VEGFR2 (IC_{50} = 74 nM) and HDAC (IC_{50} = 2.2 nM) and shows favorable inhibitory activities against human breast tumor cells MCF-7 with an IC_{50} value of 850 nM. Unfortunately, the selectivity profile of compound **64** for HDAC family members is lacking. Recently, by incorporating pharmacophores of VEGFR inhibitor **1** and HDAC inhibitor **63**, a series of phenylurea hydroxamic acids were synthesized to evaluate their inhibitory activities against VEGFR2 and HDAC [126]. Among these compounds, molecule **65** (Fig. 9) potently inhibits HDAC6 (IC_{50} = 166 nM) and is slightly selective for HDAC6 over other HDAC1, HDAC2 and HDAC8. Furthermore, **65** exerts weak potency against VEGFR2 with an IC_{50} value

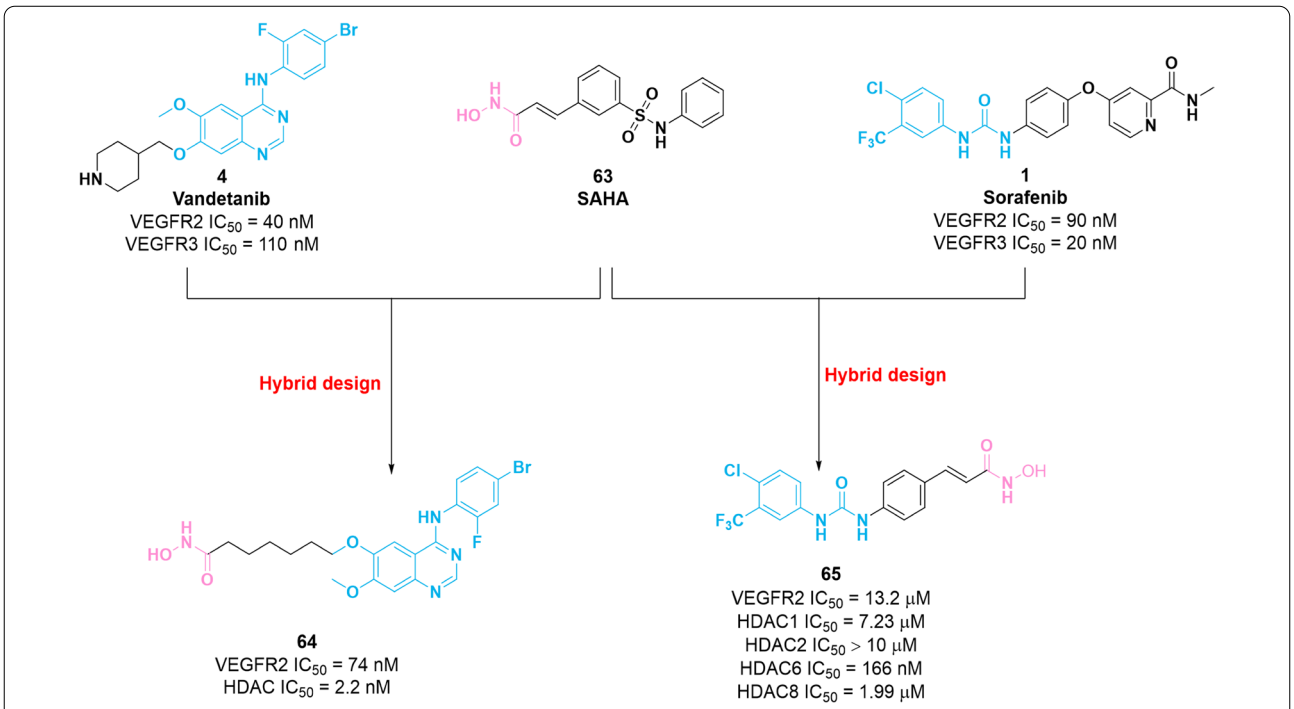


Fig. 9 Chemical structures of dual VEGFR–HDAC inhibitors **64** and **65** and their inhibitory activities against VEGFR2 and HDAC

of 13.2 μM. The co-crystal structure of **1** in complex with VEGFR2 (PDB: 3EWH) showed that the key *N*-methyl-2-pyridinecarboxamide group is of significance in the potency against VEGFR2, which is inserted into the hinge region through generating two hydrogen bonds with Cys919 [127]. Thus, unreasonable modification of this region of molecule **1** can lead to a loss of inhibitory activity.

In 2018, Zhang et al. generated two series of dual VEGFR–HDAC inhibitors using the pharmacophore of VEGFR inhibitor **3** as the CAP group and the diverse linker group, hydroxamic acid or *ortho*-aminoanilide as the ZBG [128]. Of these, compounds **67** and **68** (Fig. 10) exert potent potency against VEGFR and HDAC with IC_{50} values in the nanomolar or low micromolar concentration range. Specifically, compound **67** exerts considerable HDAC2/6 inhibitory potency and superior HDAC8 inhibition compared with that of molecule **63**. Molecule **68** also possesses comparable efficacy against HDAC1/2/3 compared with that of molecule **66** (Entinostat). Additionally, compared with compound **3** (VEGFR2 IC_{50} = 32 nM), **67** and **68** show similar inhibitory activities against VEGFR2 with IC_{50} values of 35 nM and 37 nM, respectively. Other kinases (VEGFR1, VEGFR3, PDGFRβ, FGFR, C-Fms and c-Kit), which are tumor-related targets inhibited by **3**, could be potently inhibited by molecules **67** and **68**. SAR of **67** and **68** can be briefly summarized as follows: (i) structural modification of the solvent-exposed phenyl moiety of molecule **3** was well tolerated for its kinase inhibition profile; and (ii) substitution in position 4 of the solvent-exposed phenyl group is favorable for the potency against HDAC and VEGFR2. In addition, compound **68** exerts desirable pharmacokinetic profiles (***F***% = 72) and moderate in vivo antitumor effects (TGI = 40%) in a HT-29 xenograft model. In 2022, compounds **69** and **70** (Fig. 11) were developed based on structure optimization of **67**, which have promising potency for HDACs and stronger inhibitory activities against VEGFRs compared with that of molecule **67** [129]. Furthermore, molecules **69** and **70** showed favorable antiproliferative activities against different types of tumor cells with IC_{50} values in the micromolar concentration range.

Dual VEGFR2–tubulin inhibitors

The effect of combination therapy with tubulin inhibitors and VEGFR2 inhibitors has been confirmed by several studies [130, 131]. Presently, several dual VEGFR2–tubulin inhibitors have been identified to exert potent antitumor activities. Their chemical structure, in vitro potency and optimization are illustrated in Fig. 11.

In 2014, a series of VEGFR2–tubulin inhibitors were prepared by introducing the 7-benzyl moiety of RTK inhibitor **72** into the core of tubulin inhibitor **71** [132]. Among them, molecule **73** exerts significant inhibitory efficacy against VEGFR2 and tubulin polymerization

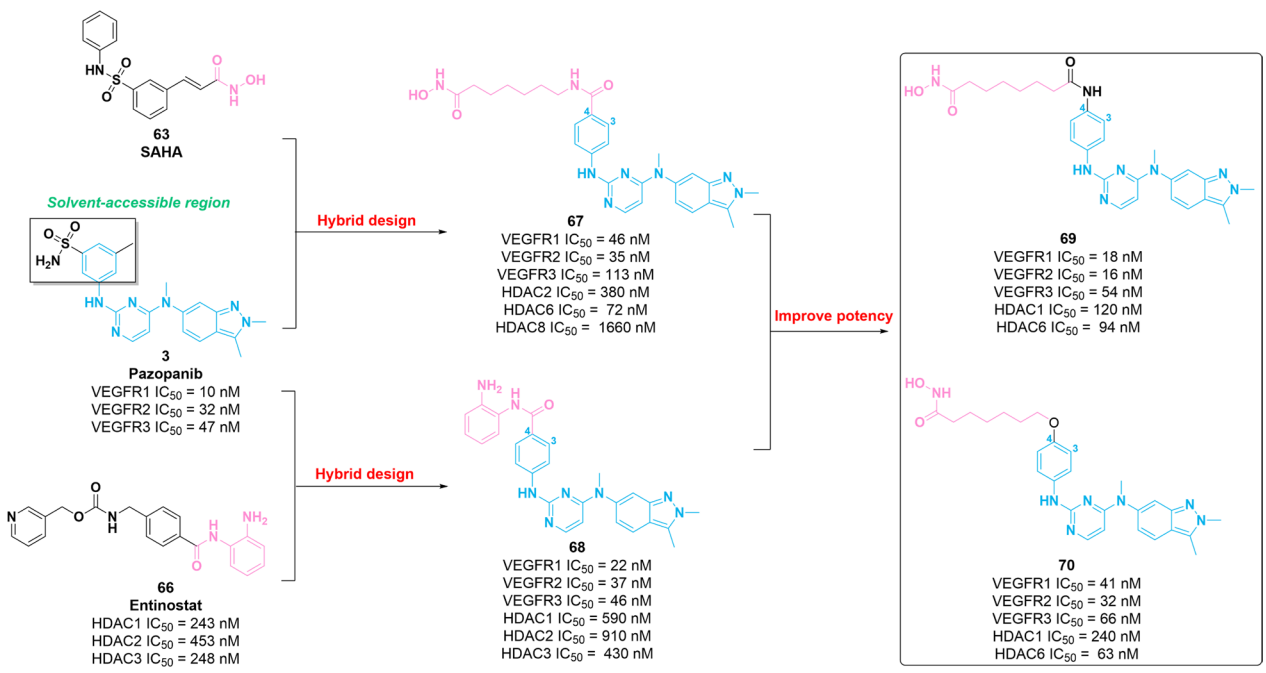


Fig. 10 Chemical structures of dual VEGFR–HDAC inhibitors **67–70** and their inhibitory activities against VEGFR2 and HDAC

