Natural Products as Angiogenesis Modulators

K.A. El Sayed*

Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monore, Monore, LA 71209, USA

Abstract: Cancer remains one of the major causes of death worldwide. Anti-angiogenic therapy is one of the new approaches to anticancer therapy. Out of 22 angiogenesis inhibitors currently under clinical trials there are 11 natural products or were modeled on a natural product parent. This review shows the potential of natural products for the discovery of new anti-angiogenic leads.

Keywords: Cancer, angiogenesis modulators, plant, marine, microbial natural products.

INTRODUCTION

Angiogenesis Definition and Background

Cancer remains one of the major causes of death worldwide [1-4]. In 2000, cancer accounted for over 7 million deaths (13% of total mortality) and there were more than 10 million new cancer cases worldwide.

More than 60% of cancer deaths and approximately half of new cases occurred in developing regions [1-3]. Lung cancer alone accounted more than 1/4 of the cancer mortality. In 2001, the cancer death toll is 553,400, including 157,400 deaths from lung cancer; 56,700 from cancers of the colon/rectum; 40,200 from female breast cancer; and 31,500 from prostate cancer [3]. Cancer death toll is expected to increase by 50% to 15 million new cases by the year 2020 [3]. The high incidence and death rate of various cancer types emphasize the need for new strategies to combat this pandemic disease [4]. Nonetheless, most current anti-tumor drugs cause severe side effects and the safe and side effects-free anti-tumor drug is yet to be discovered.

Cancer spreads by metastasis, which is the ability of cancer cells to penetrate lymphatic and blood vessels, circulate through the bloodstream, and then invade and grow in normal body tissues somewhere else. Hence, metastasis is the spread of cancer to other tissues and organs that makes cancer a potentially life-threatening disease. Metastasis requires the growth of a new network of blood vessels. This process of forming new blood vessels is called angiogenesis [5-8]. Angiogenesis is defined as the recruitment of new blood vessels. Tumor angiogenesis is the proliferation of a network of blood vessels that penetrates into cancerous growths, supplying nutrients and oxygen and removing waste products. Tumor angiogenesis actually starts with cancerous tumor cells releasing molecules that send signals to surrounding normal host tissue. This signaling activates certain genes in the host tissue that, in turn, make proteins to encourage growth of new blood vessels [5-8].

Angiogenesis is a strictly controlled process in the healthy, adult human body. It is regulated by a variety of endogenous angiogenic and angiostatic factors [5]. Angiogenesis is normally turned on during certain instances. Pathological angiogenesis occurs usually in cancer, chronic inflammation, or atherosclerosis [6]. The early discovery of angiogenesis was based on the fact that cancerous tissue cannot grow more than a few millimeters or metastasize without the development of new blood vessels, which supply tissues with oxygen and nutrients necessary for survival and growth (tumor-induced angiogenesis) [9-11]. Angiogenesis inhibition represents an active area of cancer drug discovery, with several agents and approaches now entering late stage clinical development [7].

Mechanism of Angiogenesis

In normal tissue, new blood vessels are formed during tissue growth and repair, and the development of the fetus during pregnancy [9]. In a mother's womb human fetus must create the vast network of arteries, veins, and capillaries that are found in the human body through vasculogenesis, which creates the primary network of vascular endothelial cells that will become major blood vessels [9]. Angiogenesis modify this network later into the small new blood vessels or capillaries that complete the child's circulatory system. Proliferation of new blood vessels occasionally occurs in adults. Blood vessel walls are formed by vascular endothelial cells [9]. These cells only divide once every 3 years on average. However, when the situation requires it, angiogenesis can stimulate them to divide. Endothelial cells are the source of new blood vessels and have the ability to divide and migrate. The creation of new blood vessels occurs by a series of several steps in order.

An endothelial cell forming the wall of an existing small blood vessel becomes activated, secretes enzymes that degrade the extracellular matrix, invades the matrix, and begins dividing. Filaments of new endothelial cells organize into hollow tubes, generating new networks of blood vessels that facilitate tissue growth and repair. New capillary growth is tightly controlled by a finely tuned balance between factors that activate endothelial cell growth and those that inhibit it. During the menstrual cycle in adult females, angiogenesis is activated for few days each month and new blood vessels form in the uterus lining. Angiogenesis is also necessary for the repair or regeneration of damaged tissue during wound healing and can rarely stimulate vascular

*Address correspondence to this author at the Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monore, Monore, LA 71209, USA; Tel: 318-342-1725; Fax: 318-342-1737; E-mail: Elsayed@ulm.edu

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endothelial cells to regenerate the walls of major blood vessels [12-15].

Tumor cells release molecules to activate the process of angiogenesis. Many proteins and small molecules are released by tumors as signals for angiogenesis. Examples of these molecules are the proteins: vascular endothelial growth factor (VEGF), epidermal growth factor, angiogenin, tumor necrosis factor-α, interleukin 8, acidic, and basic fibroblast growth factor (bFGF), which are produced by many kinds of cancer and normal cells [9-11]. Examples of angiogenic small molecules are: adenosine, nicotinamide, prostaglandins E1, and E2. VEGF and bFGF are first synthesized inside tumor cells and then released into the surrounding normal tissues [9]. Once they encounter endothelial cells, they bind to specific protein receptors on the outer surface of the cells, which activates a series of relay proteins that transmits a signal into the nucleus of the endothelial cells. The nuclear signal stimulates certain genes to make products needed for new endothelial cell growth. Activated endothelial cells produce matrix metalloproteinases (MMPs), which are enzymes that facilitate the degradation of the extracellular matrix. This process permits the migration of endothelial cells into the surrounding tissues, begin to divide, and they finally organize into hollow tubes that gradually develop into a mature network of blood vessels. Hence, both angiogenesis and metastasis require MMPs during blood vessel formation and tumor invasion, respectively [9]. Meanwhile, the presence of VEGF and bFGF is not enough to begin angiogenesis. These activator molecules must overcome an array of natural angiogenesis inhibitors that normally restrain blood vessel growth. The naturally occurring angiogenesis inhibitor proteins include angiostatin, interferons, tissue inhibitors of metalloproteinase-1-3, endostatin, platelet factor 4, interleukins 1 and 12, and thrombospondin. The balance between the concentration of angiogenesis inhibitors and activators decides whether a tumor can induce the growth of new blood vessels. Angiogenesis is predominant if the production of activators exceeds the production of inhibitors [12-15]. Angiogenesis is controlled by the coordinated production of endogenous angiogenic and angiostatic factors [12]. Hence, angiogenesis may be induced by activation (increased production) of angiogenic factors or reduction (suppressed production) of angiostatic factors [12]. Table **1** illustrates major angiogenic and angiostatic factors [8,12].

Since angiogenesis requires migration of vascular endothelial cells through the extracellular matrix toward tumors, it shares many features with tumor invasion. Hence, inhibition of extracellular matrix invasion is a major chemotherapeutic target to prevent metastasis and angiogenesis. Fortunately, steps required for metastasis and angiogenesis are similar for all tumors regardless of their genetic origin therefore blocking metastasis and angiogenesis by inhibiting invasion is useful for all tumors of different origin [14,15]. Generally there are four strategies used by investigators to design anti-angiogenesis agents (Fig. **1**). Blocking the factors that stimulate the formation of blood vessels, using natural inhibitors of angiogenesis, which act through the receptor integrin αvβ3, block molecules and MMPIs that allow newly forming blood vessels to invade surrounding tissue, incapacitate newly dividing endothelial cells, and induce endothelial cells apoptosis.

Angiogenesis Applications

1- Oncology

Tumor progressive growth and metastatic shedding is absolutely dependent on angiogenesis [13]. The maximum tumor growth without angiogensis is 1-2 mm, which can be reached when tumor is solely depending on diffusion for oxygen and nutrient supply [13]. In the past decade, anti-angiogenic drug development attracted more research interest and now over 20 such drugs are currently undergoing evaluation in phase I, II or III clinical trials either alone, or in various combinations with conventional cytotoxic therapeutics [15]. Angiogenesis inhibitors were found to restrain the growth of primary tumors and reduce their metastasis rate by 20-folds. Certain tumor dormancy for several years is attributed to the lack of angiogenesis. Also some primary tumors secrete the angiogenesis inhibitor angiostatin, which inhibits blood vessel growth at other sites and prevents microscopic metastases. On February 26, 2004, the FDA approved bevacizumab (Avastin®; Genentech) for treatment of metastatic colorectal cancer [16]. Bevacizumb, a humanized anti-VEGF monoclonal antibody, is the first anti-angiogenic product to be approved [16].

Unlike standard cancer chemotherapy drugs, angiogenesis inhibitors target dividing endothelial cells rather than tumor cells and other angiogenesis mediators (Fig. **1**). Anti-angiogenic drugs are not likely to cause bone marrow suppression, gastrointestinal symptoms, or hair loss side effects. Also, since anti-angiogenic drugs may not necessarily kill tumors, but rather hold them in check indefinitely, the endpoint of early clinical trials may be different than for standard therapies (Fig. **1**). Rather than

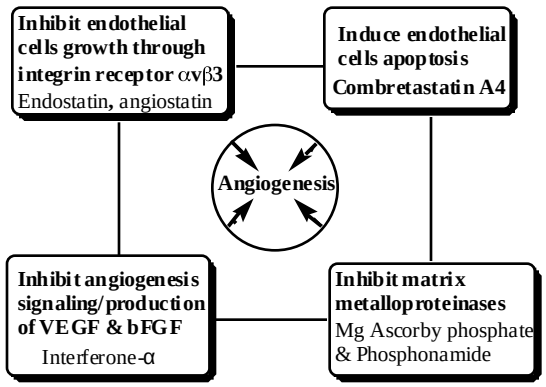


Fig. (1). Mechanisms of anti-angiogenic drugs.

looking only for tumor response, it may be appropriate to evaluate increases in survival and/or time of disease progression. Drug resistance is a major problem with conventional cancer chemotherapy. This is because most cancer cells are genetically unstable, are more susceptible to mutations and hence, they likely tend to produce drug resistant cells. Since angiogenic drugs target normal endothelial cells, which are genetically stable cells, drug resistance may not develop. So far, resistance has not been a major problem in long-term animal studies or in clinical trials. Significant problems remain in the clinical application of angiogenesis inhibitors to be resolved. For example, there is a need for alternative markers to monitor the effects of anti-angiogenic drugs when they do not cause tumor

Table 1. Angiogenic and Angiostatic Factors [9,12]

Angiogenic factors	Angiostatic factors
Angiogenin	Angiostatin (plasminogen cleavage product)
Growth factors	
Acidic fibroblast growth factor (aFGF-1)	**Cytokines**
Basic fibroblast growth factor (bFGF-2)	Interferon α (INF-α)
Epidermal growth factor	IL-12
Granulocyte colony-stimulating factor (G-CSF)	Platelet factor 4
Granulocyte macrophage CSF (GM-CSF)	TGF-β, high dose
Hepatocyte GF	TNF-α, high dose
Insulin-like growth factor-1 (IGF-1)	
Placental growth factor	Endostatin (collagen breakdown product)
Platelet-derived growth factor (PDGF)	
Transforming growth factor α (TGF-α)	Prolactin 16kD fragment
Transforming growth factor β (TGF-β), low dose	
Tumor necrosis factor α (TNF-α), low dose	Serine proteases
Vascular endothelial growth factor (VEGF)	Plasminogen inhibitor (PAI)
Cytokines	SPARC peptides 2.1 and 4.2
Interleukin 1 (IL-1)	
Interleukin 6 (IL-6)	Thrombospondin-1
Interleukin 8 (IL-8)	
	Tissue inhibitor of metalloproteinases 1, 2, and 3 (TIMP-1, 2, 3)
Prostaglandins	
Prostaglandin E_1 (PGE_1)	
Prostaglandin E_2 (PGE_2)	
Matrix metalloproteases	
Elastase	
Gelatinase	
Collagenase	
Platelet activating factor (PAF)	
Scatter factor	
Stromelysin	
Serine proteases	
Plasmin	
Urokinase	
Tissue plasminogen activator (t-PA)	
Small molecules	
Adenosine	
1-Butyl glycerol	
Nicotinamide	
Secreted protein acidic and rich in cysteine (SPARC) and bioactive SPARC peptides	

regressions. The chronic use of anti-angiogenic drugs may lead to delayed toxic side effects in humans, which do not appear in experimental short-term animal studies [15,17]. Generally the goal of anti-angiogenic therapy is to stop tumor growth which reduces the tumor burden of the body and may increase the efficacy of other concomitantly used adjuvant therapies [13]. If tumor number is small, chemotherapy, hyperthermia, radiation, and immune therapy are more effective and hence their combination with anti-angiogenic therapy is indicated [13].

2- Other Applications

Angiogenesis modulators also have other potential therapeutic applications (Fig. **2**). For example, some ocular diseases, especially diabetic retinopathy, neovascular glaucoma, trachoma, and retrolental fibroplasias are directly related to angiogenesis [12,13]. Delayed wound healing could be improved by angiogenic drugs. Vascular disorders, e.g., vascular adhesions and atherosclerotic plaque, chronic inflammatory diseases, e.g., hypertrophic scars, psoriasis, and pyogenic granuloma, as well as pediatric hemangioma could be improved by angiogenesis modulators [12,13]. Some angiogenic inhibitors also show potent *in vitro* inhibition of growth of chloroquine-sensitive and resistant strains of *P. falciparum* and *Leishmania donovani*, through inhibition of methionine aminopeptidase 2 (MetAP2), which is responsible for the hydrolysis of the initiator methionine molecule from the majority of newly synthesized proteins [18-20].

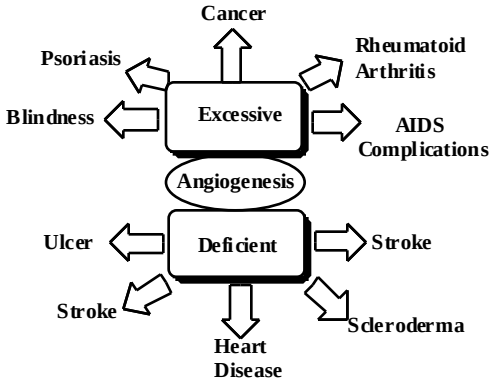


Fig. (2). Angiogenesis and diseases.

NATURAL PRODUCTS AS ANGIOGENESIS MODULATORS

Since the ancient times, natural products have served as a major source of drugs. About 50% of today's pharmaceutical drugs are derived from natural origin [21,22]. The growing interest in natural products as a source of new drugs can be attributed to many factors including urgent therapeutic needs, the wide range of both chemical structures and biological activities of natural secondary metabolites, the adequacy of bioactive natural products as biochemical and molecular probes, the development of recent techniques to accurately detect, isolate and structurally characterize the bioactive natural products, and advances in solving the demand for supply of complex natural products [21-23]. Historically, the majority of the natural product-based drugs including cyclosporin, paclitaxel, and camptothecin derivatives were first discovered by traditional cell-based *in vitro* assays before their real molecular biological targets were identified [22]. These cellular biological responses of natural products are likely to be associated with the inherent properties of secondary metabolites for the defense of their producing organisms [22,23]. Natural products are documented excellent probes for cellular targets that when modulated, may well have a selective lethal effect on the tumor cell cycle through the conventional cell cycle [23]. Out of 22 angiogenesis inhibitors currently under active clinical trials there are 11 natural products or natural-based compounds (Table **2**) [24]. This clearly shows the potential of natural products for the discovery of new lead entities as angiogenesis modulators [24].

