Quinones derived from plant secondary metabolites as anti-cancer agents

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Abstract
Quinones are plant-derived secondary metabolites that present some anti-proliferation and anti-metastasis effects in various cancer types both in vitro and in vivo. This review focuses on the anti-cancer prospects of plant-derived quinones, namely, aloe-emodin, juglone, β-lapachol, plumbagin, shikonin, and thymoquinone. We intend to summarize their anti-cancer effects and investigate the mechanism of actions to promote the research and development of anti-cancer agents from quinones.

Key words: quinone, plant, secondary metabolites, tumor

ABBREVIATIONS
5-FU: 5-fluorouracil; AR, androgen receptor; Bcl-2: B-cell lymphoma 2; CDK2: cyclin-dependent kinase 2; COX-2: cyclooxygenase-2; DR-5: death receptor-5; EGFR: epidermal growth factor receptor; FAK: focal adhesion kinase; GSH: glutathione; HER-2: human epidermal growth factor receptor-2; IGF-1, insulin-like growth factor 1; JNK, c-Jun N-terminal kinase; MDR: multi-drug resistance; MMP: matrix metalloproteinase; NF-κB: nuclear factor κ-light-chain-enhancer of activated B cells; p38 MAPK: p38 mitogen-activated protein kinase; PKC, protein kinase C; PPAR-γ, peroxisome proliferator-activated receptor γ; PTEN, phosphatase and tensin homolog; ROS: reactive oxygen species; STAT3, the signal transducer and activator of transcription 3; TopI: topoisomerase I; TopII: topoisomerase II; TRAIL: TNF-related apoptosis-inducing ligand
INTRODUCTION

Quinones, a type of plant-derived secondary metabolites, is a class of compounds with the quinone structure and can be mainly divided into four types, namely, benzoquinone, naphthoquinone, phenanthrenequinone, and anthraquinone, according to the number of benzene rings in the structural skeleton and fused [1]. Quinones are widely distributed in the plant kingdom and mainly exist in higher plants, such as those from the Polygonaceae, Rubiaceae, Leguminosae, Rhamnaceae, Labiatae, and Boraginaceae families, among others [1]. Moreover, a number of quinones present significant biological activities, such as the purgative effect of quinones isolated from *Rheum officinale*, as well as the anti-bacterial and anti-cancer activities of juglone and plumbagin isolated from Juglandiphyllum and sundew, respectively [1-4].

This review focuses on the progress of natural plant-derived quinones, such as aloe-emodin, juglone, β-lapachol, plumbagin, shikonin, and thymoquinone (Fig. 1), with anti-cancer activities and intends to investigate, as well as summarize, its mechanism of actions to promote further anti-cancer drug discovery from quinones.

ALOE-EMODIN

Aloe-emodin is a bioactive hydroxyanthraquinone that exists in several traditional medical plants, such as *R. palmatum* L., and displays laxative, anti-fungal, anti-viral, and hepatoprotective effects [5-8]. Aloe-emodin also has specific *in vitro* and *in vivo* anti-cancer activities in various human carcinoma cells, including leukemia, lung cancer, colon cancer, neuroectodermal tumor, nasopharyngeal carcinoma, and hepatocellular carcinoma [9-14]. Aloe-emodin-induced cancer cell proliferative inhibition is caused by prolonged G1, S, or G2/M phase cell cycle arrest or apoptosis depending on the cell types and treatment procedure [12, 15-19]. This quinone remarkably inhibits chorioallantoic membrane angiogenesis at low concentrations and inhibits tubule formation of endothelial cells on matrigel [13, 20]. Aloe-emodin also suppresses cancer cell migration, invasion, and metastasis [21, 22], in addition to its potential anti-proliferative effects when combined with cisplatin, doxorubicin, 5-fluorouracil (5-FU), and the tyrosine kinase inhibitor STI571 [23].