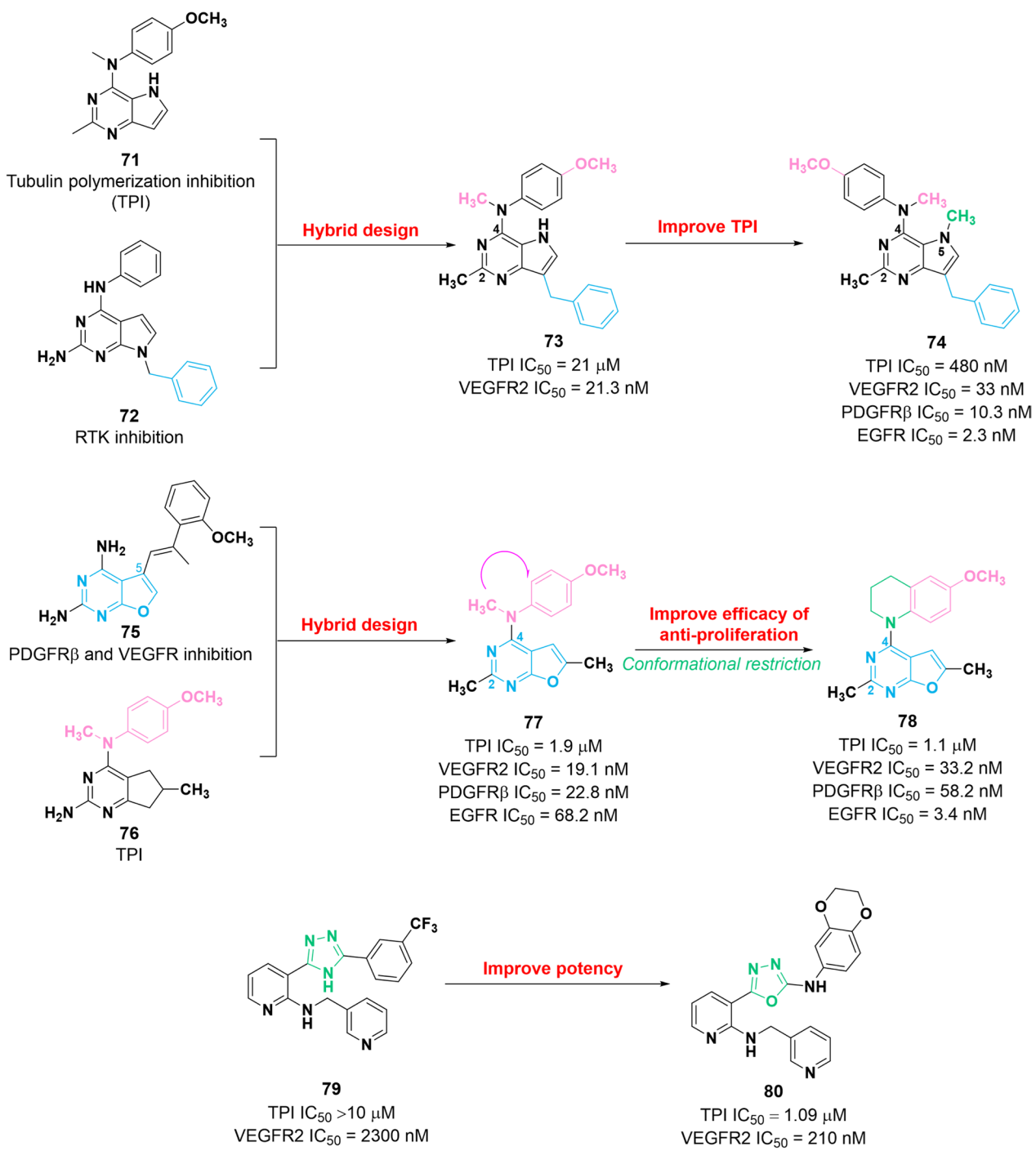


Fig. 11 Chemical structures of dual VEGFR–tubulin inhibitors **73**, **74**, **77**, **78**, **79** and **80** and their inhibitory activities against VEGFR2 and tubulin polymerization (TPI)

with IC_{50} values of 21.3 nM and 21 μM, respectively. Preliminary SAR studies showed that (i) 7-benzyl fragment plays a key role in the maintenance of potency against VEGFR2; and (ii) N–CH_3 and O–CH_3 are essential for the inhibitory activity against VEGFR2 and microtubule. In vitro assays showed that **73** possesses potent antiangiogenic and antiproliferative activity. Specifically, **73** presents remarkable inhibitory activities against βIII-tubulin-overexpressing HeLa cells (IC_{50} = 280 nM) and P-gp-overexpressing ADR-RES cells (IC_{50} = 700 nM),

thereby theoretically reversing βIII-tubulin- and P-gp-overexpression-induced drug resistance. Additionally, **73** shows potent antitumor and antimetastasis effects in in vivo tumor models. In 2017, compound **74** was developed based on the structure of **73**, which presents a potent potency against VEGFR2 (IC_{50} = 33 nM), tubulin polymerization (IC_{50} = 480 nM), EGFR (IC_{50} = 2.3 nM) and PDGFRβ (IC_{50} = 10.3 nM) in vitro [133]. Furthermore, **74** presents superior cytotoxicity activities against βIII-tubulin-overexpressing (IC_{50} = 250 nM) and P-gp-overexpressing (IC_{50} = 70 nM) tumor cells compared with that of **73**. Further SAR studies demonstrated that the N4-CH_3 and N5-CH_3 groups play a key role in the inhibitory potency against tubulin polymerization. Structurally, the N5-CH_3 group is thought to favor the formation of conformational rigidity, thereby improving efficacy. Moreover, the 2-CH_3 group was substituted with a 2-amino moiety, leading to decreased inhibitory activity against tubulin polymerization.

In another report, hybridization of the pharmacophores of RTK inhibitor **75** and tubulin inhibitor **76** in a single molecule facilitated the discovery of compound **77** [134]. This molecule exerts favorable potency against EGFR, VEGFR2, PDGFRβ, and tubulin polymerization with IC_{50} values of 68.2 nM, 19.1 nM, 22.8 nM and 1900 nM, respectively. Furthermore, in vitro and in vivo pieces of evidence proved the superior potency of compound **77** on proliferation inhibition and repression of tumor angiogenesis compared with that of docetaxel. Based on the structure of compound **77** and further ligand design, compound **78** was further discovered to be more potent than compound **77** for tubulin polymerization (IC_{50} = 1100 nM) and EGFR (IC_{50} = 3.4 nM) [135]. However, **78** exerts inferior potency against VEGFR2 (IC_{50} = 33.2 nM) and PDGFRβ (IC_{50} = 58.2 nM). SAR studies demonstrated that the introduction of tetrahydroquinoline ring fragment is beneficial to improve EGFR inhibition. Moreover, **78** significantly inhibits the growth of drug resistance HeLa and SK-OV-3 cells with IC_{50} values of 9.1 nM and 19.4 nM, respectively.

Compound **80** containing 1,3,4-oxadiazole fragment is developed based on the structure optimization of weak VEGFR2 inhibitor **79**, which has a promising potency for tubulin polymerization (TPI IC_{50}> 10 μM) and VEGFR2 (IC_{50} = 2300 nM) compared with that of molecule **80** (TPI IC_{50}= 1090 nM, VEGFR2 IC_{50} = 210 nM) [136]. Additionally, **80** can block cell cycle progression in the G2/M-phase. Acute and repeat dose oral toxicity studies demonstrated that **80** has a favorable safety profile.

Dual VEGFR2–ERα inhibitors

Combination therapy with SERMs and VEGFR inhibitors has been identified as an effective therapeutic strategy to retard SERM resistance tumor growth [137]. A number of dual *VEGFR2*–ERα inhibitors with significant anti-breast tumor activities were obtained. Their chemical structures, in vitro potency and optimization are illustrated in Fig. 12.

In 2014, based on the structures of VEGFR inhibitor **2** and SERM **81** (acolbifene), compound **83** is further discovered to present considerable potency against ERα (IC_{50} = 1.3 μM) and VEGFR2 (IC_{50} = 1.4 μM) [138]. Biological studies revealed that **83** exerts antiproliferative activity against MCF-7 cells with an IC_{50} value of 2.73 μM and possesses potential antiangiogenesis efficacy in vivo. In 2016, a series of VEGFR2/ERα inhibitors containing aryl-indenoisoquinolone core were prepared based on the structures of **2** and **81** [139]. The analogue **84** was obtained, showing a significant potency for VEGFR2 and ERα with IC_{50} values of 99 nM and 7.2 μM, respectively. Moreover, **84** shows favorable cytotoxicity activities against MCF-7, MDA-MB-231, Ishikawa and HUVEC cell lines with IC_{50} values of 1.2 μM, 0.5 μM, 8.2 μM and 800 nM, respectively. Further in vitro studies demonstrated that **84** inhibits the growth of MDA-MB-231 cells through negatively regulating VEGFR2 and the signaling transduction of the RAF-1/MAPK/ERK pathway.

In 2017, through hybridization of bioactive pharmacophores of **81** and **82**, compound **85** containing 4,6-diaryl-2-pyrimidinamine scaffold was reported as a potential agent for breast tumor therapy. It exerts favorable inhibitory activities against MCF-7, HUVEC and Ishikawa cells [140]. Chick chorioallantoic membrane (CAM) assay showed that **85** exerts significant antiangiogenesis activity in vivo. Further optimization has been performed based on the structure of molecule **85**, and as a result, compound **86** is identified as a potent inhibitor of VEGFR2 (IC_{50} = 85 nM) and ERα (IC_{50} = 1.64 μM) [141]. Furthermore, **86** possesses remarkable antiestrogenic property through downregulating the expression of progesterone receptor (PgR) mRNA in MCF-7 cells and exerts significant antiangiogenesis efficacy in vitro and in vivo.

Compound **87** (SP500263), a coumarin-based SERM, exerts a high affinity for ERα and significantly inhibits the growth of estrogen-dependent MCF-7 cells [142]. In 2017, compound **88** was developed based on the structural optimization of **87**, with potential potency against ERα (IC_{50} = 6.64 μM) and weak inhibitory activity against VEGFR2 [143]. To improve the potency, molecule **89** with favorable potency against ERα (IC_{50} = 2.19 μM) has been identified [143]. SAR studies showed that the introduction of bioisosteric O atom at 4-position of coumarin core is essential for enhancing the ERα inhibition. Compared with molecule **88**, **89** exerts superior inhibitory activities against MCF-7 and Ishikawa cells. In