A- Plant-Derived Angiogenesis Modulators

1- Catechols, Flavonoids, Tannins, and Aromatic Compounds

Combretastatin A-1 (**1**), B-1 (**2**), and A-4 (**3**) are catechol natural products, isolated from the South African shrub, *Combretum caffrum*, Combretaceae [25-27]. These compounds are found to be among the most potent antimitotic agents tested so far against a series of cancer cell lines [27]. The high potency of combretastatin A-1, A-4, and their disodium phosphate prodrugs as angiogenesis inhibitors offers a new hope and approach to cancer treatment [25-28].

Upon binding of combretastatins to tubulin, they prevent tumor cells from metastasizing by inhibiting their ability to grow new blood vessels. Combretastatins awarded more than ten patents during the past few years and combretastatin A-4 phosphate prodrug has successfully passed phase I clinical trials [27,28]. A phase II clinical study of intravenous combretastatin A-4 phosphate is currently in progress after successfully passing phase I study [29]. Combretastatin A-1 (**1**) diphosphate salt (Oxi 4503) was also successful in preclinical evaluation against the murine breast adenocarcinoma CaNT [26]. At a dose of 1 mg/kg, Oxi 4503 induced a more than 50% reduction in functional vascular volume of CaNT, which increased to 80% or more following doses of 10, 25 and 50 mg/kg [26]. In addition to these vascular effects, Oxi 4503 at doses of 100, 200 and 400 mg/kg induced a significant retardation in the growth of established CaNT tumors [26].

The consumption of a plant-based diet rich in flavonoids can prevent the development and progression of chronic diseases associated with extensive neovascularization, including the progression and growth of solid malignant tumours [30]. Flavonoids have been proposed to act as chemopreventive agents in many epidemiological studies and have been shown to inhibit angiogenesis and proliferation of tumor cells and endothelial cells *in vitro* [31]. Genistein (**4**) is an isoflavone abundant in the

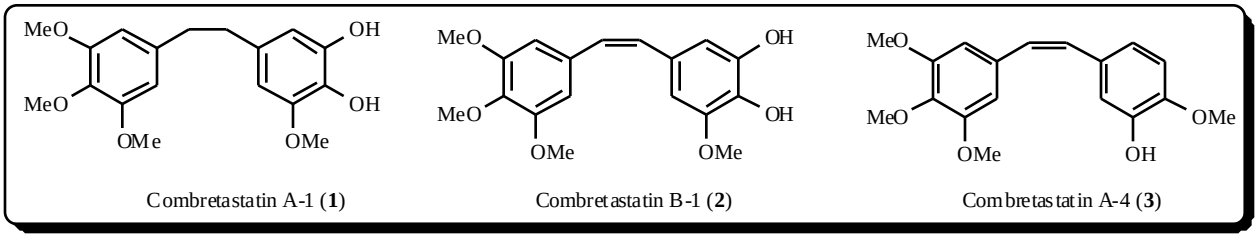
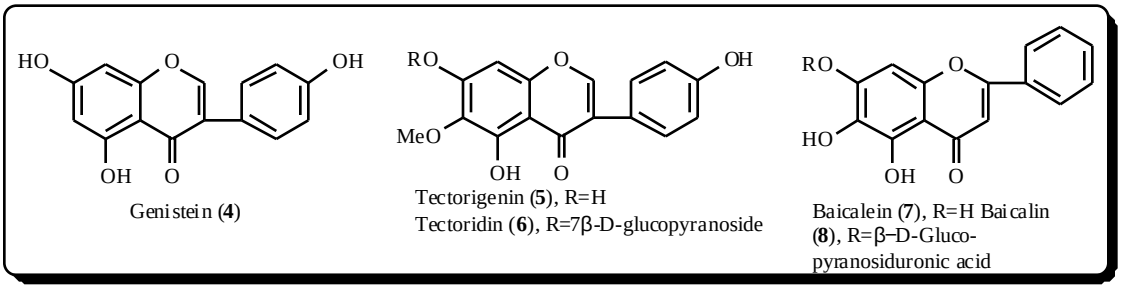


Table 2. Angiogenesis Inhibitors in Clinical Trials [24]

Drug	Angiogenesis Mechanism/Target	Origin
BMS-275291	Matrix breakdown	Synthetic
Dalteparin (Fragmin)	Matrix breakdown	Natural origin
Suramin	Matrix breakdown	Synthetic
2-Methoxyestradiol	Endothelial cells	Natural product
Thalidomide	Endothelial cells	Synthetic
CC-5013 (thalidomide analog)	Endothelial cells	Synthetic
Combretastatin A4 phosphate	Endothelial cells	Natural product
LY31761	Endothelial cells/ Protein kinase C β inhibitor	Synthetic
Genistein (Soy isoflavone)	Endothelial cells	Natural product
AE-941 (Neovastat)	Angiogenesis activators	Natural product
Anti-VEGF antibody (Bevacizumab, Avastin), Approved on February 26, 2004[16]	Angiogenesis activators	Natural product
Interferon-α	Angiogenesis activators	Natural product
PTK787/ZK 222587	Angiogenesis activators	Synthetic
VEGF-Trap	Angiogenesis activators	Natural product
ZD6474	Angiogenesis activators	Synthetic
EMD 121974	Endothelial-specific integrin/survival signaling	Natural origin
Anti-Anb integrin antibody (Medi-522, Vitaxin)	Endothelial-specific integrin/survival signaling	Natural product
Carboxyamidotriazole	No specific mechanism	Synthetic
Celecoxib (Celebrex)	COX-2 inhibitor/no specific angiogenesis mechanism	Synthetic
Rofecoxib (Vioxx)	COX-2 inhibitor/no specific angiogenesis mechanism	Synthetic
Halofuginone HBr (Tempostatin)	No specific mechanism	Synthetic
Interleukin-12	No specific mechanism	Natural product

Leguminosae, e.g. soy (*Glycine max*) and dyer's broom (*Genista tinctoria*) which is currently in clinical trial phase II as angiogenesis inhibitor [24,32,33]. Genistein inhibited endothelial cell proliferation and *in vitro* angiogenesis at half maximal concentrations of 5 and 150 μmol/L, respectively. Genistein also inhibited the proliferation of various tumor cells [33]. Genistein (**4**) could shift the matrix metalloproteinase/tissue inhibitor of matrix metalloproteinase (MMP/TIMP) proteolytic balance towards proteolysis inhibition in media or ascitic fluid conditioned by actively growing tumor cells [31,34]. Genistein blocked VEGF/bFGF-stimulated increase in TIMP-1 expression and decrease in TIMP-2 expression [31,34].

Recently, the isoflavones tectorigenin (**5**) and tectoridin (**6**) isolated from the rhizomes of *Belamcanda chinensis* (Iridaceae) are shown to inhibit angiogenesis *in vitro* and *in vivo* [35]. Tectorigenin and tectoridin decreased angiogenesis of both chick embryos in the chorioallantoic membrane assay and basic fibroblast growth factor-induced vessel



Genistein (**4**)

Tectorigenin (**5**), R=H
Tectoridin (**6**), R=7β-D-glucopyranoside

Baicalein (**7**), R=H Baicalin (**8**), R=β–D-Gluco-pyranosiduronic acid

formation in the mouse Matrigel plug assay [35]. Both compounds also reduced the proliferation of calf pulmonary arterial endothelial (CPAE) cells and found to possess relatively weak gelatinase/collagenase inhibitory activity *in vitro* [35]. Tectorigenin exhibited a much stronger anti-proliferative activity than its glycoside, tectoridin and was almost equipotent to genistein [35].

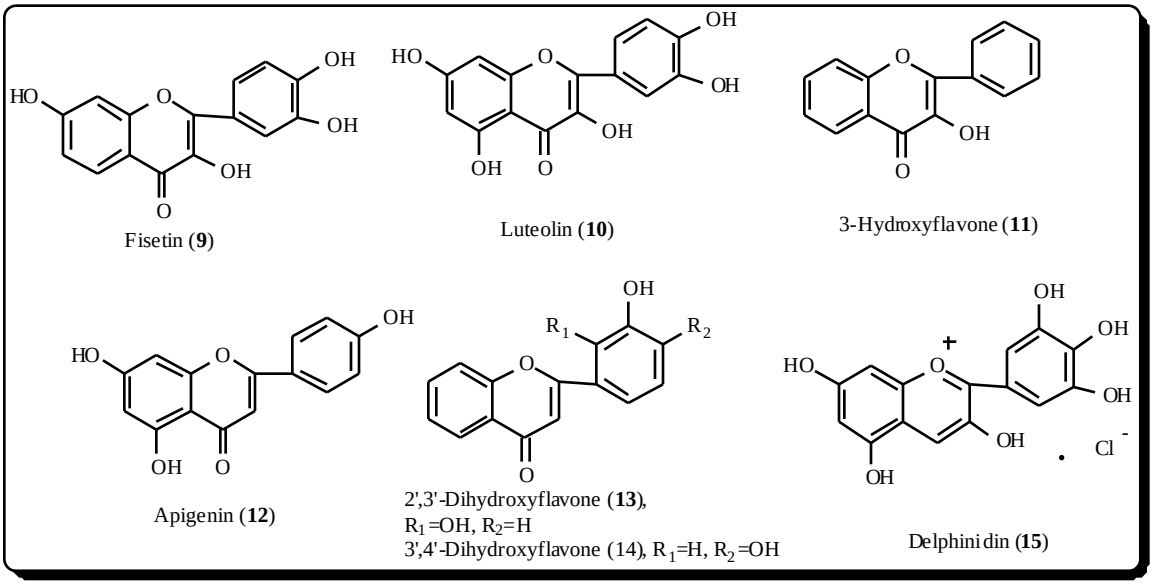
Baicalein (**7**) and baicalin (**8**) are flavones isolated from *Scutellaria baicalensis* (Lamiaceae) and recently shown to exhibit potent anti-angogenic activity *in vivo* and *in vitro* against human umbilical vein endothelial cells (HUVECs) [36]. Both **7** and **8** exhibited dual anti-proliferative (at a low dose) and apoptogenic (at a high dose) effects on HUVECs. The migration of endothelial cells and the differentiation of endothelial cells into branching networks of tubular structures *in vitro* were also inhibited [36].

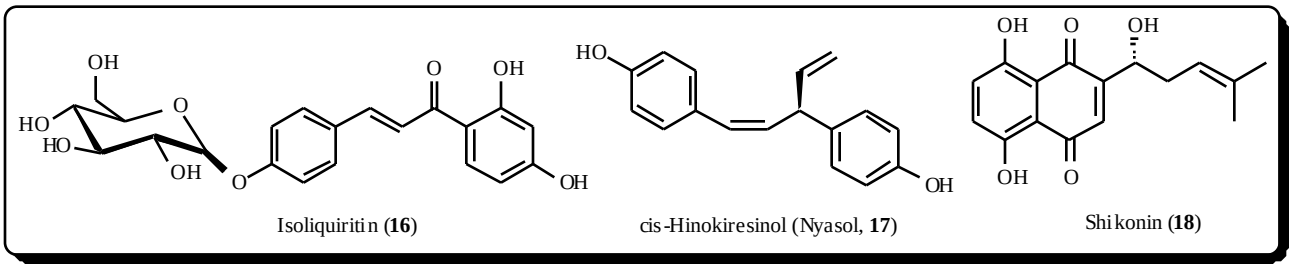
Flavanols and flavones are abundant plant polyphenols which inhibit some matrix-proteases instrumental in inflammation and cancer invasion, such as leukocyte elastase (LE) and gelatinases [37]. The structure activity relationship of flavonoids was recently reported based on their potential in blocking LE and gelatinase activities [37]. A crucial role in inhibition might be played by a galloyl moiety or hydroxyl group at C3, three hydroxyl groups at B ring, one hydroxyl group at C4', and a 2,3-double bond [37]. Gelatinase inhibition activity showed that three hydroxyl groups at the A or B ring, or, for non-planar molecules, a galloyl moiety at C3 could be determinant [37]. This could be utilized as a basis for designing new molecules with enhanced anti-proteolytic activities, and/or reduced side-effects, for use in hindering inflammation, cancer invasion and angiogenesis [37].

The flavonols fisetin (**9**), luteolin (**10**) and the isoflavone genistein (**4**) significantly inhibited corneal neovascularization *in vivo* [38]. Corneal blood vessels were induced by intrastromal implantation of pellets containing bFGF [38]. Fisetin had the strongest effect followed by genistein and luteolin. No significant topical side effects were observed which suggests that flavonoids may contribute to the preventive effect of a plant-based diet on neovascular disease of the eye [38]. 3-Hydroxyflavone (**11**) and apigenin (**12**) decreased TIMP-1 expression below basal level and completely abolished TIMP-2 expression [31]. VEGF and bFGF stimulation also significantly induced urokinase-type plasminogen activator (uPA) expression, the level of 33 kDa uPA, and increased the expression of PA inhibitor (PAI)-1. Both compounds effectively blocked the generation of 33 kDa uPA, and further decreased the activity of the 55 kDa uPA and the expression of PAI-1 below the basal level. This indicates that apigenin and 3-hydroxyflavone inhibit *in vitro* angiogenesis, in part *via* preventing VEGF/bFGF-induced MMP-1 and uPA expression and the activation of pro-MMP-2, and *via* modulating their inhibitors, TIMP-1 and -2, and PAI-1 [31]. The flavonoids 3-hydroxyflavone (**11**), apigenin (**12**), 2',3'-dihydroxyflavone (**13**), and 3',4'-dihydroxyflavone (**14**), inhibit the proliferation of normal and tumor cells as well as *in vitro* angiogenesis at half-maximal concentrations in the lower micromolar range. The anti-angiogenic activity of these compounds is even superior to the activity of genistein [33]. The anti-angiogenic and anti-mitotic activities of these common flavonoids, suggest that they may contribute to the preventive effect of a plant-based diet on chronic diseases, including solid tumors [33].

The anti-angiogenic activity and mechanism of the common plant anthocyanin delphinidin (**15**) is recently reported [39]. Delphinidin reverses the vascular endothelial growth factor-induced decrease in expression of cyclin-dependent kinase inhibitor p27kip1 and the vascular endothelial growth factor-induced increase of cyclin D1 and cyclin A, which are necessary to achieve the G1-to-S transition [39]. Delphinidin also inhibits neovascularisation *in vivo* in chorioallantoic membrane model [39].

