Trends in Organic Chemistry

Review

Natural products used as candidates for angiogenesis inhibitors in cancer therapy

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ABSTRACT

Angiogenesis is the formation of new blood vessels from existing ones. This process is important in several physiologic processes, such as embryonic development, placenta formation, and wound healing. However, angiogenesis also occurs in numerous pathologic conditions, including cancer, rheumatoid arthritis, obesity, diabetic retinopathy, age-related macular degeneration (AMD) and neurological disorders such as Parkinson and Alzheimer's disease. Recently a number of agents that inhibit angiogenesis have been approved to treat diseases such as cancer and AMD. In this context, several studies have been conducted, showing the antiangiogenic effect of natural products, which makes them a potential candidate in the development of new antiangiogenic drugs. The purpose of this review is to describe the studies of natural products, focusing on the quinones, with antiangiogenic activity.

KEYWORDS: angiogenesis, natural products, quinones

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ABREVIATIONS

AMD	Age related macular degeneration
BAEC	Bovine aortic endothelial cell
b-FGF	Basic fibroblast growth factor
CAM	Chicken chorioallantoic membrane
EGFR	Endothelial growth factor receptor
HGF	Hepatocyte growth factor
FGF-2	Basic fibroblast growth factor
HUVEC	Human umbilical vein endothelial cells
LLC	Lewis lung carcinoma
MTD	Maximum tolerated dose
MVB	Microvessel density
PBS	Phosphate-buffered saline
PDGF	Platelet-derived growth factor
PKC	Protein kinase c
RCC	Renal cell carcinoma
TGF-β	Tumor growth factor- β
TNF-α	Tumor necrosis factor- α
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

1. INTRODUCTION

1.1. Angiogenesis: concept, regulation and clinical implications

Angiogenesis is when a new capillary is developed from a pre-existing blood vessel [1, 2, 3]. It is an important event for reproduction, development and repair processes [3]. In these conditions, the angiogenic response is limited and strictly controlled, so that it is activated for a short

period of time, where it is then fully inhibited [3, 4] as soon as the growing tissue's metabolic demands are met by the vascular network newly formed [5].

Angiogenesis is, in fact, a complex and orderly process that involves soluble mediators, cell-cell and cell- extracellular matrix interactions, in addition to biomechanical forces [5]. It is controlled by a tenuous balance between promoting endogenous factors (angiogenic) and inhibiting (angiostatic) endogenous factors. The conditions of the endothelial cell are then determined by the balance between these antagonistic functions. Therefore, when positive regulators prevail, the endothelial cell is activated, where it is able to proliferate and migrate (angiogenic phenotype), whereas, when negative regulators prevail the endothelial cell remains quiescent: a mechanism that determines the angiogenic switch [6, 7]. Therefore the activation of angiogenesis may involve a decrease in the concentration of inhibitory factors and/or the increase in the levels of stimulatory factors. The strategy used by different tissues to induce angiogenesis is determined by their physiological characteristics [6]. The approach to explain the activation process of angiogenesis was initially used to describe, within the tumor growth context, the transition from a prevascular state to a vascular phase, characterized by rapid growth and increase in metastatic potential. However, such concept can also be applied to embryonic, physiologic and pathologic angiogenesis [6, 7].

In physiologic conditions, the various mechanisms that regulate the angiogenic process behave in a concerted manner, affecting vascular growth positively or negatively, in a way to assure that the angiogenic response is proportional to the metabolic demands of the growing or repair tissue [5]. However, certain pathologic conditions change the control mechanisms to favor the expansion of the disease, as observed during tumor angiogenesis. In these cases, abnormal angiogenesis – excessive or deficient – contributes to the pathogenesis of several diseases, which include inflammatory, infectious, immunologic, ischemic and malignant disorders: the angiogenesis dependent diseases [2, 3].

The advances achieved through the years regarding the comprehension of molecular and cellular mechanisms of the angiogenic process enabled the development of therapeutic strategies to fight against the angiogenesis-dependent diseases. Several proangiogenic factors were identified, such as vascular endothelial growth factor (VEGF) [8, 9], basic fibroblast growth factor (FGF-2) [10] and angiopoietins [11]; furthermore, several angiogenesis-inhibiting agents were discovered, including angiostatin, endostatin, vasostatin and, recently, VEGF inhibitors [12]. Through these discoveries, it was determined that intervention in the vascular development process could be an interesting therapeutic approach in the management of diseases arising from insufficient or excessive angiogenesis [13].

Therapeutic angiogenesis involves the clinical use of proangiogenic factors in situations where angiogenesis is insufficient, such as chronic cutaneous ulcer, bone setting delay and myocardial, brain and peripheral ischemic disease. In such conditions, insufficient angiogenesis arises from an inadequate production of proangiogenic factors and/or an excessive quantity of inhibitors. The aim of therapeutic angiogenesis is to restore and maintain tissue perfusion through neovascularization stimuli in the ischemic region [13, 14, 15].

On the other hand, angiogenic therapy refers to the clinical use of antiangiogenic agents in conditions characterized by excessive angiogenesis, such as cancer, macular degeneration in age related retinopathy diabetes, rheumatoid arthritis, psoriasis and endometriosis. In these diseases, a large amount of proangiogenic factors are released, such as VEGF and FGF-2, which supercede the effects of the endogenous inhibitors, such as angiostatin, endostatin and thrombospondin [2, 13, 16].