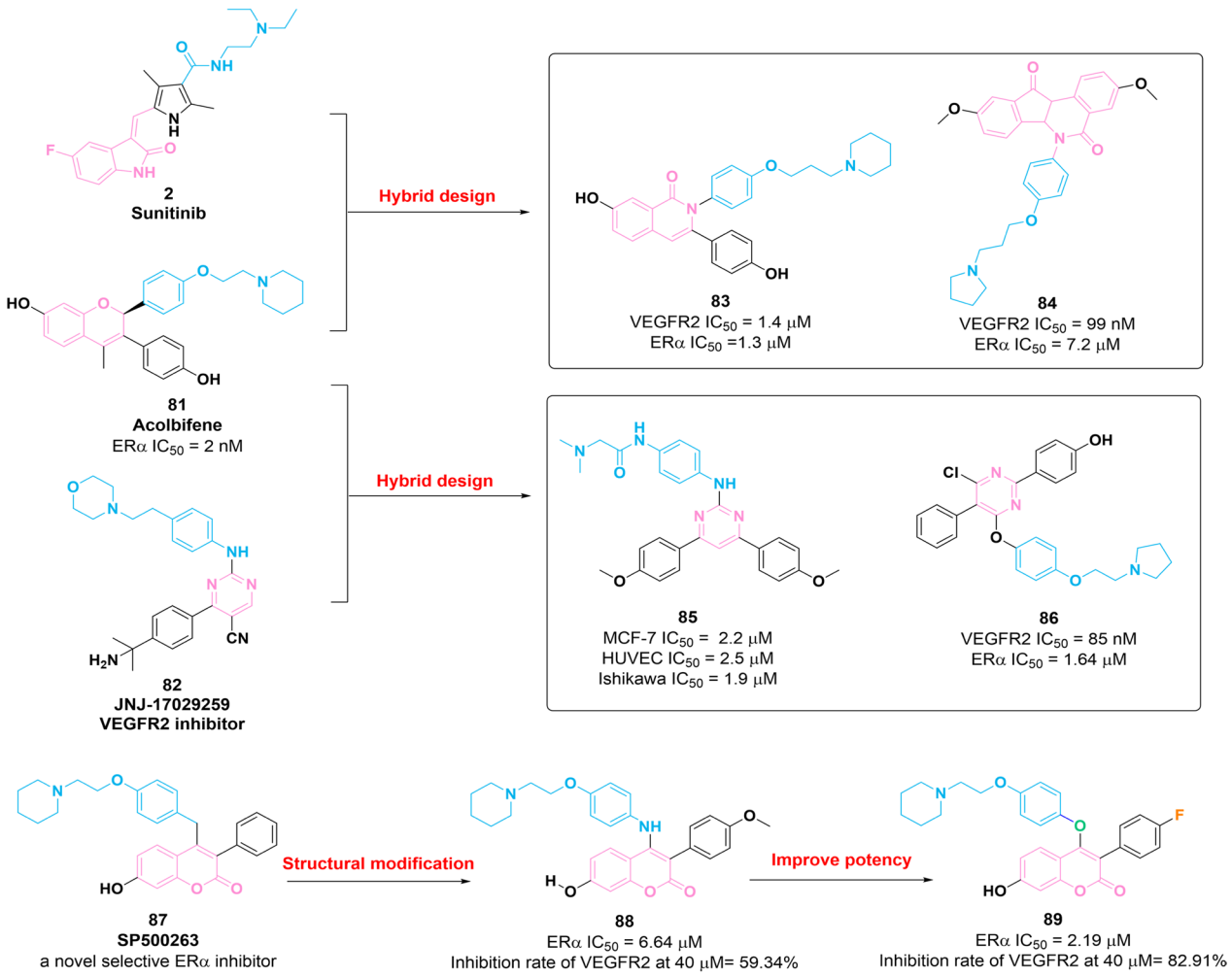


Fig. 12 Chemical structures of dual VEGFR–ERα inhibitors **83–86**, **88** and **89** and their inhibitory activities against VEGFR2 and ERα

MCF-7 cells, **89** induces cell apoptosis and a prolonged G0/G1-phase and inhibits proliferation and migration through negatively regulating the expression of VEGFR2 and the signaling transduction of RAF-1/MAPK/ERK pathway. Collectively, the structure of the VEGFR2/ERα inhibitor is characterized by the presence of an aromatic scaffold and flexible side chain with a tertiary amine substituent at the end. The introduction of the above two pharmacophores is beneficial to the inhibitory activities against ERα and VEGFR.

Dual VEGFR2–PIM1 inhibitors

The expression of PIM-1 kinase has been noted as a new resistance mechanism to VEGFR inhibitors [144]. Thus, a combination therapy involving PIM1 kinase and VEGFR inhibitor has been identified as an effective therapeutic strategy to sensitize tumor cells. In 2019, a series of PIM1/VEGFR2 dual inhibitors containing thieno[2,3-*b*]pyridine core were prepared via molecular hybridization between VEGFR inhibitors (compounds **5** and **90**) and PIM1 inhibitors (Fig. 13) [145]. Among these compounds, **91** was found to show the most potent inhibitory activities against PIM1 and VEGFR2 with IC_{50} values of 5873 nM and 7948 nM, respectively. In vitro assays showed that **91** exerted inhibitory potency against different types of tumor cells (HepG-2, Caco-2, MCF-7 and PC-3) with IC_{50} values in the nanomolar concentration range. Furthermore, **91** can positively regulate the expression of caspase 3/7 and induce apoptosis in tumor cells. Real-time PCR analysis demonstrated that **91** presents superior therapeutic potential in regulating the expression of VEGF, p53 and cyclin D compared to doxorubicin (Fig. 13).

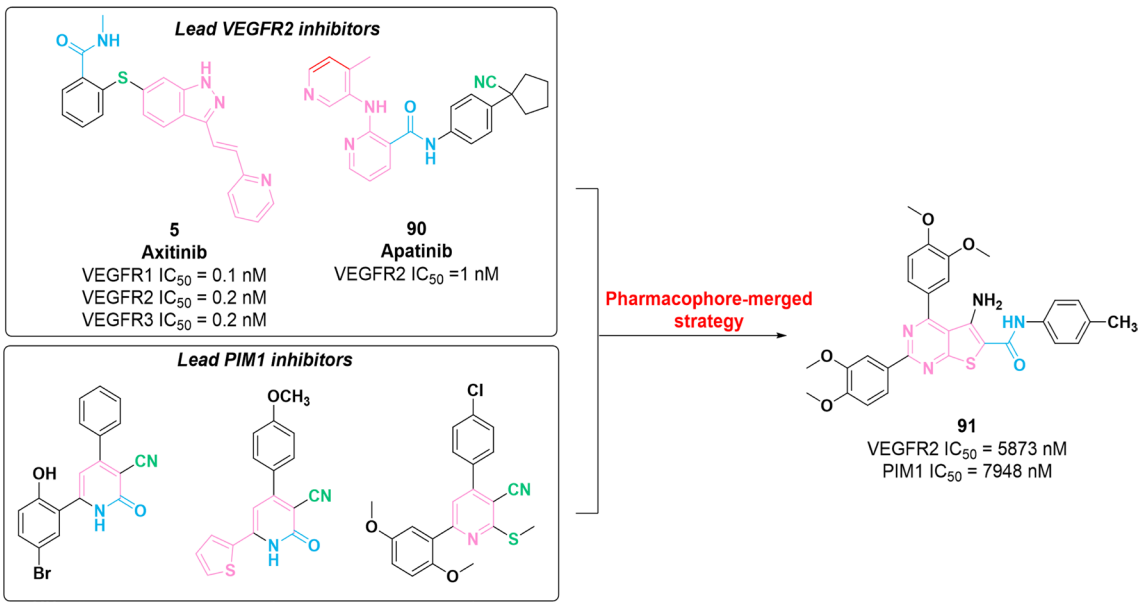


Fig. 13 Chemical structure of dual VEGFR–PIM1 inhibitor **91** and its inhibitory activities against VEGFR2 and PIM1

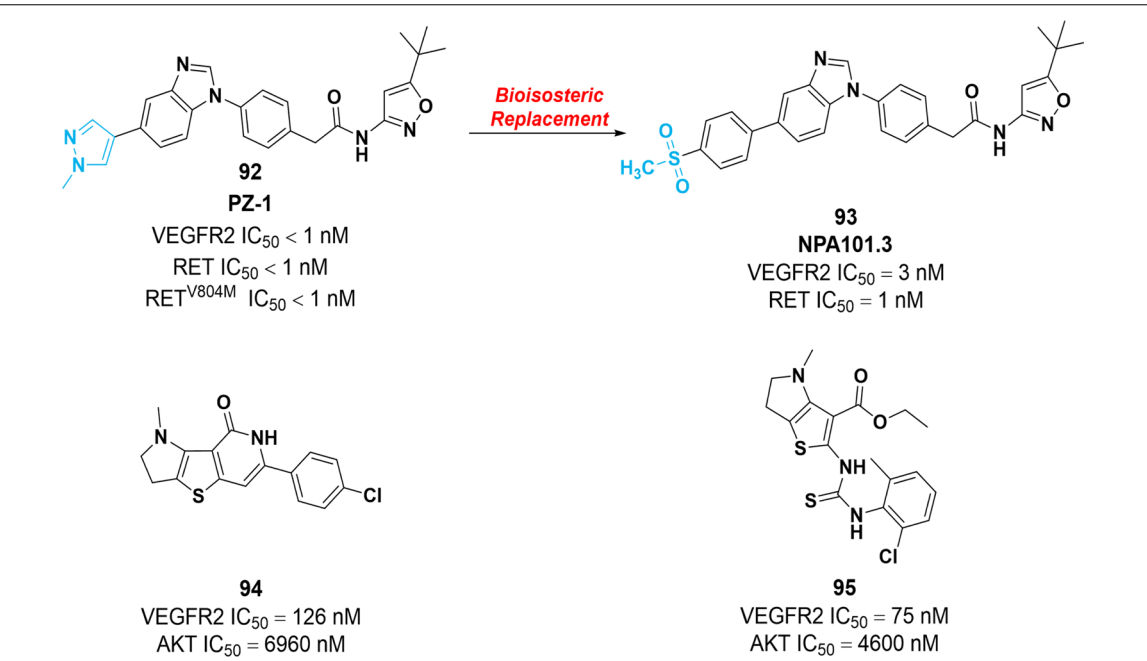


Fig. 14 Chemical structure of dual VEGFR–RET inhibitors **92**, **93,** and VEGFR–AKT inhibitor **94** as well as their inhibitory activities against their target proteins

Dual inhibitors of VEGFR2 and other antitumor targets

Currently, several dual inhibitors of VEGFR2 and other antitumor targets were identified through serendipity or using typical design strategies, and they exerted superior potency to corresponding single-target molecules. These dual inhibitors are frequently utilized as tool compounds for investigating the synergetic interactions of VEGFR2 and other antitumor targets. In addition, they can be used as potential novel leads to discover novel dual-target antitumor agents.

Under normal physiological conditions, rearranged during transfection (RET) plays an important role in the development of the kidney and nervous system. Under pathological conditions, RET rearrangements lead to the generation of chimeric genes. Mechanistically, these genes are formed by the fusion of the RET tyrosine kinase domain with the N-terminal region of other genes. Structurally, VEGFR2 and RET share a high similarity regarding their ATP-binding site. Therefore, several multi-kinase inhibitors targeting the VEGFRs, RET and other kinases are widely used in clinical practice, including **1**, **4**, **6** and **7**. In order to enhance the selectivity and reduce side effects, Brendan et al. discovered a dual pan-RET/VEGFR2 kinase inhibitor **92** (Pz-1) through the fragment-based chemical screen, which possessed remarkable inhibition activity with nanomolar potency against RET, VEGFR2 and RET^{V804M}. Notably, in vivo results confirmed the favorable safety profile and the significant tumor growth inhibition role of **92** in nude mice implanted with RET- or RAS-transformed NIH3T3 fibroblasts [146]. In 2020, to further enhance the metabolic stability of **92**, compound **93** (NPA101.3) was identified by applying bioisosteric substitution of the molecule **92** site susceptible to demethylation (Fig. 14). Enzymatic inhibition assay showed that **93** possessed a notable inhibitory potency against both RET and VEGFR2 with IC_{50} values of 1 nM and 3 nM, respectively. Furthermore, in vitro study revealed that **93** could suppress the phosphorylation of RET oncoproteins and VEGFR2. It also remarkably inhibits the proliferation of RET-transformed Ba/F3 cells with IC_{50} values in the low nanomolar concentration range. In vivo pieces of evidence proved that compound **93** completely prevented the formation of tumors induced by RET^{C634Y}-transformed cells [147].

