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Review

# Newly discovered angiogenesis inhibitors and their mechanisms of action

Ze-hong MIAO*, Jian-ming FENG, Jian DING*

*Division of Antitumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

In the past decade, the success of angiogenesis inhibitors in clinical contexts has established the antiangiogenic strategy as an important part of cancer therapy. During that time period, we have discovered and reported 17 compounds that exert potent inhibition on angiogenesis. These compounds exhibit tremendous diversity in their sources, structures, targets and mechanisms. These studies have generated new models for further modification and optimization of inhibitory compounds, new information for mechanistic studies and a new drug candidate for clinical development. In particular, through studies on the antiangiogenic mechanism of pseudolaric acid B, we discovered a novel mechanism by which the stability of hypoxia-inducible factor 1α is regulated by the transcription factor c-Jun. We also completed a preclinical study of AL3810, a compound with the potential to circumvent tumor drug resistance to a certain extent. All of these findings will be briefly reviewed in this article.

**Keywords:** cancer therapy; angiogenesis inhibitor; antiangiogenic mechanism; hypoxia-inducible factor 1α; pseudolaric acid B; AL3810

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## Introduction

Angiogenesis drives tumor progression; in particular, angiogenesis is critical for the growth and metastasis of solid tumors[1–4]. The inhibition of tumor angiogenesis has therefore become an important strategy for cancer therapy. Several inhibitors of tumor angiogenesis, such as bevacizumab (Avastin), sorafenib (Nexavar) and sunitinib (Sutent), have been successfully used in the clinic to treat solid tumors[4]. This success has led to increased efforts to discover new angiogenesis inhibitors with different mechanisms of action and/or distinct chemical structures. During the past 10 years, we have discovered and reported on 17 compounds that demonstrate potent inhibition of angiogenesis (Table 1). In contrast to the inhibitors that are already employed for clinical purposes, these newly discovered antiangiogenic agents display intriguing diversity in their sources, chemical structures, antiangiogenic mechanisms, and molecular targets. This review will focus on these diverse characteristics of the 17 newly discovered antiangiogenic compounds, as well as the representative findings that have been produced by the mechanistic investigation and preclinical development of these compounds.

* To whom correspondence should be addressed.
E-mail zhmiao@mail.shcnc.ac.cn (Ze-hong MIAO);
jding@mail.shcnc.ac.cn (Jian DING)
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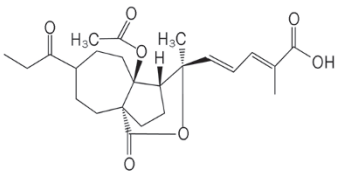
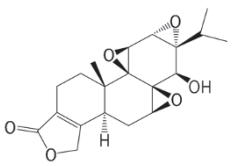
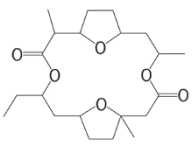
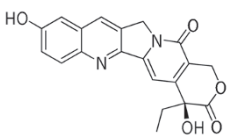
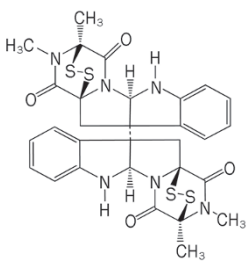
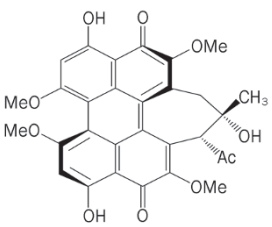
## An overview of the 17 antiangiogenic compounds

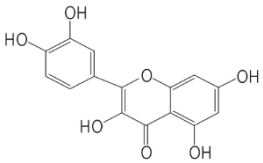
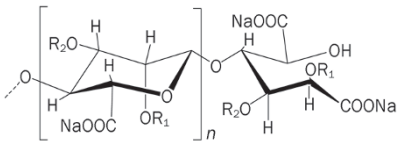
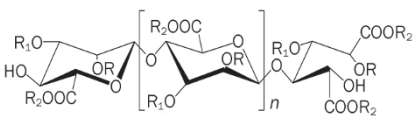
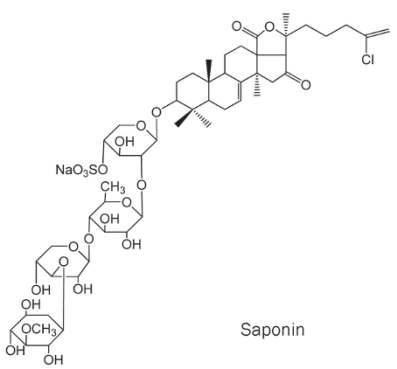
In a search for new angiogenesis inhibitors, we performed extensive evaluations of thousands of natural products and synthetic or semi-synthetic compounds over the course of the past 10 years. From this process, 17 compounds were discovered to possess antiangiogenic activity (Table 1). The mechanisms of action of several of these compounds have been investigated, and one of these compounds, namely, AL3810, has been registered as an IND (investigational new drug) for cancer therapy in China.