Several clinical trials have evaluated or are currently evaluating the efficacy of various antiangiogenic agents for the treatment of angiogenesis-dependant diseases, especially regarding anticancer therapy [16, 17, 18]. Preliminary data suggest that angiogenic therapy alone is limited, regarding the complexity of the angiogenic process [2, 16]. On the other hand, the combination of angiogenic therapy with conventional chemotherapy seems more promising. For example, combined therapy with bevacizumab, a monoclonal anti-VEGF antibody which inhibits

the activity of all isoforms of this factor, with traditional chemotherapy in the treatment of metastatic colorectal cancer showed positive results [16, 18, 19].

Satisfactory results were also revealed after the treatment of certain tumors with small molecules defined as tyrosine kinase inhibitors. Some of these agents block, but not selectively, the tyrosine kinase activity of the VEGF receptors, in this way being angiogenesis inhibitors. Amongst them, sunitinib and sorafenib stand out, as they are orally administered and inhibit the tyrosine kinase activity of the three VEGF receptors [20, 21]. Sunitinib is used in the treatment for advanced renal cell carcinoma (RCC) and gastrointestinal stromal tumor [20, 22], while sorafenib is also used as treatment for advanced renal cell carcinoma (RCC) [21].

The emergence of antiangiogenic agents has provided new therapeutic options for angiogenesis-dependent ophthalmic diseases, mainly in the neovascularization of macular degeneration related to age. In this condition, studies have demonstrated clear benefits of the treatment, via intravitreal injection of the following VEGF inhibitors: pegaptanib, a ribonucleic acid aptamer with 28 nitrogenous bases (oligonucleotide), which selectively binds to the VEGF165 isoform, blocking its activity [23, 24]; ranibizumab, a monoclonal anti-VEGF antibody fragment, which inhibits the activities of all VEGF isoforms [25, 26]; and also bevacizumab [27].

However, despite promising results, the challenge still remains to extend our knowledge of the molecular basis of angiogenesis-dependent diseases, particularly cancer, in order to provide the development of safer and more efficacious antiangiogenic therapies.

1.2. Tumor angiogenesis

Solid tumors form structures similar to an organ, consisting of neoplastic cells and stromal cells from the host, scattered in an extracellular matrix and nourished by a vascular network [28]. They are formed by a population of cells that have lost the ability to regulate their growth with consequent increase in cell proliferation. Additionally, neoplastic cells are prone to mutations, so they can progressively acquire several phenotypes [5, 29]. Similar to normal tissue, tumors need an adequate supply of oxygen and nutrients, and also an effective mechanism for waste elimination. To accomplish this, they are able to induce their own blood supply from the host, in a process that simulates normal angiogenesis. Therefore, angiogenesis is an important process for tumor growth and subsequent metastasis [5, 12].

In most solid primary tumors, an avascular phase is observed, a time of apparent quiescence, in which the tumor mass can reach the maximum size of a spheroid of 1 to 2 millimeters in diameter [13, 30]. Such dimensions are defined by the capacity to obtain oxygen and nutrients necessary for tumor cell growth, through simple passive diffusion. During this phase, the rate of cancer cell proliferation is balanced by the rate of apoptosis, in such a way that the tumor remains clinically undetectable for months to years, characterizing a state of tumor quiescence [3, 31]. A similar phenomenon is also observed in micrometastasis, in which the tiny tumors also remain quiescent and clinically undetectable [31].

To grow beyond the above mentioned maximum dimensions, the tumor induces the formation of a vascular network to supplement the expanding cells' metabolic demands. In fact, the transition from the pre-vascular phase to vascular phase (angiogenic trigger) is characterized by the acquisition of an angiogenic phenotype by a clone of tumor cells and involves changes in the local equilibrium between positive and negative vascular growth regulators [3, 6]. The process involves the sprouting of new capillaries from the host's blood vessels adjacent to the tumor, which grow and infiltrate the tumor mass [13].

The development of new blood vessels by the tumor is mediated by several factors, some of which also participate in physiologic angiogenesis. The vascular endothelial growth factor (VEGF) is without a doubt the main promoting agent of tumor angiogenesis, particularly VEGF 165 isoform [32]. Other molecules also deserve to be emphasized, such as basic fibroblast growth factor (FGF-2), angiopoetin-2, platelet derived growth factor (PDGF) and the matrix's metalloproteinase [5, 12]. Such factors can be produced in different ways: by the neoplastic cells themselves after

acquiring the angiogenic phenotype; through tumor stromal cells, such as fibroblasts, macrophages and leucocytes; through the host's tissue cells surrounding the tumor; or by macrophages and fibroblasts recruited from distant tissues [29, 13]. Furthermore, products of tumor cells that influence the angiogenic process may also act in distant tissues besides in situ. Thus, several cell types derived from the bone marrow, including endothelial cell precursors, are recruited to the tumor microenvironment, where they amplify the angiogenic response [29, 32].

Tumor blood vessels differ from normal vessels by structural and functional alterations or abnormalities. They are sorted in a disorganized, chaotic manner, showing a heterogeneous spatial distribution; they are tortuous, dilated with sacs; they possess excessive ramifications, with varied diameters, as well as numerous anastomosis [28, 33, 34]. The vascular wall is exceptionally permeable, due to the presence of fenestrations and transcellular openings, vesicles, as well as the lack of a normal basal membrane [5]. Furthermore, tumor cells share with endothelial cells spaces in the vascular wall, creating the so-called mosaic vessels. Such architectural abnormalities follow the pathologic character associated with the vascular generation process [5, 34].