VEGF binding to the VEGFR can lead to AKT activation, improving the proliferation, migration and invasion capacity of tumor cells. Additionally, a few studies have suggested that the resistance to VEGFR inhibitors is contributed to acquired mutations in AKT. Therefore, dual inhibition of VEGFR2 and AKT may trigger apoptosis at different focal points. In 2022, a series of VEGFR–AKT dual inhibitors containing thienopyrrole or pyrrolothienopyrimidine scaffold is prepared by Abdelnaby et al. Among them, compounds **94** and **95** showed better inhibitory activities against AKT (IC_{50} = 6.96 μM and 4.60 μM, respectively) and VEGFR2 (IC_{50} = 126 nM and 75 nM, respectively) (Fig. 14). In HepG2 cells, **94** and **95** could aggravate apoptosis by inhibiting cell proliferation and arresting cell growth in the S-phase, resulting in cell apoptosis [148].

Conclusion and future direction

Currently, antiangiogenesis therapy based on inhibition of VEGFR is considered to be an effective clinical strategy for the treatment of solid tumors. Although VEGFR inhibitors showed prospective efficacy in clinical application, there are still barriers and challenges to surmount, such as the moderate clinical efficacy, mechanism-related toxicities and the occurrence of clinical resistance. Encouragingly, great advances have been made in identifying novel combination treatment strategies due to the progressed technologies in structural biology and pharmacochemistry. Multi-target, especially dual-target drug design, is one of the hottest areas in tumor treatment. Compared with combination chemotherapy, multi-target drugs have the advantages of synergistic antitumor effect and improved pharmacokinetic properties. Given the critical role of VEGFR in the development of tumor angiogenesis, dual-target drug design for VEGFR has become a hot topic in the drug research and development field. Several studies have proven the favorable efficacy and safety of VEGFR inhibitors and inhibitors of other tumor-associated targets (including EGFR, FGFR, BRAF, c-Met, HDAC, tubulin, ERα and PIM1) combination therapy in patients with tumors.

In general, the hybrid design strategy integrates the active group of a VEGFR inhibitor with the pharmacophore of another inhibitor of tumor-associated targets into one molecule to identify novel and potent agents. In this review, we summarize VEGFR-based dual-target inhibitors, which provide a rationale for the future design of dual-target inhibitors involving VEGFR. Clinical practice and research have demonstrated that VEGFR inhibitors have synergistic effects with various inhibitors of other tumor-associated targets [149]. However, the dual-target drug design approach has not yet been extensively applied for several targets, such as poly ADP-ribose polymerase (PARP), which possesses synergistic effects with VEGFR inhibitors [150]. Notably, several clinical studies have confirmed the efficacy and safety of VEGFR-based dual-target drugs (such as compounds **23** and **26**) for the treatment of different types of tumors. The above studies confirmed the feasibility of the VEGFR-based dual-target drug design strategy.

Yet where there are opportunities there are challenges. Firstly, identifying rational target combinations based on the correlation between reported targets and tumors is a major challenge in identifying dual-target VEGFR inhibitors. Nowadays, this is typically realized through clinical investigations and phenotype-based screening for combination treatment. Moreover, the clinical success of dual-target VEGFR inhibitors depends on the optimization of efficacy, pharmacokinetic properties and toxicity. To meet these demands, obtaining highly potent dual-target

lead compound with excellent pharmacokinetic properties can serve as a starting point. A better procedure is to maximize the overlap of pharmacophores of maternal molecules to generate smaller molecules with desirable functionalities that have competent chemical space for structural optimization. Specifically, maintaining low lipophilicity and avoiding superfluous structural enlargement are the main issues to consider when optimizing the pharmacokinetic properties of dual-target VEGFR inhibitors. The pharmacophores of active parent molecules share a high degree of structural similarity. However, the dual-target molecules obtained by merging pharmacophores are not necessarily effective. Secondly, most of the potent VEGFR inhibitors in clinical studies are multi-targeted, such as compounds **1**–**12**. It is noteworthy that these drugs are limited to a certain extent due to poor selectivity, potential toxicity or low metabolic stability, which seriously affects their clinical application. Thus, there is a pressing need to develop highly selective VEGFR inhibitors. Although highly potent and selective single-target drugs can temporarily solve these problems, these drugs are limited by drug resistance caused by the activation of compensatory signaling pathways. A superior approach is to identify dual-target VEGFR inhibitors with favorable selectivity and dual inhibitory potency that simultaneously inhibit at least two synergistic targets.

Reassuringly, besides the traditional drug discovery strategies described above, a number of novel approaches have been used for rational and efficient drug design of dual-function inhibitors. Particularly, computation-based approaches provide an opportunity to develop new dual-target VEGFR inhibitors. These strategies promote the identification of potentially rational target combinations of dual-target VEGFR inhibitors via predicting structural similarity between active sites of VEGFR and other tumor-related targets or reliable analyses of relevant signaling pathways. Additionally, structure- and ligand-based drug designs (SBDD and LBDD) have been widely applied in the development of dual-target lead compounds containing novel scaffolds and the molecular optimization of dual-target inhibitors [151]. Notably, artificial intelligence (AI) is an emerging trend in drug discovery. With the advanced development of technologies of AI, multiple approaches such as high-quality datasets, new hypotheses and machine learning models, and new algorithms have been developed and applied in the identification of dual-target VEGFR inhibitors [152]. Finally, the field of structural biology has encountered numerous technological breakthroughs. Consequently, a number of high-resolution structures of ligand–protein complexes have recently been obtained and provide a comprehensive overview of the molecular mechanisms of ligand–protein interactions. These findings afford insight into the structural modification via structure-based drug discovery and provide a structural basis for the identification of dual-target inhibitors.

Collectively, we highlighted the progress made in the development of dual-target VEGFR inhibitors to assess the physiological functions and morbid implications of relevant targets and discussed challenges and future directions in the discovery and rational design of more potent dual-target inhibitors.

Abbreviations

AI: Artificial intelligence; AKT: Protein kinase B; BC: Breast cancer; BRAF: B-rapidly accelerated fibrosarcoma; DDIs: Drug–drug interactions; EGFR: Epidermal growth factor receptor; ERα: Estrogen receptor alpha; ERK1/2: Extracellular signal-regulated kinase ½; FDA: Food and Drug Administration; FGFR: Fibroblast growth factor receptor; HDAC: Histone deacetylase; LBDD: Ligand-based drug design; PARP: Poly ADP-ribose polymerase; PDGFR: Platelet-derived growth factor receptor; PIM1: Recombinant Pim-1 oncogene; SAR: Structure–activity relationship; SERM: Estrogen receptor modulator; TNBC: Triple-negative breast cancer.

Acknowledgements

Not applicable.

Author contributions

FW, JZ and LC conceived the project and supervised the project. YL, YL and YW summed up the literature, drafted the manuscript and drew the figures. LC and DZ collected and organized the inhibitors. JC and FW proofread the structures and figures. YO, JZ and LC revised the manuscript. All authors approved the final manuscript.

Funding

This review was supported by the National Natural Science Foundation of China (81903502, 81922064, 81874290); Sichuan Science and Technology Program (2019YFS0003); and National Major Scientific and Technological Special Project for Significant New Drugs Development (2018ZX09201018-021).

Availability of data and materials

The material supporting the conclusion of this review has been included within the article.

Declarations

Ethics approval and consent to participate

This is not applicable for this review.

Consent for publication

This is not applicable for this review.

Competing interests

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author details

[1]Department of Neurology, Joint Research Institution of Altitude Health, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China. [2]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital of Sichuan University, Chengdu 610041, Sichuan, China. [3]Targeted Tracer Research and Development Laboratory, Frontiers Science Center for Disease-Related Molecular Network, Institute of Respiratory Health, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China. [4]Department of Pharmacy, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China.