Analyses of these 17 compounds indicate their diverse sources (Table 1). In particular, 7 of these inhibitors are derived from terrestrial sources, and 5 of these 7 compounds (pseudolaric acid B, triptolide, 10-hydroxycamptothecin, 11,11′-dideoxyverticillin and shiraiachrome A) are used in traditional Chinese medicine (TCM). It is also notable that quercetin is present both in many fruits and vegetables and in olive oil, red wine and tea. Of the remaining 10 compounds that were identified, 5 are marine-derived inhibitors, 3 of which [JG3, MDOS and *Grateloupia longifolia* polysaccharide (GLP)] are saccharides and 2 of which are saponins from sea cucumber. The final 5 inhibitors are chemically synthesized; these synthesized compounds are also representative of a great diversity of chemical structures, including terpenoids, alkaloids, flavonoids, saccharides, saponins and pyridopyrimidines, as shown in Table 1.

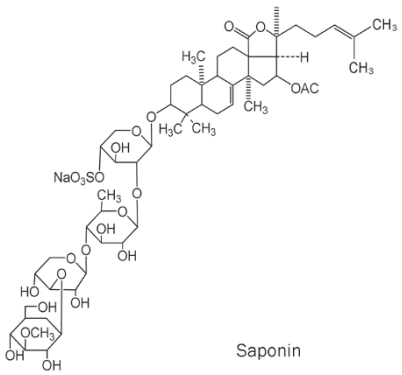
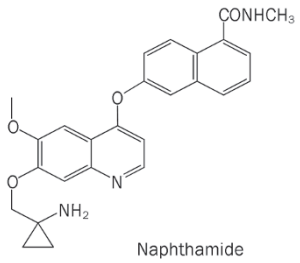
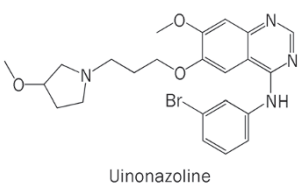
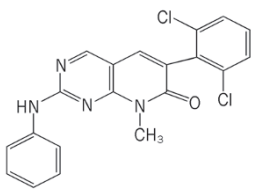
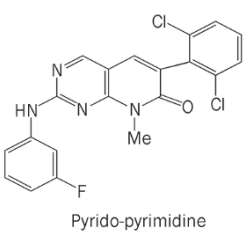
**Table 1.** Angiogenesis inhibitors discovered in the Shanghai Institute of Materia Medica since 2001.

| No | Inhibitors | Chemical structure | Origin | Targets | Antiangiogenic mechanisms | Refs |
|---|---|---|---|---|---|---|
| **Terrestrial natural products** | | | | | | |
| 1 | Pseudolaric acid B |  Iterpenoid | The root bark of *Pseudolarix amabilis*, a TCM | Microtubulin | 1. To increase the phosphorylated c-Jun while reducing the non-phosphorylated c-Jun at Ser63/73, which impairs its function in stabilizing HIF-1α;<br>2. To reduce HIF-1α protein by promoting its proteasome-mediated degradation;<br>3. To abrogate hypoxia-induced VEGF secretion via reducing HIF-1α protein. | [16, 18, 22, 23] |
| 2 | Triptolide |  Diterpenoid triepoxide | *Tripterygium wilfordii* Hook F, a TCM | XPB; RNAP II | 1. To increase the levels of HIF-1α mRNA, but to reduce its transcriptional function;<br>2. To decrease mRNA levels of HIF-1α target genes including VEGF, BNIP3, and CAIX;<br>3. To lower the secretion of VEGF protein, and to reduce sprout outgrowth. | [20, 21] |
| 3 | MFTZ-1 |  Macrolide | An endophyte *Streptomyces* sp. Is9131 of *Magnolia hookeri* | Topoisomerase II | 1. To reduce HIF-1α accumulation, irrelevant to its topoisomerase II inhibition;<br>2. To abrogate the HIF-1α-driven increase in VEGF mRNA;<br>3. To reduce constitutive, HIF-1α-independent VEGF secretion and concurrently antagonize inducible, HIF-1α-dependent VEGF secretion. | [17, 25] |
| 4 | 10-Hydroxy-camptothecin |  Alkaloid | *Camptotheca acuminata*, a TCM | Topoisomerase I | 1. To inhibit proliferation, migration and tube formation of HMEC cells;<br>2. To inhibit angiogenesis in CAM assays;<br>3. To elicit apoptosis in HMEC cells. | [19] |
| 5 | 11,11′-di-deoxyverti-cillin |  Indole alkaloid | The fungus *Shiraia bambusicola*, a TCM | VEGF<br>VEGFR | 1. To antagonize the antiapoptotic effects of VEGF, and to inhibit VEGF-induced HUVEC migration and tube formation;<br>2. To completely block VEGF-induced microvessel sprouting and vessel growth;<br>3. To decrease VEGF secretion and to suppress VEGF-induced tyrosine phosphorylation of Flt-1 and KDR/Flk-1. | [6] |
| 6 | Shiraia-chrome A |  Naphthoquinone | The fungus *Shiraia bambusicola*, a TCM | VEGFR-2; FGFR; PDGFR; EGFR | 1. To suppress the autophosphorylation of VEGFR-2, FGFR, PDGFR, and EGFR;<br>2. To inhibit the proliferation, migration, and tube formation of HMEC;<br>3. To inhibit the formation of new microvessels in a rat aorta culture model as well as in the CAM assay. | [7] |