The abnormal architecture of tumor vessels leads to heterogeneous and disordered microcirculation. In fact, in the tumor vascular network, permeability is increased and blood flow is chaotic, distributed irregularly in a way that in some regions the perfusion is minimal or absent. These alterations, associated with the absence of functional lymphatic vessels, promote an increase in interstitial fluid pressure. They also promote the emergence of a hostile microenvironment, characterized by hypoxia and acidosis. Such conditions reduce the bioavailability and effectiveness of agents aimed at the tumor, as in chemotherapy, immunotherapy and radiotherapy, as well as contributing to the selection of neoplastic cells that are more aggressive and prone to metastasize [28, 33, 34].

Treatment with antiangiogenic agents, such as VEGF inhibitors promotes a "normalization" of tumor vessels. Such phenomenon is marked by a reduction in the vessels' density and diameter, as well as a cutback in vascular permeability, leading to an improvement of perfusion, reduction of interstitial fluid pressure and increase in the oxygen partial pressure, even if in a transitory manner – the so-called "normalization window." Thus, during this period, an increase in the bioavailability of chemotherapeutic agents is observed, improving the effectiveness of chemotherapy. Thus, a synergistic effect is observed when antiangiogenic therapy is combined with a cytotoxic treatment [28, 32].

2. Some natural products with antiangiogenic activity

Natural products have played a significant role in the development of new bioactive small molecules and have provided leads for drug development. Several natural compounds have shown many biological activities as anti-viral, anti-bacterial, and anti-cancer agents [35].

Since the inhibition of angiogenesis is considered to be one of the most promising strategies leading to the development of new antineoplastic therapies [36], many natural products have been tested for their antiangiogenic potential.

The Vinca alkaloids isolated from *Catharanthus roseus*, for example, possess many therapeutic effects including antitumor activity. These alkaloids have been widely used as clinical antitumor agents for the treatment of leukemias, lymphomas, and some solid tumors [37]. These compounds represent one of the main classes of tubulin-binding agents.

The first group of Vinca alkaloids with antitumor activity identified were vincristine, vinblastine and vindesine [38]. These drugs disrupt the mitotic spindle assembly through their interaction with tubulin, and kill actively dividing cells by inhibiting their progression through mitosis [39].

Much later, several new alkaloids were obtained through structural modification studies. Some modifications in the "upper" portion of the velbenamine structure, for example, created vinorelbine obtained by C' ring contraction of anhydrovinblastine [40]. Another novel drug of the second generation of Vinca alkaloids, vinflunine, a bis-fluorinated vinorelbine derivative, has been synthesized by superacid chemistry and showed antiangiogenesis and delayed multi-drug resistance development. The binding of vinflunine to tubulin

and subsequent cellular arrest in mitosis is the core mechanism of this antineoplastic agent. In addition, its potential vascular-disrupting and antiangiogenic activities support further clinical development of this compound.

Colchicine, like vinflunine, is another drug developed from a natural product with tubulin-interacting potential associated with vascular effects. However, colchicines and vinflunine only have antiangiogenic activity at doses approaching the maximum tolerated dose (MTD). In this form, some authors suggest that these drugs can be used in combination therapies with other drugs at doses below their MTD, with synergistic antitumor activity with minimal toxicity [41].

Other research has shown that many plants possess antiangiogenic activity. The use of juice, peel and oil of *Punica granatum*, for example, has also been shown to possess anticancer activity, including interference with tumor cell proliferation, cell cycle, invasion and angiogenesis. This plant has been the subject of classical reviews for over 100 years for its use for tanning, dyeing, and as a foodstuff. Furthermore, it has anthelminthic properties, is used for arresting hemorrhage and healing ulcers, and serves as an astringent in diarrhea [42].

However, recently, the importance of the oily phase of the seed was discovered, where studies showed that this plant possesses the ability to inhibit the development of new blood vessels. Recent research has also indicated that pomegranate appears to contain components capable of suppressing tumor cell invasion. A phase II clinical trial was conducted to investigate the relative anticancer health benefits of pomegranate [43].

Ganoderma tsugae (also known as Lingzhi or Reishi) has been one of the most popular chemopreventive mushrooms in East Asia [44]. Among the many bioactive components identified from *Ganoderma*, polysaccharides and triterpenoids are the two major ingredients for the treatment of various diseases, including cancer. The antitumor effect of polysaccharides isolated from the aqueous extract of this mushroom was observed *in vivo* against sarcoma 180 at a dose of 10mg/kg/day [45]. The methanol extract of this mushroom also inhibited colorectal cancer cell proliferation with cells accumulating in G_2/M phase. This extract also inhibited the expression of EGFR and VEGF *in vitro* and *in vivo*, and inhibited capillary tube formation by human umbilical vein endothelial cells (HUVECs) [44, 46]. The VEGF (vascular endothelial growth factor) is considered to be the most powerful and specific growth factor responsible for tumor angiogenesis [47]. The blockage of EGFR (endothelial growth factor receptor) activation causes the downregulation of VEGF and inhibition of angiogenesis [48, 49].

Honokiol, a biphenyl extract from *Magnolia obovata* bark, has been reported to induce apoptosis in a number of tumor cell lines [50, 51, 52, 53]. Furthermore, inhibition of transformed endothelial SVR cell growth [54] and tube formation by human umbilical vein endothelial cells suggest its antiangiogenic effect [55]. Li *et al.* (2008) investigated the effects of honokiol on ovarian tumor cells and observed significant inhibition of cell proliferation and induction of apoptosis, confirmed by DNA ladder and Hoechst staining assays, with G1 arrest in the cell cycle [56]. The Li *et al.* (2008) studies demonstrated that the honokiol-dependent apoptosis was accompanied by a significant increase in Bad and decrease of Bcl-XL [56]. It is well known that Bad promotes apoptosis by hetero-dimerization with Bcl-2 or Bcl-XL, and sequential release of free Bax [57].