Received: 3 May 2022 Accepted: 30 June 2022
Published online: 07 July 2022

References

1. Viallard C, Larrivée B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis. 2017;20:409–26.
2. Sato Y. Persistent vascular normalization as an alternative goal of anti-angiogenic cancer therapy. Cancer Sci. 2011;102:1253–6.
3. Li Y, Yang G, Zhang J, Tang P, Yang C, Wang G, Chen J, Liu J, Zhang L, Ouyang L. Discovery, synthesis, and evaluation of highly selective vascular endothelial growth factor receptor 3 (VEGFR3) inhibitor for the potential treatment of metastatic triple-negative breast cancer. J Med Chem. 2021;64:12022–48.
4. Li Y, Wang G, Liu J, Ouyang L. Quinolizidine alkaloids derivatives from Sophora alopecuroides Linn: bioactivities, structure-activity relationships and preliminary molecular mechanisms. Eur J Med Chem. 2020;188: 111972.
5. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to modulate antitumor immunity. Front Immunol. 2018;9:978.
6. Liu G, Chen T, Ding Z, Wang Y, Wei Y, Wei X. Inhibition of FGF-FGFR and VEGF-VEGFR signalling in cancer treatment. Cell Prolif. 2021;54: e13009.
7. Li Y, Yang G, Yang C, Tang P, Chen J, Zhang J, Liu J, Ouyang L. Targeting autophagy-related epigenetic regulators for cancer drug discovery. J Med Chem. 2021;64:11798–815.
8. Yang JG, Wang LL, Ma DC. Effects of vascular endothelial growth factors and their receptors on megakaryocytes and platelets and related diseases. Br J Haematol. 2018;180:321–34.
9. Iwamoto M, Saso W, Sugiyama R, Ishii K, Ohki M, Nagamori S, Suzuki R, Aizaki H, Ryo A, Yun JH, Park SY, Ohtani N, Muramatsu M, Iwami S, Tanaka Y, Sureau C, Wakita T, Watashi K. Epidermal growth factor receptor is a host-entry cofactor triggering hepatitis B virus internalization. Proc Natl Acad Sci U S A. 2019;116:8487–92.
10. Imoukhuede PI, Popel AS. Quantification and cell-to-cell variation of vascular endothelial growth factor receptors. Exp Cell Res. 2011;317:955–65.
11. Estrada CC, Maldonado A, Mallipattu SK. Therapeutic inhibition of VEGF signaling and associated nephrotoxicities. J Am Soc Nephrol. 2019;30:187–200.
12. Roskoski R Jr. Vascular endothelial growth factor (VEGF) and VEGF receptor inhibitors in the treatment of renal cell carcinomas. Pharmacol Res. 2017;120:116–32.
13. Roskoski R Jr. Properties of FDA-approved small molecule protein kinase inhibitors: a 2021 update. Pharmacol Res. 2021;165: 105463.
14. Bhanumathy KK, Balagopal A, Vizeacoumar FS, Vizeacoumar FJ, Freywald A, Giambra V. Protein tyrosine kinases: their roles and their targeting in leukemia. Cancers. 2021;13(2):184. https://doi.org/10.3390/cancers13020184.
15. Liao M, Zhang J, Wang G, Wang L, Liu J, Ouyang L, Liu B. Small-molecule drug discovery in triple negative breast cancer: current situation and future directions. J Med Chem. 2021;64:2382–418.
16. Abdeldayem A, Raouf YS, Constantinescu SN, Moriggl R, Gunning PT. Advances in covalent kinase inhibitors. Chem Soc Rev. 2020;49:2617–87.
17. Abdel-Mohsen HT, Abd El-Meguid EA, El Kerdawy AM, Mahmoud AEE, Ali MM. Design, synthesis, and molecular docking of novel 2-arylbenzothiazole multiangiokinase inhibitors targeting breast cancer. Arch Pharm (Weinheim). 2020;353: e1900340.
18. Somwar R, Hofmann NE, Smith B, Odintsov I, Vojnic M, Linkov I, Tam A, Khodos I, Mattar MS, de Stanchina E, Flynn D, Ladanyi M, Drilon A, Shinde U, Davare MA. NTRK kinase domain mutations in cancer variably impact sensitivity to type I and type II inhibitors. Commun Biol. 2020;3:776.
19. Staben ST, Feng JA, Lyle K, Belvin M, Boggs J, Burch JD, Chua CC, Cui H, DiPasquale AG, Friedman LS, Heise C, Koeppen H, Kotey A, Mintzer R, Oh A, Roberts DA, Rouge L, Rudolph J, Tam C, Wang W, Xiao Y, Young A, Zhang Y, Hoeflich KP. Back pocket flexibility provides group II p21-activated kinase (PAK) selectivity for type I 1/2 kinase inhibitors. J Med Chem. 2014;57:1033–45.
20. Tuley A, Fast W. The taxonomy of covalent inhibitors. Biochemistry. 2018;57:3326–37.
21. Khanna P, Soh HJ, Chen CH, Saxena R, Amin S, Naughton M, Joslin PN, Moore A, Bakouny Z, O'Callaghan C, Catalano P, Signoretti S, McKay R, Choueiri TK, Bhasin M, Walther T. Bhatt RS. ACE2 abrogates tumor resistance to VEGFR inhibitors suggesting angiotensin-(1–7) as a therapy for clear cell renal cell carcinoma. Sci Transl Med. 2021;13:eabc0170.
22. Liang W, Zheng Y, Zhang J, Sun X. Multiscale modeling reveals angiogenesis-induced drug resistance in brain tumors and predicts a synergistic drug combination targeting EGFR and VEGFR pathways. BMC Bioinformatics. 2019;20:203.
23. De Lisi D, De Giorgi U, Lolli C, Schepisi G, Conteduca V, Menna C, Tonini G, Santini D, Farolfi A. Lenvatinib in the management of metastatic renal cell carcinoma: A promising combination therapy? Expert Opin Drug Metab Toxicol. 2018;14:461–7.
24. Liu T, Wang Y, Wang J, Ren C, Chen H, Zhang J. DYRK1A inhibitors for disease therapy: current status and perspectives. Eur J Med Chem. 2022;229: 114062.
25. Zhu AX, Kang YK, Rosmorduc O, Evans TR, Santoro A, Ross P, Gane E, Vogel A, Jeffers M, Meinhardt G, Peña CE. Biomarker analyses of clinical outcomes in patients with advanced hepatocellular carcinoma treated with sorafenib with or without erlotinib in the SEARCH Trial. Clin Cancer Res. 2016;22:4870–9.
26. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, Pouliot F, Alekseev B, Soulières D, Melichar B, Vynnychenko I, Kryzhanivska A, Bondarenko I, Azevedo SJ, Borchiellini D, Szczylik C, Markus M, McDermott RS, Bedke J, Tartas S, Chang YH, Tamada S, Shou Q, Perini RF, Chen M, Atkins MB, Powles T. KEYNOTE-426 Investigators. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med. 2019;380:1116–27.
27. Motzer RJ, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J, Nathan P, Staehler M, de Souza P, Merchan JR, Boleti E, Fife K, Jin J, Jones R, Uemura H, De Giorgi U, Harmenberg U, Wang J, Sternberg CN, Deen K, McCann L, Hackshaw MD, Crescenzo R, Pandite LN, Choueiri TK. Pazopanib versus sunitinib in metastatic renal-cell carcinoma. N Engl J Med. 2013;369:722–31.
28. Wells SA Jr, Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, Baudin E, Elisei R, Jarzab B, Vasselli JR, Read J, Langmuir P, Ryan AJ, Schlumberger MJ. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol. 2012;30:134–41.
29. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, Pouliot F, Alekseev B, Soulières D, Melichar B, Vynnychenko I, Kryzhanivska A, Bondarenko I, Azevedo SJ, Borchiellini D, Szczylik C, Markus M, McDermott RS, Bedke J, Tartas S, Chang YH, Tamada S, Shou Q, Perini RF, Chen M, Atkins MB, Powles T. KEYNOTE-426 investigators. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med. 2019;380:1116–27.
30. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, Cicin I, Merle P, Chen Y, Park JW, Blanc JF, Bolondi L, Klümpen HJ, Chan SL, Zagonel V, Pressiani T, Ryu MH, Venook AP, Hessel C, Borgman-Hagey AE, Schwab G, Kelley RK. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. N Engl J Med. 2018;379:54–63.
31. Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, Habra MA, Newbold K, Shah MH, Hoff AO, Gianoukakis AG, Kiyota N, Taylor MH, Kim SB, Krzyzanowska MK, Dutcus CE, de las Heras B, Zhu J, Sherman SI. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med. 2015;372:621–30.
32. Fukuoka S, Hara H, Takahashi N, Kojima T, Kawazoe A, Asayama M, Yoshii T, Kotani D, Tamura H, Mikamoto Y, Hirano N, Wakabayashi M, Nomura S, Sato A, Kuwata T, Togashi Y, Nishikawa H, Shitara K. Regorafenib plus Nivolumab in patients with advanced gastric or colorectal cancer: an open-label, dose-escalation, and dose-expansion phase Ib trial (REGONIVO, EPOC1603). J Clin Oncol. 2020;38:2053–61.
33. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre PD, Paquette R, Chuah C, Nicolini FE, Apperley JF, Khoury HJ, Talpaz M, DeAngelo DJ, Abruzzese E, Rea D, Baccarani M, Müller MC, Gambacorti-Passerini C, Lustgarten S, Rivera VM, Haluska FG, Guilhot F, Deininger MW, Hochhaus A, Hughes TP, Shah NP, Kantarjian HM. Ponatinib efficacy and safety in Philadelphia chromosome-positive leukemia: final 5-year results of the phase 2 PACE trial. Blood. 2018;132:393–404.