The aqueous extract of licorice root inhibits granuloma angiogenesis in adjuvant-induced chronic inflammation and tube formation from vascular endothelial cells [40]. Isoliquiritin (**16**, 0.31-3.1 mg/kg), a licorice-derived flavonoid, identified as the main anti-angiogenic ingredient, which inhibited the carmine content of granuloma tissue and tube formation 50-fold greater than licorice extract at a dose





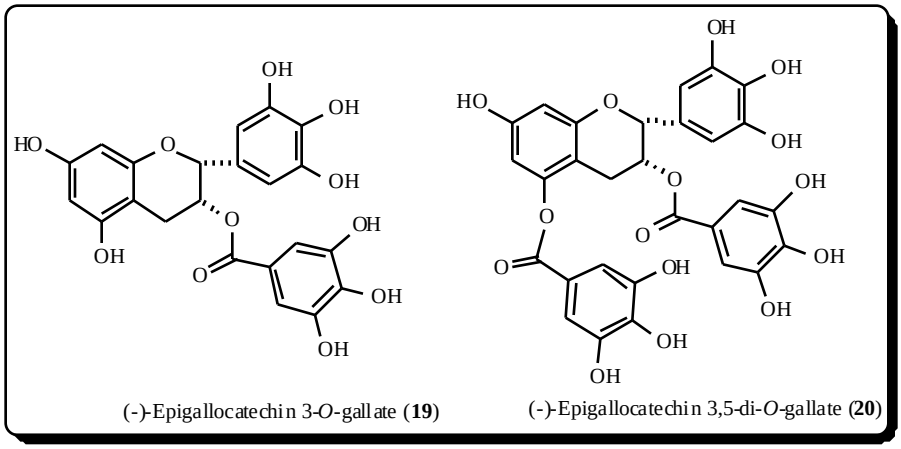
of 4.2 μg/ml [40]. Unlike **16**, licorice saponins glycyrrhizin and glycyrrhetinic acid increased tube formation [40].

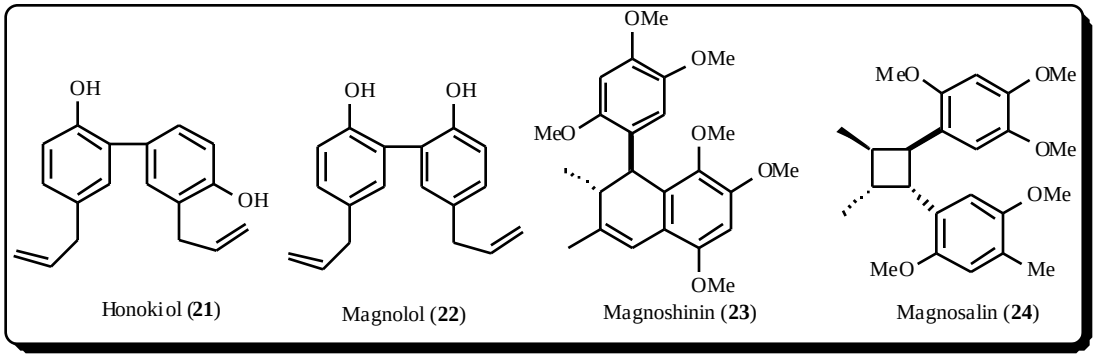
cis-Hinokiresinol (Nyasol, **17**) is a phenylpropanoids isolated from rhizomes of *Anemarrhena asphodeloides*, and its epimer from the tubers of *Asparagus cochinchinensis*, Liliaceae [41,42]. *cis*-Hinokiresinol and its salts are claimed as angiogenesis inhibitors for treatment of related diseases [41]. Compound **17** also showed anti-proliferative effect on human endothelial cells [41].

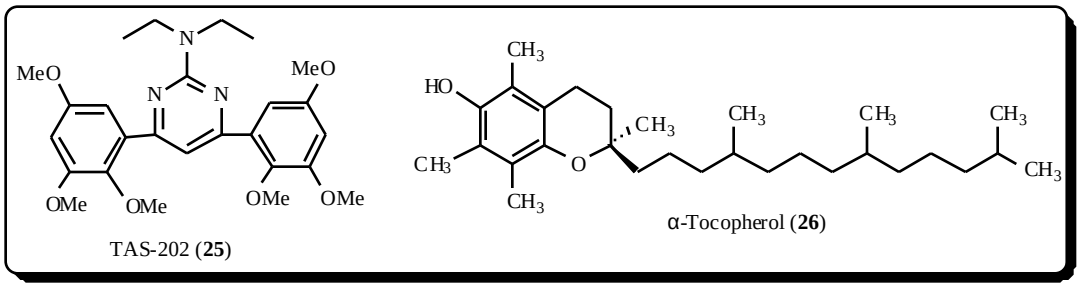
The naphthoquinone pigment, shikonin (**18**), isolated from *Lithospermum erythrorhizon* (Boraginaceae) is a Chinese herbal remedy used for the treatment of burns, tumors, and inflammation in China since the 5th century [43]. Shikonin effectively inhibits tumor necrosis factor-α-induced and B16 melanoma-induced angiogenesis in mice and normal developmental angiogenesis in the yolk-sac membranes of chick embryos [44]. Shikonin also inhibited proliferation and migration of endothelial cells in culture and network formation by endothelial cells on Matrigel *in vitro* [44]. Its mechanism is proposed to be through prevention of network formation by endothelial cells *via* blocking integrin αvβ3 expression [44].

Studies have indicated that the consumption of green tea is associated with a reduced risk of developing certain forms of cancer and angiogenesis [45,46]. Membrane-type 1 matrix metalloproteinase (MT1-MMP), which generates an active form of MMP-2 from proMMP-2, are deeply involved in angiogenesis, tumor cell migration, and metastasis. (-)-Epigallocatechin 3-*O*-gallate (EGCG, **19**) followed by (-)-epigallocatechin 3,5-di-*O*-gallate (**20**) and epitheaflagallin 3-*O*-gallate, were found to have potent and distinct inhibitory activity against MT1-MMP [45].

Vascular endothelial (VE)-cadherin, an adhesive molecule located at the site of intercellular contact, is involved in cell-cell recognition during vascular morphogenesis [46]. The extracellular domain of VE-cadherin mediates initial cell adhesion, whereas the cytosolic tail binding with β-catenin is required for interaction with the cytoskeleton and junctional strength [46]. Therefore, the cadherin-catenin adhesion system is implicated in cell recognition, differentiation, growth, and migration of capillary endothelium [46]. The vascular endothelial growth factor (VEGF)-induced tube formation is inhibited by anti-VE-cadherin antibody and dose-dependently by green tea







TAS-202 (**25**) α-Tocopherol (**26**)

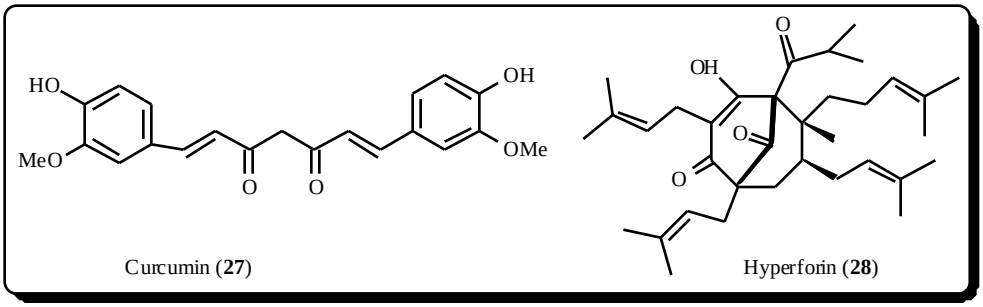
EGCG [46]. Hence, EGCG inhibits tumor angiogenesis through multiple mechanisms.

The aqueous extract of *Magnolia grandiflora* exhibit potent antiproliferative and anti-angiogenic activities [47]. The neolignans honokiol (**21**), magnolol (**22**), magnoshinin (**23**), and magnosalin (**24**) were identified as the anti-angiogenic ingredients of this extract [47-50]. Honokiol exhibited potent anti-proliferative activity against SVR cells *in vitro* and preferentially inhibited primary human endothelial cells compared with fibroblasts [47-48]. This inhibition was antagonized by antibodies against TNFα-related apoptosis-inducing ligand [47]. *In vivo*, honokiol was highly effective against angiosarcoma in nude mice and without toxicity. Both **21**, **22**, and their analogs were patented and proposed as potential anti-angiogenic chemotherapeutic agents [47-48]. Magnosalin (**23**) and magnoshinin (**24**) are the first magnolia neolignans that showed anti-angiogenic activity [49,50]. Compounds **23** and **24** inhibited fetal bovine serum (FBS)-stimulated tube formation in a concentration-dependent manner, with IC_{30} 0.51 and 8.14 μM, respectively [49]. The positive drug control in this study was cortisone, which showed IC_{30} 3.65 μM [49]. Both compounds also inhibited interleukin (IL)-1α-stimulated tube formation with IC_{50} of 1.22 and 0.74 μM, respectively [49]. Magnoshinin (**23**) and magnosalin (**24**) selectively inhibited adjuvant-induced angiogenesis and granuloma formation in the mouse pouch [50]. The recently reported magnosalin semisynthetic analog TAS-202 [**25**, 4-(3,4,5-trimethoxyphenyl)-6-(2,4,5-trimethoxyphenyl)-2-diethylaminopyrimidine] showed potent anti-angiogenic and anti-rheumatic activities [51]. TAS-202 inhibited the proliferation of vascular endothelial cells more potently than magnosalin, and when given orally it inhibited basic fibroblast growth factor (bFGF)-induced angiogenesis and collagen-induced arthritis in mice [51].

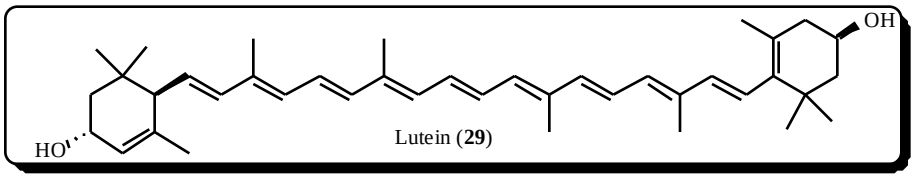
Long-term use of α-tocopherol (**26**) reduced prostate cancer through suppression of tumor angiogenesis, and tumor growth [52]. The long-term supplementation with α-tocopherol (50 mg daily) induced 11% reduction in serum vascular endothelial growth factor (VEGF) levels, a cytokine integrally involved in angiogenesis, in men who were not diagnosed with cancer [52].

Curcumin (**27**) is the major diaryl heptanoid yellow coloring material of turmeric rhizomes (*Curcuma longa*, Zingiberaceae) [32]. Curcumin showed potent *in vitro* and *in vivo* anti-angiogenic activity [53,54]. CD13/aminopeptidase N (APN) is a membrane-bound, zinc-dependent metalloproteinase that plays a key role in tumor invasion and angiogenesis [54]. Curcumin was shown to bind to APN and irreversibly inhibits its activity [54]. The direct interaction between curcumin with APN was confirmed both *in vitro* and *in vivo* [54]. Curcumin and other known APN inhibitors strongly inhibited APN-positive tumor cell invasion and basic fibroblast growth factor-induced angiogenesis. However, curcumin did not inhibit the invasion of APN-negative tumor cells, suggesting that the antiinvasive activity of curcumin against tumor cells is attributable to the inhibition of APN [54].

The anti-depressant and antibacterial prenylated phloroglucinol hyperforin (**28**) of St John's Wort (*Hypericum perforatum*, Hypericaceae) is reported to have a potent anticancer activity *in vitro* and *in vivo* [55,56]. The antiproliferative potential of hyperforin in 16 different human and rat cancer cell lines was reported and patented [55,56]. Hyperforin inhibited the growth of several cancer cell lines including some resistant strains to vincristine, paclitaxel, and camptothecin [55,56]. Hyperforin induces apoptosis that could be blocked by the caspase inhibitor zVAD.fmk and resulted in the induction of caspase-3 and caspase-9 but not caspase-8, suggesting that hyperforin could have an effect on an intrinsic, mitochondria-mediated cell death pathway. Hyperforin acts by facilitating the release of cytochrome c (and perhaps other pro-apoptotic molecules) from mitochondria which in turn activates the apoptosome-associated caspase-9 and triggers the caspase cascade [55,56]. Hyperforin is proposed as a good drug candidate because it



Curcumin (**27**) Hyperforin (**28**)



Lutein (**29**)

has a wide spectrum of activity, it has low toxicity, and it can be easily obtained in large quantities [55,56].

2- Carotenes and Rotenoids

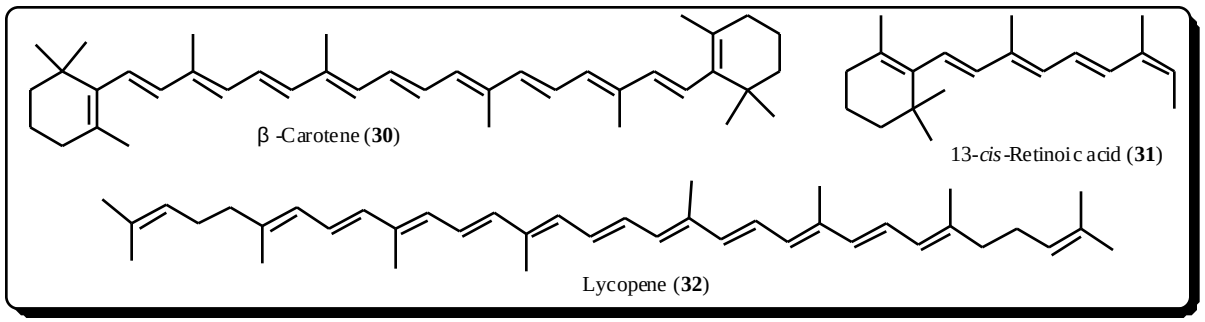
The common dietary carotene lutein (**29**) was shown to inhibit mouse mammary tumor growth by regulating angiogenesis and apoptosis [57]. Female BALB/c mice fed lutein had tumors that were 30 to 40% smaller on day 50 post-inoculation compared to unsupplemented mice. Final tumor volume was lowest in mice fed 0.002% lutein [57]. Mice fed lutein had higher apoptotic activity in the tumors but lower apoptotic activity in blood lymphocytes. Lutein-fed mice also had lower angiogenic activity in the tumors [57].

The dietary β-carotene (**30**), administered orally to mice, caused a decrease in angiogenesis evoked by HPV-transformed tumorigenic cell lines (SKv-t, HeLa) [58]. It did not affect angiogenesis induced by the non-tumorigenic SKv cell line, and increased lymphocyte-induced immune angiogenesis [58]. Hence, the anti-cancer effect of **30** is due to its inhibitory effect on formation of new blood vessels within the tumor mass [58].

for transforming growth factor-α (TGF-α) [59]. A recent patent was awarded for the anti-angiogenic activity of tomato carotene hydrocarbon lycopene (**32**) [60]. Lycopene, is used in combination with vitamin E and/or C for the primary and secondary prevention of angiogenesis-associated pathologies as coadjuvant treatment [60]. A tablet for the coadjuvant treatment of prostate carcinoma is formulated to contain 5 mg of lycopene, 200 mg of vitamin E, 250 mg of vitamin C, 37.5 mg of resveratrol, and 50 mg of quercetin [60]. The daily dosage is two tablets [60].