Besides its potent activities in inducing apoptosis, honokiol could also be demonstrated to exert antitumor effects through some other mechanisms, such as enhancement of retinoid-induced differentiation of leukemia cells [52], induction of mitochondrial permeability transition pore [58], activation of poly-adenosine diphosphate ribose polymerase [59], etc.

The antitumor effect of honokinol *in vivo* was also evident using a SKOV3 tumor xenograft nude mice model assay [56]. The dose and schedule of honokiol administration were well tolerated by all mice and honokiol showed a significant suppression of tumor growth. To determine the potential role of honokiol in angiogenesis, microvessel density (MVD) was examined in dissected tumor tissues by anti-CD31 immunostaining. The results showed

that MVD was significantly decreased when treated with honokiol ($p < 0.05$), in comparison with the control (PBS), suggesting that the potent antitumor efficiency of honokiol *in vivo* should be partially due to the antiangiogenic activities.

In order to determine the role of VEGF in the antiangiogenic effect of honokinol, the authors examined the expression of VEGF in tumor tissues. A significant decrease in VEGF expression was observed in the honokiol-treated group, accompanied by a decrease in MVD. Another similar study also indicated that this decrease involved the modulation of the nuclear factor-kappaB activation pathway [60]. Additionally, other studies showed that honokiol inhibited angiogenesis by interfering with VEGFR2 auto-phosphorylation [54] and subsequently blocking VEGF-induced Rac activation [61], which is required for endothelial cell migration and proliferation [62]. In addition, regulation of platelet-derived endothelial cell growth factor and TGF-b expression [63], inhibition of nitric oxide synthesis and TNF-a [64] could be involved in this event. Further studies will be conducted to elucidate the precise mechanisms of action of honokiol in angiogenesis.

These data show that honokiol, a natural compound, has remarkable activities both *in vivo* and *in vitro* on ovarian tumors. Honokiol significantly suppressed cell proliferation and induced apoptosis in ovarian tumor cells, and restrained tumor growth and inhibited angiogenesis *in vivo*, with no cytotoxicity. Thus, honokiol appears to have both antitumor and antiangiogenesis properties.

R-(–)-b-O-methylsynephrine (OMe-Syn) is a natural compound isolated from a plant of the *Rutaceae* family, which has a unique chemical structure that is distinct from known angiogenesis inhibitors. Studies carried out by Kim *et al.* (2010) demonstrated that OMe-Syn effectively inhibited VEGF-induced angiogenesis both *in vitro* and *in vivo* [65]. Initially, the authors showed a stronger growth inhibition of human umbilical vein endothelial cells (HUVECs) by OMe-Syn. In addition, it was demonstrated that OMe-Syn inhibits tube formation, the invasion of HUVECs using the invasion assay with Matrigel and tube formation *in vivo* using the chick embryo chorioallantoic membrane (CAM) assay. OMe-Syn also inhibited expression of proangiogenic factors such as VEGF, HGF, and bFGF under VEGF-induced conditions in HUVECs [65].

Marine ecosystems possess the greatest biodiversity, with potential sources for biotechnology that are virtually unlimited. Seas and oceans have also been a promising source of cytotoxic substances, isolated from plants or marine animals. Some of these are already undergoing clinical trials such as the angiogenesis inhibitor Neovastat®. Produced by the Canadian company Æterna Zentaris, Neovastat® is a standardized liquid extract of the fraction <500 kDa cartilage of the shark *Squalus acanthias,* and its antiangiogenic effect was recently characterized, having completed phase III studies for the treatment of metastatic renal cell carcinoma and non-small cell lung cancer (stages IIIA and IIIB; sponsored by the US National Cancer Institute), in combination with other chemotherapy or radiotherapy [66].

Both preclinical and clinical studies demonstrate the nontoxic oral bioavailability of Neovastat. A recent analysis of a phase II trial using two doses of Neovastat administered to patients with metastatic renal cell cancer demonstrated a dose-survival relationship [67].

It has been shown that the antiangiogenic mechanism of action is correlated with the inhibition or blocking of VEGF. Neovastat is able to block the VEGF-dependent microvessel sprouting from Matrigel-embedded rat aortic rings, and it also blocked VEGF-induced endothelial cell tubulogenesis *in vitro*. *In vivo* studies showed that Neovastat specifically inhibited VEGF-induced plasma tissue extravasation. At the molecular level, Neovastat was shown to compete in the binding of VEGF to its receptor in endothelial cells and significantly inhibited the VEGF-dependent tyrosine phosphorylation of VEGF receptor-2 [68].

Substances with antitumor activity can also act by modulating the activity of kinases. From marine animal Bugula neritina a group of special substances, the bryostatins, were isolated, which together with fouling organisms negatively influenced human activities such as navigation and mariculture, [69]. Today this species has been

the object of study in biochemistry and biomedicine, and since their discovery, bryostatins have created a wide interest in the scientific community. The bryostatins comprise a large series of compounds, and this class has been recognized as a candidate to be used in the chemotherapeutic treatment of cancer, due to antineoplastic activity combined with low toxicity [70].

