

## ADVANCES OF PLANT-DERIVED NATURAL PRODUCTS IN OVARIAN CANCER THERAPY

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### ABSTRACT

Ovarian cancer is the leading cause of death in the gynaecologic cancers within the UK and US. Presently the standard treatment for ovarian cancer entails the use of chemotherapy drugs paclitaxel and carboplatin after aggressive surgical reduction in order to prolong the patient's life for multiple years. However, prolonged use of platinum-based chemotherapy often leads to drug resistance, which causes the ovarian cancer patient to relapse and potential death. Therefore there is an urgent medical need for breakthrough drugs with an effective therapeutic impact on ovarian cancer. Phytochemicals (plant-derived natural products) have been used for thousands of years as treatment for various diseases, because of their huge chemical diversity and wide range of biological activities.

In this review, the role of phytochemicals as chemo-preventive compounds, potential sources of new drugs for ovarian cancer and the benefits of their adoption as mono-therapeutic agents or as chemosensitizers when used in-conjunction with the conventional anti-cancer drugs is highlighted. We will describe the phytochemicals: 1) clinically approved drugs such as paclitaxel and camptothecin including its semi-synthetic derivatives topotecan and irinotecan; 2) currently in clinical trials such as epipodophyllotoxin derivatives etoposide and teniposide, ventfolide, phenoxodiol, and combretastatins; 3) in preclinical trials such as quercetin, baicalein, baicalin, thymoquinone, betulinic acid and tetrandrine; and recently discovered compounds which have high potency ( $IC_{50}$  less than  $10 \mu M$ ) and have been discovered recently (last 15 years). In particular, several new compounds including bufatrienolides, ipomoeassin D, 2'-(R)-O-acetylglauucarubinone, and molvizarin have  $IC_{50}$ s lower than 100 nM in ovarian cancer cells and might have different mechanisms of action from those of platinum derivatives/paclitaxel, therefore providing potential ways to attack multidrug resistance in ovarian cancer without jeopardising the patient's treatment.

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## 1. INTRODUCTION

### Ovarian Cancer

Ovarian cancer is the ninth most prevalent cancer in the US and is the leading cause of death in gynaecologic cancers in the UK and US [1]. Ovarian cancer forms in tissues of the ovary and most of them are either ovarian epithelial carcinomas (cancer that begins in the cells on the surface of the ovary) or malignant germ cell tumors (cancer that begins in egg cells). The major cause of death in women from ovarian cancer is largely due to poor diagnosis because there is a lack of any clear early detection or screening test. About 70% of cases are not diagnosed until they have reached advanced stages [2].

A number of interventions are currently in use or in trials for the treatment of ovarian cancer. These include surgery, radiotherapy, hyperthermia, laser therapy, gene therapy and chemotherapy. Conventional treatment mainly involves a combination of these interventions with surgery, radiotherapy and chemotherapy. However these interventions are not without disadvantages and limitations, which have been recognized by the scientific community. Some chemotherapeutic drugs employed in the treatment of cancers are cisplatin and carboplatin (Figure 1). Cisplatin is one of the platinum-based drugs used in the treatment of several cancers, such as testicular, small cell lung and ovarian cancers.

