Antiangiogenic Alkaloids from Plants

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Contents

Abstract

Angiogenesis, the formation of new blood vessels from preexisting capillaries, is an important research field. As the understanding of this process increases, this new knowledge will have a significant impact on several angiogenesis-dependent diseases. A wide variety of plants are rich in alkaloids, and these

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compounds have traditionally been of interest due to their pronounced effects on various physiological activities in animals and humans. Nowadays, it is known that many alkaloids obtained from plants exhibit antiangiogenic activity, and these alkaloids may act through different mechanisms to inhibit angiogenesis. Herein, we will discuss the most important alkaloids obtained from plants, focusing especially in their antiangiogenic activity. Because of the great diversity of plants, certainly, there are many antiangiogenic alkaloids that have yet to be discovered.

Keywords

Angiogenesis • antiangiogenic activity • angiogenesis-dependent diseases • natural products • plants • alkaloids

Abbreviations

Akt	AKT8 virus oncogene cellular homologue
AP-1	Activator protein 1
APL	Promyelocytic leukemia
ATF-2	Activating transcription factor 2
BAEC	Bovine aortic endothelial cells
Bax	B cell lymphoma-associated X
Bcl-2	B cell lymphoma 2 protein
bFGF	Basic fibroblast growth factor
BrdU	Bromodeoxyuridine
CAM	Chorioallantoic membrane
CD-31	Cluster of differentiation 31
CDK-2	Cyclin-dependent kinase-2
CDK-4	Cyclin-dependent kinase-4
COL1α2	Collagen type 1, alpha 2
COX-2	Cyclooxygenase-2
CREB-1	cAMP response element-binding protein
ECM	Extracellular matrix
ECs	Endothelial cells
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ERK1/2	Extracellular-signal-regulated kinases 1 and 2
FBS	Fetal bovine serum
FCA	Freund's complete adjuvant
FGF	Fibroblast growth factor
G6PDH	Glucose-6-phosphate dehydrogenase
GFR-Matrigel	Growth factor reduced-Matrigel
GM-CSF	Granulocyte macrophage colony-stimulating factor

HCC	Hepatocellular carcinoma cells
HIF-1	Hypoxia-inducible factor-1
HIF-1α	Hypoxia-inducible factor-1 – subunit alpha
HIF-1β	Hypoxia-inducible factor-1 – subunit beta
Hsp70	Heat shock p70
HUVEC	Human umbilical vein endothelial cells
IC_{50}	Half maximal inhibitory concentration
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-12	Interleukin-12
iNOS	Inducible oxide nitric synthase
IUPAC	International Union of Pure and Applied Chemistry
JAG2	Jagged-2
MAPK	Mitogen-activated protein kinase
MDS	Myelodysplastic syndrome
MMP-2	Matrix metalloproteinase-2
MMP-13	Matrix metalloproteinase 13
MMPs	Matrix metalloproteinases
MVD	Microvessel density
NFκB	Nuclear factor kappa B
NSCLC	Non-small cell lung cancer
p38 MAPK	p38 mitogen-activated protein kinase
p42/p44 MAPK	p42/p44 mitogen-activated protein kinase
$p125^{FAK}$	p125 focal adhesion kinase
PDB ID	Protein data bank identifier
PDGF-ββ	Platelet-derived growth factor subunits beta beta
PLCγ1	Protein kinase C-gamma 1
PlGF	Placental growth factor
pRb	Retinoblastoma protein
RT-PCR	Reverse transcription-polymerase chain reaction
TGF-β1	Transforming growth factor beta1
TIMP	Tissue inhibitor of metalloproteinase
TNF-α	Tumor necrosis factor-alpha
TrpRS	Tryptophanyl-tRNA synthetase
TRPV-1	Transient receptor potential vanilloid-type 1
TUNEL	Terminal transferase dUTP nick end labeling
VASP	Vasodilator-stimulated phosphoprotein
VE-cadherin	Vascular endothelial cadherin
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor-A
VEGFR-2	Vascular endothelial growth factor receptor subtype 2

1 Introduction

Angiogenesis research has a solid scientific foundation. It started with the theory of Judah Folkman (1933–2008) that tumor growth is always accompanied by neovascularization. He postulated that in order to survive and grow, tumors require blood vessels, and that by blocking the blood supply, a cancer could be starved into remission [1, 2].

In the past, the blood vessels in the body have long been considered to basically function as a transport compartment of the blood. Nowadays, it is recognized that the vasculature is one of the main organs in the body, extending more than 900 m^2 and playing a major role in supporting the body's integrity in various ways [3].

During the last decade, the field of angiogenesis has been characterized by an enormous increase in research activity [4]. The knowledge of endothelial cells (ECs) physiology and tumor angiogenesis improved, and it provided the necessary background to develop effective antiangiogenic strategies [5].

Nowadays, it is known that many alkaloids obtained from plants exhibit antiangiogenic activity and they can act through different mechanisms to inhibit angiogenesis. In this chapter, we will discuss the most important alkaloids obtained from plants, focusing especially in their antiangiogenic activity.

1.1 Angiogenesis

Angiogenesis, the sprouting of new capillaries from preexisting vessels, is a complex multistep process. It requires extensive interactions between a variety of cells and molecules and is controlled by various peptides and other modulating factors [4, 6].

In order for vascular sprouting to occur, a cascade of events must be concluded. First, extracellular matrix degradation must occur, a process that is facilitated by the activity of matrix metalloproteinases (MMPs). Next, chemoattractants and mitogens are activated in order to assist EC migration and proliferation, respectively. Finally, the tube formation occurs and requires inhibitory signals as well as those that enable formation of junctional complexes and reconstitution of a basement membrane. Formation of the basement membrane, which signals the onset of vessel maturation, involves recruitment by ECs of pericytes that embed within the basement membrane. If this microvessel is to become a larger vessel with a medial layer, then appropriate signals are required for the recruitment of smooth muscle cells [4, 6, 7]. It is known that during angiogenesis, vascular ECs interact with pericytes and such cell–cell contact inhibits EC proliferation. Thus, lack of sufficient number of pericytes around an EC layer may result in an uncontrolled development of microvasculature [8].

Imbalanced expression of pro- and antiangiogenic factors and their receptors on EC may determine the development or regression of new blood vessels. More than 200 pro-angiogenic factors have been identified. Among those, the most important factors include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF),

angiopoietins, cytokines, chemokines, and angiogenic enzymes. These factors initiate angiogenesis by modulating the migration and/or proliferation of ECs and the formation of neovasculature [8, 9].

The significance of the angiogenic process increases considerably every year as new findings reveal novel mechanisms through which this process can modulate disease development and recovery [10].

1.1.1 Angiogenesis, Inflammation, and Immune System

It is widely accepted that angiogenesis is the result of a net balance between the activities exerted by positive and negative regulators. This balance is conceptually very similar to that of the pro- and anti-inflammatory mediators that modulate an appropriate and specific inflammatory response [11].