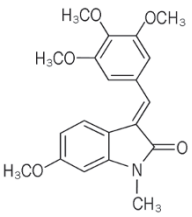
(To be continued)

| No | Inhibitors | Chemical structure | Origin | Targets | Antiangiogenic mechanisms | Refs |
|---|---|---|---|---|---|---|
| 7 | Quercetin |  Flavonoid | Many fruits and vegetables, as well as olive oil, red wine, and tea | MMP-2 | 1. To inhibit proliferation, migration, and tube formation of HMEC and HUVEC;<br>2. To display an antiangiogenic effect *in vivo*;<br>3. To decrease the expression and activity of MMP-2. | [29] |
| **Marine-derived natural products** | | | | | | |
| 8 | JG3 |  Sulfated oligosaccharide | Marine oligomannurarate blocks | Heparanase | 1. To combat heparanase activity via binding to the KKDC and QPLK domains of the heparanase molecule;<br>2. To abolish heparanase-driven invasion, and to inhibit the release of heparan sulfate–sequestered bFGF from the extracellular matrix, and to repress subsequent angiogenesis;<br>3. To inactivate bFGF-induced bFGFR and ERK1/2 phosphorylation and to block bFGF-triggered angiogenic events by binding to bFGF. | [27, 42] |
| 9 | MDOS |  R=$SO_3Na$, $R_1$=$SO_3Na$ or H, $R_2$=$CH_2CH(OH)CH_3$ or Na, *n*=2–8 Sulfated oligosaccharide | Marine oligomannurarate blocks | HER2; EGFR; VEGFR; PDGFR; c-Kit; FGFR1; c-Src | 1. To directly inhibit HER2, EGFR, VEGFR, PDGFR, c-Kit, FGFR1 and c-Src, with little impact on FGFR2;<br>2. To inhibit phosphorylation of PTKs, exemplified by HER2, EGFR and VEGFR2, and downstream molecules of Erk1/2 and AKT;<br>3. To act as an ATP competitive inhibitor via directly binding to the residues of entrance rather than those of the ATP-binding pocket. | [8] |
| 10 | *Grateloupia longifolia* polysaccharide | Sulphated polysaccharide (MW: $1.8\times10^6$) | The marine alga *G longifolia* | Tissue factor | 1. To decrease tissue factor at both mRNA and protein levels;<br>2. To inhibit proliferation of HMECs and HUVEC and tube formation and to reduce the number of migratory cells in a VEGF-independent manner;<br>3. To reduce new vessel formation and the vessel density in Matrigel plugs implanted in mice. | [30] |
| 11 | Philinopside E |  Saponin | Sea cucumber (*pentacta quadrangularis*) | KDR | 1. To inhibit KDR phosphorylation and downstream signaling;<br>2. To specifically interact with KDR extracellular domain, and to block its interaction with VEGF and the downstream signaling;<br>3. To markedly suppresses αvβ3 integrin-driven downstream signaling and as a result, to disturb the physical interaction between KDR and αvβ3 integrin in HMECs, followed by disruption of the actin cytoskeleton organization and decreased cell adhesion to vitronectin. | [9] |