3- Steroids, Terpenes, and Saponins

Angiosatic steroids are among the most early anti-angiogenic compounds and they are not of plant origin [13,61,62]. These steroids are lacking glucocorticoid and mineralocorticoid activity and some of them are patented for potential anti-angiogenic activity [63]. 2-Methoxyestradiol (**33**), an endogenous estrogen metabolite of previously unknown function, is a potent inhibitor of endothelial cell proliferation and migration as well as angiogenesis *in vitro* [64-66]. Compound **33** strongly inhibited the neovascularization in solid tumors and suppressed their

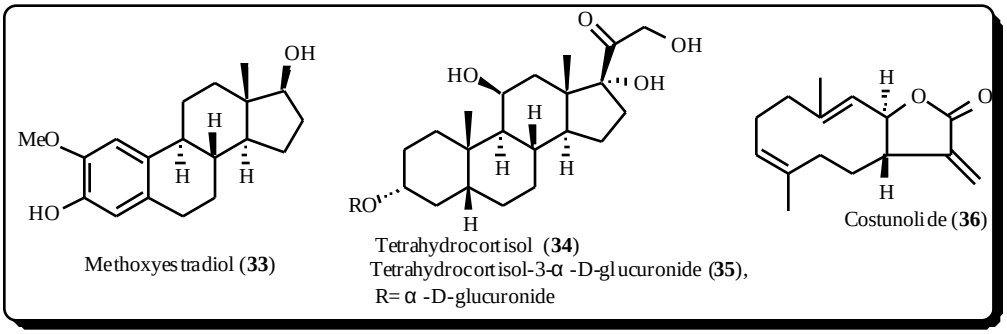

β -Carotene (**30**)

13-*cis*-Retinoic acid (**31**)

Lycopene (**32**)

Other study showed that β-carotene and canthaxanthin and the retinoid 13-*cis*-retinoic acid (**31**) have inhibited oral carcinogenesis in the hamster cheek pouch (1.4 mg/kg) induced by a 0.5% solution of 7, 12-dimethylbenz-[*a*]anthracene (DMBA) [59]. However, **31** at a higher dose (> 2.0 mg/kg per treatment) increased squamous cell carcinoma growth [59]. β-Carotene, **31**, and canthaxanthin administered to 60 hamsters (16 weeks, 3 times/week, 10 mg/kg) altered neovascularization characterized by immunohistochemistry

growth in mice and currently under clinical trials (Table 2)[24]. Unlike the angiostatic steroids of corticoid structure, it does not require the co-administration of heparin or sulfated cyclodextrins for activity like other angiostatic steroids, e.g. tetrahydrocortisol (**34**) or tetrahydrocortisol-3-α-D-glucuronide (**35**)[13,64-66]. Hence, 2-methoxyestradiol is the first steroid to have a high anti-angiogenic activity by itself [66].

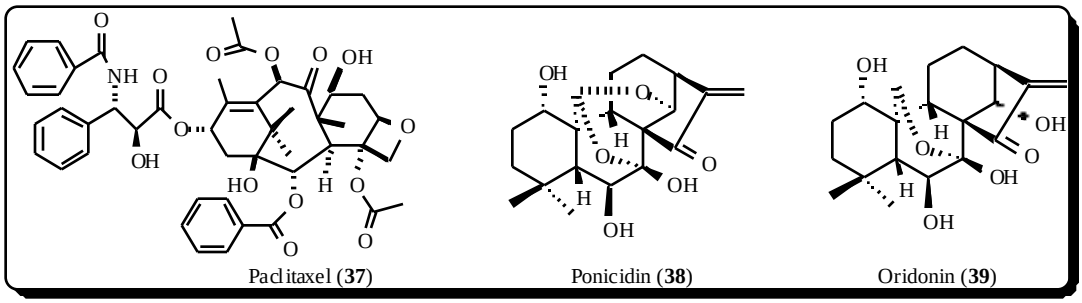


Methoxyestradiol (**33**)

Tetrahydrocortisol (**34**)

Tetrahydrocortisol-3-α-D-glucuronide (**35**), R= α -D-glucuronide

Costunolide (**36**)

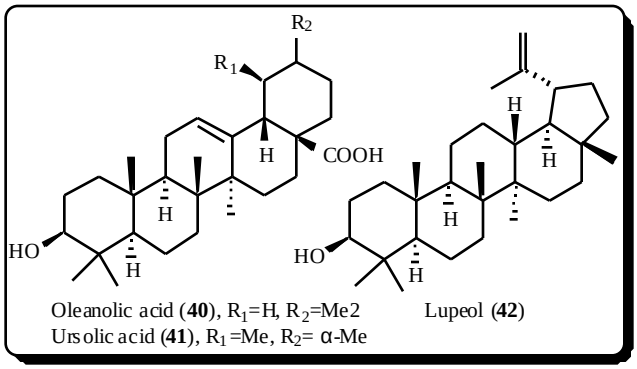


Costunolide (**36**), a sesquiterpene lactone isolated from *Saussurea lappa* (Asteraceae), is proposed as a possible anti-angiogenic lead [67]. Costunolide selectively inhibited the endothelial cell proliferation induced by vascular endothelial growth factor (VEGF) [67]. Compound **36** also inhibited the VEGF-induced chemotaxis of human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner [67]. Its anti-angiogenic mechanism is suggested to be through blocking the angiogenic factor-signaling pathway [67]. Costunolide also reduced VEGF-induced neovascularization in a mouse corneal micropocket assay [67].

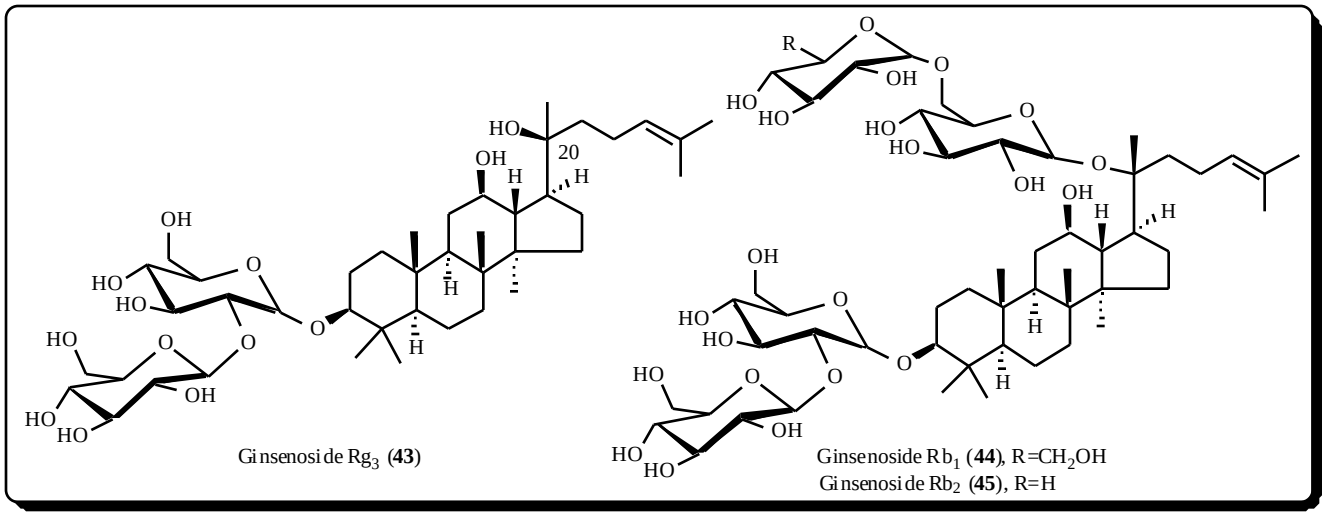
Paclitaxel (Taxol, **37**) is a diterpene ester isolated from the Yew tree *Taxus brevifolia* and *T. bacatta*, Taxaceae [32]. Paclitaxel is a potent microtubule depolymerization disruptor and currently considered the first choice prescription drug for many ovarian, breast, and non-small cell lung cancers [32]. A recent study pointed the potential anti-angiogenic and anti-migration effects of paclitaxel and other microtubule disruptors on tumor cells at a non-cytotoxic concentration [68]. The same report also indicates the possible use of some abandoned highly toxic microtubule-affecting agents as efficient anti-migratory without being too cytotoxic [68]. Concomitant use of paclitaxel and thalidomide in the treatment of highly vascular colorectal tumors in mice xenograft model also showed effective decreased expression of angiogenic growth factors and increased apoptotic index [69].

Anti-angiogenic activity of the aqueous ethanolic extract of *Rabdosia rubescens* (Lamiaceae), a component of the anti-prostate cancer dietary supplement PC SPES was attributed to the diterpenoids ponicidin (**38**) and oridonin (**39**) [70]. Both compounds inhibited network formation of HMEC-1 at a subtoxic concentration of 1.5 and 2.5 μg/ml, respectively [70]. The cytotoxic concentration of these compounds on HMEC-1 was higher than 4 μg/ml [70].

The common triterpene acids oleanolic acid (**40**) and ursolic acid (**41**) are reported to express anti-angiogenic activities by using the chick embryo chorioallantoic membrane (CAM) assay with ID_{50} 40 μg and 5 μg, respectively [71,72]. Both compounds effectively inhibited the proliferation of bovine aortic endothelial cell with IC_{50} values of 20 and 5 μM, respectively [71]. Both compounds were patented for their anti-angiogenic and anti-proliferative activities [72].



The methanol extract of barks of *Bombax ceiba*, Bombaceae, was found to exhibit a significant *in vitro* anti-angiogenic activity on tube formation of HUVEC [73]. Bioactivity-guided fractionation afforded the triterpene alcohol lupeol (**42**) as an active principle [73]. At 50 and 30 μg/ml, lupeol showed a marked inhibitory activity on



HUVEC tube formation but it did not affect the growth of SK-MEL-2, A549, and B16-F10 melanoma cells [73].

More than 25 dammarane-type tetracyclic triterpenoid saponins have been isolated from ginseng, the root and rhizome of *Panax ginseng*, Araliaceae [32, 74]. Ginsenoside Rg_3 (**43**) and its 20*R* epimer were found potent anti-angiogenic and effectively inhibit cancer cell invasion and metastasis, at a concentration of 10-100 μg/ml, by down regulating the expression of VEGF mRNA and protein and reducing microvascular density [13,74-76].

An intestinal bacterial metabolite of ginsenoside Rb_1 (**44**) inhibited the growth of implanted tumor and its intrahepatic metastasis following implantation of a small fragment of colon 26-L5 tumor into the mice liver [77]. The same compound eliminated the ability of colon 26-L5 cells CM-L5 to promote the migration of hepatic sinusoidal endothelial cells on Matrigel-coated substrates [77]. Intravenous administration of ginsenoside Rb_2 (**45**) to mice after B16-BL6 melanoma tumor cells inoculation achieved a remarkable reduction in the vessels number oriented toward the tumor mass, but did not cause a significant inhibition of tumor growth [78]. The anti-angiogenic effect was dose-dependent ranging from 10-500 μg/mouse [78]. Intra-tumoral or oral administration of ginsenoside Rb_2 caused a marked inhibition of both neovascularization and tumor growth [78]. These results suggest that the inhibition of tumor-associated angiogenesis by **45** may contribute to the inhibition of lung tumor metastasis [78].

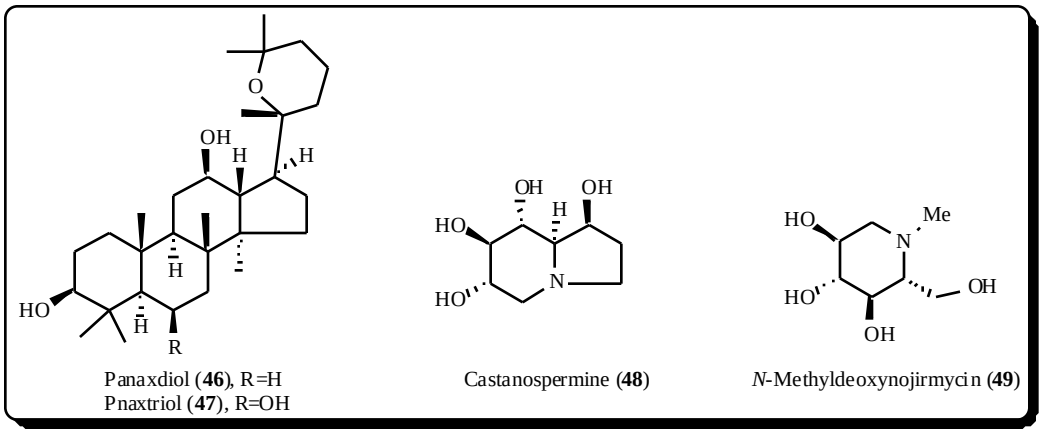
In contrast to ginsenosides **43**-**45**, ginseng saponins panaxdiol (**46**), panaxtriol (**47**), and dihydroginsenoside Rb_1 were patented in 2002 due to their potent stimulatory effect on angiogenesis [79-81]. Panaxadiol and panaxtriol are claimed to treat conditions requiring stimulation of angiogenesis but not stimulation of chemoinvasion [79]. They significantly stimulate tube formation by endothelial cells (10-100 μg/ml), slightly enhancing endothelial cell proliferation, and effectively stimulating its migration [13]. A skin preparation composed mainly of dihydroginsenoside Rb_1 was claimed useful in promoting vascular regeneration and reconstruction or preventing, treating and remedying diseases causing blood flow failures [81]. Dihydroginsenoside Rb_1 works *via* the activation of a transcription factor STAT5 and/or a transcription factor HIF-1 [81].

4- Alkaloids

The indolizidine alkaloid castanospermine (**48**) isolated from *Castanospermum australe*, Leguminosae, is a potent antiviral and glucosidase I inhibitor that convert protein *N*-linked high mannose carbohydrates to complex oligosaccharides [32, 82]. Castanospermine shows potent inhibition of tumor growth in nude mice [82]. Angiogenesis to basic fibroblast growth factor in castanospermine-treated C57/BL mice was similarly reduced [82]. Castanospermine and the *N*-containing sugar glucosidase inhibitor *N*-methyldeoxynojirmycin (**49**) prevented the morphological differentiation of endothelial cells *in vitro* [82]. Cultured endothelial cells showed a reduced ability to migrate and to invade basement membrane gels *in vitro* and an increased tendency to form aggregates that was inhabitable by D-mannose [82]. Hence, certain cell surface oligosaccharides are required for angiogenesis and glucosidase inhibitors that alter these structures on endothelial cells are able to inhibit tumor growth [82].

Tetrandrine (**50**) is a *bis*-benzylisoquinoline alkaloid isolated from the Chines medicinal herb *Stephania tetrandra*, Menispermaceae, and traditionally has been used for the treatment of congestive circulatory disorder and inflammatory diseases [83,84]. Tetrandrine, together with a few of its structural analogs, has long been demonstrated to have antihypertensive action in clinical and animal studies [84]. Tetrandrine potently inhibited choroidal angiogenesis and air-pouch granuloma angiogenesis in the diabetic state [83]. Tetrandrine (10-30 nM) non-competitively inhibited the tube formation stimulated by interleukin-1α and platelet-derived growth factor even greater than the positive control FBS-stimulated tube formation [83]. Hence, tetrandrine reduces the tube formation of endothelial cell in the angiogenic process through inhibition on the post-receptor pathway of interleukin-1α and platelet-derived growth factor in chronic inflammation [83,84]. Tetrandine could then be a potentially useful lead for the development as anti-inflammatory, anti-fibrogenic agent, and for the treatment of lung silicosis, liver cirrhosis, and rheumatoid arthritis [83,84].