Inflammatory mediators can also, either directly or indirectly, promote angiogenesis. Angiogenesis, in turn, contributes to inflammatory pathology. New blood vessels maintain the chronic inflammatory state by enabling the transport of inflammatory cells to the site of inflammation and supplying nutrients and oxygen to the proliferating inflamed tissue. The increased endothelial surface area also creates a massive capacity for the production of cytokines, adhesion molecules, and other inflammatory stimuli. Although inflammation is essential to protect the body against pathogens, it has adverse effects on surrounding tissue. Some of these effects induce angiogenesis. Inflammation and angiogenesis are thereby linked processes [12].

Therefore, it is well established that the immune system plays an important role in the regulation of angiogenesis. Next to the regulation of angiogenesis by leukocytes, angiogenic processes can influence the cells of the immune system and the development of an immune response as well. Normal EC contributes to the recruitment of immune cells to the site of inflammation by the expression of adhesion molecules. Some authors suggest that several advantages of antiangiogenic therapy for chronic inflammatory diseases can be visualized analogous to those of tumor growth antiangiogenic strategies. First of all, suppression of blood vessel growth leads to a decreased nutrient supply to the metabolically active cells present in inflamed tissue. Second, by preventing blood vessel formation, the entry route of inflammatory cells into the tissue becomes blocked. A third possible advantage of inhibiting EC activation, proliferation, and vascular remodeling in chronically inflamed lesions is the inhibition of the production of EC-derived soluble factors such as MMPs and cytokines [3].

It is also important to mention that monocytes, macrophages, platelets, mast cells, and lymphocytes, cells that are frequently infiltrated in chronic inflammatory sites, directly release or stimulate other cells to produce angiogenic factors [8]. Furthermore, it is well accepted that hypoxia is a potent stimulus for abnormal angiogenesis in these chronic inflammatory sites [13, 14].

1.1.2 Angiogenesis-Dependent Diseases

Angiogenesis occurs in physiologic and pathologic processes. In the physiological condition, the activity of inducers and inhibitors of angiogenesis maintains it in

equilibrium. Normal tissue growth, such as in embryonic development, wound healing, and menstrual cycle, is characterized by dependence on new vessel formation for the supply of oxygen and nutrients as well as removal of waste products. However, persistent and upregulated angiogenesis is often found to be a critical causal factor in several diseases, including cancer (both solid and hematologic tumors), cardiovascular diseases (atherosclerosis), chronic inflammation-associated disorders (rheumatoid arthritis, Crohn's disease), diabetes (diabetic retinopathy), psoriasis, endometriosis, and adiposity. These diseases may benefit from therapeutic inhibition of angiogenesis [15–18].

Pathological angiogenesis is closely associated with almost all of the major diseases afflicting human life [19]. Angiogenesis is therefore necessary for tumors and their metastases to grow beyond a microscopic size, and it can give rise to bleeding, vascular leakage, and tissue destruction. These consequences of pathological angiogenesis can be responsible, directly or indirectly, for the symptoms, incapacitation, or death associated with a broad range of "angiogenesis-dependent diseases." In fact, according to Folkman, the concept of angiogenesis-dependent diseases originated in 1972 with the recognition that certain nonneoplastic diseases depend on chronic neovascularization to provide a conduit for the continual delivery of inflammatory cells to the inflammatory site. Examples of such diseases include cancer, autoimmune diseases, age-related macular degeneration, and atherosclerosis. Subsequently, other nonneoplastic disorders were recognized to be in part angiogenesis dependent, for example, infantile hemangiomas, peptic ulcers, ocular neovascularization, and rheumatoid arthritis [20].

1.1.3 The Inhibition of Angiogenesis

The term "antiangiogenesis" was introduced to describe treatments designed to prevent the induction of new blood vessels and perhaps reduce the number of those already present [21]. Angiogenesis inhibition has been proposed as a general strategy to fight cancer and other angiogenesis-dependent diseases [15].

In the treatment of cancer, the inhibition of angiogenesis is a major area of therapeutic development. Whereas conventional chemotherapy, radiotherapy, and immunotherapy are directed against tumor cells, antiangiogenic therapy is aimed at the vasculature of a tumor and will either cause total tumor regression or keep tumors in a state of dormancy [2]. Angiopreventive drugs may have the potential to repress angiogenesis during early steps of carcinogenesis where they might retard the angiogenic switch, preventing unrestrained tumor growth [22].

Moreover, in the treatment of angiogenesis-dependent disease, angiogenesis inhibitors may be useful adjuncts to conventional therapies. When all conventional therapy has failed, an angiogenesis inhibitor may be successfully used as has been demonstrated in the treatment of multiple myeloma by thalidomide [23].

Definitely, angiogenesis can be targeted at several stages. The strategies for therapies are targeting growth factors and their receptors, interrupting the downstream signaling, blocking matrix degrading enzymes, using endogenous inhibitors, or attempting to mimic naturally occurring angiogenesis inhibitors, such as thrombospondin [24, 25].

1.1.4 Evaluating Potential Antiangiogenic Agents Obtained from Plants

Since angiogenesis mainly depends on proper activation, proliferation, adhesion, migration, and maturation of ECs, most approaches to modulate angiogenesis are focused on these endothelial functions during blood vessel formation [3].

Various in vitro and in vivo assays have been utilized to analyze angiogenesis experimentally. In vitro, ECs such as human umbilical vein endothelial cells (HUVECs) cultures are widely used. Migration or invasion of HUVECs could be assayed by transwell, and proliferation could be determined by bromodeoxyuridine (BrdU) incorporation. Differentiation is determined by tube formation assay using HUVECs seeded on Matrigel with or without angiogenic activator. In vivo, the Matrigel plug assay is widely performed in mice subcutaneously inoculated with angiogenic factor treated Matrigel, and the content of hemoglobin is measured in the Matrigel plugs to indirectly quantify functional new blood vessel formation. Taken together, these angiogenesis experimental tools can be useful in studies focused on antiangiogenic activity screening of plants used in traditional folk medicine [10, 26].

Some authors believe that bioactive compounds from natural sources may be used as regulatory agents in the formation of new blood vessels offering an enormous potential for therapeutic intervention of many disorders. An increasing number of bioactive compounds from natural sources and whose chemical structures have already been elucidated are reported as potential inhibitors of angiogenesis [27].

Ng et al. consider that naturally occurring bioactive compounds may be good candidates for the prevention of angiogenic disease, when standard therapy is unsuitable or lacking. They suggest that these products can also complement chemotherapy or radiotherapy, in combination with other anticancer drugs for therapy at different stages, and increase the survival time of the patients [28].

According to Tai-Ping Fan et al., the identification of new drugs from plants has a long and successful history, and certain pro-angiogenic and antiangiogenic plant components have been used in traditional Chinese medicine for thousands of years. Thus, it is rational to explore these medicinal plants as a source of novel angiomodulators [29].