(To be continued)

| No | Inhibitors | Chemical structure | Origin | Targets | Antiangiogenic mechanisms | Refs |
|---|---|---|---|---|---|---|
| 12 | Philinopside A |  Saponin | Sea cucumber (*pentacta quadrangularis*) | VEGFR; FGFR1; PDGFRβ; EGFR | 1. To inhibit the proliferation, migration and tube formation of HMECs;<br>2. To suppress the formation of new microvessels in cultured rat aorta and angiogenesis in CAM assays;<br>3. To inhibit VEGFR, FGFR1, PDGFRβ, and EGFR. | [10] |
| **Synthetic inhibitors** | | | | | | |
| 13 | AL3810 |  Naphthamide | Synthetic | VEGFR1; VEGFR2; FGFR1; PDGFRβ | 1. To inhibit VEGFR1, VEGFR2, FGFR1 and PDGFRβ;<br>2. To inhibit the autophosphorylation of VEGFR2, PDGFRβ, and FGFR1 in endothelial cells;<br>3. To exhibit potent antiangiogenesis activity, manifested by significant inhibition of microvessel outgrowth of rat arterial ring and CAM in *ex vivo* angiogenesis models. | [11, 31] |
| 14 | BB |  Uinonazoline | Synthetic | EGFR | 1. To selectively inhibit EGFR;<br>2. To abrogate autophosphorylation of the EGF-stimulated EGFR and phosphorylation of its key downstream signaling molecules ERK and AKT in A549 cells;<br>3. To exhibit antiangiogenesis activity, as evidenced by antagonizing EGF-induced HMECS migration *in vitro*, blocking HMECS tube formation, and inhibiting microvessel sprouting from rat aortic rings. | [12] |
| 15 | TKI-28 |  Pyrido-pyrimidine | Synthetic | ErbB-2; EGFR; KDR; PDGFRβ; c-kit; c-Src | 1. To inhibit ErbB-2, EGFR, KDR, PDGFRβ, c-kit and c-Src in cell-free systems;<br>2. To block their autophosphorylation and subsequently to downregulate phosphorylation of many downstream signaling proteins at the cellular level;<br>3. To inhibit cell proliferation driven by EGF, VEGF and PDGF, and cell migration and tube formation in HMECs. | [13] |
| 16 | TKI-31 |  Pyrido-pyrimidine | Synthetic | VEGFR2; PDGFRβ; c-kit; c-Src | 1. To inhibit VEGFR2, PDGFRβ, c-kit and c-Src, showing no activity against VEGFR1 and EGFR;<br>2. To repress VEGF-induced phosphorylation of VEGFR2 in endothelial cells and PDGFBB-induced phosphorylation in fibroblast cells, leading to the inhibition of PI3K/Akt/mTOR, MAPK42/44 (ERK) and paxillin; | [14] |

(To be continued)

| No | Inhibitors | Chemical structure | Origin | Targets | Antiangiogenic mechanisms | Refs |
|---|---|---|---|---|---|---|
| | | | | | 3. To suppress VEGF-induced endothelial cells proliferation, migration and their differentiation into capillary-like tube formation. | |
| 17 | C9 |  Indolinone | Synthetic | Microtubulin | 1. To inhibit proliferation, migration and tube formation of endothelial cells, and angiogenesis in aortic ring and CAM assays;<br>2. To induce disassembly of microtubules in endothelial cells and to downregulate Raf-MEK-ERK signaling activated by pro-angiogenic factors;<br>3. To disrupt capillary-like networks and newly formed vessels *in vitro* and to rapidly decrease perfusion of neovasculature *in vivo*, and to induce endothelial cell contraction and membrane blebbing in neovasculature dependent on the Rho/Rho kinase pathway. | [24] |

Abbreviations: BNIP3, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3; CAIX, carbonic anhydrase IX; CAM, chick chorioallantoic membrane; EGFR, epidermal growth factor receptor; ERK, extracellular signal regulated kinase; bFGF, basic fibroblast growth factor; FGFR1, FGF receptor 1; HER2, human epidermal growth factor receptor-2; HIF-1α, hypoxia-inducible factor 1alpha; HMEC, human dermal microvasculature endothelial cells; HUVEC, human umbilical vein endothelial cells; KDR, receptor for vascular endothelial growth factor; MEK, MAPK/ERK kinase; MMP-2, matrix metalloproteinase-2; PDGFBB, platelet derived growth factor BB; PDGFR, platelet-derived growth factor receptor; RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; TCM, traditional Chinese medicine.