Aloe-emodin induces DNA damage and inhibits DNA repair gene expression in cancer cells probably related to the induction of reactive oxygen species (ROS) [11, 24]. This molecule downregulates cyclin A, cyclin-dependent kinase 2 (CDK2), protein kinase C (PKC), and c-Myc as well as upregulates cyclin B1, CDK1, p53, p21, and p27 [15, 16, 18, 19]. The p38 mitogen-activated protein kinase (p38 MAPK) pathway, p53 pathway, c-Jun N-terminal kinase (JNK) activation [25], Fas/death-receptor, and caspase activation are mechanistically involved in aloe-emodin-induced apoptosis [19, 26, 27]. Aloe-emodin also performs the following activities: decreases the levels of urokinase; reduces protein and mRNA expressions of focal adhesion kinase (FAK) [20]; suppresses the expression of matrix metalloproteinase 2 (MMP-2), MMP-9 [12, 21, 22]; and restrains nuclear translocation as well as DNA binding of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) [13].

Other natural hydroxyanthraquinones, such as emodin and rhein, also have anti-cancer activities and mechanisms similar to aloe-emodin [28-32].

JUGLONE
Juglone is a naturally occurring naphthoquinone isolated from walnut trees and has various biological activities, such as anti-fungal, anti-viral, and anti-bacterial activities. This quinone also lowers the blood glucose and is a potent anti-cancer agent that inhibits proliferation, mediates G2 phase cell cycle arrest, and induces apoptosis, among others [33-37]. Juglone inhibits intestinal carcinogenesis induced by azoxymethane in rats and might be a promising chemopreventive agent in human intestinal neoplasia [38]. This quinone also shows potent immunostimulating activities in tumorous tissues [39]. Treatment of cells with juglone synergistically enhances the cytotoxicity of etoposide when etoposide treatment precedes juglone exposure [40]. However, most of the data reported are based on in vitro cellular toxicity assays. Concrete information based on mouse models of cancer is insufficient, and there is a dearth of clinical evidence.

The anti-cancer effects of juglone can be attributed to the induction of oxidative stress, depletion of glutathione, and damage of cell membrane, and so on, leading to cell death by both apoptosis and necrosis [4, 41-45]. Juglone is also an inhibitor of peptidyl-prolyl isomerase Pin1 that is overexpressed in many human cancer tissues [46, 47]. This quinone also markedly suppresses the expression of activator protein-2α and the human epidermal growth factor receptor-2 promoter activity [48], downregulates p53 protein level [49], alters the levels of B-cell lymphoma 2 (Bcl-2) and Bax [41, 42, 50], activates PARP [41], and induces subsequent caspase activation [41, 45, 51].

β-LAPACHONE

β-Lapachone is a natural naphthoquinone derived from Tabebuia avellanedae (Bignoniaceae) [52]. This molecule exhibits a wide spectrum of pharmacological activities, including anti-fungal, anti-bacterial anti-trypanocidal, and anti-cancer activities [53-55]. β-lapachone, a potent anti-cancer agent, can selectively induce cell death in several human cancer cells without killing non-transformed cells [27]. This quinone also delays cell cycle progression at the G1/S transition. The in vivo anti-cancer effect of β-Lapachone has been demonstrated in animal xenografts [56-59]. The combination of β-lapachone and radiotherapy has potential for the treatment of human cancers. Thus, β-Lapachone is currently in multiple phase II clinical trials for use in monotherapy and in combination with other drugs [60].

β-lapachone inhibits the activity of topoisomerase I (Topo I) through a mechanism in contrast to that of typical topoisomerase inhibitors [61]. This quinone induces topoisomerase II (Topo II) α-mediated DNA breaks, instead of Topo I-mediated DNA breaks [62]. β-lapachone also inhibits eukaryotic DNA polymerase-α [63], activates the Mre11p-Tel1p checkpoint pathway by inducing DNA double strand breaks [64], activates MAPKs [65], and induces caspase activation [66], among others. NAD(P)H:quinone oxidoreductase (NQO1) is an intracellular target for β-lapachone in tumor cells [67]. NQO1 catalyses the reduction of β-lapachone and causes a futile cycling between its quinone and hydroquinone forms. This cycle results in a severe loss in reduced NAD(P)H [67] and a concomitant rise in cytosolic Ca2+, finally inducing apoptosis [68]. The metabolism of β-lapachone by NQO1 also leads to ROS generation, DNA breaks, and γ-H2AX foci formation [69, 70]. β-lapachone sensitizes cells to radiation by interrupting the involvement of NQO1 in radiation-induced activation of NF-κB [71] and inhibiting sub-lethal radiation damage repair [72].