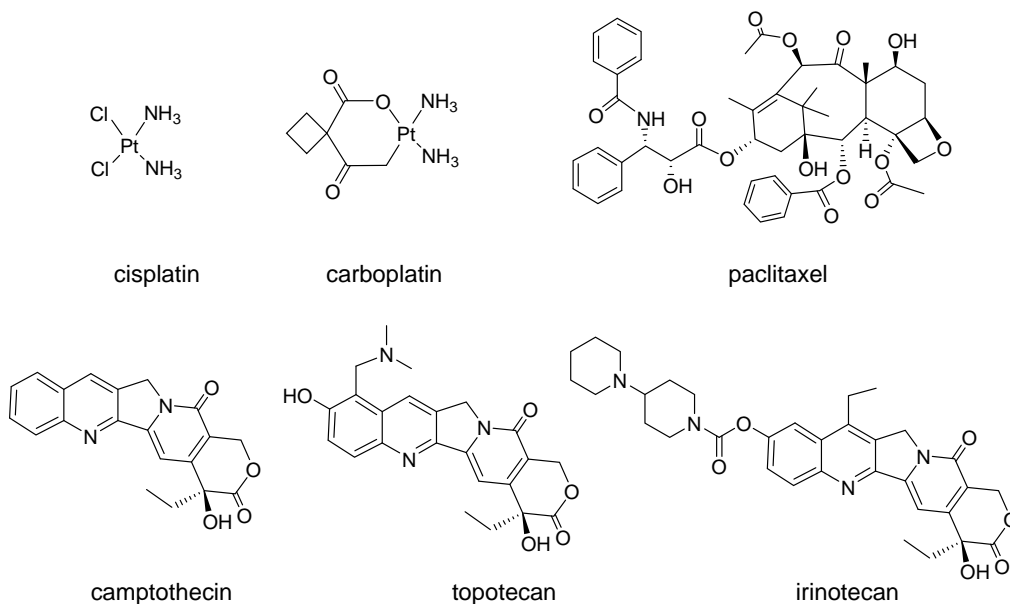


Figure 1. Structures of plant-derived natural products (drugs) and platinum-derived drugs used for the treatment of ovarian cancer.

The drug is very toxic and could cause damage to kidneys and nerves. Other adverse effects of cisplatin include loss of hearing, vomiting and bone marrow suppression resulting in anemia. Due to these adverse side effects, cisplatin is used mainly in conjunction with other chemotherapeutic agents [3, 4]. Carboplatin is another platinum-based drug used in the

treatment of lungs, head, neck and ovarian cancers. Unlike cisplatin, carboplatin is less toxic and thus has fewer side effects compared with cisplatin but the disadvantage is that it's less effective. Also, as with all platinum drugs, platinum resistance has been advancing which may cause the cancer to re-emerge [5, 6]. Thus, there is an urgent medical need for breakthrough drugs that have an effective therapeutic impact on ovarian cancer.

## Natural Products in Cancer Treatment

Since ancient times natural products (mainly plants) have been used for the treatment of various diseases. Natural products sources of drugs comprises of plants, marine/aquatic, terrestrial microbial/fungi, terrestrial animal and unspecified organism.

However, most drugs have been derived from plant sources from focused research, i.e., higher plants mainly because there is a great interest in investigating the medicinal plants across the continents [7]. Most of populations (80%) in the developing countries depend on traditional medicine in their health care systems based on the WHO survey [8]. It is estimated that there are about 250,000 known higher plant species in the world, of which only 5-15% have been studied for biological usefulness (bio-activity) [9]. About 200,000 natural products have been reported in plants [10]. Some of the important phytochemical constituents, found in plants, include alkaloids (atropine, quinine, etc.), flavonoids, tannins, terpenes, terpenoids, steroids, glycosides, saponins, phenolics, and quinones. The bio-activities of medicinal plants have been linked to the presence of one or more of the various classes of phytochemicals, with isoprenoids, phenolic compounds and alkaloids being the most commonly biosynthesised [7]. Investigations into the anticancer property of plants have been for about 60 years and are fairly recent, with even fewer plants being screened [11].

The process of discovering anticancer drugs derived from plants includes preclinical and clinical studies. The preclinical study includes random or an ethanopharmacological screening of plant extracts, isolation, structural elucidation of bioactive compounds, and toxicological and effectiveness tested on animals. If the compound passes all the testing, the results are submitted to the Food and Drug Administration (FDA) in US or a comparable agency in other countries before clinical studies. In the clinic, there are generally three phases of clinical trials. Phase I involves the evaluation of safety of a drug in healthy volunteers; Phase II includes the testing of efficacy and dose range in patients; while Phase III is to further validate the efficacy and safety in thousands of patients. If the tested compound passes all the evaluations and is approved by FDA after thorough review, the new drug can be offered for clinical use [12].

Within 1981-2010, about 50 natural products derived anti-cancer drugs were approved, either as un-modified compounds, semisynthetic analogues, or synthetic compounds based on natural product leads, with 5 drugs namely: romidepsin, cabazitaxel, eribulin, mifamurtide and vinflunine developed in 2010 alone. This underlines the importance of plants as sources of new cancer chemotherapeutic agents [13, 14].