Work by a number of groups, has established that bryostatin binds to and activates protein kinases C (PKC) in a manner that mimics, and competes with, the phorbol esters but has different physiologic effects [71]. This modulation is characterized by the initial activation followed by rapid down-regulation of PKC [72]. The macrocyclic lactone bryostatin 1, for example, was found to act on protein kinase C, and its effects on kinases may antagonize tumor functions such as invasion, angiogenesis and cell adhesion.

The PKC target may be important in targeting tumor angiogenesis and is considered to be part of a major intracellular pathway involved in differentiation and growth of endothelial cells. A phase I trial with bryostatin evaluated its ability to inhibit this pathway and the production of transforming growth factor β [73], preventing the establishment and maintenance of vessel wall integrity in the angiogenic process. Currently, the most interesting of studies with bryostatin 1 is in anticancer chemotherapy, already accounting for more than 80 phase 1 or 2 clinical trials, completed or in progress [66].

Following the line of marine organisms, several chemical and biochemical studies of sea squirts, also called ascidians, have led to the characterization of a series of compounds with pharmacological properties. The staurosporin derivatives isolated from ascidians have been mainly studied as antitumor agents. STI412 (CGP41251; *N*-benzoyl staurosporine) was originally identified as an inhibitor of PKC. *In vivo* studies indicated that STI412 exhibits dose- dependent tumor growth inhibition in various human tumor types. In addition, STI412 inhibited angiogenesis stimulated by VEGF, but not by FGF (fibroblast growth factor), suggesting that the antitumor properties of STI412 may be due to both its antiangiogenic effects on RTKs and its inhibitory effect on PKCs. Phase 1 and 2 clinical trials have evaluated the dose and effects of STI412 on patients with advanced solid tumors for the treatment of various human cancers [74].

Brominated natural products are common in marine sponges, but the ecological function of these products has not yet been elucidated, but they may play a role in chemical defense and deterrence [75]. Some brominated compounds can be found in sponges of the *Aplysina* species, which were derived from dibromotyrosine, such as aeroplysinin-1, fistularin-3, agelorins A and B, and verongiaquinol, as well as many others [76]. Analogues inspired by marine secondary metabolites (using dibromotyrosine as precursor) were tested by Sallam *et al.* (2010) using biological models to determine the antiangiogenic effects, including the CAM assay, Wound-healing assay and BME cell invasion. Using these assays, the author observed that these analogues have antiangiogenic potential and are inhibitors of prostate cancer cell proliferation and migration [76].

The vast field of research of drugs derived from natural products with antiangiogenic activity, allows us a glimpse of a search with a happy ending, since several of these inhibitors of angiogenesis are currently in clinical evaluation.

3. Quinones with antiangiogenenic activity

Quinones are an important class of naturally occurring compounds widely distributed among all respiring organisms [77]. This class of compounds plays an important part in various reactions, including photosynthesis, respiration and blood coagulation, with an essential role in the biochemistry of living cells [78]. Quinones have been used as antimalarial, antibacterial and anticancer agents, and their effects can be attributed to their redox-cycling capacity. However, in addition of this capacity, they have been found to influence various signaling pathways that can affect cell proliferation, apoptosis and migration. In particular, it has been demonstrated in the literature that some quinones can alter the angiogenic response, which makes this class of compounds promising in cancer research [79].

Quinones have been studied for their antiangiogenic activity using some experimental models, including

tests using endothelial cells. One quinone that was studied using this approach was HU-331 (cannabidiol hydroxyquinone, Figure 1) a quinone isolated from cannabidiol, one of the most abundant cannabinoids of *Cannabis sativa* [79]. This compound is an anthracycline antibiotic, belonging to a class of quinones with a antiangiogenic potential as described in the literature [80, 81]. The anthracycline antibiotics affect vascular tone through complex mechanisms, such as the modulation of Ca2+ homeostasis and of the expression of membrane proteins and enzymes that are involved in the control of smooth muscle contraction. These quinones also alter the generation of autoregulatory mediators, such as nitric oxide and endothelin [81].

Using bovine aortic endothelial cells, Kogan *et al.* (2006) found that HU-331 inhibits the proliferation of these cells [80]. In addition, these authors observed that HU-331 also inhibited angiogenesis in the *ex-vivo* rat aortic ring assay [80]. Similar to this work, mitomycin C (Figure 2), a naphthoquinone, also inhibited the proliferation of cortex-derived bovine microvascular endothelial (BME) cells [82] with IC_{50} values of 0.05 μg/ml (0.15 μM). Furthermore, the authors also demonstrated that mitomycin C inhibited invasion and tube formation by these cells on a collagen matrix [82].

Other quinones tested were eight analogs (LYR-1 to LYR-8, Figure 3) of synthetic hexahydro-cannabinols, which are structurally similar to natural cannabinoid THC [83]. The drugs were tested using the tube formation assay, where endothelial cells were plated on a Matrigel-coated surface in the presence of VEGF, a well-known factor that induces endothelial cell growth and is widely expressed in most cancers. All analogs (5 μM) tested blocked *in vitro* endothelial tube formation. In addition, Thapa *et al.* (2011) observed that LYR-7 and LYR-8 delayed VEGF-induced migration at 10 h after HUVEC wound injury and that both analogs significantly inhibited chemotactic migration of HUVECs using the Transwell assay [83].