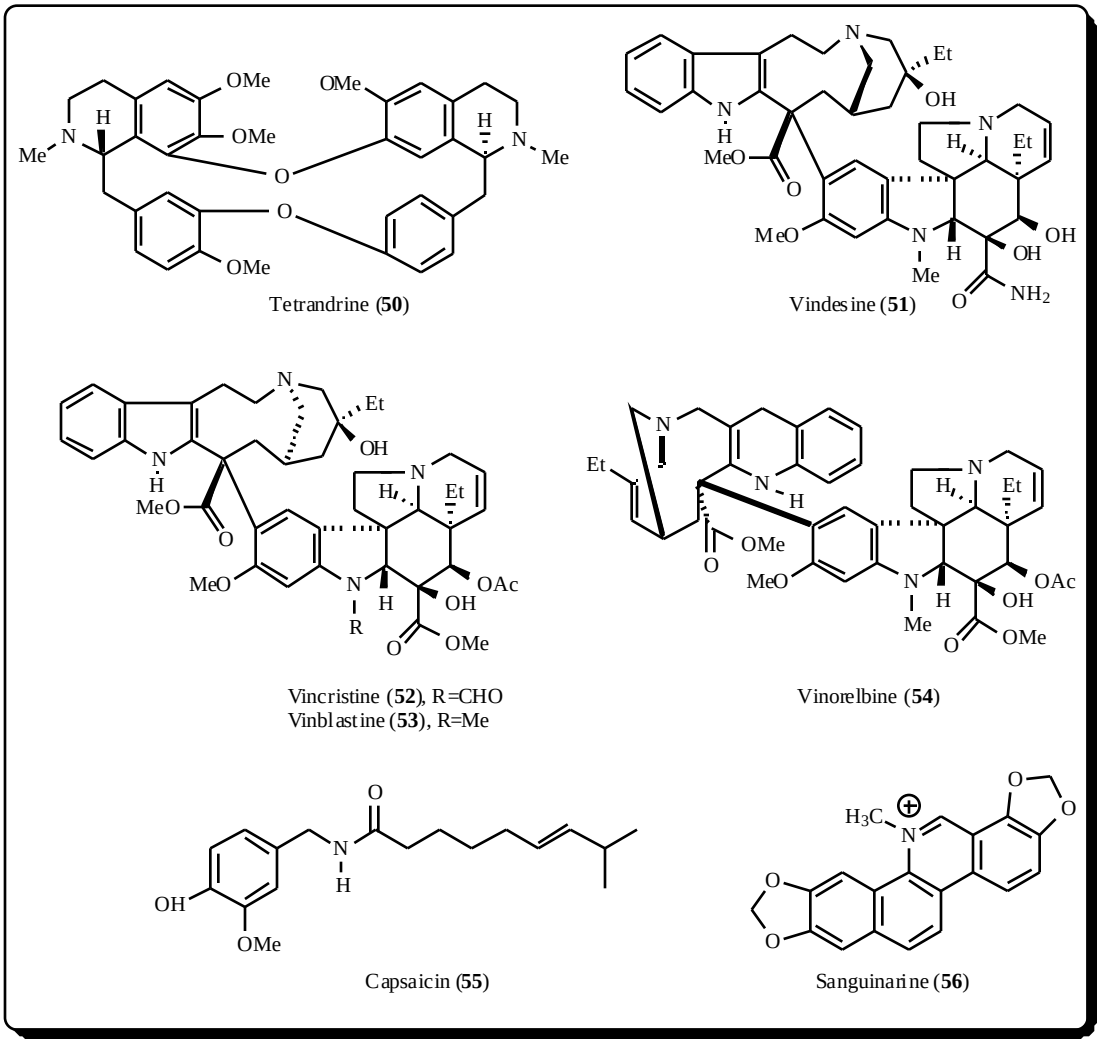
The known anti-mitotic alkaloids of *Catharanthus roseus* (Apocynaceae) vindesine (**51**) and vincristine (**52**) behave similarly in their ability to reduce the capillary network formation by HUVEC cells cultured on Matrigel



Panaxdiol (**46**), R=H
Pnaxtriol (**47**), R=OH

Castanospermine (**48**)

N-Methyldeoxynojirmycin (**49**)



[32,68]. This anti-angiogenic effect appears at a non-cytotoxic concentration. In contrast, vinblastine (**53**) and vinorelbine (**54**) produce apparent anti-angiogenic effects by direct cytotoxicity [68]. Microtubule-affecting agents are also able to significantly reduce the level of migration of tumor cells at non-cytotoxic concentrations, some of these effects may occur *via* modifications of the actin cytoskeleton organization [68]. Some microtubule-affecting agents abandoned in pharmacological assays could turn out to be potent anti-migratory drugs acting on tumor cells, though without being too cytotoxic [68].

Capsaicin (**55**) is a natural alkaloid of *Capsicum annum var. minimum* and *C. frutescens* (Solanaceae) fruits [32]. It is known to induce excitation of nociceptive terminals involved in pain perception. *In vitro*, capsaicin inhibited VEGF-induced proliferation, DNA synthesis, chemotactic motility, and capillary-like tube formation of primary cultured human endothelial cells [85]. Capsaicin inhibited both VEGF-induced vessel sprouting in rat aortic ring assay and VEGF-induced vessel formation in the mouse Matrigel plug assay [85]. It also suppressed tumor-induced angiogenesis in CAM assay [85]. Capsaicin induces G1 arrest in endothelial cells. This effect correlated with the down-regulation of the expression of cyclin D1 that led to inhibition of cyclin-dependent kinase 4-mediated phosphorylation of retinoblastoma protein [85]. Capsaicin inhibits VEGF-induced p38 mitogen-activated protein kinase, p125FAK, and AKT activation [85].

Sanguinarine (**56**) is a benzophenanthridine alkaloid derived from the root of *Sanguinaria canadensis* [32, 86]. It is used as antibacterial, antifungal, and anti-inflammatory agent in dental products [32, 86]. Sanguinarine effectively inhibited VEGF-induced endothelial cell migration, sprouting, and survival *in vitro* in a dose-dependent manner at nanomolar concentrations [86]. It also potently suppressed blood vessel formation *in vivo* in mouse Matrigel plugs and CAM [86]. Sanguinarine (**56**) strongly suppressed basal and VEGF-induced Akt phosphorylation (activation), while it did not produce any changes in VEGF-induced activation of ERK1/2 and PLCγ 1 [86].

B. Marine-Derived Angiogenesis Modulators

The importance of marine life in human's short-term needs can hardly be overestimated. The contribution of marine primary producers to the Earth's fixed carbon is similar to that of terrestrial plants. The sea is a net oxygen producer, through irreversible carbon dioxide fixation. Oceans nearly possess 80% of Earth's animal life [87]. Marine macrofauna includes abundant and diverse

macroscopic sessile or moving animals. Invertebrate marine animals, e.g., sponges, corals, cnidarians, bryozoans, brachiopods, echinodermis, crustaceans, chelicerates, ascidians, and mollusks, are known of their efficient ability to produce diverse secondary metabolites, in part as a main chemical defense tools against their natural predators. Only a few thousand compounds have been reported from marine origin [87,88]. These compounds displayed a wide array of biological activities and chemical entities. This could be attributed to the ability of marine organisms to release secondary metabolites as their own chemical defense weapons to survive in extreme temperature, salinity, and pressure and to resist their own predators [88].

1- Bromotyrosine and Aromatic Compounds

(+)-Aeroplysinin-1 (**57**) is a bromotyrosine derivative produced by many Verongide sponges [87,88]. Aeroplysinin-1 inhibited the growth of endothelial cells in culture and induced endothelial cell apoptosis [89]. Capillary tube formation on Matrigel was completely abrogated by addition of a micromolar concentration of aeroplysinin-1 [89]. Aeroplysinin-1 also exhibited a clear inhibitory effect on the migration capabilities of endothelial cells and decreases the concentration of matrix metalloproteinase-2 and urokinase in conditioned medium from endothelial cells [89]. It also shows a dose-dependent inhibitory effect on the *in vivo* chorioallantoic membrane assay, showing potent apoptosis-inducing activity in the developing endothelium [89].

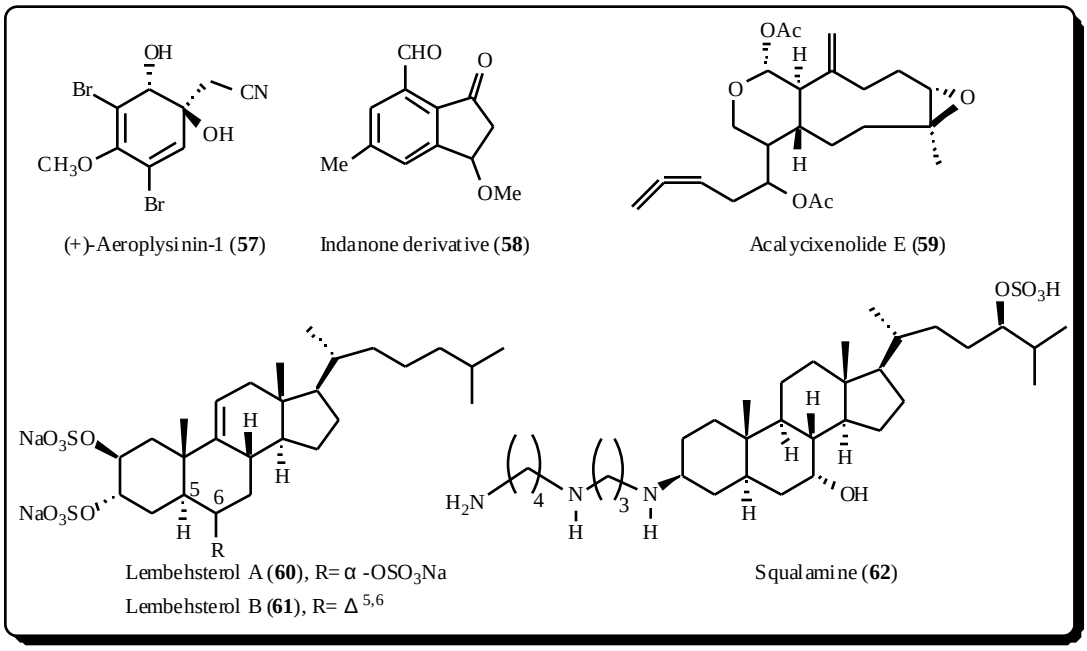
The indanone derivative (**58**) isolated from the filamentous marine cyanobacterium *Lyngbya majuscula*, inhibits hypoxia-induced activation of the VEGF gene promoter in Hep3B human liver tumor cells, *in vitro* [90].

2- Terpenes and Steroids

Acalycixenolide E (**59**), a norditerpene isolated from the marine organism *Acalycigorgia inermis* exhibits a potent anti-angiogenic activity both *in vitro* and *in vivo* [91]. It inhibits the bFGF-induced proliferation of HUVECs in a dose dependent manner, along with the bFGF-induced migration, invasion, and tube formation [91]. Acalycixenolide E (**59**) potently inhibits the *in vivo* neovascularization of CAM and suppresses the expression of metalloproteases 2 and 9 [91].

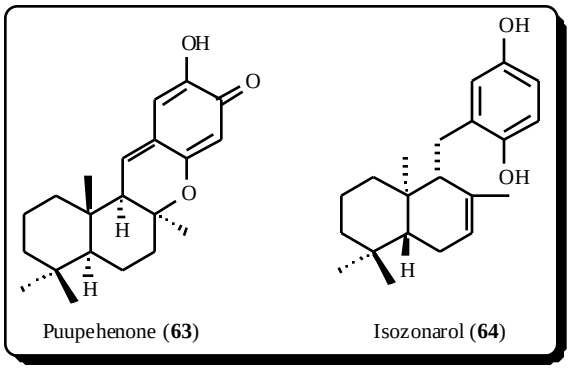
Lembehsterols A (**60**) and B (**61**) are two sulfated sterols reported from the marine sponge *Petrosia strongylata* [92]. Both sterols showed inhibitory activity against thymidine phosphorylase, which is an enzyme related to angiogenesis in solid tumors [92].

Squalamine (**62**) is sulfated aminosterol isolated from the dogfish shark *Squalus acanthias* [93]. Originally identified as an antimicrobial substance, squalamine has now been shown to be an angiostatic steroid in several *in vitro* assays and *in vivo* [93]. Squalamine differs in structure from previously described angiostatic steroids. It does not interact with glucocorticoid or mineralocorticoid receptors, and operates by a previously undescribed mechanism for steroids that modulate angiogenesis [93]. It inhibited angiogenesis and tumor growth in multiple animal models [93]. This effect is mediated, at least in part, by blocking mitogen-induced proliferation and migration of endothelial cells, thus preventing neovascularization of the tumor [93]. Squalamine has no observable effect on unstimulated endothelial cells, is not directly cytotoxic to tumor cells, does not alter mitogen production by tumor cells, and has no obvious effects on the growth of newborn vertebrates [93]. Squalamine was also found to have remarkable effects on the primitive vascular bed of the chick chorioallantoic membrane, which has striking similarities to tumor capillaries [93]. A Phase I study of squalamine was conducted in patients with advanced cancers [94]. On the basis of preclinical evidence of squalamine synergy with cytotoxic agents and demonstration of human safety from this trial, additional clinical trials have been initiated with squalamine in combination with chemotherapy for patients with late stage lung cancer and ovarian cancer [94]. Squalamine is well tolerated at a dose of 500 mg/day and results in plasma concentrations at least an



order of magnitude higher than those required for prominent anti-angiogenic effects in preclinical studies [94]. Squalamine is progressing to higher clinical trials as a successful example of angiostatic natural product.

Puupehenone (**63**) is a cytotoxic and antituberculosis shikimate-derived sesquiterpene produced by sponges of the orders Verongida and Dictyoceratida [95]. The potential anti-angiogenic activities of puupehenone and 11 related compounds were reported using cell growth and differentiation assays on bovine aorta endothelial cells [96]. The differentiation of endothelial cells into tubular structures was completely inhibited by **63** and 6 other related compounds at concentrations ≤ 3 μM [96]. The sesquiterpene hydroquinone isozonarol (**64**), 8-epipuupehedione and 8-*epi*-9,11-dihydropuupehedione, completely inhibited the *in vivo* angiogenesis in the CAM assay at doses ≤ 30 nmol/egg [96]. These 3 terpenes also inhibited endothelial cell production of urokinase and invasion. Only 8-*epi*-puupehedione inhibited endothelial cell migration in a dose-dependent manner [96].