The literature on 5 plant extracts and 96 natural products isolated from higher plants and microorganisms before 2001 and with potential anticancer activity against ovarian neoplasia was reviewed [15]. In the current review, we will discuss plant-derived natural products and their derivatives, which have been approved in clinics, in clinical trials and in preclinical investigation for the treatment of ovarian cancer. In particular we list novel phytochemical

structures with potent *in vitro* anti-cancer activity in ovarian cancer cell lines (Table 1), which were discovered from 2001 to 2014.

## 2. PHYTOCHEMICALS APPROVED FOR THE TREATMENT OF OVARIAN CANCER

### Camptothecin

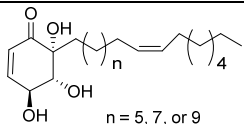
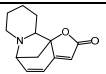
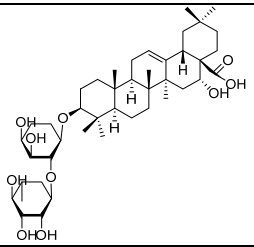
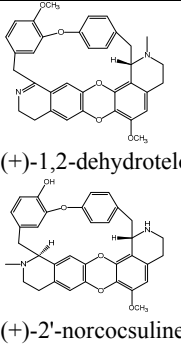
Camptothecin (Figure 1) was isolated from the Chinese tree *Camptotheca acuminata* (family *Cornaceae*; [www.theplantlist.org](http://www.theplantlist.org)) by Dr. Monroe E. Wall and Dr. Mansukh C. Wani of Research Triangle Institute [16]. The mechanism of action of camptothecin involves the inhibition of DNA topoisomerase I and DNA relaxation. It can specifically stabilize a covalent binary complex formed between DNA and topoisomerase I [17]. Camptothecin has been shown to have significant antitumor activity against: lung, ovarian, breast, pancreas and stomach cancers. To improve its water solubility and pharmacological properties, various semi-synthetic analogues of camptothecin have been made. Topotecan and Irinotecan (CPT-11) (Figure 1) are used for the treatment of ovarian and colon cancers, respectively [18].

### Paclitaxel

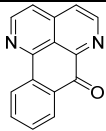
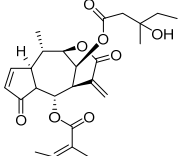
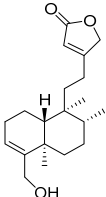
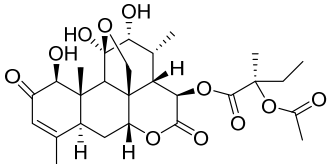
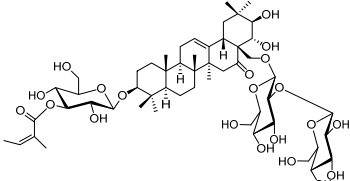
Paclitaxel (Figure 1) was originally discovered from the bark of the Pacific yew tree, *Taxus brevifolia* Nutt. (family *Taxaceae*) also by Dr. Monroe E. Wall and Dr. Mansukh C. Wani [19]. The mechanism of action of paclitaxel is to bind to beta-tubulin subunits of microtubule, therefore stabilizing the microtubule and protecting it from disassembly, which could cause dysfunctions in chromosome segregation, mitotic spindle assembly, and cell division [20]. The drug has been approved for use in the treatment of breast, lung, non-small cell lung and ovarian cancers. Paclitaxel is used in the first-line and second-line treatment of ovarian cancer.

Cremophor EL is used as the vehicle in the delivery of paclitaxel due to its poor water solubility. However, this has led to increasing clinical toxicity of paclitaxel. Thus, paclitaxel in combination with other agents, such as carboplatin, which are less toxic, is often used. Furthermore, a number of its derivatives are explored for clinical trials. An albumin-paclitaxel called Abraxane is a water-soluble formulation where paclitaxel is covalently bound to albumin nano-particles. Abraxane was approved by FDA in 2005, which exhibited enhanced paclitaxel tissue distribution and tumor penetration with fewer side effects in multiple tumor types [21]. Paclitaxel poliglumex is another water soluble conjugate of paclitaxel and poly(L-glutamic acid) which can reduce hypersensitivity reactions. Current pre-clinical pharmacokinetic studies and key Phase I and II clinical trial in ovarian cancer indicates that this derivative is active in ovarian cancer, but has not been shown to have a better safety profile than a standard taxane-based therapy [22].