Another naphthoquinone extensively studied is vitamin K. Matsubara *et al.* (2008), while studying

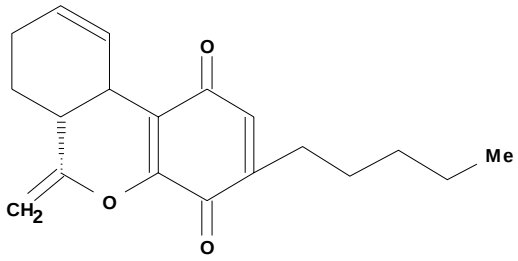


Figure 1. Chemical structure of HU-331 [79].

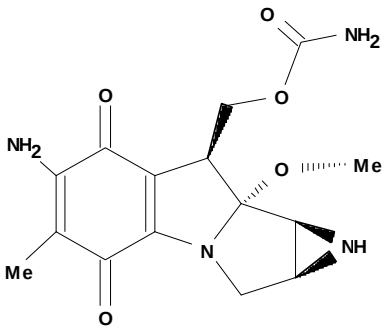


Figure 2. Chemical structure of mitomycin C [82].

vitamin K3 (2-methyl-1,4-naphthoquinone), showed that this quinone suppressed angiogenesis in a rat aortic ring model [84]. This napthoquinone also inhibited the growth, tube formation on reconstituted basement membrane, and chemotaxis of human vein endothelial cells (HUVECs). In addition, Yoshiki *et al.* (2005) observed that vitamin K2 also inhibited endothelial cell proliferation and tubular formation [85]. The chemical structures of vitamin K2 and K3 are shown in Figure 4, indicating that both molecules are naphtoquinone derivatives. This suggests that this class of compounds could have anti-cancer and antiangiogenic activities. In fact, other naphthoquinone derivatives have demonstrated anti-cancer effects, such as S-(1,4-naphthoquinon-2-yl)-mercaptoalkanoic acid amides [86], shikonin [87], lapachol, and β-lapachone and its derivatives [88]. In this circumstance, Kayashima *et al.* (2009) examined the anti-cancer and antiangiogenic effects of 1,4-naphthoquinones and structurally related compounds as shown in Figure 5 [89]. In a rat aortic ring assay, it was observed that 1,4-naphtoquinone (compound 1) showed the strongest antiangiogenic activity. 1,4-Benzoquinone

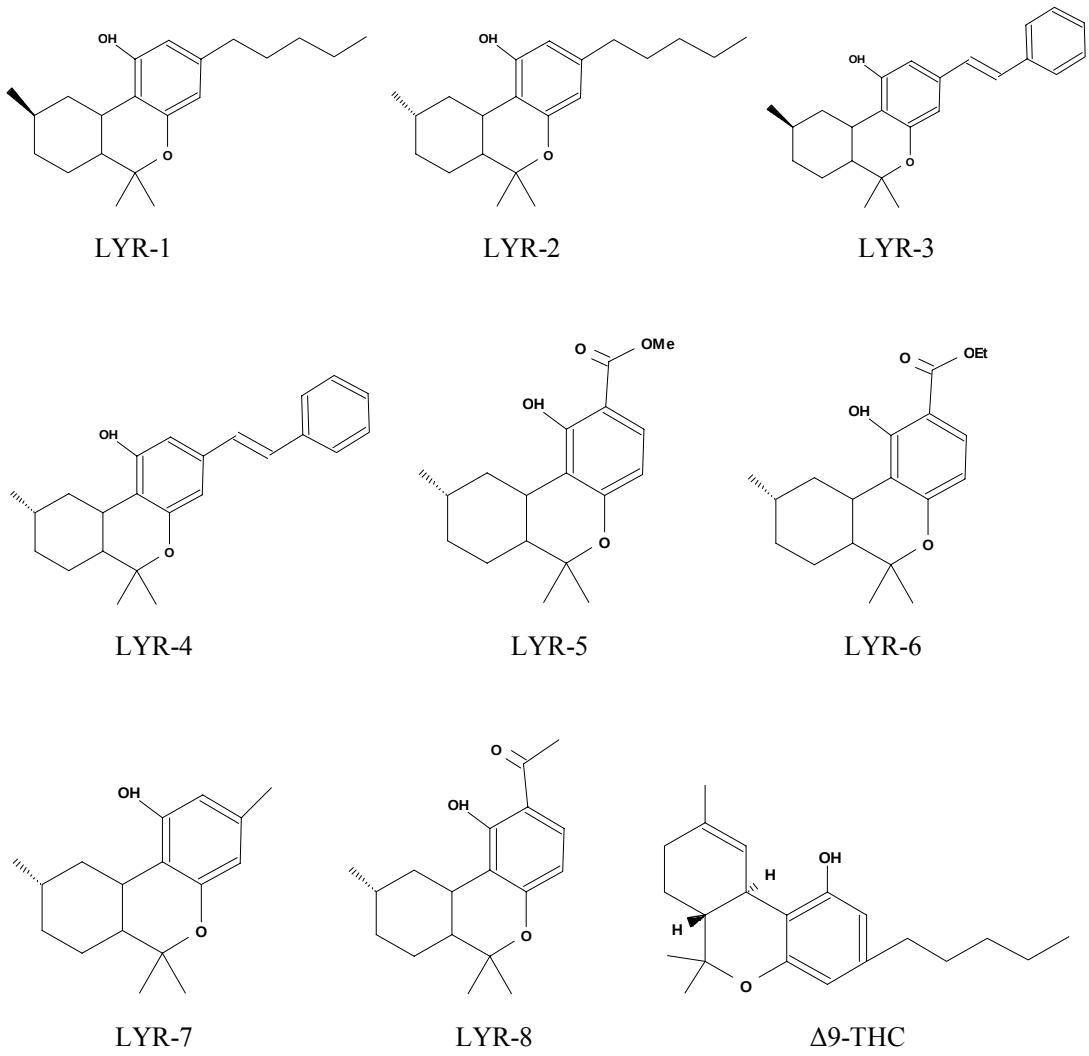


Figure 3. Chemical structure of LYR analogs (LYR-1 to LYR-8). We can see that these analogs are structurally related to classical cannabinoid Δ9-tetrahydrocannabinol (THC) [83].