2 Antiangiogenic Alkaloids from Plants

Plant metabolites bearing different structural patterns have showed potent effects on angiogenesis process [30–32]. Several of these substances are alkaloids, and their structures and biological effect on angiogenesis process are detailed below. In this chapter, we have used a broad concept to the alkaloid term, which was based on IUPAC recommendations. Its definition includes any plant metabolite containing basic nitrogen or clearly derived from an amino acid and still retaining its nitrogen atom, whether it demonstrates basicity or not [33]. Herein, the alkaloids have been separated according to their hypothetical amino acid precursor.

2.1 Alkaloids Derived from Tyrosine

2.1.1 Berberine

Berberine (**2**) is a quaternary ammonium salt from the protoberberine class. It is found in several occidental and oriental medicinal plants, including *Hydrastis canadensis* L. (Ranunculaceae) and *Coptis chinensis* Franch var. *chinensis* (Ranunculaceae), also known as Goldenseal and Huanglian, respectively [34].

Berberine has exhibited cytotoxicity against several cancer cell lines from different histological origins, including bone, breast, brain, colon, esophagus, liver, lung, and stomach. This activity was correlated to two main effects: (1) antimitotic, through protein and DNA synthesis inhibitory activities, and (2) proapoptotic, by regulating apoptotic gene expression and decreasing mitochondrial transmembrane potential [35].

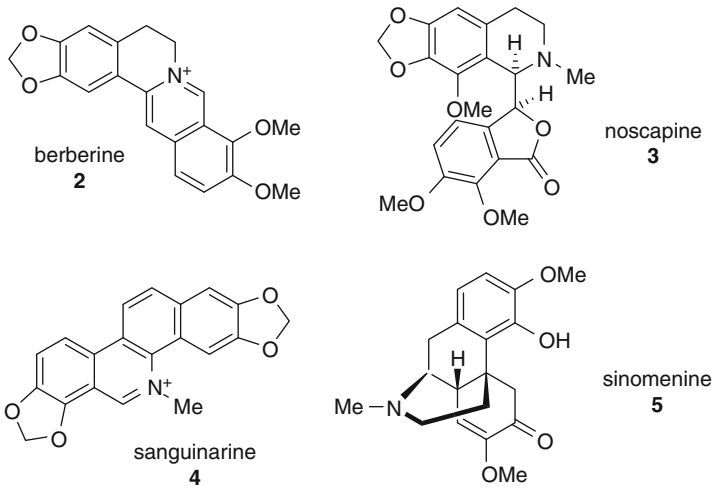
Low micromolar concentrations of **2** reduced the capacity of hepatocellular carcinoma cells (HCC) to stimulate HUVEC proliferation, migration, and endothelial tube formation. In addition, **2** prevented secretion of VEGF from HCC and decreased VEGF mRNA expression. Altogether, these data indicated that **2** showed antiangiogenic properties, which could influence the cross talk between HCC cells and HUVEC [36].

In other experiments, **2** decreased directly the ability of HUVEC migration and capillary tube formation and inhibited gastric adenocarcinoma cancer cell line SC-M1 to stimulate HUVEC migration. The influence of **2** on the cross talk between HUVEC and SC-M1 was studied under hypoxic culture conditions. These experiments indicated that **2** caused a downregulation on the expression of VEGF and hypoxia-inducible factor-1alpha (HIF-1α), two key factors in mediating tumor angiogenesis [37].

2.1.2 Noscapine

Noscapine or narcotine (**3**) is a phthalideisoquinoline alkaloid from plants of the Papaveraceae family. It is an important alkaloid from the opium poppy (*Papaver somniferum* L.), occurring in variable quantities (level ranges from 2% to 10%) [38]. Unlike opioid morphinane drugs, noscapine lacks hypnoanalgesic, euphoric, and respiratory depressant properties. Its main therapeutic application is as oral antitussive drug, which is indicated to treat nonproductive coughs [39, 40].

Noscapine displayed HIF-1 inhibitory activity on human glioma cells under different types of hypoxic stress. This activity was determined through variations in the HIF-1α expression. The reductions of the HIF-1α expression were due to accumulation in the nucleus and its degradation through the proteasome pathway [41]. Considering that the expression of HIF-1α protein promotes transcriptional activation of VEGF gene [42, 43], noscapine decreased VEGF production through upregulation of VEGF gene. However, noscapine inhibited the ability of HUVEC to form capillary-like structures in vitro, corroborating its potential as antiangiogenic agent [41].



2.1.3 Sanguinarine

Sanguinarine or pseudochelerythrine (**4**) is quaternary ammonium salt belonging to the benzo[*c*]phenanthridine class. It constitutes approximately 50% of redroot latex of *Sanguinaria canadensis* L. (Papaveraceae), an herbaceous medicinal plant popularly known as bloodroot [44]. The main pharmacological application of sanguinarine is in dental formulations, including mouthwashes and toothpastes. It shows antimicrobial and anti-inflammatory properties, reducing gingival inflammation and supragingival plaque formation [45].

Sanguinarine possess antiproliferative activity against several human cancer cell lines, exhibiting differential apoptosis-inducing effect in tumorigenic cells *versus* normal cells [46]. In preclinical experiments, oral treatment with sanguinarine suppressed murine tumor growth in a syngeneic host (B16 melanoma 4A5 cells implanted in C57BL/6 mice) and in a human melanoma tumor xenograft (A37) in athymic mice [47].

Sanguinarine has been recognized as a potent antiangiogenic agent, suppressing VEGF-induced migration, sprouting, and survival in primary cultured ECs, at nanomolar levels. On the other hand, sanguinarine was a relatively mild inhibitor of VEGF-induced DNA synthesis. In vivo assays, using mouse Matrigel plug and chorioallantoic membrane (CAM) of chick embryos, evidenced its potent action on vasculogenesis. Preliminary biochemical experiments revealed that sanguinarine strongly suppressed basal and VEGF-induced Akt (AKT8 virus oncogene cellular homologue) phosphorylation and not interfered on VEGF-induced ERK1/2 (extracellular-signal-regulated kinases 1 and 2) and PLCγ1 (protein kinase C-gamma 1) phosphorylations [48]. More recently,

the effects of sanguinarine on Akt phosphorylation inhibition were confirmed by immunoassays [49].

Considering that physiological angiogenesis is a crucial step to ovarian follicle development, under normal conditions, sanguinarine attenuated VEGF production by swine granulosa cells, interfering negatively on female reproductive performance [50].

2.1.4 Sinomenine

Sinomenine or cocculine (**5**) is a morphinane alkaloid identified as antirheumatic chief component from the *Sinomenium acutum* Rehder and Wilson (Menispermaceae). The whole plant has been used to treat joint pain and arthritis for over 2,000 years, and its preparations have been adopted by traditional Chinese medicine [51, 52].