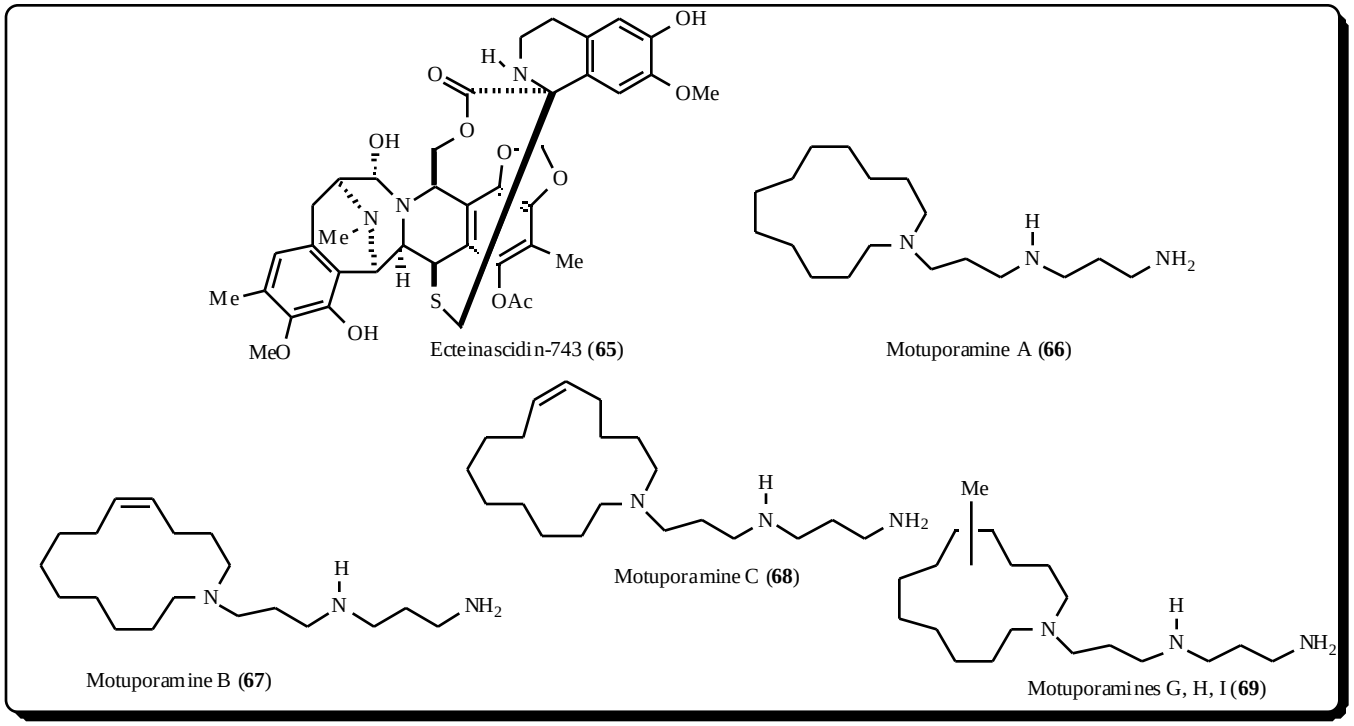


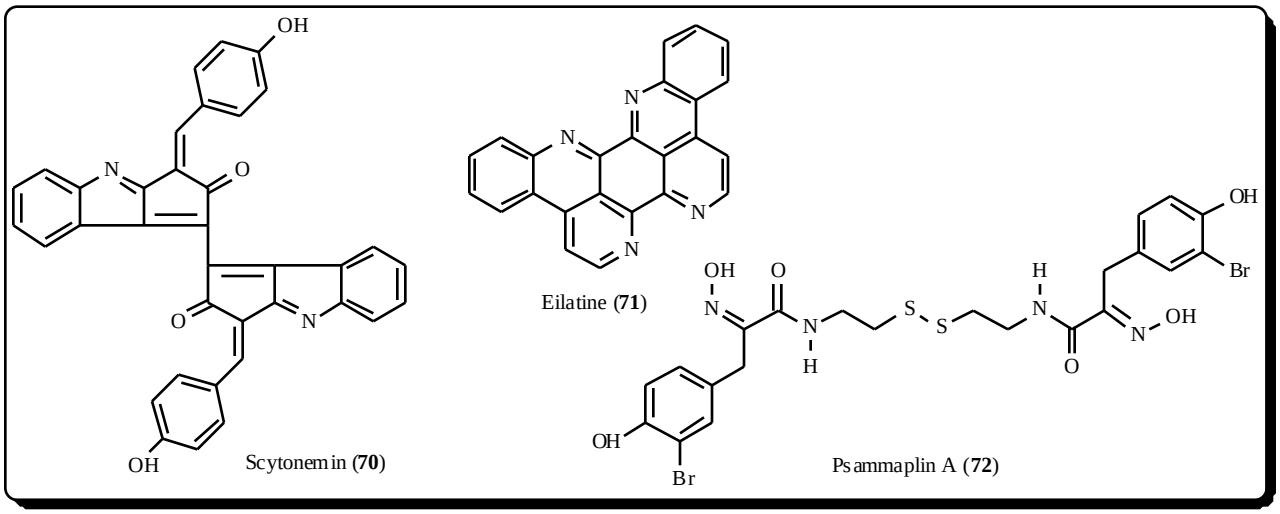
Puupehenone (**63**) Isozonarol (**64**)

3- Alkaloids

The marine alkaloid ecteinascidin-743 (**65**), isolated from the colonial tunicate *Ecteinascidia turbinate* is a perfect example for the potential marine natural products in the area of anticancer [97]. This potent antiproliferative alkaloid uniquely interacts with DNA and it has a reversible alkylation mechanism with guanine N2 in the DNA minor groove [98]. Ecteinascidin-743 is a selective transcription inhibitor, which has the unique characteristic of poisoning transcription-coupled nucleotide excision repair [98]. It is currently in phase II/III clinical trials for soft tumors in the US and Europe [99]. A combination of **65** and plasminogen-related protein B, which antagonizes various endothelial cell activities was effective at slowing the growth of primary human chondrosarcoma (CHSA) [100]. The combination of the two agents resulted in only a modest further repression of tumor growth over that associated with **65** treatment alone, as measured by tumor volume (82% versus 76% inhibition, respectively) [100]. However, analysis of the extent of tumor necrosis and vascularization of the tumor revealed that the coadministration of the two compounds was clearly more effective, eliciting a 2.5-fold increase in tumor necrosis relative to single-agent treatment [100]. This combination was also effective at antagonizing tumor-associated microvessel formation, suggesting that combination therapy may hold promise for treating CHSA [100]. Tumor necrosis produced by combination therapy of **65** and recombinant plasminogen-related protein B was also significantly greater than that produced by conventional doxorubicin treatment [100].

Motuporamines A (**66**), B(**67**), and C(**68**) and the mixture of G, H, and I (**69**), isolated from the marine sponge *Xestospongia exigua* (Kirkpatrick), were found to have potent anti-invasion activity in micromolar concentration in basement membrane gels by MDA-231 breast carcinoma, PC-3 prostate carcinoma, and U-87 and U-251 glioma cells [101,102]. Motuporamine C inhibits cell migration in monolayer cultures and impairs actin-mediated membrane





ruffling at the leading edge of lamellae [101,102]. Motuporamine C also reduces β1-integrin activation and inhibits angiogenesis in an *in vitro* sprouting assay with human endothelial cells and an *in vivo* chick chorioallantoic membrane assay. The motuporamines show little or no toxicity or inhibition of cell proliferation, and their simple structure and synthesis render them significant anticancer leads [101,102].

Scytonemin (**70**) is a yellow-green pigment isolated from the extracellular sheath of cyanobacteria [103]. It showed potent anti-inflammatory and anti-proliferative activities, through dual kinases inhibitory activity [103]. It offers a novel pharmacophore, which may serve as a template for synthesizing more potent and selective angiogenesis inhibitors [103].

Eilatin (**71**) is a novel marine alkaloid, isolated from the Red Sea purple tunicate *Eudistoma* sp. It shows antileukemic activity against *in vitro* Philadelphia chromosome-positive (Ph+) cells and may be used in conjunction with currently available agents, e.g. interferon-α and Ara-C, for chronic myelogenous leukemia patients in conjunction with autologous bone marrow transplantation [104].

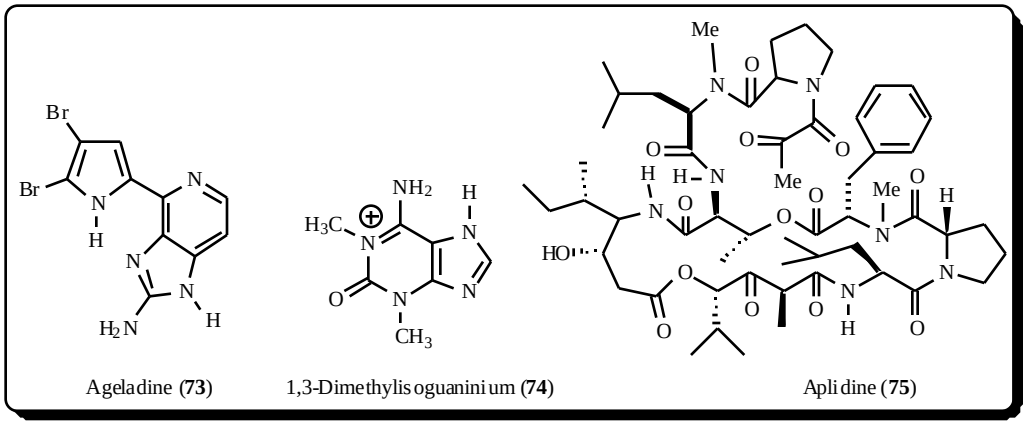
Psammaplin A (**72**) is a phenolic brominated alkaloid isolated from a marine sponge. It inhibits mammalian aminopeptidase N that plays a key role in tumor cell invasion and angiogenesis [105]. Psammaplin inhibited aminopeptidase N activity with an IC_{50} of 18 μM in a non-competitive manner [105]. It also potently inhibited the proliferation of several cancer and endothelial cells [105]. Compound **72** suppressed the invasion and tube formation of endothelial cells stimulated by basic fibroblast growth factor [105].

Ageladine A (**73**) is a bromopyrrole alkaloid from the marine sponge *Agelas nakamurai*, which showed potent anti-angiogenic and matrix metalloproteinase inhibitory activities [106].

1,3-dimethylisoguaninium (**74**) is an anti-angiogenic purine alkaloid from the Okinawan sponge *Amphimedon paraviridis* [107]. Compound **74** exhibited specific inhibition of the bFGF-induced proliferation of bovine aorta endothelial cells (BAECs) and reduced the tube formation of BAECs in a time-dependent manner [107].

4- Peptides and Proteins

Aplidine (dehydrodidemnin B, **75**) is a naturally occurring cyclic depsipeptide isolated from the Mediterranean tunicate *Aplidium albicans* [108]. Aplidine displays promising *in vitro* and *in vivo* antitumor activities against various solid human tumor xenografts and hence it is currently under clinical testing [108]. Aplidine inhibits vascular endothelial growth factor (VEGF) secretion, blocks VEGF-VEGFR-1 (flt-1) autocrine loop, and induces apoptosis in human leukemia cells MOLT-4 [108,109].



A shark cartilage angiogenesis inhibitor-a (SCAI-a), which composed of a peptide with molecular mass of 12600, was specifically active in CAM assays [110].

5- Carbohydrates and Sulfated Carbohydrates

Fucoidans are sulfated polysaccharides extracted from brown marine algae. A purified fucoidan fraction exhibits the same venous antithrombotic activity as heparin in rabbits, but with a lower anticoagulant effect [111]. Fucoidan had no modulatory effect on the mitogenic activity of FGF-2, but significantly increased tubular structure induced by FGF-2. In the presence of FGF-2, fucoidan enhanced $\alpha 6$, $\beta 1$ and PECAM-1 and inhibited $\alpha v\beta 3$ integrin expression. Heparin had no effect in these systems [111]. Natural and chemically modified oversulfated fucoidan are also reported to have a similar activity [111].

Neovastat (AE-941) is an anti-angiogenic polysaccharide isolated from marine cartilage and currently under clinical investigation (Table 2) [112,113]. It selectively competes for the binding of vascular endothelial growth factor to its receptor, stimulates tissue plasminogen activator enzymic activities, interferes with several steps associated with the development of angiogenesis through its ability to induce endothelial cell apoptosis, and to inhibit matrix metalloproteinase activities and vascular endothelial growth factor-mediated signaling pathways. This suggested that Neovastat behaves as a multifunctional anti-angiogenic drug [113,114]. Neovastat is orally bioavailable, and shows significant antitumor and antimetastatic properties in animal models [113,114]. Neovastat is proved clinically safe for long-term use, either alone or in combination with other anticancer therapies and hence it is currently under evaluation in three pivotal clinical studies with two-phase III clinical trials in patients with lung and renal carcinoma, and a phase II clinical trial in patients with multiple myeloma [113,114]. AE-941 is reported to be well tolerated in patients with advanced lung cancer, which may confer a survival benefit [112].

A series of marine-derived sulfated polysaccharides including genuine carrageenans and modified carrageenans were tested for their ability to inhibit the formation of new blood capillaries with good success, however, they had considerable side effects. Better results were obtained with a series of modified galactans [115]. Hence, β-1,4-galactansulfates of relatively low degree of polymerization with a distinct degree of sulfation have better anti-angiogenic activity, which entitles them for further development as angiogenesis inhibitor lead structures [115].

C- Microbial-Derived Angiogenesis Modulators

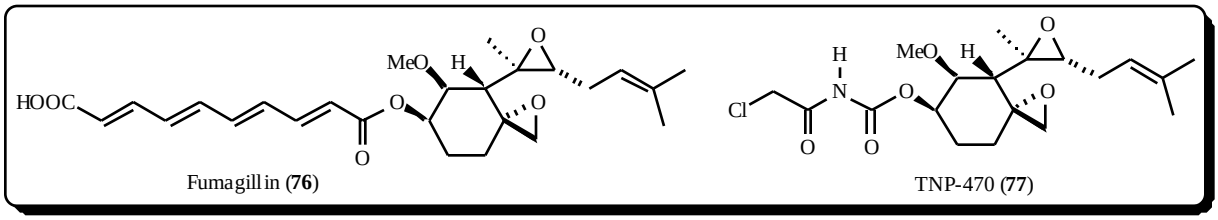
Every surface on earth rapidly becomes covered with a biofilm. Our environment, body surfaces and cavities support a rich microbial flora [116]. This usually causes us no harm as long as we are not overexposed to unusual quantities or unusual microbial strains. Humankind has been subject to infections by pathogenic microbes since the dawn of recorded history [116]. The use of microorganisms as useful biocatalysts is as old as human history. Ancient civilizations used the microbes to make vinegar, wine, and cheese. Modern civilizations extended the friendly relationship with microbes through using them as unique resources to produce indispensable antibiotics, essential hormones, and to produce many important chemicals and pharmaceuticals. In the year 2000, worldwide commerce in antibiotics is estimated in multiple tons and valued in excess of $10 billion [116]. Half of these are associated with the β-lactam antibiotics. Hence, microbes and microbial natural products have a huge impact on the human civilization. Many microbial natural products show promising angiogenesis modulation activities. Examples of these compounds are presented below.