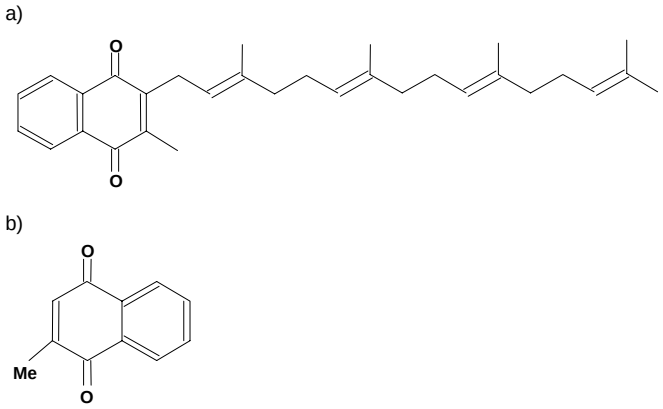


Figure 4. Chemical structure of vitamin K2 (a) and vitamin K3 (b) [84].

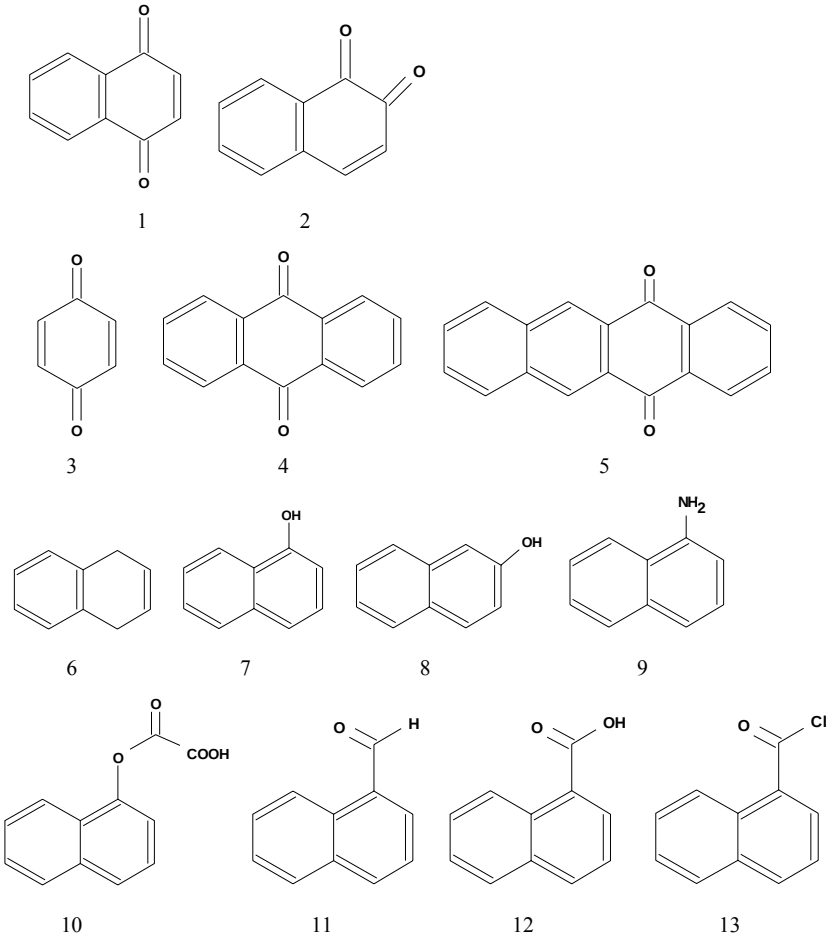
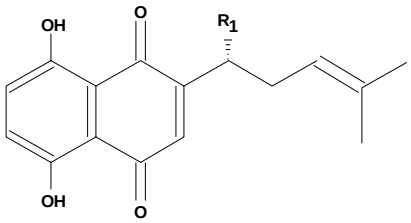


Figure 5. Chemical structures of naphthoquinones (compounds 1 and 2) and their structurally related compounds (compounds 3–13). Compounds 1: 1,4- naphthoquinone, 2: 1,2-naphthoquinone, 3: 1,4-benzoquinone, 4: anthraquinone, 5: 5,12- aphthacenequinone, 6: naphthalene, 7: 1-naphthol, 8: 2-naphthol, 9: 1-naphthylamine, 10: 1-naphthylacetic acid, 11: 1-naphthaldehyde, 12: 1-naphthoic acid, 13: 1-naphthoyl chloride [89].

(compound 3), 1-naphthylacetic acid (compound 10) and 5,12-naphthacenequinone (compound 5) also inhibited angiogenesis, but the other compounds studied had no effect in this assay. These results demonstrate that the 1,4-naphthoquinone structure is important for the inhibitory effect of vitamin K2 and K3 on angiogenesis [89]. This theory was confirmed by Kayashima *et al.* (2009), who tested 1,4-naphthoquinone against HUVEC tube formation on reconstituted basement membrane and proliferation [89].