Sinomenine inhibited basic fibroblast growth factor (bFGF)-induced in vitro angiogenesis, acting through four different modes of action on HUVEC: (1) inhibiting of proliferation, (2) arresting their cell cycle in G1 phase, (3) suppressing chemotactic mobility, and (4) disrupting tube formation. Furthermore, in vivo and ex vivo antiangiogenic assays corroborated its in vitro results. It reduced the neovascularization and microvascular outgrowth, which are measured by Matrigel plug and rat aorta ring sprouting experiments, respectively [53].

Recent studies revealed that sinomenine showed modulatory effects on gene expression of the IL-1β activated in human synovial sarcoma using Hs701.T cell line. Sinomenine suppressed the expression of genes associated with angiogenesis and vascular remodeling, such as JAG2 (jagged-2), a transmembrane ligand of notch receptor; COL1α2 (collagen, type 1, alpha 2); MMP-13 (matrix metalloproteinase 13); and P1GF (placental growth factor), a member of VEGF family [54].

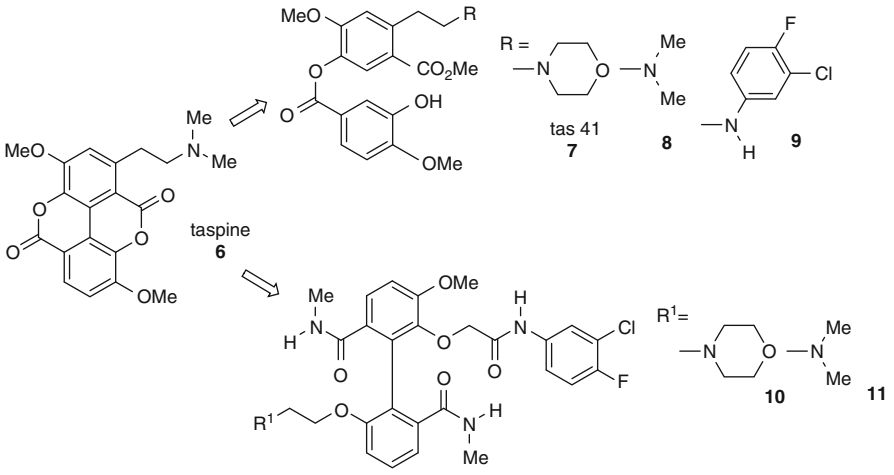
2.1.5 Taspine and Its Synthetic Analogues

Taspine (**6**) is unusual alkaloid with two lactones, resembling ellagic acid, and a *N*, *N*-dimethylaminoethyl side chain, which hypothetically arisen from tyrosine metabolism [55]. It has been showed several biological activities, such as cicatrizing capacity and acetylcholinesterase inhibition [56, 57].

Taspine has displayed several in vitro antiangiogenic properties on HUVEC, including reduction on its VEGF secretion. It exhibited HUVEC growth inhibition by inducing its apoptosis in a concentration-dependent manner. Its apoptosis-inducing activity was confirmed by transmission electron microscopy, which showed chromatin agglutination, nuclear karyopyknosis, and typical apoptotic bodies. Immunoassays indicated a decreased in the Bcl-2 (B cell lymphoma 2 protein) expression, as well as an increased in the Bax (B cell lymphoma-associated X) expression, anti-apoptotic and pro-apoptotic proteins, respectively [58]. Considering that Bcl-2 overexpression is closely related to the angiogenesis process [59], the dual action of taspine on this target and VEGF could constitute its mechanism of antiangiogenic activity [58]. More recently, taspine showed

antiangiogenic and apoptosis-inducing activities against epidermoid carcinoma cells in a manner closely related to HUVEC. Additionally, taspine has increased the expression of caspase-3, cleaved caspase-3, CDK2 (cyclin-dependent kinase-2), and CDK4 (cyclin-dependent kinase-4) [60].

The potential antiangiogenic of taspine encouraged the synthesis of novel analogues (**7**–**9**). Tas 41 (**7**) showed potent antiproliferative activity against human umbilical endothelial (ECV304 cell line) and human epithelial colorectal adenocarcinoma cells (Caco-2 cell lineage), exhibiting IC_{50} values of 2.67 nM and 52.5 nM, respectively. These data indicated that tas 41 was more effective against ECV304 than Caco-2 (approximately 19-fold), suggesting this compound may inhibit vascular endothelial growth factor receptor subtype 2 (VEGFR-2). Docking studies of tas 41 in the active site of VEGFR-2 (PDB ID: 3C7Q), using Sybyl/FlexX, showed its binding mode. It included hydrogen bond interactions to the Asn 923, Arg 1032, and Cys 913 residues [61]. Further studies confirmed the antiangiogenic properties of tas 41, which showed effects closely related to the taspine [62]. More recently, biphenyl taspine analogues (**10** and **11**) showed inhibitory activity against cell lines express abnormally high levels of EGFR (epidermal growth factor receptor). Their predicted physicochemical properties, including distribution and partition coefficients, indicated these compounds were more water-soluble than taspine. Altogether, these studies led to pivotal structural features of ring-opened analogues, including the importance of lactone ring B for antiangiogenic activity. On the other hand, the cleavage of lactone ring A did not interfere to biological activity [63, 64].



2.1.6 Tetrandrine and Its Synthetic Analogues

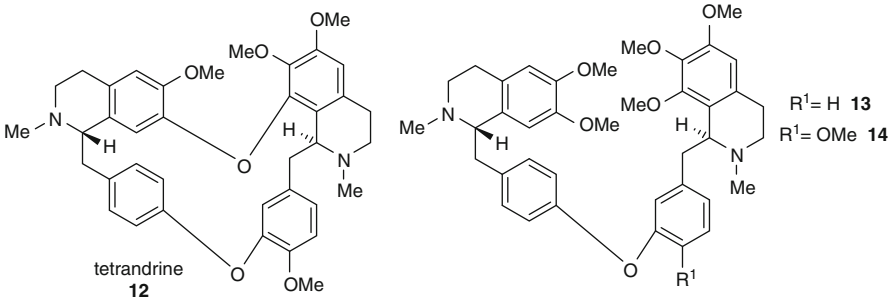
Tetrandrine (**12**) is a *bis*-benzylisoquinoline alkaloid identified as bioactive chief alkaloid from *Stephania tetrandra* S. Moore (Menispermaceae) [65, 66].

It has been traditionally used for the treatment of inflammatory process by several oriental systems of herbal medicine. In the traditional Chinese medicine, this species is among 50 fundamental herbs used, belonging to the Pharmacopoeia of the People's Republic of China [67]. Tetrandrine showed potent antiproliferative activity against a broad panel of human cell lines, including those derived from hepatocellular carcinoma, colorectal, and esophageal cancers [68, 69].

The potential antiangiogenic of tetrandrine was evaluated by several experiments. Tetrandrine was able to decrease the proliferation of HUVEC primary culture and LoVo cells (human colorectal carcinoma). Also, its in vitro activities on HUVEC included migration and tube formation impairment, apoptosis induction, and DNA synthesis suppression. Additionally, in vivo experiments using LoVo cells xenograft in nude mice indicated that tetrandrine significantly reduced the number of hot spots of neo-angiogenesis, which was measured by immunohistochemistry techniques [70].