Lapachol, among the analogs and derivatives of β-lapachone studied and is present in T. avellanedae along with β-lapachone, shows a number of pharmacologic properties [52] and has
highly significant activity against tumors [73]. However, further studies on lapachol as an anti-neoplastic were terminated in 1970 because of its toxicity [74].

**PLUMBAGIN**

Plumbagin, an organic yellow dye, was originally isolated from the roots and aerial parts of *Dyerophytum africanum, Plumbago pearsonii*, and in roots of *P. auriculata* [75]. Multiple pharmacologic properties of plumbagin, such as anti-inflammatory, analgesic, anti-oxidant, anti-cancer, anti-bacterial, and anti-fungal activities, have been documented in various cellular and animal models [3, 76]. The anti-cancer effect of this quinone has been gradually confirmed in the past decade. Plumbagin significantly inhibits cancer cell proliferation in *in vitro* cell models in dose- and time-dependent manners with IC50 at micromolar level depending on the type of cells and duration of treatment [77-79]. The inhibitory effect of plumbagin on tumor growth in *in vivo* animal models was also observed in solid tumor and Ehrlich ascites model [80], C57BL/6J mice bearing B16F1 melanoma [36], and hormone-refractory prostate cancer [81]. Moreover, plumbagin suppresses tumor angiogenesis and tumor growth in human colon carcinoma, as well as in prostate cancer xenograft mouse models [82], in addition to its enhancement of radiation induced cytogenetic in mouse Ehrlich ascites carcinoma *in vivo* [83]. Interestingly, plumbagin inhibits ultraviolet radiation-induced development of squamous cell carcinomas suggesting that this quinone may be a novel agent for the prevention of skin cancer [84].

Plumbagin induces cell cycle arrest at G2/M phase in cancer cell lines by regulating p53, p21, cyclinB1, Cdc25B, cyclin A, Cdc2, Cdc25C, and Chk2, among others [85-88]. The inhibitory effect of this molecule on cancer cell proliferation can be attributed mainly to apoptosis [77-79, 89, 90] mediated by induction of intracellular ROS formation [77, 89-92], upregulation of p53 [90], inactivation of NF-κB [78], downregulation of cyclooxygenase-2 (COX-2) [79], activation of JNK [87], among others, and subsequent activation of caspases and Bcl-2 family proteins. Plumbagin induced ROS generation might be mainly from mitochondria since plumbagin was identified as a NOX4 inhibitor of NAD(P)H oxidase [93]. ROS mediated inhibition of Topo II is another important mechanism contributing to plumbagin-induced apoptosis [94]. Plumbagin is a potent inhibitor of the NF-κB activation pathway that leads to the suppression of NF-κB-regulated gene products, such as IAP, Bcl-2, Bcl-xL, survivin, cyclinD1, COX-2, and MMP-9, among others [95]. This molecule also inhibits the invasion and migration of breast, as well as gastric, cancer cells by downregulating the expression of chemokine receptor CXCR4 mediated by NF-κB inhibition [96]. In addition, plumbagin is a substrate for the multidrug resistance (MDR) linked ATP binding cassette drug transporter ABCG [97], suggesting its potential role in MDR.

**SHIKONIN**

Shikonin, a naphthoquinone pigment, is an active component isolated from the roots of Chinese herbal plant *Lithospermum erythrorhizon*. This medicinal plant has been broadly used to treat burns, sore throats, measles, carbuncles, macular eruptions, and allergic disease in China for thousands of years [98]. In addition to its well-established anti-inflammatory and anti-oxidant activities, recent studies have focused on the anti-cancer effects of shikonin, which includes inhibition of cell proliferation, induction of apoptosis, and retardation of invasion, among others. The values of IC50 for anti-proliferation vary depending upon the cancer cell type and treatment time, and approximately at micromolar levels. The anti-cancer effects of shikonin were also
determined in both allografts and xenografts animal models \textit{in vivo} \cite{99}.

ROS generation, inactivation of NF-κB, regulation of epidermal growth factor receptor and insulin-like growth factor 1, and activation of caspases may be involved in the anti-cancer mechanisms of shikonin \cite{32, 100-107}. This quinone inhibits Topo I/II activity and results in DNA damage in cancer cells \cite{108}. Shikonin also has anti-cancer effects as an anti-estrogen agent by reversing NQO1 expression \cite{109, 110} and acts as a selective estrogen enzyme modulator by downregulating the expression of steroid sulfatase, important for the biosynthesis of estrogen \cite{111}. In addition, pyruvate kinase-M2 and proteasome are also inhibited by shikonin or its analogs \cite{112}.