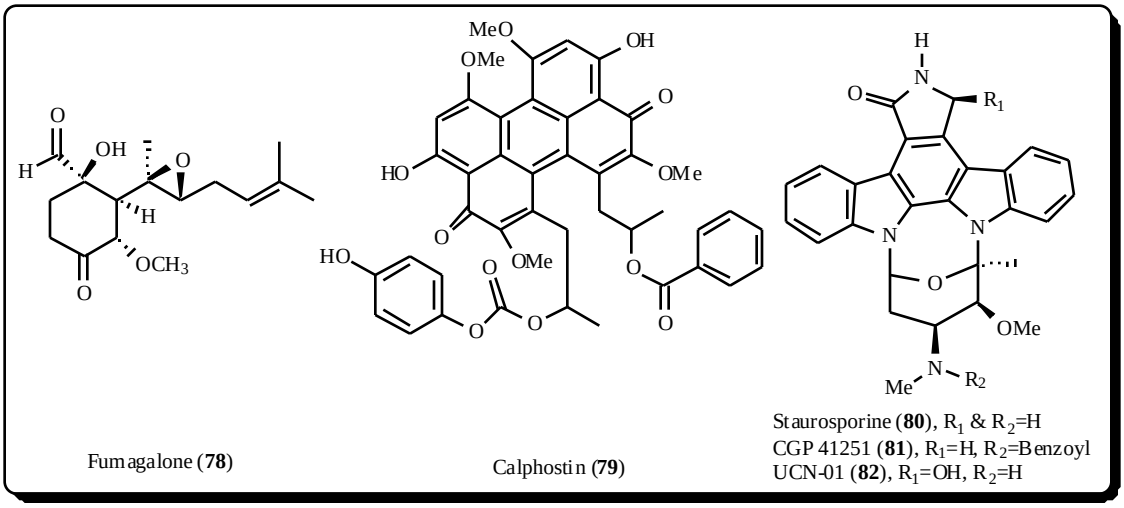
1- Fumagillin and Analogs

The antibiotic fumagillin (**76**) is isolated from the pathogenic fungi *Aspergillus fumigatus* [13]. Fumagillin and its semisynthetic analog TNP-470 (**77**) are known to inhibit endothelial cell proliferation selectively [117,118]. Fumagillin inhibited angiogenesis at a concentration of 2 μg/pellet in CAM assay [117,118]. Fumagillin also potentiates angiogenesis inhibition by heparin and sulfated cyclodextrins, such as β-cyclodextrin tetradecasulfate [117,118]. The Na salt of **76** inhibited vascularization of the chick embryo chorioallantoic membrane at a 10-50 μg [117,118]. Both compounds induced potent anti-tumor and anti-prolactin activities in the experimentally estrogen-induced pituitary adenoma, which is mediated indirectly through the inhibition of angiogenesis [117]. TNP-470 is more potent, less toxic, and after oral administration it was able to inhibit the growth of solid tumors, e.g., Lewis lung carcinoma in mice [119]. The CNS toxicity of TNP-470 (**77**) limited its clinical trials at phase II [13,119]. Attempts to synthesize new fumagillin analogs continued. Recently, fumagalone (**78**) was synthesized, in which fumagillin's spiroepoxide group is replaced with an aldehyde [120]. Fumagalone inhibits type 2 methionine aminopeptidase with an IC_{50} of 8 μM and endothelial cell proliferation with an IC_{50} of 52 nM. It was unambiguously proved that binding of fumagalone to methionine aminopeptidase is reversible [120].

2- Protein kinase C Inhibitors

Vascular endothelial growth factor (VEGF) promotes angiogenesis by a variety of mechanisms including stimulation of endothelial cell proliferation and migration and increasing vascular permeability [121-123]. VEGF mitogenic activity is mediated primarily by the β2-isoforms



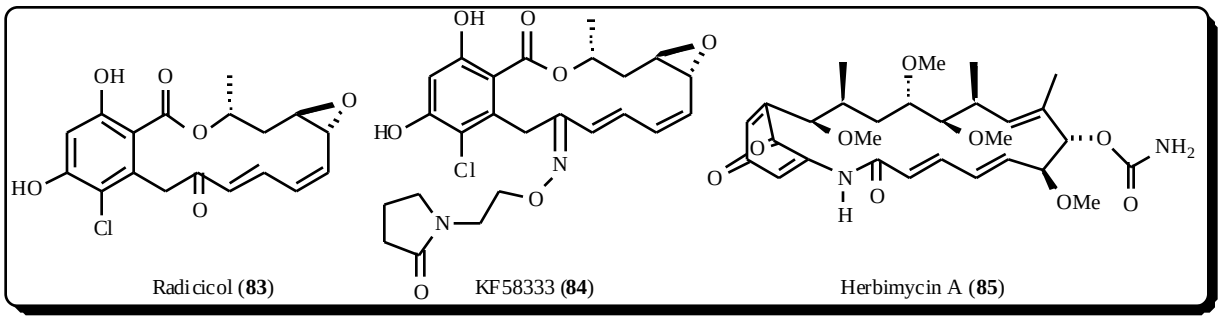


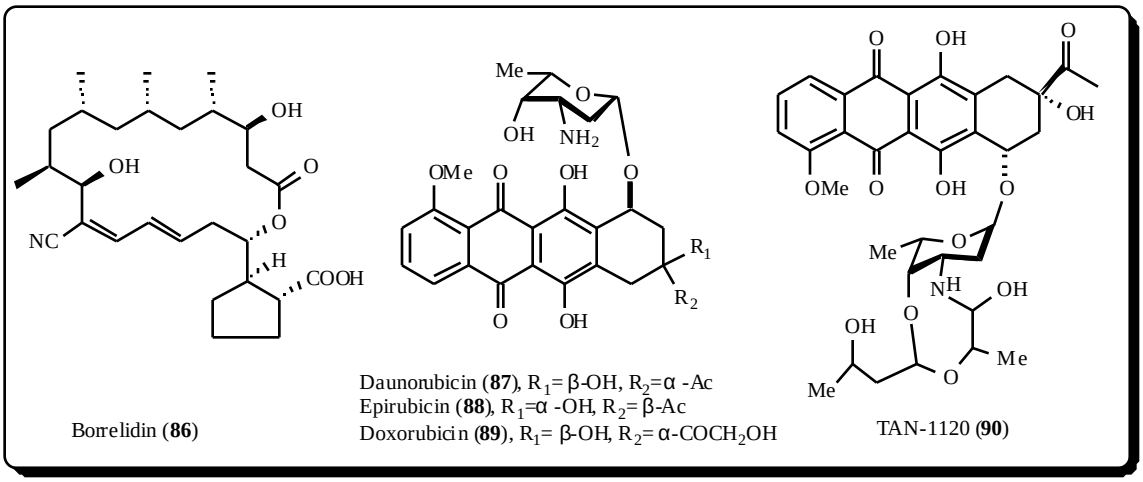
of protein kinase C (PKC) [121-123]. The conventional PKC inhibitor calphostin C (**79**) isolated from *Cladosporium cladosporioides* inhibited angiogenesis potentiated by integrin $\alpha_v\beta_5$ and not by integrin $\alpha_v\beta_3$. It also inhibited neovascularization induced by FGF-2, IL-1α, and TNF-α [13,121−123]

Staurosporine (**80**) is another PKC inhibitor antibiotic isolated from a *Streptomyces* species [124]. It inhibits angiogenesis in CAM assay with an IC_{50} of 33 μg/egg and inhibits VEC with an IC_{50} of 0.88 nM [124]. Staurosporine semisynthetic analogues *N*-benzoylstaurosporine (**81**, CGP 41251) and 7-hydroxystaurospine (**82**, UCN-01) inhibit endothelial cell proliferation and angiogenic hypoxic response [125-128]. They compete with ATP, even though the exact action by which they inhibit PKC is more complicated. Staurosporine analogues do not exhibit specificity for particular PKC isoenzymes, but they inhibit 'conventional' PKC isoenzymes [125-128]. They also interfere directly with the cell cycle machinery. Both CGP 41251 and UCN-01 are currently progressing through clinical evaluation [125]. CGP 41251 was shown to inhibit the vascular endothelial growth factor (VEGF) receptor kinase insert domain-containing receptor, which is involved in angiogenesis [125,126]. CGP 41251 inhibits reversibly intracellular PKC activity and the corresponding activation of the mitogen-activated protein kinase induced by either tumor promoting phorbol esters, platelet-derived growth factor, or basic fibroblast growth factor, but not by the epidermal growth factor [125,126]. CGP 41251 also inhibited the ligand-induced autophosphorylation of the receptors for platelet-derived growth factor, stem cell factor, and VEGF. CGP 41251 showed broad antiproliferative activity against various tumor and normal cell lines *in vitro*, and is able to reverse the P-glycoprotein-mediated multidrug resistance of tumor cells *in vitro* [126,127]. CGP 41251 showed *in vivo* antitumor activity as single agent and inhibited angiogenesis *in vivo*. Hence, CGP 41251 may suppress tumor growth by inhibiting tumor angiogenesis (*via* its effects on the VEGF receptor tyrosine kinases) in addition to directly inhibiting tumor cell proliferation (*via* its effects on PKCs) [127].

3- Macrolides and Ansamycins

Radicicol (**83**) is a differentiation modulating macrolide antibiotic isolated from strains of *Neocosmospora tenuicristata* and *Nectaria radicola* [129]. In a dose-dependent fashion, radicicol inhibited chick embryonic angiogenesis with ID_{50} value of 200 μg/egg [129]. Radicicol also inhibited both the proliferation of vascular endothelial cells and the production of urokinase-type plasminogen activator by these cells in nM dose [129]. An oxime derivative of radicicol, KF58333 (**84**), binds to the heat shock protein 90 (Hsp90) and destabilizes its associated signaling molecules, which is critical for growth inhibition of tumor cells [130]. KF58333 dose-dependently inhibited the growth and vascular endothelial growth factor (VEGF) secretion, concomitantly with a decrease in VEGF mRNA expression, in two human breast cancer cell lines, KPL-1 and KPL-4, both *in vitro* and *in vivo*. Compound **84** suppressed the increase of VEGF secretion and expression induced by hypoxia. The antitumor activity of **84** may be partly mediated by decreasing VEGF secretion from tumor





cells and inhibiting tumor angiogenesis [130].

Herbimycin A (**85**) is an ansamycin antibiotic isolated from *Streptomyces hygroscopius* [131]. It inhibits angiogenesis in the chick embryo chorioallantoic membrane with an ID_{50} value of 150 ng/egg, and inhibits tumor angiogenesis in rabbit corneas [131]. The name angiostatic antibiotic is proposed for herbimycin A [131]. It is proposed to selectively reduce the activity of certain oncogene products related to tyrosine kinase [13,131]. Herbimycin A also showed anti-proliferative activity on cultured capillary endothelial cells, and inhibited angiogenesis the new capillary sprouts induced by crude tumor angiogenesis factor (TAF) in rabbit cornea [132]. Herbimycin A showed hemorrhagic necrosis of tumor tissue in herbimycin A treated mice [132]. A semisynthetic derivative of herbimycin A, 17-cyclopropylaminoherbimycin A dose-dependently inhibited embryonic angiogenesis with ID_{50} value of 0.1 μg/egg [133]. Structure activity relationship study of **85** revealed that C-19 position, amino group between positions C-1 and C-20 and carbamoyl group in C-7 are essential for the anti-angiogenic activity [133].

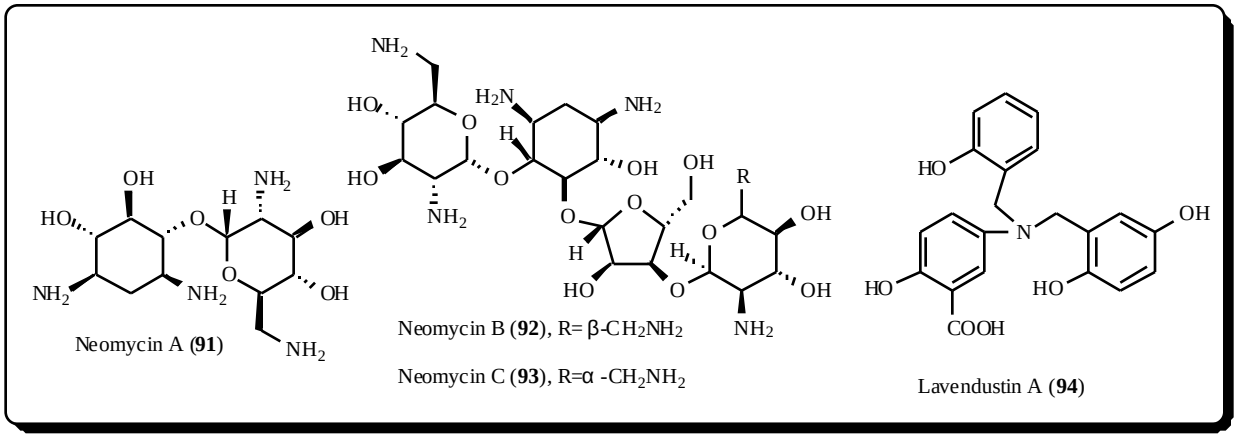
Borrelidin (**86**) is another anti-angiogenic antibiotic reported from *Streptomyces rochi* [134,135]. It suppresses new capillary tube formation and collapses formed capillary tubes in a rat aorta culture model [134]. It selectively inhibits threonyl-tRNA synthetase [134]. High concentration of threonine (1 mM) attenuated the ability of borrelidin to inhibit both capillary tube formation in the rat aorta culture model and human umbilical vein endothelial cells (HUVEC) proliferation but did not affect the ability of borrelidin to collapse formed capillary tubes or to induce apoptosis in HUVEC [134].

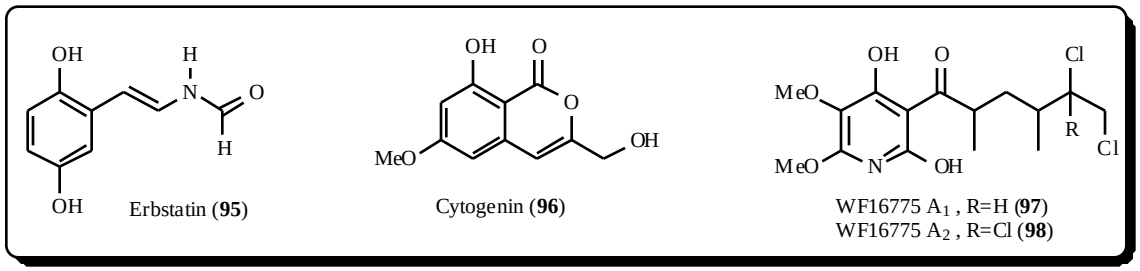
Borrelidin activated caspase-3 and -8, and inhibitors of both caspase-3 and -8 suppressed borrelidin-induced apoptosis in HUVEC [134]. Hence, the anti-angiogenic activity of borrelidin was proved to be mediated through threonine-dependent and the other threonine-independent mechanisms [134]. Borrelidin induces apoptosis in endothelial cells *via* the caspase-8/-3 pathway [134,135]. It was also reported to induce the transcription of amino acid biosynthesis enzymes through a GCN4-dependant pathway [135].