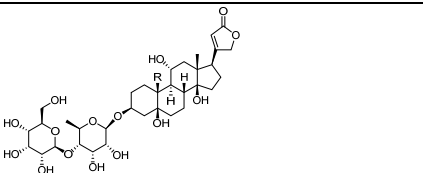

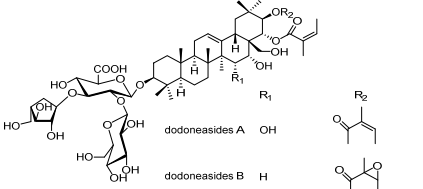

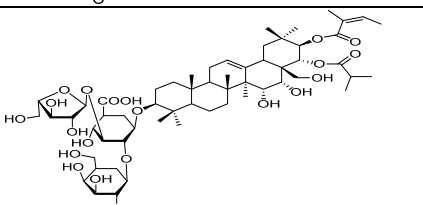
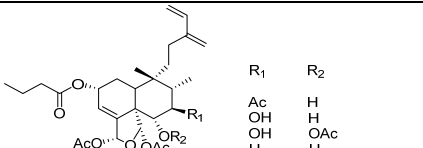
**Table 1. A list of novel and potent plant natural products against ovarian cancer cells discovered between 2001 and 2014 (the order of the compounds is arranged according to the year in which they were reported)**

Compound name	Structure	Plant name (family)	IC <sub>50</sub> (A2780 ovarian cancer cell line)	Reference
trihydroxyalkylcyclohexenones	 n = 5, 7, or 9	<i>Pleiogynium timoriense</i> (A. DC.) Leenh. ( <i>Anacardiaceae</i> )	0.8, 0.7, and 0.8 μM, respectively.	[81]
securinine		<i>Margaritaria discoidea</i> (Baill.) G. L. Webster ( <i>Euphorbiaceae</i> )	3-16 μM (OVCAR-8, A2780 and A2780cis) <sup>a</sup>	[77]
3-β-[(O-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranosyl)oxy]-16-α-hydroxyolean-12-en-28-oic acid		<i>Polyscias duplicate</i> (Thouars ex Baill.) Lowry and G. M. Plunkett ( <i>Araliaceae</i> )	2.8 μM	[82]
(+)-1,2-dehydrotelobine and (+)-2'-norcocculine	 (+)-1,2-dehydrotelobine (+)-2'-norcocculine	<i>Anisocyclus grandidieri</i> Baill. ( <i>Menispermaceae</i> )	4.1 ± 0.3 and 2.7 ± 0.3 μM, respectively.	[83]

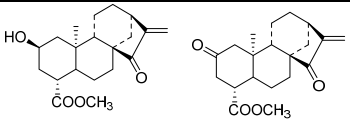
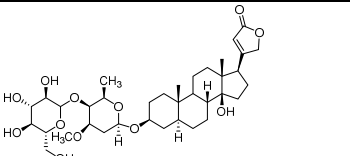
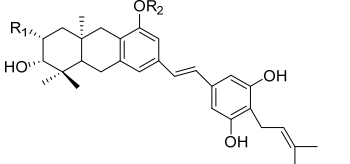
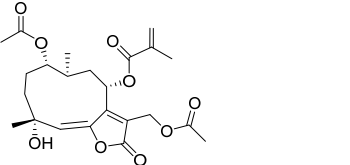
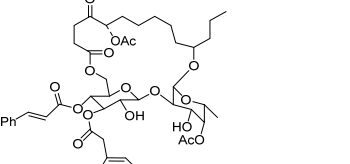