In other experiments, tetrandrine exhibited inhibitory effect on tube formation of rat vascular ECs, which were stimulated by IL-1α (interleukin-1 alpha) and PDGF-ββ (platelet-derived growth factor subunits beta beta), and FBS (fetal bovine serum). Nanomolar concentrations of tetrandrine were more effective against tube formation stimulated by IL-1α and PDGF-ββ than FBS. In vivo experiments indicated that tetrandrine inhibited air-pouch granuloma angiogenesis, induced by Freund's complete adjuvant (FCA) with croton oil. Similarly to in vitro experiments, tetrandrine exhibited potency similar to the hydrocortisone. These pharmacological results support the traditional use of *S. tetrandra* as anti-inflammatory plant for chronic inflammations and **12** as its responsible biologically active compound [71].

Tetrandrine and its synthetic analogues (**13** and **14**) displayed potent inhibitory effect on FBS-stimulated angiogenesis of cultured choroidal tissues explanted of streptozotocin-diabetic Wistar rats and in vivo air-pouch granuloma angiogenesis in diabetic mice. This study indicated that *bis*[tetrahydroisoquinoline] unit connected by oxy-*bis*[phenylenemethylene] and 2,2-dimethyl groups was important structural feature for antiangiogenic activity. Analogue **13** may be considered as lead compound to treat diabetes-associated choroidopathy and retinopathy [72].



2.2 Alkaloids Derived from Tryptophan

2.2.1 Brucine

Brucine (**15**) is a bitter substance closely related to strychnine, which is mainly isolated from the seeds of *Strychnos nux-vomica* L. (Loganiaceae). These compounds are monoterpenoid indole alkaloids and have been classified into type Ib category (or strychnines) [73]. Despite structural similarity between these compounds, brucine was not found be a potent toxic agent [74]. Brucine and strychnine exhibit distinguished historical relevance and have been extensively used as modern research tools, including their application as chiral deriving agents for racemic mixture resolutions [75].

Brucine inhibited tumor angiogenesis process, which was elucidated by in vitro and in vivo assays. It was able to inhibit human breast adenocarcinoma cell proliferation, when administered along VEGF polyclonal antibody, and reduced their pro-angiogenic factors secretion, including VEGF and tumor necrosis factor-alpha (TNF-α). This experiment suggests that brucine binds with VEGF receptors, inhibiting cell proliferation. In vivo experiments, using two Ehrlich tumor models, proved to confirm in vitro results, indicating that brucine downregulated VEGF and TNF-α levels, and increased interleukin-12 (IL-12), another antiangiogenic factor [76].

Brucine showed potent effects on inflammatory angiogenesis process, which was evaluated by in vivo cannulated sponge implant model in Swiss albino mice. It indicated that brucine decreased wet weight granuloma tissue and its hemoglobin content. Also, brucine exhibited inhibitory activity on inflammatory, angiogenic, and fibrogenic cytokines, such as cluster of differentiation 31 (CD-31), transforming growth factor beta 1 (TGF-β1), and VEGF [77].

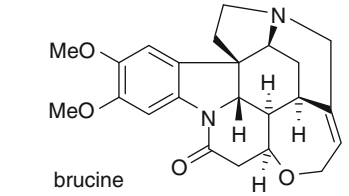
2.2.2 Evodiamine

Evodiamine (**16**) is an indoloquinazoline alkaloid of mixed biosynthetic origin and possesses three nitrogen atoms arising from tryptophan and *N*-methyl-anthranilic acid residues [78]. It is chief alkaloid from fruits of *Evodia rutaecarpa* Bentham (Rutaceae), a plant widely used by traditional Chinese medicine [79].

Pharmacologically, evodiamine has been widely studied as innovative anti-obesity agent and was classified as a full TRPV-1 (transient receptor potential vanilloid-type 1) agonist. This receptor is a nonspecific ion channel and is activated by not only chemical compounds containing vanillyl moiety but also heat ($\geq$45 °C) and acid (micromolar range) stimuli. It exhibited binding affinity similar to capsaicin in Chinese hamster ovary cell model. Despite similar in vitro and in vivo anti-obese activities between these compounds, evodiamine has not perceptible taste, including a peppery hot taste [80, 81].

Several evidences demonstrated that evodiamine possesses in vitro and in vivo anticancer properties, such as antiproliferative, anti-metastatic, apoptosis-inducing, and antiangiogenic activities [82]. Evodiamine was able directly to reduce HUVEC capillary tube formation and invasion, using Matrigel assay. In vivo assay, using

CAM of chick embryo and local administration of evodiamine, confirmed the suppression on tube formation process. Western blot and northern blot experiments, using HUVEC, indicated that evodiamine reduced VEGF and p42/p44 mitogen-activated protein kinases (p42/p44 MAPKs) expression and the mRNA expression of VEGF, respectively. In other experiments, using human lung adenocarcinoma cell lines (CL-1 cells), evodiamine inhibited markedly CL-1-induced tube formation in ECs and their VEGF expression [83].



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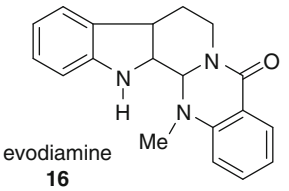

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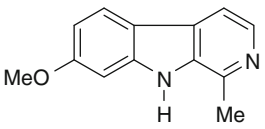

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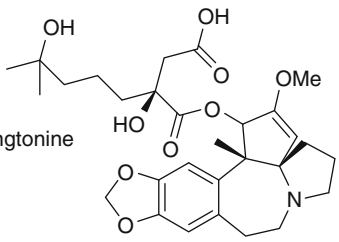


homoharringtonine
18

2.2.3 Harmine

Harmine (**17**) belongs to the β-carboline alkaloid class, which occurring in Malpighiaceae family, mainly in South American plants, such as *Banisteriopsis caapi* (Spruce ex Griseb) C. V. Morton [84]. This species is popularly known as "ayahuasca" (Ecuador, Peru), and "caapi" (Brazil) has been used in hallucinogenic preparations. *Banisteriopsis caapi* stems and *Psychotria viridis* Ruiz & Pav. leaves (Rubiaceae) have been widely used in mystic-religious ceremonies. Psychopharmacological and neuropharmacological properties of these infusions and decoctions were related to a complex alkaloid mixture, including harmine [85].

Harmine exhibits potent antineoplastic activity against tumors with high metastatic capacity, including HepA, Lewis lung cancer, and sarcoma 180. It has showed antiproliferative activity against cells of different histological origins, such as epidermoid carcinoma of nasopharynx, osteosarcoma, lung carcinoma, glioblastoma, and breast cancer [86].