4- Anthracycline Antibiotics

Daunorubicin (**87**), epirubicin (**88**), and doxorubicin (**89**) are cytotoxic anthracycline antibiotics isolated from *Streptomyces caeruleorubidus* or *Streptomyces peuceticus* var. *caesius.* They inhibited angiogenesis in the CAM assay and the lumen formation of endothelial cells in matrigel assay, at a non-toxic concentration, with IC_{50} of 5-20 μg/pellet and 2.5-15 μg/ml, respectively [136,137]. Compounds **87**-**89** are reported to modulate the angiogenic phenotype of endothelial cells by a mechanism that is not related to inhibition of metalloproteinases [136].

TAN-1120 (90) is another potent angiogenesis-inhibitor anthracycline produced by *Streptomyces triangulatus*





subspecies *angiostaticus* [138]. It strongly inhibited proliferation of vascular endothelial cells but did not prevent capillary cord formation *in vitro* by the endothelial cells on extracellular matrix-coated plates [138]. TAN-1120 is proposed as one of the most potent angiostatic agents reported [138].

5- Aminoglycosides

The topical aminoglycoside antibiotics neomycins A (**91**), B (**92**), C (**93**), and others, isolated from *Streptomyces fradiae* were recently patented for their potent anti-angiogenic activity [139]. A dose of 20 ng neomycins/ embryo or higher completely inhibited angiogenin-induced angiogenesis in CAM assay [139]. Neomycins inhibit angiogenin-induced angiogenesis mainly through inhibition of nuclear translocation of angiogenin [139].

6- Miscellaneous Microbial Metabolites

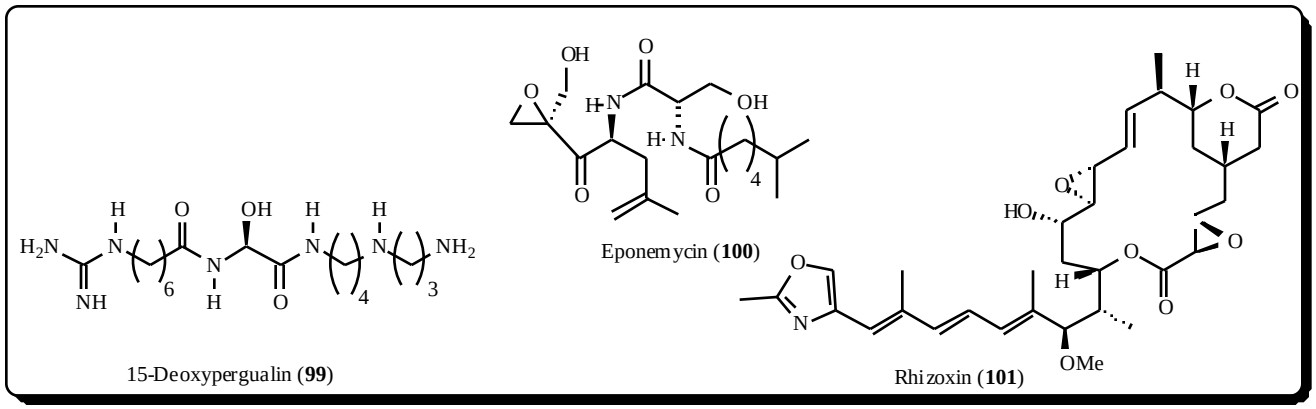
Lavendustin A (**94**) is a phenolic metabolite of *Streptomyces griseolavendus* [140]. It showed potent and selective inhbition of tyrosine kinase enzyme. It also inhibited VEGF- and FGF-2-induced angiogenesis in the sponge implants in rats at a concentration of 10 μg/sponge/ day [140].

Erbstatin (**95**) is a specific tyrosine kinase inhibitor isolated from a *Streptomyces* species [141]. Erbstatin produced a dose-dependent inhibitory action on CAM embryonic angiogenesis [141]. This inhibition occurred at a dose of 10 ng/egg and ID_{50} value of 80 ng/egg [141]. Erbstatin affected the proliferation of vascular endothelial cells, in a dose-dependent manner with an IC_{50} value of 3.6 μM [141].

Cytogenin (**96**, 8-hydroxy-3-hydroxymethyl-6-methoxyisocoumarin) is a microbial product of *Streptoverticillium eurocidicum* and has antitumor and antirheumatoid arthritis activities [142]. Both neoplasia and rheumatoid arthritis are angiogenesis-dependent diseases. Cytogenin at ≤100 μg/egg did not affect embryonic angiogenesis when topically placed on the surface of the chorioallantoic membrane, suggesting that it has no effect on the physiological (normal) angiogenic response [142]. Systemic administration of cytogenin (100 mg/kg/day, orally, for 5 days) suppressed angiogenesis induced by malignant tumor cells (S-180), a model of pathological neovascularization, in a mouse dorsal air sac assay system [142]. Cytogenin had a little effect on plasminogen activator secretion, tube formation or the proliferation of endothelial cells. Hence, it is proposed as a potent oral anti-angiogenic agent with potential therapeutic value for cancer, rheumatoid arthritis and other angiogenesis-dependent disorders such as diabetic retinopathy [142]. 2-(8-Hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl)propionic acid (NM-3) is a more stable and active semisynthetic analog of cytogenin, which modified collagen-induced arthritis in mice [143].

WF 16775 A_1 (**97**) and A_2 (**98**) are halogenated pyridine analogs which were isolated from the fungus *Chaetasbolisia erysiophoides* [144]. They effectively inhibited angiogenesis in CAM assay and show preferential cytostatic effect on endothelial cells [144].

Spergualin is spermidine-containing antibiotic reported from the broth of the bacterium *Bacillus laterosporus* [13,145]. Its semisynthetic analog 15-deoxyspergualin (**99**) exhibited anti-angiogenic activity in the CAM assay with an IC_{50} value of 480 ng/egg [145]. 15-Deoxyspergualin is a tyrosine kinase inhibitor and inhibited proliferation of VEC by 40% at a concentration of 10^{-5} M [145,146]. 15-Deoxyspergualin did not inhibit the production of urokinase-type plasminogen activator or type IV collagenase by vascular endothelial cells [145,146]. 15-Deoxyspergualin inhibited both spontaneous and epidermal growth factor (EGF)-stimulated migration of human omental



microvascular endothelial cells [146]. Treatment of cells with this agent resulted in a decreased expression of tissue-type plasminogen activator (PA), but did not affect expression of PA inhibitor [146]. 15-Deoxyspergualin (3 mg/kg, i.p.) effectively inhibited human glioma cells induced angiogenesis the mouse dorsal air sac [146].

Eponemycin (**100**) is a novel antibiotic isolated from *Streptomyces hydroscopicus* [13,147]. Eponemycin powerfully and dose-dependently inhibited angiogenesis in the CAM assay [147]. This powerful inhibition was detectable at a dose of 0.1 ng/egg and an ID_{50} value of 3.3 ng/egg, suggesting that eponemycin is the strongest angiogenesis inhibitor identified so far [147].

Rhizoxin (**101**) is an antifungal macrolide antibiotic isolated from *Rhizopus chinensis* Rh-2 as a pathogen of rice seedling bright [148]. It binds to β-tublin, causing either inhibition of polymerization or depolymerization of tubulin [148]. Rhizoxin caused dose-dependent inhibition of embryonic angiogenesis, with an ID_{50} value of 2 ng/egg [149]. It also significantly suppressed neovascularization induced by M5076 mouse tumor cells in a mouse dorsal air sac assay system at an i.p. dose of 2 mg/kg [149].

Depudecin (**102**) is a fungal metabolite isolated from *Altermaria brassiciicola* and *Xylaria* species [150]. It inhibited embryonic angiogenesis in a dose-dependent manner with an ID_{50} value of 320 ng/egg [150]. It also inhibited the growth of vascular endothelial cells [150].

The immunomodulating protein-bound polysaccharide PSK (Krestin) is obtained from cultured mycelia of *Coriolus versicolor* in basidiomycetes [151]. PSK inhibited the proliferation of HUVECs in the absence of bFGF but inhibited more effectively in its presence [152]. PSK also suppressed the bFGF-dependent MAP kinase phosphorylation [152]. Intravenous PSK injection (5 mg/kg) reduced the bFGF-induced angiogenesis in an *in vivo* rat cornea assay [152]. PSK suppressed pulmonary metastasis of methylcholanthrene-induced sarcomas, human prostate cancer DU145M, and lymphatic metastasis of mouse leukemia P388 [151]. It also prolonged the survival period in spontaneous metastasis models [151]. PSK cancer metastasis inhibitory mechanism includes suppression of intravasation through the inhibition of tumor invasion, adhesion and production of cell matrix-degrading enzymes, suppression of tumor cell attachment to endothelial cells through the inhibition of tumor cell-induced platelet aggregation, suppression of tumor cell migration after extravasation through the inhibition of tumor cell motility [151].

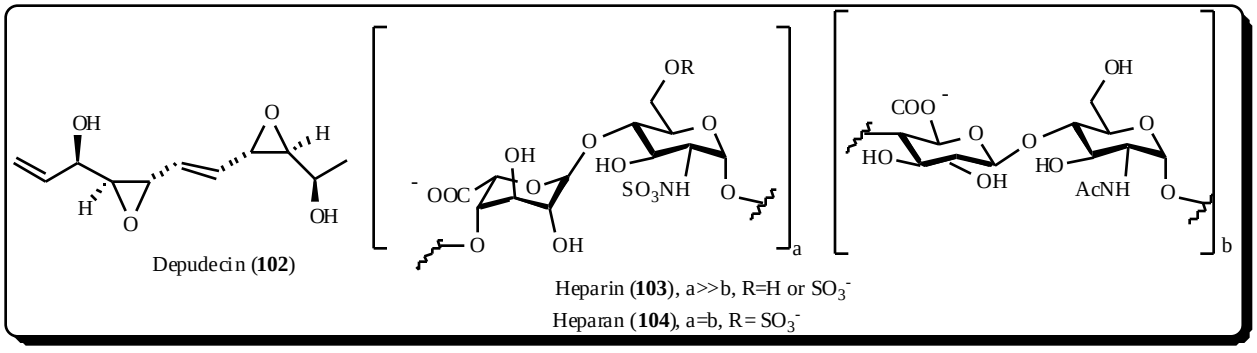
Tecogalan (DS-4152, SP-PG) is a sulfated polysaccharide-peptidoglycan complex obtained from the cell wall of *Athrobacter* species [13,153]. The anti-angiogenic effect of tecogalan in CAM assay is enhanced by cortisone acetate [13]. It is proved to inhibit FGF-2-induced angiogenesis in corneal rabbits at a concentration of 100 μg-200 μg/pellet [13]. Tecogalan inhibited the *in vitro* endothelial cell growth at 250 μg/ml and FGF-2-induced migration at 64 μg/ml. Tecogalan also inhibited Kaposi's sarcoma angiogenesis at 2.5 mg/kg dose [13]. Tecogalan markedly inhibited the *in vitro* angiogenic activity of retinal vascular endothelial cells (RECs) although the growth inhibition of RECs was small [153]. Tecogalan decreased the cell-associated urokinase-type plasminogen activator (uPA) activity and matrix metalloprotease 1 (MMP-1) activity even in the presence of anti-bFGF IgG [153].

A recent patent was awarded for a composition containing a yeast-origin components NBG or ImmutolTM having a β 1,3/1,6 glucan structure, due to their efficacious IL-12 inducing activity [154]. ImmutolTM activates NK cells and NKT cells and inhibits tumor angiogenesis, when orally administered [154].

D- Animal-Derived Angiogenesis Modulators

1- Heparin and Heparan

Heparin (**103**) is a glycosaminoglycan (GAG) that is extracted from porcine intestinal tissues and is widely used as anticoagulant [155]. It is biosynthesized as a proteoglycan and stored exclusively in mast cells and is partially degraded to peptidoglycan and GAG upon immunologic activated mast cell degranulation [155]. In contrast, the structurally related heparan sulfate (**104**), is the polysaccharide portion of a ubiquitous proteoglycan, localized on cell surface and in the extracellular matrix of all animal tissues [155]. Heparins affect the proliferation of endothelial cells by interaction with growth factors and influence the angiogenesis *in vitro* by altering structure and mechanism of the fibrin network [156]. Structural alterations of the fibrin matrix by unfractionated heparin enhance invasion of the matrix by capillary-forming endothelial cells, while low mol. wt. heparin reduces this process [156]. Vascular endothelial growth factor (VEGF) regulates blood vessel formation by binding to the receptor tyrosine kinases VEGF receptor-1 or VEGF receptor-2 and to the structurally unrelated neuropilins [157]. Isoforms of VEGF binding to heparin resulting in angiogenesis modulation. Heparin decreased binding of 125I-VEGF to 50% at 5 μg/mL and crosslinking of 125I-VEGF to VEGF receptor-1 on intact cells was



similarly decreased [157]. The systemic effect of heparin fractions with mean molecular masses of 2.5, 5.0, and 16.4 kD on angiogenesis induced by vascular endothelial growth factor was studied [158]. Treatment with the 5.0-kD fraction suppressed angiogenesis significantly compared with treatment with 2.5- and 16.4-kD heparins [158]. This result suggested that the systemic angiosuppressive effect of heparin in different mammalian angiogenic reactions is distinctly related to molecular size [158]. The effective angiostatic activity of low molecular weight heparan sulfate (**104**) is reported [159]. A 6-days treatment using 2.5% solution of **104** in carboxymethyl cellulose markedly reduced vascular network and bFGF immunoreactivity in rat corneal assay [159].

2- Protamine

Protamine is a natural 43 kD arginine-rich cationic protein isolated from rat and other mammals epididymal sperm cell nuclei and testes of salmons and bonitos [160,161]. Protamine inhibits migration and proliferation of endothelial cells, interfering with growth factor and production of angiogenic factors [162]. Protamine inhibits angiogenesis and growth of C6 rat glioma and potentiate the therapeutic effects of nitrosoureas for the treatment of glial tumors [163].

3- Obtustatin

Obtustatin is a short disintegrin (containing 41 amino acids) isolated from the venom of the *Vipera lebetina obtusa viper* [164]. Obtustatin is a potent and selective inhibitor of $\alpha 1\beta 1$ integrin and does not inhibit integrin $\alpha 2\beta 1$ [164]. Obtustatin potently inhibited angiogenesis *in vivo* in the CAM assay and in the Lewis lung syngeneic mouse model [164]. It reduced tumor development by half, confirming and extending previous results on the relevance of $\alpha 1\beta 1$ integrin to angiogenesis [164].

CONCLUSION

Malignant tumors are angiogenesis-dependent diseases. Several studies suggest that primary tumor growth, invasiveness, and metastasis require neovascularization [165]. Research studies suggest that modulation of angiogenic activity is associated with tumor regression in animals with different types of neoplasia [165]. Anti-angiogenic therapy represents one of the more promising new approaches to anticancer therapy [165]. Out of 22 angiogenesis inhibitors currently under active clinical trials there are 11 natural products or were modeled on a natural product parent (Table 2) [24]. This clearly shows the potential of natural products for the discovery of new lead entities as angiogenesis modulators [23,24]. It is estimated that only 5-15% of nearly the known 250,000 higher plant species have been systematically examined for their bioactive secondary metabolites [23]. Marine and microbial natural products are also additional wealthy resources for novel unexplored compounds. Natural products represent an enormous resource for the discovery of angiogenic modulation leads waiting for inspired discoverers.

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