**THYMOQUINONE**

Thymoquinone is an active ingredient isolated from \textit{Nigella sativa} and has been investigated for its anti-oxidant, anti-inflammatory, and anti-cancer activities in both \textit{in vitro} and \textit{in vivo} models since its first extraction in 1960s \cite{113-115}. The anti-cancer effects of thymoquinone are mediated through different modes of action, including anti-proliferation, apoptosis induction, cell cycle arrest, anti-metastasis, and anti-angiogenesis \cite{113, 116, 117}. The anti-tumor effects of thymoquinone have also been investigated in tumor xenograft mice models for colon, prostate, pancreatic, and lung cancer, among others \cite{113, 118}. Thymoquinone greatly inhibits doxorubicin-resistant human breast cancer MCF-7/DOX cell proliferation \cite{119} without exhibiting cytotoxicity to normal human intestinal FHs74Int cells \cite{117}. The combination of thymoquinone and conventional chemotherapeutic drugs, such as 5-FU, gemcitabine, cisplatin, oxaliplatin, and doxorubicin, could produce greater therapeutic effect \textit{in vitro} and/or \textit{in vivo} in different cancer types \cite{113, 120-122}. Sub-toxic doses of thymoquinone sensitize PEL cells to TNF-related apoptosis-inducing ligand via upregulation of death receptor-5 (DR-5) \cite{123}.

This quinone exhibits anti-cancer activity through the modulation of multiple molecular targets, including F-actin, α and β tubulin, p53, p73, phosphatase and tensin homolog, the signal transducer and activator of transcription 3, NF-κB, peroxisome proliferator-activated receptor γ, polo-like kinase 1, polo-box domain, androgen receptor, E2F-1, Bcl-2, focal adhesion kinase, and MMPs among others \cite{113, 118, 119, 121, 123-130}. Thymoquinone also generates ROS, induces DNA damage, and inhibits telomerase activity \cite{117, 131}. Most recently, thymoquinone treatment has been demonstrated to inhibit strongly CXCL12-mediated chemotaxis in multiple myeloma cells, but not normal peripheral blood mononuclear cells, and significantly downregulate CXCR4 expression and CXCL12-mediated CXCR4/CD45 association \cite{132}.

**SUMMARY**

We summarized the recent progress of several anti-cancer quinones from plant-derived secondary metabolites as well as the characteristics of these quinones. Based on previous studies, sources of the anti-cancer quinones derived from plant secondary metabolites are fewer than alkaloids, terpenoids, and flavone. Certain quinone antibiotics, such as mitomycins, have also been used in clinical treatments of malignant diseases \cite{133}. However, there are few plant-derived quinones that have entered clinical trials thus far. One promising compound, salvicine, synthesized by the structural modification of a natural product isolated from \textit{Salvia prionitis lance}, has already entered phase II clinical trial in China \cite{60, 134, 135}. Therefore, obtaining promising anti-cancer quinones from plants is possible, but appropriate structural modification is also necessary.
Given that oxidative damage appears to be a common characteristic of quinones to achieve the anti-cancer effects [25, 41, 117], more than one type of cellular molecule, especially those sensitive to oxidative stress such as enzyme with thiol [107, 136], will be targeted after quinone treatment. These anti-cancer quinones have multiple molecular targets, and the mechanisms still require further clarification. Thus, we propose that more high-throughput technologies, such as proteomics, high content screening, and biochip, among others, [137-139] be employed to explore the anti-cancer mechanisms of quinones. The toxicity and characteristics of drug metabolism of these quinones should also be studied urgently to develop successfully anti-cancer agents.

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**Figure Legends**

Figure 1. The chemical structures of aloe-emodin, juglone, lapachol, plumbagin, shikonin, thymoquinone.

Figure 2. A schematic diagram of molecular machinery for anti-neoplastic properties of aloe-emodin, juglone, lapachol, plumbagin, shikonin, thymoquinone. All of them have multiple anti-cancer effects through direct cell toxicity, anti-proliferation and anti-invasion pathways *etc.*