The apparent differences in the sources and chemical structures of the 17 inhibitors are reflected in their distinct (potential) targets. These targets include angiogenic kinases (angiokinases), extracellular matrix (ECM) components and the hypoxia-inducible factor 1α (HIF-1α)-vascular endothelial growth factor (VEGF) axis, among others (Table 1, Figure 1). Moreover, the antiangiogenic activities of these compounds revealed in the *in vitro*, *ex vivo* and/or *in vivo* experimental models are mediated by distinct molecular signaling pathways (Figure 1).

### Angiokinase inhibitors

Although many protein tyrosine kinases (PTKs) contribute to the angiogenic process, the VEGF-VEGF receptor (VEGFR) axis is the main target for clinical applications of antiangiogenic therapy[5]. Of the 17 compounds listed in Table 1, 9 compounds, namely, 11,11′-dideoxyverticillin, shiraiachrome A, MDOS, philinopside A, philinopside E, AL3810, BB, TKI-28, and TKI-31, were found to directly inhibit this axis[6–14] (Table 1 and Figure 1). The former 5 compounds are derived from natural products, whereas the latter 4 inhibitors are synthetic. These 9 compounds display different profiles of PTK inhibition, as they have distinct selectivity against various receptor and/or non-receptor tyrosine kinases, including human epi-

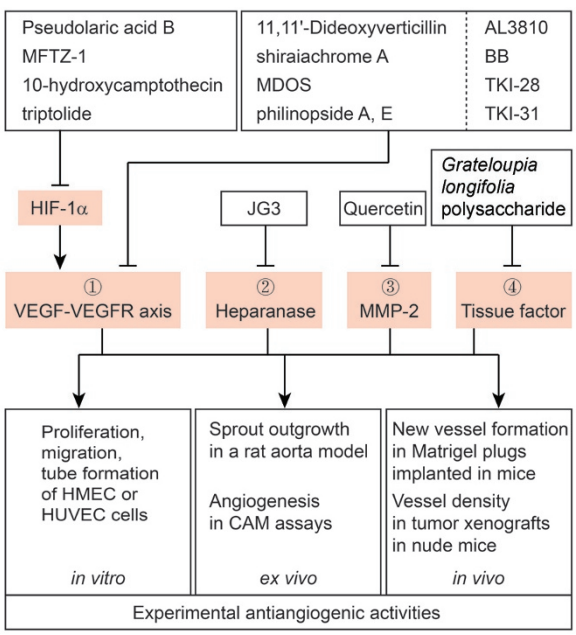



**Figure 1.** A schematic overview of molecular signaling that possibly mediates experimental antiangiogenic activities of the compounds discussed here.

dermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), VEGFR, platelet-derived growth factor receptor (PDGFR), c-Kit, fibroblast growth factor receptor 1 (FGFR1) and/or c-Src. Nevertheless, all 9 of these compounds can directly suppress the critical angiokinase VEGFR, producing significant experimentally observed antiangiogenic effects as a result (Table 1 and Figure 1). In particular, AL3810 has been in clinical trials because it not only displays excellent anticancer and antiangiogenic activities but also demonstrates good pharmacokinetics and toxicity in preclinical studies[15, 16].

### HIF-1α-VEGF axis inhibitors

We also found that of the 17 identified antiangiogenic compounds, 4 compounds, namely, pseudolaric acid B, MFTZ-1, 10-hydroxycamptothecin and triptolide, can indirectly inhibit the VEGF-VEGFR axis by decreasing cellular HIF-1α accumulation and thereby reducing VEGF expression and secretion[15–21] (Figure 1). We define these compounds to be HIF-1α-VEGF axis inhibitors. HIF-1α is a critical transcription factor that impacts tumor angiogenesis by regulating the expression of VEGF. HIF-1α has thus been proposed as a promising anticancer target.

The HIF-1α-VEGF axis inhibitors reduce the cellular amount of HIF-1α in different ways. Pseudolaric acid B targets microtubulin and causes its depolymerization[22, 23]. Pseudolaric acid B inhibits angiogenesis by reducing the stability of HIF-1α and thereby downregulating the VEGF-VEGFR axis[15, 16, 18]. However, there has been no direct evidence indicating any association between its antiangiogenic activity and its inhibition of microtubulin[23, 24]. By contrast, MFTZ-1 does not affect either the degradation of HIF-1α protein or the level of HIF-1α mRNA. Instead, MFTZ-1 can abrogate the HIF-1α-driven increase in VEGF mRNA and VEGF protein secretion, producing antiangiogenic effects. Specifically, MFTZ-1 can reduce constitutive, HIF-1α-independent VEGF secretion and concurrently antagonize inducible, HIF-1α-dependent VEGF secretion, in an effect that is independent of its inhibition of its primary target, topoisomerase II[17, 25]. The inhibition of angiogenesis by 10-hydroxycamptothecin[19] may be associated with this drug's suppression of HIF-1α expression, which occurs *via* the repression of topoisomerase I-dependent transcription. This mechanism of action is possibly similar to the mechanism that is used by topotecan, another camptothecin derivative[26]. In contrast to the previous 3 inhibitors, triptolide may enhance the levels of cellular HIF-1α mRNA and protein[20]. However, triptolide also causes the downregulation of VEGF expression and secretion[20], possibly because it binds to XPB (which is also known as ERCC3) and causes the degradation of RNA polymerase II[21], disrupting the transcriptional function of HIF-1α.