Harmine proved to be a potent angiogenic inhibitor, which was extensively evaluated by in vitro and in vivo experiments. In vitro, it was able to reduce different steps of neo-vessel formation by VEGF-induced HUVEC, including proliferation, motility, invasion, and tube formation. Additionally, harmine showed inhibitory effect on transcription factors related to crucial tumor development and angiogenesis process. It reduced the translocation and activation of

nuclear factor kappa B (NF-κB) subunits (p65, p50, and c-Rel) and nuclear translocation of important AP-1 (activator protein 1) factors, including c-fos, ATF-2 (activating transcription factor 2), and CREB-1 (cAMP response element-binding protein). Secretion of other factors by B16F-10 melanoma cells, which are involved in angiogenesis, including cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and matrix metalloproteinase was also reduced by treatment with harmine. Ex vivo assay, using rat aortic rings and conditioned medium from B16F-10 melanoma cells, demonstrated that harmine inhibited microvessel outgrowth. Experiments using B16F-10 cells intradermally injected on the shaven ventral skin and intraperitoneal administration of harmine (10 mg kg^{-1} body weight) in mice indicated potent reduction of tumor specific neo-vessel formation. The level of serum pro-angiogenic factors of VEGF and nitric oxide, as well as pro-inflammatory cytokines (IL-1β, interleukin-6 (IL-6), TNF-α, and granulocyte macrophage colony-stimulating factor (GM-CSF)), was effectively decreased by harmine treatment. Furthermore, harmine significantly enhanced the levels of interleukin-2 (IL-2) and tissue inhibitor of metalloproteinase (TIMP); both are important antiangiogenic factors [87].

2.2.4 Homoharringtonine

Homoharringtonine (**18**) is an alkaloid of unusual structure and belongs to harringtonine class, which is structurally characterized as cephalotaxine esters. These compounds have been exclusively isolated from *Cephalotaxus* plants, including *C. fortunei* Hook F and *C. harringtonia* K. Koch *var. harringtonia* [88].

Cephalotaxine and its esters are of particular chemical and medical interest. Among these, **18** and harringtonine are most promising anticancer agents [89]. Homoharringtonine has been submitted to extensive phase I/II clinical studies in patients with different solid tumors, such as malignant melanoma, sarcoma, head and neck carcinoma, breast carcinoma, and colorectal carcinoma. Also, several clinical trials include studies efficacy in patients with acute leukemia, myelodysplastic syndrome (MDS), acute promyelocytic leukemia (APL), and chronic myeloid leukemia [90].

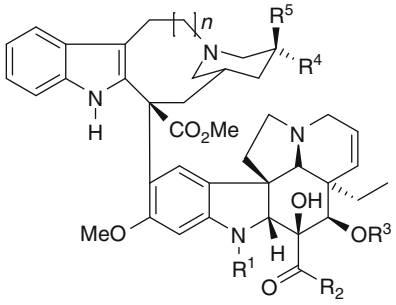
Homoharringtonine exhibited in vitro antiangiogenic effects on endothelial and leukemic cells, ECV304 and K562 cells, respectively. Its effects on ECV304 include proliferation inhibition and apoptosis induction. For leukemic cells, **18** downregulated VEGF mRNA expression and inhibited VEGF protein production, which were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and western blot, respectively [91]. Another in vitro study indicated VEGF antisense oligodeoxynucleotide increased the sensitivity of myeloid leukemia cells to **18** [92]. Experiments using gelatin sponges implanted on top of chick embryo CAM demonstrated that **18** significantly reduced microvessel density (MVD), confirming its in vitro antiangiogenic properties [93]. Studies involving RNA interference and transfected K562 cells suggested that inhibition of H1F-1α expression, at both mRNA and protein after RNAi H1F-1α, decreases VEGF, under hypoxic conditions. Moreover, this HIF-1α inhibition was able to increase K562 cell chemosensitivity to **18**. Altogether, these observations suggested that inhibition of H1F-1α

expression is related to antiangiogenic activity through two complementary mechanisms, inhibition of VEGF expression and increase of leukemic cells susceptibility to **18**, an antiangiogenic agent [94].

2.2.5 Vinca Alkaloids and their Semisynthetic Analogues

Vinca alkaloids constitute a group of indole–indoline dimeric compounds from *Catharanthus roseus* (L.) G. Don. (formerly named as *Vinca rosea* L.). It is a pantropical species and belongs to the Apocynaceae family and is popularly known as Madagascar periwinkle [95]. Among these, vinblastine (or vincaleucoblastine, **19**) and vincristine (or leurocristine, **20**) are of clinical oncology interest [96]. Vincristine and vinblastine have been commonly administered in combination with other anticancer drugs in the treatment of hematological malignancies (leukemias and lymphomas) and solid tumors (bladder and breast cancers), respectively [97].

Soon after clinical impact of these phytochemicals in curative and palliative cancer chemotherapies, a number of studies focused on design of their semisynthetic derivatives were developed. These efforts led to important anticancer agents, including vindesine (**21**), vinorelbine (**22**), and vinflunine (**23**). Vinorelbine has been administered for treating non-small cell lung cancer (NSCLC), metastatic breast cancer, and rhabdomyosarcoma [98]. Vindesine is usually used to treat leukemia, lymphoma, melanoma, and breast and lung cancers. Vinflunine is a fluorinated vinblastine derivative and has been submitted to phase III clinical studies in patients with NSCLC and bladder cancer [99].



vinblastine **19** R^1= Me, R^2= OMe, R^3= COMe, R^4= Et, R^5= OH, n = 1
vincristine **20** R^1= CHO, R^2= OMe, R^3= COMe, R^4= Et, R^5= OH, n = 1
vindesine **21** R^1= Me, R^2= NH_2, R^3= H, R^4= Et, R^5= OH, n = 1
vinorelbine **22** R^1= Me, R^2= OMe, R^3= COMe, R^4= Et, R^5= H, n = 0
vinflunine **23** R^1= Me, R^2= OMe, R^3= COMe, R^4= CF_2Me, R^5= H, n = 0

Vinblastine exhibited in vitro antiangiogenic activity. It was able to interfere in HUVEC angiogenic phenotype, at noncytotoxic concentrations, including