34. Hui R, Pearson A, Cortes J, Campbell C, Poirot C, Azim HA Jr, Fumagalli D, Lambertini M, Daly F, Arahmani A, Perez-Garcia J, Aftimos P, Bedard PL, Xuereb L, Scheepers ED, Vicente M, Goulioti T, Loibl S, Loi S, Pierrat MJ, Turner NC, Andre F, Curigliano G. Lucitanib for the treatment of HR^{+}/$HER2^{-}$ metastatic breast cancer: results from the multicohort phase II FINESSE study. Clin Cancer Res. 2020;26:354–63.
35. De Boer RH, Kotasek D, White S, Koczwara B, Mainwaring P, Chan A, Melara R, Ye Y, Adewoye AH, Sikorski R, Kaufman PA. Phase 1b dose-finding study of motesanib with docetaxel or paclitaxel in patients with metastatic breast cancer. Breast Cancer Res Treat. 2012;135:241–52.
36. Jost LM, Gschwind HP, Jalava T, Wang Y, Guenther C, Souppart C, Rottmann A, Denner K, Waldmeier F, Gross G, Masson E, Laurent D. Metabolism and disposition of vatalanib (PTK787/ZK-222584) in cancer patients. Drug Metab Dispos. 2006;34:1817–28.
37. Colli LM, Machiela MJ, Zhang H, Myers TA, Jessop L, Delattre O, Yu K, Chanock SJ. Landscape of combination immunotherapy and targeted therapy to improve cancer management. Cancer Res. 2017;77:3666–71.
38. Choueiri TK, Motzer RJ. Systemic therapy for metastatic renal-cell carcinoma. N Engl J Med. 2017;376:354–66.
39. Finn RS, Zhu AX. Evolution of systemic therapy for hepatocellular carcinoma. Hepatology. 2021;73:150–7.
40. Martin NT, Bell JC. Oncolytic virus combination therapy: killing one bird with two stones. Mol Ther. 2018;26:1414–22.
41. Zhu P, Hu C, Hui K, Jiang X. The role and significance of VEGFR2+ regulatory T cells in tumor immunity. Onco Targ Ther. 2017;10:4315–9.
42. Zhao L, Chen HY, Lu L, Wang L, Zhang XK, Guo XL. New insights into the role of co-receptor neuropilins in tumour angiogenesis and lymphangiogenesis and targeted therapy strategies. J Drug Target. 2021;29:155–67.
43. Prasad CB, Singh D, Pandey LK, Pradhan S, Singh S, Narayan G. VEGFa/VEGFR2 autocrine and paracrine signaling promotes cervical carcinogenesis via β-catenin and snail. Int J Biochem Cell Biol. 2022;142:106122.
44. Gao F, Yang C. Anti-VEGF/VEGFR2 monoclonal antibodies and their combinations with PD-1/PD-L1 inhibitors in clinic. Curr Cancer Drug Targets. 2020;20:3–18.
45. Lai S, Chen JN, Huang HW, Zhang XY, Jiang HL, Li W, Wang PL, Wang J, Liu FN. Structure activity relationships of chrysoeriol and analogs as dual c-Met and VEGFR2 tyrosine kinase inhibitors. Oncol Rep. 2018;40:1650–6.
46. Marijon H, Faivre S, Raymond E. Thérapies ciblées des carcinomes hépatocellulaires: progrès récents et futurs développements [Targeted therapies in hepatocellular carcinomas: recent results and future development]. Bull Cancer. 2009;96:553–61.
47. Shi L, Zhou J, Wu J, Shen Y, Li X. Anti-angiogenic therapy: Strategies to develop potent VEGFR-2 tyrosine kinase inhibitors and future prospect. Curr Med Chem. 2016;23:1000–40.
48. Won E, Basunia A, Chatila WK, Hechtman JF, Chou JF, Ku GY, Chalasani SB, Boyar MS, Goldberg Z, Desai AM, Tuvy Y, Berger MF, Tang L, Kelsen DP, Schattner M, Ilson DH, Capanu M, Solit DB, Schultz N, Janjigian YY. Efficacy of combined VEGFR1-3, PDGFα/β, and FGFR1-3 blockade using nintedanib for esophagogastric cancer. Clin Cancer Res. 2019;25:3811–7.
49. Moehler M, Gepfner-Tuma I, Maderer A, Thuss-Patience PC, Ruessel J, Hegewisch-Becker S, Wilke H, Al-Batran SE, Rafiyan MR, Weißinger F, Schmoll HJ, Kullmann F, von Weikersthal LF, Siveke JT, Weusmann J, Kanzler S, Schimanski CC, Otte M, Schollenberger L, Koenig J, Galle PR. Sunitinib added to FOLFIRI versus FOLFIRI in patients with chemorefractory advanced adenocarcinoma of the stomach or lower esophagus: a randomized, placebo-controlled phase II AIO trial with serum biomarker program. BMC Cancer. 2016;16:699.
50. Kurzrock R, Ball DW, Zahurak ML, Nelkin BD, Subbiah V, Ahmed S, O'Connor A, Karunsena E, Parkinson RM, Bishop JA, Ha Y, Sharma R, Gocke CD, Zinner R, Rudek MA, Sherman SI, Azad NS. A phase I trial of the VEGF receptor tyrosine kinase inhibitor pazopanib in combination with the MEK inhibitor trametinib in advanced solid tumors and differentiated thyroid cancers. Clin Cancer Res. 2019;25:5475–84.
51. Zebisch A, Caraffini V, Sill H. RAF kinase inhibitor protein in myeloid leukemogenesis. Int J Mol Sci. 2019;20:5756.
52. Yaeger R, Corcoran RB. Targeting alterations in the RAF-MEK pathway. Cancer Discov. 2019;9:329–41.
53. Moore M, Hirte HW, Siu L, Oza A, Hotte SJ, Petrenciuc O, Cihon F, Lathia C, Schwartz B. Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43–9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. Ann Oncol. 2005;16:1688–94.
54. Brose MS, Cabanillas ME, Cohen EE, Wirth LJ, Riehl T, Yue H, Sherman SI, Sherman EJ. Vemurafenib in patients with BRAF(V600E)-positive metastatic or unresectable papillary thyroid cancer refractory to radioactive iodine: a non-randomised, multicentre, open-label, phase 2 trial. Lancet Oncol. 2016;17:1272–82.
55. Izar B, Sharfman W, Hodi FS, Lawrence D, Flaherty KT, Amaravadi R, Kim KB, Puzanov I, Sosman J, Dummer R, Goldinger SM, Lam L, Kakar S, Tang Z, Krieter O, McDermott DF, Atkins MB. A first-in-human phase I, multicenter, open-label, dose-escalation study of the oral RAF/VEGFR-2 inhibitor (RAF265) in locally advanced or metastatic melanoma independent from BRAF mutation status. Cancer Med. 2017;6:1904–14.
56. Tavallai S, Hamed HA, Grant S, Poklepovic A, Dent P. Pazopanib and HDAC inhibitors interact to kill sarcoma cells. Cancer Biol Ther. 2014;15:578–85.
57. Chan D, Zheng Y, Tyner JW, Chng WJ, Chien WW, Gery S, Leong G, Braunstein GD, Koeffler HP. Belinostat and panobinostat (HDACI): in vitro and in vivo studies in thyroid cancer. J Cancer Res Clin Oncol. 2013;139:1507–14.
58. Fu S, Hou MM, Naing A, Janku F, Hess K, Zinner R, Subbiah V, Hong D, Wheler J, Piha-Paul S, Tsimberidou A, Karp D, Araujo D, Kee B, Hwu P, Wolff R, Kurzrock R, Meric-Bernstam F. Phase I study of pazopanib and vorinostat: a therapeutic approach for inhibiting mutant p53-mediated angiogenesis and facilitating mutant p53 degradation. Ann Oncol. 2015;26:1012–8.
59. Aggarwal R, Thomas S, Pawlowska N, Bartelink I, Grabowsky J, Jahan T, Cripps A, Harb A, Leng J, Reinert A, Mastroserio I, Truong TG, Ryan CJ, Munster PN. Inhibiting histone deacetylase as a means to reverse resistance to angiogenesis inhibitors: phase I study of abexinostat plus pazopanib in advanced solid tumor malignancies. J Clin Oncol. 2017;35:1231–9.
60. Chen H, Lin Z, Arnst KE, Miller DD, Li W. Tubulin inhibitor-based antibody-drug conjugates for cancer therapy. Molecules. 2017;22:1281.
61. Cesca M, Morosi L, Berndt A, Fuso Nerini I, Frapolli R, Richter P, Decio A, Dirsch O, Micotti E, Giordano S, D'Incalci M, Davoli E, Zucchetti M, Giavazzi R. Bevacizumab-induced inhibition of angiogenesis promotes a more homogeneous intratumoral distribution of paclitaxel, improving the antitumor response. Mol Cancer Ther. 2016;15:125–35.
62. Patel RR, Sengupta S, Kim HR, Klein-Szanto AJ, Pyle JR, Zhu F, Li T, Ross EA, Oseni S, Fargnoli J, Jordan VC. Experimental treatment of oestrogen receptor (ER) positive breast cancer with tamoxifen and brivanib alaninate, a VEGFR-2/FGFR-1 kinase inhibitor: a potential clinical application of angiogenesis inhibitors. Eur J Cancer. 2010;46:1537–53.
63. Xu J, Zhang T, Wang T, You L, Zhao Y. PIM kinases: an overview in tumors and recent advances in pancreatic cancer. Future Oncol. 2014;10:865–76.
64. Casillas AL, Toth RK, Sainz AG, Singh N, Desai AA, Kraft AS, Warfel NA. Hypoxia-inducible PIM kinase expression promotes resistance to antiangiogenic agents. Clin Cancer Res. 2018;24:169–80.
65. Toth RK, Solomon R, Warfel NA. Stabilization of PIM kinases in hypoxia is mediated by the deubiquitinase USP28. Cells. 2022;11:1006.
66. Hu L, Fan M, Shi S, Song X, Wang F, He H, Qi B. Dual target inhibitors based on EGFR: promising anticancer agents for the treatment of cancers (2017-). Eur J Med Chem. 2022;227: 113963.
67. Meric-Bernstam F, Larkin J, Tabernero J, Bonini C. Enhancing anti-tumour efficacy with immunotherapy combinations. Lancet. 2021;397:1010–22.
68. Wang X, Song K, Li L, Chen L. Structure-based drug design strategies and challenges. Curr Top Med Chem. 2018;18:998–1006.
69. Langedijk J, Mantel-Teeuwisse AK, Slijkerman DS, Schutjens MH. Drug repositioning and repurposing: terminology and definitions in literature. Drug Discov Today. 2015;20:1027–34.
70. Seidel T, Schuetz DA, Garon A, Langer T. The pharmacophore concept and its applications in computer-aided drug design. Prog Chem Org Nat Prod. 2019;110:99–141.

71. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015;20:13384–421.
72. Yang SY. Pharmacophore modeling and applications in drug discovery: challenges and recent advances. Drug Discov Today. 2010;15:444–50.
73. Basak SC, Bhattacharjee AK. Computational approaches for the design of mosquito repellent chemicals. Curr Med Chem. 2020;27:32–41.
74. Paul MD, Hristova K. Interactions between ligand-bound EGFR and VEGFR2. J Mol Biol. 2021;433: 167006.
75. Sangande F, Julianti E, Tjahjono DH. Ligand-based pharmacophore modeling, molecular docking, and molecular dynamic studies of dual tyrosine kinase inhibitor of EGFR and VEGFR2. Int J Mol Sci. 2020;21:7779.
76. Khan MI, Rath S, Adhami VM, Mukhtar H. Hypoxia driven glycation: mechanisms and therapeutic opportunities. Semin Cancer Biol. 2018;49:75–82.
77. Wei H, Duan Y, Gou W, Cui J, Ning H, Li D, Qin Y, Liu Q, Li Y. Design, synthesis and biological evaluation of novel 4-anilinoquinazoline derivatives as hypoxia-selective EGFR and VEGFR-2 dual inhibitors. Eur J Med Chem. 2019;181: 111552.
78. Bang KC, Song TH, Park YJ, Lee JS, Kim HH. Synthesis of 4-anilinoquinazoline-derivative dual kinase inhibitors targeting EGFR and VEGFR2. B Korean Chem Soc. 2018;39:123–5.
79. Sun S, Zhang J, Wang N, Kong X, Fu F, Wang H, Yao J. Design and discovery of quinazoline- and thiourea-containing sorafenib analogs as EGFR and VEGFR-2 dual TK inhibitors. Molecules. 2017;23:24.
80. Abd El-Meguid EA, Naglah AM, Moustafa GO, Awad HM, El Kerdawy AM. Novel benzothiazole-based dual VEGFR-2/EGFR inhibitors targeting breast and liver cancers: synthesis, cytotoxic activity, QSAR and molecular docking studies. Bioorg Med Chem Lett. 2022;58: 128529.
81. Mourad AAE, Farouk NA, El-Sayed EH, Mahdy ARE. EGFR/VEGFR-2 dual inhibitor and apoptotic inducer: design, synthesis, anticancer activity and docking study of new 2-thioxoimidazolidin-4one derivatives. Life Sci. 2021;277: 119531.
82. Brands RC, Knierim LM, De Donno F, Steinacker V, Hartmann S, Seher A, Kübler AC, Müller-Richter UDA. Targeting VEGFR and FGFR in head and neck squamous cell carcinoma in vitro. Oncol Rep. 2017;38:1877–85.
83. Katoh M. FGFR inhibitors: effects on cancer cells, tumor microenvironment and whole-body homeostasis (Review). Int J Mol Med. 2016;38:3–15.
84. Bhide RS, Cai ZW, Zhang YZ, Qian L, Wei D, Barbosa S, Lombardo LJ, Borzilleri RM, Zheng X, Wu LI, Barrish JC, Kim SH, Leavitt K, Mathur A, Leith L, Chao S, Wautlet B, Mortillo S, Jeyaseelan R Sr, Kukral D, Hunt JT, Kamath A, Fura A, Vyas V, Marathe P, D'Arienzo C, Derbin G, Fargnoli J. Discovery and preclinical studies of (R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5- methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan- 2-ol (BMS-540215), an in vivo active potent VEGFR-2 inhibitor. J Med Chem. 2006;49:2143–6.
85. Cai ZW, Zhang Y, Borzilleri RM, Qian L, Barbosa S, Wei D, Zheng X, Wu L, Fan J, Shi Z, Wautlet BS, Mortillo S, Jeyaseelan R Sr, Kukral DW, Kamath A, Marathe P, D'Arienzo C, Derbin G, Barrish JC, Robl JA, Hunt JT, Lombardo LJ, Fargnoli J, Bhide RS. Discovery of brivanib alaninate ((S)-((R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate), a novel prodrug of dual vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1 kinase inhibitor (BMS-540215). J Med Chem. 2008;51:1976–80.
86. Hofman J, Sorf A, Vagiannis D, Sucha S, Kammerer S, Küpper JH, Chen S, Guo L, Ceckova M, Staud F. Brivanib exhibits potential for pharmacokinetic drug-drug interactions and the modulation of multidrug resistance through the inhibition of human ABCG2 drug efflux transporter and CYP450 biotransformation enzymes. Mol Pharm. 2019;16:4436–50.
87. Bello E, Colella G, Scarlato V, Oliva P, Berndt A, Valbusa G, Serra SC, D'Incalci M, Cavalletti E, Giavazzi R, Damia G, Camboni G. E-3810 is a potent dual inhibitor of VEGFR and FGFR that exerts antitumor activity in multiple preclinical models. Cancer Res. 2011;71:1396–405.
88. Wei M, Peng X, Xing L, Dai Y, Huang R, Geng M, Zhang A, Ai J, Song Z. Design, synthesis and biological evaluation of a series of novel 2-benzamide-4-(6-oxy-N-methyl-1-naphthamide)-pyridine derivatives as potent fibroblast growth factor receptor (FGFR) inhibitors. Eur J Med Chem. 2018;154:9–28.
89. Holmström TH, Moilanen AM, Ikonen T, Björkman ML, Linnanen T, Wohlfahrt G, Karlsson S, Oksala R, Korjamo T, Samajdar S, Rajagopalan S, Chelur S, Narayanan K, Ramachandra RK, Mani J, Nair R, Gowda N, Anthony T, Dhodheri S, Mukherjee S, Ujjinamatada RK, Srinivas N, Ramachandra M, Kallio PJ. ODM-203, a selective inhibitor of FGFR and VEGFR, shows strong antitumor activity, and induces antitumor immunity. Mol Cancer Ther. 2019;18:28–38.
90. Bono P, Massard C, Peltola KJ, Azaro A, Italiano A, Kristeleit RS, Curigliano G, Lassen U, Arkenau HT, Hakulinen P, Garratt C, Ikonen T, Mustonen MVJ, Rodon JA. Phase I/IIa, open-label, multicentre study to evaluate the optimal dosing and safety of ODM-203 in patients with advanced or metastatic solid tumours. ESMO Open. 2020;5: e001081.
91. Yan W, Wang X, Dai Y, Zhao B, Yang X, Fan J, Gao Y, Meng F, Wang Y, Luo C, Ai J, Geng M, Duan W. Discovery of 3-(5′-Substituted)-Benzimidazole-5-(1-(3,5-dichloropyridin-4-yl)ethoxy)-1H-indazoles as potent fibroblast growth factor receptor inhibitors: design, synthesis, and biological evaluation. J Med Chem. 2016;59:6690–708.
92. Carvalho B, Lopes JM, Silva R, Peixoto J, Leitão D, Soares P, Fernandes AC, Linhares P, Vaz R, Lima J. The role of c-Met and VEGFR2 in glioblastoma resistance to bevacizumab. Sci Rep. 2021;11:6067.
93. Zhang Q, Zheng P, Zhu W. Research progress of small molecule VEGFR/c-Met inhibitors as anticancer agents (2016-Present). Molecules. 2020;25:2666.
94. Nakagawa T, Tohyama O, Yamaguchi A, Matsushima T, Takahashi K, Funasaka S, Shirotori S, Asada M, Obaishi H. E7050: a dual c-Met and VEGFR2 tyrosine kinase inhibitor promotes tumor regression and prolongs survival in mouse xenograft models. Cancer Sci. 2010;101:210–5.
95. García-Quiroz J, Cárdenas-Ochoa N, García-Becerra R, Morales-Guadarrama G, Méndez-Pérez EA, Santos-Cuevas C, Ramírez-Nava GJ, Segovia-Mendoza M, Prado-García H, Avila E, Larrea F, Díaz L. Antitumoral effects of dovitinib in triple-negative breast cancer are synergized by calcitriol in vivo and in vitro. J Steroid Biochem Mol Biol. 2021;214: 105979.
96. Salgia NJ, Zengin ZB, Pal SK. Tivozanib in renal cell carcinoma: a new approach to previously treated disease. Ther Adv Med Oncol. 2020;12:1758835920923818.
97. Kasikara C, Davra V, Calianese D, Geng K, Spires TE, Quigley M, Wichroski M, Sriram G, Suarez-Lopez L, Yaffe MB, Kotenko SV, De Lorenzo MS, Birge RB. Pan-TAM tyrosine kinase inhibitor BMS-777607 enhances anti-PD-1 mAb efficacy in a murine model of triple-negative breast cancer. Cancer Res. 2019;79:2669–83.
98. Padda S, Neal JW, Wakelee HA. MET inhibitors in combination with other therapies in non-small cell lung cancer. Transl Lung Cancer Res. 2012;1:238–53.
99. Torres MA, Raju U, Molkentine D, Riesterer O, Milas L, Ang KK. AC480, formerly BMS-599626, a pan her inhibitor, enhances radiosensitivity and radioresponse of head and neck squamous cell carcinoma cells in vitro and in vivo. Invest New Drugs. 2011;29:554–61.
100. Dong L, Meng F, Wu L, Mitchell AV, Block CJ, Zhang B, Craig DB, Jang H, Chen W, Yang Q, Wu G. Cooperative oncogenic effect and cell signaling crosstalk of co-occurring HER2 and mutant PIK3CA in mammary epithelial cells. Int J Oncol. 2017;51:1320–30.
101. Liu L, Siegmund A, Xi N, Kaplan-Lefko P, Rex K, Chen A, Lin J, Moriguchi J, Berry L, Huang L, Teffera Y, Yang Y, Zhang Y, Bellon SF, Lee M, Shimanovich R, Bak A, Dominguez C, Norman MH, Harmange JC, Dussault I, Kim TS. Discovery of a potent, selective, and orally bioavailable c-Met inhibitor: 1-(2-hydroxy-2-methylpropyl)-N-(5-(7-methoxyquinolin-4-yloxy)pyridin-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxamide (AMG 458). J Med Chem. 2008;51:3688–91.
102. Álvarez R, Aramburu L, Puebla P, Caballero E, González M, Vicente A, Medarde M, Peláez R. Pyridine based antitumour compounds acting at the colchicine site. Curr Med Chem. 2016;23:1100–30.
103. Qiang H, Gu W, Huang D, Shi W, Qiu Q, Dai Y, Huang W, Qian H. Design, synthesis and biological evaluation of 4-aminopyrimidine-5-cabaldehyde oximes as dual inhibitors of c-Met and VEGFR-2. Bioorg Med Chem. 2016;24:3353–8.
104. Zhao Y, Zhang J, Zhuang R, He R, Xi J, Pan X, Shao Y, Pan J, Sun J, Cai Z, Liu S, Huang W, Lv X. Synthesis and evaluation of a series of pyridine and pyrimidine derivatives as type II c-Met inhibitors. Bioorg Med Chem. 2017;25:3195–205.
105. Gu W, Dai Y, Qiang H, Shi W, Liao C, Zhao F, Huang W, Qian H. Discovery of novel 2-substituted-4-(2-fluorophenoxy) pyridine derivatives