### ECM component inhibitors

ECM components including heparanase and matrix metalloproteinase (MMP) are critically involved in the metastatic and angiogenic capabilities of tumor cells. Inhibitors targeting ECM components are increasingly emerging as promising agents for cancer therapy. We have discovered a series of compounds that inhibit tumor angiogenesis by targeting heparanase and MMP. Oligomannurarate sulfate (JG3), a novel oligosaccharide, was identified as a heparanase inhibitor. JG3 significantly inhibits tumor angiogenesis and metastasis, both *in vitro* and *in vivo*, by combating heparanase activity; in particular, this effect is mediated through the binding of JG3 to the KKDC and QPLK domains of heparanase. In addition, JG3 abolished heparanase-driven invasion, inhibited the release of basic fibroblast growth factor (bFGF) from the ECM and repressed subsequent angiogenesis[27]. By contrast, the antiangiogenic effects of quercetin were found to be associated with its downregulation of MMP-2[28]. In fact, however, other studies reveal complicated molecular mechanisms involved in the antiangiogenic activity of quercetin, including its inhibition of the synthesis and accumulation of the HIF-1α protein; this inhibition reduces the production and secretion of VEGF, as discussed above[29].

### Other angiogenesis inhibitors

C9 and GLP exert antiangiogenic effects through relatively unique mechanisms of action (Table 1). C9 is a new microtubule-depolymerizing agent. In striking contrast to pseudolaric acid B, C9 elicits its antiangiogenic and vascular disrupting effects by inducing microtubule disassembly, the downregulation of Raf-MEK-ERK signaling and the reorganization of actin through the Rho/Rho kinase pathway[24]. GLP is a new type of polysaccharide isolated from the alga *G longifolia*. GLP causes obvious *in vitro* and *in vivo* antiangiogenic effects that are not associated with classical VEGF-VEGFR signaling. GLP decreases tissue factor at both the mRNA and protein levels, and this effect may be involved in the inhibition of angiogenesis by GLP[30].

Our findings provide the following beneficial clues to facilitate the future discovery of angiogenesis inhibitors: (1) Natural products are an important source of angiogenesis inhibitors. In particular, through drug discovery guided by therapeutic experiences from long-established TCM practices, inhibitors of angiogenesis could be obtained that have new chemical structures and unique mechanisms of action; examples of these sorts of inhibitors include pseudolaric acid B, triptolide, 11,11'-dideoxyverticillin and shiraiachrome A (Table 1). The original medicinal materials producing these compounds have long been used to either treat various angiogenesis-related diseases, such as rheumatoid arthritis, microbial skin diseases and psoriasis[7, 21, 23], or cause the early termination of pregnancies[23]. (2) Marine-derived compounds could be another important source of angiogenesis inhibitors, and certain compounds with specific types of chemical structures, such as saccharides, should be subjected to particular scrutiny; as demonstrated by JG3[27] and GLP[30], these compounds may exhibit unique modes of antiangiogenic activity. (3) Chemical synthesis, particularly if it is based on rational designs and modifications, is a common, frequently necessary method of generating compounds with possible medical applications; this principle is demonstrated by AL3810 in this instance[11, 31]

and is generally exemplified by a variety of clinically utilized drugs. (4) The compounds that we have discovered (Table 1) can be used as chemical models for further modification and optimization to improve their therapeutic potential as angiogenesis-inhibiting drugs for clinical use. (5) The known primary target(s) of certain compounds, such as pseudolaric acid B[15, 16, 18] and MFTZ-1[17], are not necessarily related to their antiangiogenic effects, indicating that unknown and potentially novel mechanism(s) are involved in these effects; these mechanisms merit further investigation.