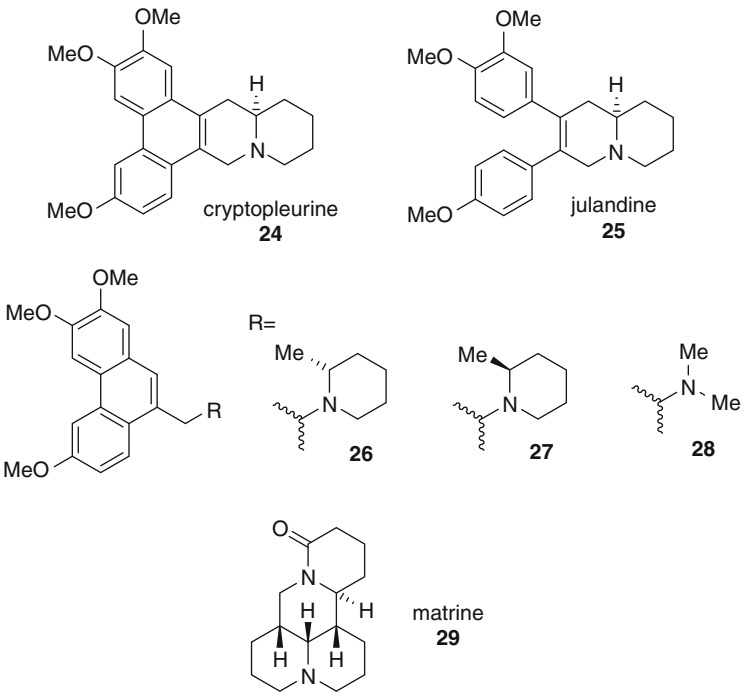
proliferation, chemotaxis, adhesion, and abnormal vessel morphology. These features were observed when HUVEC was cultured in optimal growth medium alone or supplemented with angiogenic growth factors. Electron microscopy experiments demonstrated morphology alterations caused by **19**. They indicated that the HUVEC elongation was related to thin disturbance of cytoskeleton, in the form of slight depolymerization and addensation, as well as accumulation of microfilaments [100]. In other experiments, **19** inhibited adrenomedullin-induced angiogenesis, using in vitro and in vivo experiments, such as Matrigel tube formation and CAM of chick embryo, respectively [101]. The combination between **19** and rapamycin has exhibited in vitro and in vivo synergistic effects on angiogenesis [102, 103]. Differential proteomic analysis of this combination on ECs indicated crucial variations in protein contents, which were related to angiogenesis process. Among these, downregulation of ATP synthase, annexin A2, heat shock p70 (Hsp70), glucose-6-phosphate dehydrogenase (G6PDH), vasodilator-stimulated phosphoprotein (VASP), proteasome 26S, tryptophanyl-tRNA synthetase (TrpRS), and stathmin/Op18, as well as the up-modulation of carbonyl reductase, RhoGDP-dissociation inhibitor (Rho-GDI), and histone H1.0, was observed [104]. In another in vivo experiment, metronomic vinblastine chemotherapy inhibited VEGF-A-induced-angiogenesis. The continuous vinblastine treatment with an apparently nontoxic dose was able substantially to inhibit vascularized area and microvascular length, which were evaluated by microscopy morphometry and computerized image analysis, respectively [105]. Also, **20** and **23** have been intensively investigated as antiangiogenic agents, including their synergistic effects when combined with other drugs [106–108].

2.3 Alkaloids Derived from Lysine

2.3.1 Cryptopleurine, Julandine, and their Synthetic Analogues

Cryptopleurine (**24**) and julandine (**25**) are phenanthroquinolizidine alkaloids. Together with phenanthroindolizidine alkaloids, they are constituted by over 70 compounds. These alkaloids are commonly isolated from plants of Asclepiadaceae and Moraceae families [109, 110]. They have exhibited similar biological activities, which include antiviral, antifungal, cytotoxic, and vesicant [111].

Cryptopleurine inhibited hypoxic HIF-1 activation in human gastric cancer cells (AGS cells), which were induced by hypoxia using a HIF-mediated reporter gene assay. Furthermore, **24** blocked HIF-1α protein accumulation in AGS cells, but not HIF-β protein, confirming its inhibitory effect on HIF-1 activation under hypoxic conditions [112]. Several C8c–C15 monoseco-analogues of **24** and **25** exhibited antiangiogenic activity, using rat aorta assay. Quinolizidine-ring opened analogues of cryptopleurine **26**–**28** inhibited 100% of blood vessel growth, at 100 μg mL^{-1}, and were more potent than PI-88, a heparin sulfate mimetic used as positive control [113, 114].



2.3.2 Matrine

Matrine (**29**) is a quinolizidine alkaloid isolated from several species of the genus *Sophora* (Fabaceae), mainly *S. alopecuroides* L. and *S. flavescens* Aiton. The latter has widely been used by traditional Chinese medicine, and its pharmacological properties were correlated to matrine contents [115, 116].

Matrine possesses potent antitumor activities in vitro and in vivo. It exhibits cytotoxic activity on cancer cell lines of different histological origins, including cervical cancer, gastric cancer, glioma, HCC, leukemia, lung cancer, and melanoma. Also, matrine administration reduced the growth of HCC and gastric tumor in mice [117–120].

Matrine showed potent in vivo antiangiogenic effect. Experiments, using breast cancer cell implanted in Balb/c mice, indicated that **29** reduced tumor MVD, which is measured by immunostaining techniques using anti-CD31 antibodies. Corroborating these findings, western blot assays demonstrated that **29** downregulated VEGF and VEGFR-2 expression [121].

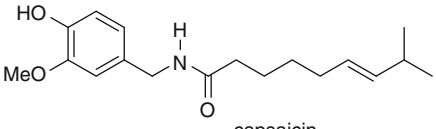
2.4 Capsaicin and its Natural Ester Analogues

Capsaicin (**30**) is an amide belonging to capsaicinoid class, which is formed by condensation between branched-chain fatty acids (C_8–C_{13}) and vanillylamine unit.

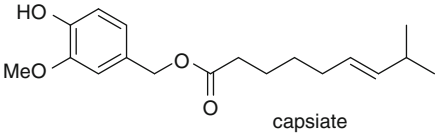
They are isolated from several varieties of *Capsicum annuum* L. and *C. frutescens* L. (Solanaceae), which are commonly named as hot peppers. Their physiological effects, such as lachrymation, coughing, and burning sensation, are directly correlated to capsaicinoid contents [122].

Pharmacologically, **30** has been intensively studied due to high TRPV-1 affinity. After TRPV-1 activation, **30** causes its long-lasting desensitization and loss of pain sensitivity. This unusual analgesia mode became **30** as valuable lead compound for design of innovative painkiller agents. The main therapeutic application of **30** is in cream-topical formulations, used to treat several algias associated with neurological origin, such as osteoarthritis, painful neuropathy linked to the trigeminal nerve, neuralgia caused by herpes infections, and painful diabetic neuropathy [123].