possessing pyrazolone and triazole moieties as dual c-Met/VEGFR-2 receptor tyrosine kinase inhibitors. Bioorg Chem. 2017;72:116–22.
106. Wang MS, Zhuo LS, Yang FP, Wang WJ, Huang W, Yang GF. Synthesis and biological evaluation of new MET inhibitors with 1,6-naphthyridinone scaffold. Eur J Med Chem. 2020;185: 111803.
107. Liu J, Nie M, Wang Y, Hu J, Zhang F, Gao Y, Liu Y, Gong P. Design, synthesis and structure-activity relationships of novel 4-phenoxyquinoline derivatives containing 1,2,4-triazolone moiety as c-Met kinase inhibitors. Eur J Med Chem. 2016;123:431–46.
108. Liu J, Yang D, Yang X, Nie M, Wu G, Wang Z, Li W, Liu Y, Gong P. Design, synthesis and biological evaluation of novel 4-phenoxyquinoline derivatives containing 3-oxo-3,4-dihydroquinoxaline moiety as c-Met kinase inhibitors. Bioorg Med Chem. 2017;25:4475–86.
109. Nan X, Jiang YF, Li HJ, Wang JH, Wu YC. Design, synthesis and evaluation of sulfonylurea-containing 4-phenoxyquinolines as highly selective c-Met kinase inhibitors. Bioorg Med Chem. 2019;27:2801–12.
110. El-Gamal MI, Anbar HS. Recent advances of pyrrolopyridines derivatives: a patent and literature review. Expert Opin Ther Pat. 2017;27:591–606.
111. Zhu WF, Wang WH, Xu S, Tang QD, Luo R, Wang M, Gong P, Zheng PW. Design, synthesis, and docking studies of phenylpicolinamide derivatives bearing 1H-pyrrolo[2,3-b]pyridine moiety as c-Met inhibitors. Bioorg Med Chem. 2016;24:812–9.
112. Zhu WF, Wang WH, Xu S, Wang JQ, Tang QD, Wu CJ, Zhao YF, Zheng PW. Synthesis, and docking studies of phenylpyrimidine-carboxamide derivatives bearing 1H-pyrrolo[2,3-b]pyridine moiety as c-Met inhibitors. Bioorg Med Chem. 2016;24:1749–56.
113. Wang W, Xu S, Duan Y, Liu X, Li X, Wang C, Zhao B, Zheng P, Zhu W. Synthesis and bioevaluation and doking study of 1H-pyrrolo[2,3-b] pyridine derivatives bearing aromatic hydrazone moiety as c-Met inhibitors. Eur J Med Chem. 2018;145:315–27.
114. Wang LX, Liu X, Xu S, Tang Q, Duan Y, Xiao Z, Zhi J, Jiang L, Zheng P, Zhu W. Discovery of novel pyrrolo-pyridine/pyrimidine derivatives bearing pyridazinone moiety as c-Met kinase inhibitors. Eur J Med Chem. 2017;141:538–51.
115. Li J, Gu W, Bi X, Li H, Liao C, Liu C, Huang W, Qian H. Design, synthesis, and biological evaluation of thieno[2,3-d]pyrimidine derivatives as novel dual c-Met and VEGFR-2 kinase inhibitors. Bioorg Med Chem. 2017;25:6674–9.
116. Shi W, Qiang H, Huang D, Bi X, Huang W, Qian H. Exploration of novel pyrrolo[2,1-f][1,2,4]triazine derivatives with improved anticancer efficacy as dual inhibitors of c-Met/VEGFR-2. Eur J Med Chem. 2018;158:814–31.
117. Huang D, Huang L, Zhang Q, Li J. Synthesis and biological evaluation of novel 6,11-dihydro-5H-benzo[e]pyrimido- [5,4-b][1,4] diazepine derivatives as potential c-Met inhibitors. Eur J Med Chem. 2017;140:212–28.
118. Zhuo LS, Xu HC, Wang MS, Zhao XE, Ming ZH, Zhu XL, Huang W, Yang GF. 2,7-naphthyridinone-based MET kinase inhibitors: a promising novel scaffold for antitumor drug development. Eur J Med Chem. 2019;178:705–14.
119. Megally Abdo NY, Milad Mohareb R, Halim PA. Uses of cyclohexane-1,3-dione for the synthesis of 1,2,4-triazine derivatives as anti-proliferative agents and tyrosine kinases inhibitors. Bioorg Chem. 2020;97: 103667.
120. El-Nassan HB. Recent progress in the identification of BRAF inhibitors as anti-cancer agents. Eur J Med Chem. 2014;72:170–205.
121. Fu Y, Wang Y, Wan S, Li Z, Wang G, Zhang J, Wu X. Bisarylureas based on 1H-Pyrazolo[3,4-d]pyrimidine scaffold as novel pan-RAF Inhibitors with potent anti-proliferative activities: structure-based design, synthesis, biological evaluation and molecular modelling studies. Molecules. 2017;22:542.
122. Wang YY, Wan SH, Li ZH, Fu Y, Wang GF, Zhang JJ, Wu XY. Design, synthesis, biological evaluation and molecular modeling of novel 1H-pyrazolo[3,4-*d*]pyrimidine derivatives as BRAFV600E and VEGFR2 dual inhibitors. Eur J Med Chem. 2018;155:210–28.
123. Abdel-Mohsen HT, Omar MA, El Kerdawy AM, Mahmoud AEE, Ali MM, El Diwani HI. Novel potent substituted 4-amino-2-thiopyrimidines as dual VEGFR-2 and BRAF kinase inhibitors. Eur J Med Chem. 2019;179:707–22.
124. Peng X, Sun Z, Kuang P, Chen J. Recent progress on HDAC inhibitors with dual targeting capabilities for cancer treatment. Eur J Med Chem. 2020;208: 112831.
125. Peng FW, Xuan J, Wu TT, Xue JY, Ren ZW, Liu DK, Wang XQ, Chen XH, Zhang JW, Xu YG, Shi L. Design, synthesis and biological evaluation of N-phenylquinazolin-4-amine hybrids as dual inhibitors of VEGFR-2 and HDAC. Eur J Med Chem. 2016;109:1–12.
126. Lee S, Wang SW, Yu CL, Tai HC, Yen JY, Tuan YL, Wang HH, Liu YT, Chen SS, Lee HY. Effect of phenylurea hydroxamic acids on histone deacetylase and VEGFR-2. Bioorg Med Chem. 2021;50: 116454.
127. Zhang Y, Chen Y, Zhang D, Wang L, Lu T, Jiao Y. Discovery of novel potent VEGFR-2 inhibitors exerting significant antiproliferative activity against cancer cell lines. J Med Chem. 2018;61:140–57.
128. Zang J, Liang X, Huang Y, Jia Y, Li X, Xu W, Chou CJ, Zhang Y. Discovery of novel pazopanib-based HDAC and VEGFR dual inhibitors targeting cancer epigenetics and angiogenesis simultaneously. J Med Chem. 2018;61:5304–22.
129. Xue X, Zhang Y, Liao Y, Sun D, Li L, Liu Y, Wang Y, Jiang W, Zhang J, Luan Y, Zhao X. Design, synthesis and biological evaluation of dual HDAC and VEGFR inhibitors as multitargeted anticancer agents. Invest New Drugs. 2022;40:10–20.
130. Chen Q, Liu J, Sawada T, Wei C, Wu S, Han F. Possible role of EphA4 and VEGFR2 interactions in neural stem and progenitor cell differentiation. Exp Ther Med. 2020;19:1789–96.
131. Czeisler C, Mikawa T. Microtubules coordinate VEGFR2 signaling and sorting. PLoS ONE. 2013;8: e75833.
132. Gangjee A, Pavana RK, Ihnat MA, Thorpe JE, Disch BC, Bastian A, Bailey-Downs LC, Hamel E, Bai R. Discovery of antitubulin agents with antiangiogenic activity as single entities with multitarget chemotherapy potential. ACS Med Chem Lett. 2014;5:480–4.
133. Pavana RK, Choudhary S, Bastian A, Ihnat MA, Bai R, Hamel E, Gangjee A. Discovery and preclinical evaluation of 7-benzyl-N-(substituted)-pyrrolo[3,2-d]pyrimidin-4-amines as single agents with microtubule targeting effects along with triple-acting angiokinase inhibition as antitumor agents. Bioorg Med Chem. 2017;25:545–56.
134. Zhang X, Raghavan S, Ihnat M, Thorpe JE, Disch BC, Bastian A, Bailey-Downs LC, Dybdal-Hargreaves NF, Rohena CC, Hamel E, Mooberry SL, Gangjee A. The design and discovery of water soluble 4-substituted-2,6-dimethylfuro[2,3-d]pyrimidines as multitargeted receptor tyrosine kinase inhibitors and microtubule targeting antitumor agents. Bioorg Med Chem. 2014;22:3753–72.
135. Zhang X, Raghavan S, Ihnat M, Hamel E, Zammiello C, Bastian A, Mooberry SL, Gangjee A. The design, synthesis and biological evaluation of conformationally restricted 4-substituted-2,6-dimethylfuro[2,3-d] pyrimidines as multi-targeted receptor tyrosine kinase and microtubule inhibitors as potential antitumor agents. Bioorg Med Chem. 2015;23:2408–23.
136. Chekler EL, Kiselyov AS, Ouyang X, Chen X, Pattaropong V, Wang Y, Tuma MC, Doody JF. Discovery of dual VEGFR-2 and tubulin inhibitors with in vivo efficacy. ACS Med Chem Lett. 2010;1:488–92.
137. Nagini S. Breast cancer: current molecular therapeutic targets and new players. Anticancer Agents Med Chem. 2017;17:152–63.
138. Tang Z, Niu S, Liu F, Lao K, Miao J, Ji J, Wang X, Yan M, Zhang L, You Q, Xiao H, Xiang H. Synthesis and biological evaluation of 2,3-diaryl isoquinolinone derivatives as anti-breast cancer agents targeting ERα and VEGFR-2. Bioorg Med Chem Lett. 2014;24:2129–33.
139. Tang Z, Wu C, Wang T, Lao K, Wang Y, Liu L, Muyaba M, Xu P, He C, Luo G, Qian Z, Niu S, Wang L, Wang Y, Xiao H, You Q, Xiang H. Design, synthesis and evaluation of 6-aryl-indenoisoquinolone derivatives dual targeting ERα and VEGFR-2 as anti-breast cancer agents. Eur J Med Chem. 2016;118:328–39.
140. Liu L, Tang Z, Wu C, Li X, Huang A, Lu X, You Q, Xiang H. Synthesis and biological evaluation of 4,6-diaryl-2-pyrimidinamine derivatives as anti-breast cancer agents. Bioorg Med Chem Lett. 2018;28:1138–42.
141. Luo G, Tang Z, Lao K, Li X, You Q, Xiang H. Structure-activity relationships of 2, 4-disubstituted pyrimidines as dual ERα/VEGFR-2 ligands with anti-breast cancer activity. Eur J Med Chem. 2018;150:783–95.
142. Okamoto Y, Shibutani S. Development of novel and safer anti-breast cancer agents, SS1020 and SS5020, based on a fundamental carcinogenic research. Genes Environ. 2019;41:9.

143. Luo G, Li X, Zhang G, Wu C, Tang Z, Liu L, You Q, Xiang H. Novel SERMs based on 3-aryl-4-aryloxy-2H-chromen-2-one skeleton-a possible way to dual ERα/VEGFR-2 ligands for treatment of breast cancer. Eur J Med Chem. 2017;140:252–73.
144. Tursynbay Y, Zhang J, Li Z, Tokay T, Zhumadilov Z, Wu D, Xie Y. Pim-1 kinase as cancer drug target: an update. Biomed Rep. 2016;4:140–6.
145. Rizk OH, Teleb M, Abu-Serie MM, Shaaban OG. Dual VEGFR-2/PIM-1 kinase inhibition towards surmounting the resistance to antiangiogenic agents via hybrid pyridine and thienopyridine-based scaffolds: design, synthesis and biological evaluation. Bioorg Chem. 2019;92: 103189.
146. Frett B, Carlomagno F, Moccia ML, Brescia A, Federico G, De Falco V, Admire B, Chen Z, Qi W, Santoro M, Li HY. Fragment-based discovery of a dual pan-RET/VEGFR2 kinase inhibitor optimized for single-agent polypharmacology. Angew Chem Int Ed Engl. 2015;54:8717–21.
147. Moccia M, Frett B, Zhang L, Lakkaniga NR, Briggs DC, Chauhan R, Brescia A, Federico G, Yan W, Santoro M, McDonald NQ, Li HY, Carlomagno F. Bioisosteric discovery of NPA101.3, a second-generation RET/VEGFR2 inhibitor optimized for single-agent polypharmacology. J Med Chem. 2020;63:4506–16.
148. Abdelnaby RM, El-Malah AA, FakhrEldeen RR, Saeed MM, Nadeem RI, Younis NS, Abdel-Rahman HM, El-Dydamony NM. *In vitro* anticancer activity screening of novel fused thiophene derivatives as VEGFR-2/AKT dual inhibitors and apoptosis inducers. Pharmaceuticals (Basel). 2022;15:700.
149. Ibrahim N, Yu Y, Walsh WR, Yang JL. Molecular targeted therapies for cancer: sorafenib mono-therapy and its combination with other therapies (review). Oncol Rep. 2012;27:1303–11.
150. Liu JF, Barry WT, Birrer M, Lee JM, Buckanovich RJ, Fleming GF, Rimel B, Buss MK, Nattam S, Hurteau J, Luo W, Quy P, Whalen C, Obermayer L, Lee H, Winer EP, Kohn EC, Ivy SP, Matulonis UA. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. Lancet Oncol. 2014;15:1207–14.
151. Nayarisseri A. Experimental and computational approaches to improve binding affinity in chemical biology and drug discovery. Curr Top Med Chem. 2020;20:1651–60.
152. Chan HCS, Shan H, Dahoun T, Vogel H, Yuan S. Advancing drug discovery via artificial intelligence. Trends Pharmacol Sci. 2019;40:592–604.

Publisher's Note