## The discovery that the stability of HIF-1α protein is regulated by c-Jun based on mechanistic studies of the antiangiogenic activity of pseudolaric acid B

Pseudolaric acid B is a diterpenoid isolated from the root bark of *Pseudolarix amabilis*[32]. Pseudolaric acid B has been demonstrated to both elicit potent anticancer effects by depolymerizing microtubulin[22, 23] and circumvent tumor multidrug resistance[22]. Detailed structure-activity studies have revealed that the components of pseudolaric acid B that are essential to its anticancer activity include a hydrophobic group (-$CO_2$ Me or -Me) at C-7, a $\Delta^7$ double bond, an acyloxy (OAc) at C-4,3 and a side chain with a conjugated double bond and a hydrophilic terminal group[33]. Based on its traditional use in Chinese folk medicine for facilitating the early termination of pregnancy, we first discovered and reported that its antiangiogenic activity occurred because it accelerates the proteasome-executed degradation of the HIF-1α protein[18].

We previously reported that the activation of the transcription factor c-Jun plays a critical role in the circumvention of tumor multidrug resistance by salvicine[34, 35]. Based on those earlier findings, we investigated the effect of pseudolaric acid B and found that it could also drive c-Jun phosphorylation[16]. During our attempts to correlate HIF-1α protein degradation with the c-Jun phosphorylation induced by pseudolaric acid B, a novel mechanism was revealed in which c-Jun in its non-phosphorylated form regulates the stability of the HIF-1α protein[15, 16] (Figure 2).

HIF-1α is a transcription factor that drives neoangiogenesis by regulating the expression of various target genes, including the proangiogenic genes VEGF and VEGFR, in response to hypoxia during the growth of solid tumors[36]. The ubiquitination-mediated, proteasome-executed degradation constitutes a critical methanism of regulating the stability of the cellular HIF-1α protein[37]. HIF-1α can be hydroxylated by an oxygen-sensitive prolyl hydroxylase at the Pro402 and Pro564 residues within its oxygen-dependent degradation domain (ODD)[38]. This hydroxylation will promote the ubiquitination of HIF-1α at Lys532, a process that is effectively mediated by the ubiquitin ligase known as the Von Hippel-Lindau tumor suppressor (pVHL)[39]. This ubiquitination then, in turn, leads to the reduced stability of HIF-1α by facilitating its degradation through the 26S proteasome[15] (Figure 2).

Our studies demonstrated that c-Jun binds to the ODD of HIF-1α protein, protecting HIF-1α from being ubiquitinated and thereby enhancing its stability by reducing its susceptibility to the proteasome-executed degradation[15] (Figure 2). Notably, this binding requires the domains of c-Jun for DNA binding and heterodimerization but is independent of Ser63/73 phosphorylation. Further investigations have clarified that only non-phosphorylated c-Jun, *ie*, c-Jun without transcriptional activity, can bind to and protect HIF-1α[15, 16]. Relatively constant total levels of c-Jun are generally maintained within the cell. Pseudolaric acid B causes the increased phosphorylation of c-Jun, thus reducing the proportion of c-Jun that is in the Ser63/73-non-phosphorylated form. Consequently, the quantity of c-Jun that is able to bind to HIF-1α decreases, impairing the ability of c-Jun to stabilize HIF-1α[16] (Figure 2). Our findings constituted the first discovery of a function for the non-phosphorylated form of c-Jun; in accordance with these results, a function-converter model of c-Jun was proposed, in which Ser63/73 phosphorylation serves as a function converter that shifts c-Jun from its non-transcriptional functions to its transcriptional functions[16].

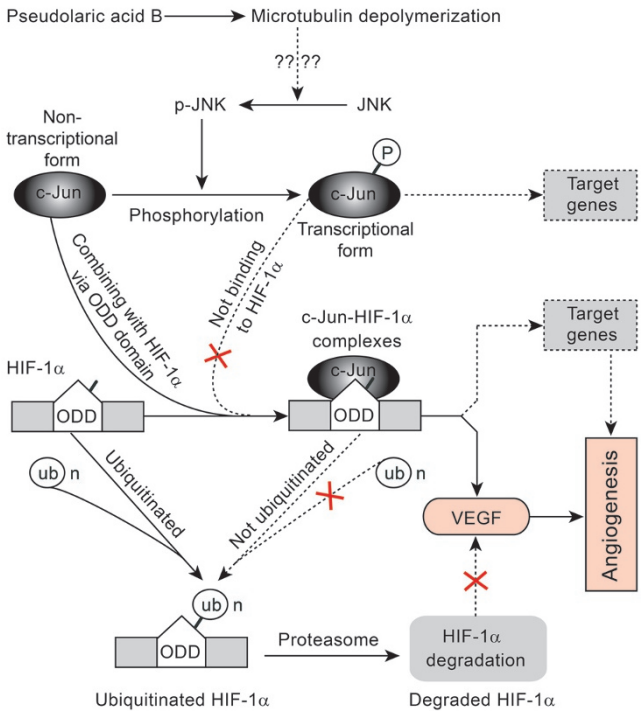



**Figure 2.** The antiangiogenic mechanism of pseudolaric acid B. Solid or broken lines indicate the relationships between the linked factors with direct experimental evidence (solid lines) or with logical possibility (broken lines) in the case of pseudolaric acid B.