Some anti-cancer naphthoquinones have been tested for an antiangionic effect, such as shikonin. Shikonin (Figure 6) is a pigment ingredient isolated from the *Lithospermum erythrorhizon* [87, 90]. Both shikonin and its derivatives have been studied for their antiangiogenic effect (Figure 6). These studies demonstrated that shikonin and its derivatives acetylshikonin and isobutyroylshikonin, decreased VEGF-induced migration of HUVEC in a concentration-dependent manner [87]. It was also demonstrated that this compound inhibited VEGF production in VEGF-treated HUVECs, indicating that these compounds could inhibit vascular endothelial cells migration and capillary-like formation. In addition, shikonin and its derivatives showed an antitumor activity *in vivo*, using LLC-bearing mice, which indicates that the antiangiogenic activity of shikonin and its derivates can mediate an *in vivo* antitumor activity [87].



R = OH (shikonin)

R = $OCOCH_3$ (acetylshikonin)

R = $OCOCH(CH_3)_2$ (isobutyroylshikonin)

Figure 6. Structures of shikonin and its derivatives [87].

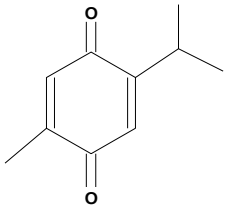


Figure 7. Chemical structure of thymoquinone [91].

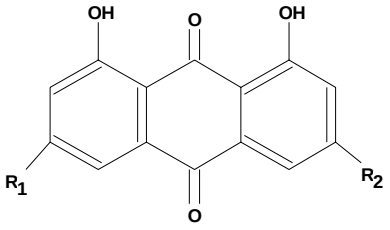


Figure 8. Structures of the five anthraquinone derivatives tested. Aloe-emodin R1 = H, R2 = CH2OH; chrysophanol R1 = H, R2 = CH3; emodin R1 = OH, R2 = CH3; physcion R1 = CH3O, R2 = CH3; rhein R1 = H, R2 = COOH [98].

Yi *et al.* (2008) studied a component derived from the medicinal plant *Nigella sativa*, thymoquinone (Figure 7) [91]. It was reported previously by Shoieb *et al.* (2003) that thymoquinone inhibits cell proliferation in some cancer lines, such as ovarian adenocarcinoma and breast adenocarcinoma [92]. Gali-Muhtasib *et al.* (2004) showed that this quinone also inhibited the proliferation of colorectal cancer via a p53-dependent mechanism [93]. Other effects of thymoquinone on tumor growth were reported, such as against human pancreatic adenocarcinoma and uterine sarcoma [94], neoplastic keratinocytes [93], human osteosarcoma [95], fibrosarcoma and lung carcinoma [96]. Complementing these findings, Yi *et al.* (2008) [91] found that thymoquinone effectively inhibits endothelial cell migration, invasion, proliferation, and tube formation, prevents angiogenesis *in vitro* and *in vivo*, and suppresses tumor angiogenesis and tumor growth *in vivo*. Moreover, it was demonstrated that thymoquinone inhibits angiogenesis by suppressing the activation of VEGF-induced ERK and AKT but it is not a VEGFR2 inhibitor [91].

One other key target in cancer therapy is the heat shock protein 90 (Hsp90), which can be one of the signaling molecules involved in angiogenesis. This process may be deregulated by Hsp90 inhibition, which makes this protein an interesting target for the development of new antiangiogenic drugs. In this context, Sanderson *et al.* (2006) [97] studied the potential of benzoquinone ansamycin Hsp90 inhibitors to modulate induction of and response to angiogenic cytokines and client protein expression in human endothelial cells. These authors demonstrated that benzoquinone ansamycin Hsp90 inhibitors have effects on the production of vascular endothelial growth factor (VEGF) by tumor cells and blocked proliferative responses of human endothelial cells at nanomolar concentrations [97]. In addition, this substance also reduces endothelial cell migration, tubular differentiation, invasion through Matrigel, and secretion of urokinase-type plasminogen activator [97].

The use of zebrafish embryos is another way to determine the antiangiogenic potential of study compounds. Using this method, He *et al.* (2009) [98] studied five anthraquinone derivatives (aloe-emodin, emodin, rhein, chrysophanol and physcion) (Figure 8) from *Rhubarb* root (Dahuang), an ingredient in traditional Chinese compound prescriptions for the treatment of inflammatory diseases [98]. The authors found that three of the five drugs tested (aloe-emodin, emodin and rhein) have antiangiogenic effect against vessel formation in zebrafish embryos. Aloe-emodin (AE) was also

studied using the *in vivo* chicken chorioallantoic membrane (CAM) assay which demonstrated its antiangiogenic potential [99]. This quinone also inhibits the growth of two endothelial cells: BAEC and HUVEC [99]. In addition, Kwak *et al.* (2006) [100] showed that emodin inhibits in a dose-dependent manner the proliferation, migration into the denuded area, invasion through a layer of Matrigel, and tube formation of HUVEC stimulated with VEGF-A. They also showed that emodin inhibits *in vivo* angiogenesis in a Matrigel plug imbedded in mice [100].

CONCLUSION

Natural products continue to provide new and important leads against different pharmacological targets including cancer. Inhibition of angiogenesis is now considered to be one of the most promising strategies leading to the development of new antineoplastic therapies. Accordingly, numerous bioactive natural compounds have been tested for their antiangiogenic potential, especially quinones, which indicates a strong potential for natural products in the discovery of new and effective antiangiogenic agents.

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