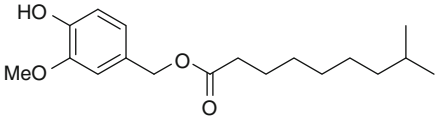
Capsaicin exhibited potent effect on cancer and inflammatory angiogenesis processes. In vitro assays demonstrated that **30** was able to inhibit the proliferation of HUVEC primary culture, which was not related to high TPRV-1 affinity. In other experiments, using HUVEC, capsaicin inhibited their migration and capillary tube formation. Further studies showed that **30** was able to inhibit DNA synthesis in HUVEC, causing G1 phase arrest. Its effect was attributed to downregulation of cyclin D1 expression, leading to inhibition of phosphorylation of retinoblastoma protein (pRb), mediated by CDK-4. Signaling experiments showed that capsaicin inhibited VEGF-induced activation of p38-MAPK (p38 mitogen-activated protein kinase), $p125^{FAK}$ (p125 focal adhesion kinase), and Akt, suggesting its possible antiangiogenic molecular mechanism. Ex vivo assay, using art aortic ring, **30** significantly reduced VEGF-induced microvessel sprouting. Two in vivo experiments confirmed the potential antiangiogenic activity of **30**. It was capable of blocking angiogenesis induced by Matrigel plug containing VEGF, subcutaneously implanted into C57BL/6 mice. In another experiment, using CAM of chick embryo assay, **30** inhibited angiogenesis induced by primate fibrosarcoma (HT1080 cells) [124]. In vivo experiments, using acetic acid-induced kissing gastric ulcer, **30** strongly inhibited the microvessels formation in the granulation tissue at the ulcer margin. Additionally, **30** decreased epidermal growth factor (EGF) levels in salivary gland, serum and gastric mucosa, suggesting its potential antiproliferative activity on several epithelial cells [125].



capsaicin
30



capsiate
31



dihydrocapsiate
32

Capsinoids, which include capsiate (**31**) and dihydrocapsiate (**32**), are esters naturally occurring in red peppers. Although they are structurally similar to capsaicin and its amide-type analogues, capsinoids were not found to be potent pungent compounds. Capsiate and dihydrocapsiate showed in vitro and in vivo antiangiogenic properties closely related to capsaicin performance [124]. Additionally, these compounds blocked VEGF-induced endothelial permeability and loss of endothelial cell–cell junctions facilitated by vascular endothelial cadherin (VE-cadherin). Capsiate was able to suppress not only VEGF-induced activation of Src kinase but also the phosphorylation of its downstream substrates, including $p125^{FAK}$ and VE-cadherin. Molecular modeling studies indicated the binding mode of **31** in Src tyrosine kinase, which interacted to its ATP-binding pocket [126].

2.5 Halofuginone

Halofuginone (**33**) is not plant-derived alkaloid. It is halogenated synthetic compound, which shows structural features based on febrifugine (**34**), a *N*3-substituted quinazolin-4-one alkaloid [127].

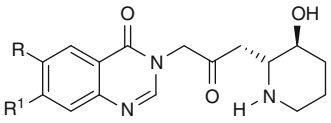
Febrifugine was first isolated from roots of *Dichroa febrifuga* Lour. (Saxifragaceae), a Chinese medicinal plant traditionally used to treat malaria fevers. This alkaloid showed potent anti-*Plasmodium* activity, which was superior to quinine, and unacceptable side effects, such as hepatotoxicity and severe emesis [128, 129]. Its properties not only precluded its use as drug but also encouraged the synthesis of safer analogues [127].

Halofuginone is of particular interest as a drug orphan candidate to treat scleroderma [130, 131]. It has been intensively investigated for control of diseases involving collagen biosynthesis and some cancer types [131, 132]. The main application of its hydrobromide salt is as a curative and prophylactic agent, used to treat protozoan veterinary infections, mainly in commercial poultry and cattle production [131].

Halofuginone was effective in critical steps of in vitro angiogenesis process, which was evaluated on bovine aortic endothelial cells (BAEC). These events include (1) antiproliferative activity, (2) inhibition of MMP-2 gelatinolytic activity, (3) decrease of invasion capacity through Matrigel containing medium conditioned by 3 T3 fibroblasts, (4) inhibition of capillary tube formation, and (5) inhibition of subendothelial extracellular matrix (ECM) deposition. Ex vivo assay, using rat aortic ring, indicated that **33** markedly reduced vascular sprouting. The most relevant antiangiogenic property was evidenced by in vivo experiments, using mouse corneal micropocket experiments. This assay revealed that **33** inhibited bFGF-induced neovascularization, both after intraperitoneally or diet administration [133].

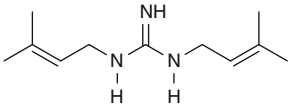
In other studies, **33** exhibited biological activities closely related to key antiangiogenic effects. A potent inhibition on MMP-2 gene transcription activity was demonstrated in human and murine bladder carcinoma cell lines [134]. Highly

specific suppression of collagen type α1 gene expression was observed in a broad panel of different cell types, including chicken, mouse, rat, and human origin [135]. Furthermore, **33** showed antiangiogenic properties in several in vivo models [136, 137].



halofuginone **33** R = Cl, R^1= Br

febrifugine **34** R = R^1= H



pterogynidine
35

2.6 Pterogynidine

Pterogynidine (**35**) belongs to guanidine alkaloid class, a quite rare plant-alkaloid category, which is most common in marine organisms. It is formed by guanidine core substituted by two isoprenylated units [138]. This compound was first isolated from *Pterogyne nitens* Tul. (Fabaceae), an ornamental species, which occurs only in South America regions [139].

Pterogynidine has showed cytotoxicity against several human cancer cell lines, including myeloblastic leukemia, glioblastoma, and malignant breast [140]. Its cytotoxic effect has been related to apoptosis-inducing activity, which was evaluated by several in vitro assays, such as Hoechst-propidium iodide, cytometry-based annexin-V, and caspase-Glo 3 and 7 [141].

Pterogynidine showed in vitro antiangiogenic properties, using HUVEC. Among these, **35** was able to decrease proliferation and invasion capacity, which was evaluated by bromodeoxyuridine (BrdU) and double-chamber assay, respectively. The terminal transferase dUTP nick end labeling (TUNEL) experiments indicated that **35** increases HUVEC apoptosis index, corroborating with the effects on cancer cells. In addition, **35** drastically reduced the number of capillary-like structures formation using HUVEC cultured on growth factor reduced-Matrigel (GFR-Matrigel) coated plates. In addition, **35** was shown to act through the inhibition of NFκB [26].

3 Conclusion

Plants are well known to be a rich source of bioactive constituents, and they can provide high potential for discovery of new and effective antiangiogenic agents. Angiogenesis is a process regulated by a balance between pro- and antiangiogenic molecules, and the inhibition of pathological angiogenesis can be important in several diseases. Rapid advances were made in the field of angiogenesis over the past years, and, more importantly, a great attention has been given to angiogenesis as a therapeutic target. The application of this strategy in the treatment of several

diseases, including cancer and chronic inflammatory disorders, has now become a reality. At the moment, there are a growing number of alkaloids obtained from plants that may have antiangiogenic properties, suggesting that these naturally occurring compounds may provide significant approaches in treatment or prevention of diseases where excessive angiogenesis is part of the pathology. It can be concluded that antiangiogenic activities can be derived from alkaloids obtained from plants; however, most of the studies using antiangiogenic alkaloids were focused on in vitro studies, and there is a need to carry out in vivo studies to establish their efficacy and evaluate their toxic potentials. Besides that, it is still necessary to continue the search for novel molecules for drug development.

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