## The development of AL3810 as a clinically promising angiokinase inhibitor

Various PTKs are aberrantly activated during tumor progression. Several of these PTKs, including VEGFR, PDGFR, and FGFR1, have been demonstrated to contribute to tumor angiogenesis[11, 31]. In recent years, we have discovered hundreds of compounds with inhibitory activities against different PTKs and have reported that 9 compounds exhibit potent antiangio-

genic effects (Table 1). Of these 9 compounds, AL3810 (also designated as E-3810)[31] demonstrates the greatest potential for clinical use.

AL3810 is a synthetic multitargeted PTK inhibitor that inhibits VEGFR1, VEGFR2, PDGFRα, PDGFRβ, and FGFR1 with $IC_{50}$ values in the nanomolar range[11, 31]. In endothelial cells, AL3810 can suppress the autophosphorylation of VEGFR2, PDGFRβ and FGFR1. AL3810 thus displays apparent antiangiogenic activity in all of the tested *in vitro*, *ex vivo,* and *in vivo* models (Figure 1). Even at millimolar concentrations, however, AL3810 demonstrates no cytotoxic effects on cancer cell lines. Nevertheless, AL3810 can elicit broad-spectrum *in vivo* antitumor activity in human kidney, pancreas and liver cancer xenograft models; its activity appears to make it more potent than several commercially available multitargeted PTK inhibitors, such as sorafenib and Sutent. Moreover, its antitumor activity is closely correlated with its antiangiogenic activity[11, 31].

Notably, it appears that tumors do not easily become resistant to AL3810, as xenografted tumors re-grown after the withdrawal of AL3810 demonstrate a response to a second cycle of AL3810 treatment that is similar to the response observed for the first treatment cycle. By contrast, tumors re-grown after the withdrawal of sunitinib treatment display reduced sensitivity to a second cycle of treatment with sunitinib. However, these sunitinib-resistant tumors remain sensitive to AL3810[31]. Although this result requires further confirmation, particularly in the clinical context, and the mechanism underlying this result also must be clarified, its potential importance is obvious, particularly given that tumors are generally prone to becoming resistant to the PTK inhibitors that are currently in clinical use[40].

The oral bioavailability of AL3810 is excellent, reaching 31% in rats[11]. Moreover, the concentration of AL3810 in different tissues is higher than the concentration of AL3810 in the plasma of tumor-bearing nude mice. In particular, a 51.7-fold increase in the concentration of AL3810 was detected in the examined tumoral tissues when compared with that in plasma, demonstrating the high affinity of AL3810 to tumors[11]. In addition, AL3810 has a relatively long terminal half-life of approximately 4 h; this extended half-life may help explain its persistent antitumor effects[31]. All of these results indicate that AL3810 possesses a favorable pharmacokinetic profile.

The prominent advantages of AL3810, which include its potent antiangiogenic effect, its broad spectrum of antitumor activity, its ability to potentially circumvent the drug resistance of tumors and its favorable pharmacokinetic profile, make it an excellent candidate for development as an anticancer drug. AL3810 has already entered into clinical trials in Europe to assess its potential use for this application, and its clinical trials in China will be launched shortly[11, 31].

## Concluding remarks

As an aspect of international efforts to explore new antiangiogenic compounds, we investigated the inhibition of angiogenesis by 17 compounds. These compounds have diverse origins, structures, primary targets and mechanisms. Our findings provide new models for the further modification and optimization of antiangiogenic compounds, and new clues related to the examination of antiangiogenic mechanisms. Tumor drug resistance poses a challenge for the antiangiogenic agents that are in current use[41]. Therefore, one of the most urgent tasks in the future will be to both demonstrate the critical molecular mechanism(s) underlying the effects of AL3810 and accelerate the clinical development of AL3810 to meet the potential clinical need for agents that can circumvent this tumor drug resistance. The detailed dissection of the structure-effect relationships of certain compounds to analyze the relationships between their antiangiogenic mechanisms and their cytotoxicity and between their primary targets and their antiangiogenic mechanisms could be another important task to accomplish, as well; the elucidation of these relationships could generate new strategies for cancer therapy.

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