Compound name	Structure	Plant name (family)	IC <sub>50</sub> (A2780 ovarian cancer cell line)	Reference
sampangine		<i>Ambavia gerrardii</i> (Baill.) Le Thomas ( <i>Annonaceae</i> )	0.58 μM	[89]
athrolide D		<i>Athroisma proteiforme</i> (Humbert) Mattf. ( <i>Compositae</i> )	0.6 μM	[90]
16,18-dihydroxykolavenic acid lactone		<i>Cyphostemma greveana</i> Desc. ( <i>Vitaceae</i> )	0.44 μM	[91]
2 <sup>l</sup> -(R)-O-acetylglucarubinone <sup>b</sup>		<i>Quassia gabonensis</i> Pierre [Syn. <i>Odyndyea gabonensis</i> (Pierre) Engl.] ( <i>Simaroubaceae</i> )	<18 nM (MSPC1, 2774-C10, HeyA8 Hoc7)	[92]
terminaliaside A		<i>Terminalia tropophylla</i> H. Perrier ( <i>Combretaceae</i> )	1.2 μM	[93]

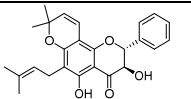
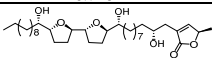
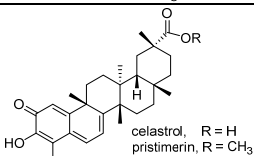
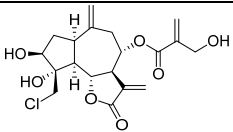
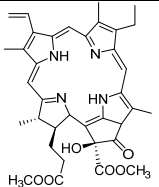
**Table 1. (Continued)**

Compound name	Structure	Plant name (family)	IC <sub>50</sub> (A2780 ovarian cancer cell line)	Reference
cardenolide glycosides <sup>b</sup>		<i>Elaeodendron alluaudianum</i> H. Perrier	70 nM	[94]
isomahubannolide-23		<i>Machilus wangchiana</i> Chun (Lauraceae)	2.66 μM	[95]
dodoneasides A and B		<i>Dodonea viscosa</i> (L.) Jacq. (Sapindaceae)	0.79 and 0.70 μM, respectively	[96]
parthenolide		<i>Magnolia kobus</i> DC. (Magnoliaceae)	9.7 μM (SK-OV-3)	[97]
xanifolia-Y <sub>0</sub>		<i>Xanthoceras sorbifolia</i> Bunge (Sapindaceae)	4.2 ± 0.7 μM (OVCAR3)	[98]
caseanigrescens A-D		<i>Casearia nigrescens</i> Tul. (Salicaceae)	0.83-1.4 μM	[99]



Compound name	Structure	Plant name (family)	IC <sub>50</sub> (A2780 ovarian cancer cell line)	Reference
atractyligenin 15 keto and atractyligenin 2,15-diketo	 <p>atractyligenin 15-keto      atractyligenin 2,15-diketo</p>	<i>Atractylis gummifera</i> L. ( <i>Asteraceae</i> )	0.3 and 0.2 μM (1A9), respectively.	[100]
boivinide A		<i>Roupellina (Strophanthus) boivinii</i> Baill. ( <i>Apocynaceae</i> )	0.17 μM	[101]
vedelianin, schweinfurthin E	 <p>schweinfurthin E, R<sub>1</sub> = OH, R<sub>2</sub> = CH<sub>3</sub> vedelianin, R<sub>1</sub> = OH, R<sub>2</sub> = H</p>	<i>Macaranga alnifolia</i> Baker ( <i>Euphorbiaceae</i> )	0.26 and 0.13 μM, respectively.	[102]
glaucolides M		<i>Vernonia pachyclada</i> Baker ( <i>Compositae</i> )	3.3 μM	[103]
Ipomoeassin D <sup>b</sup>		<i>Ipomoea squamosa</i> Choisy ( <i>Convolvulaceae</i> )	35 nM	[104]

**Table 1. (Continued)**

Compound name	Structure	Plant name (family)	IC <sub>50</sub> (A2780 ovarian cancer cell line)	Reference
isomundulinol		<i>Mundulea chapelieri</i> (Baill.) Du Puy and Labat ( <i>Leguminosae</i> )	1.2 μM	[105]
molvizarin <sup>b</sup>		Annonaceous plants	12 pM (1A9 cell)	[106]
celastrol and pristimerin	 celastrol, R = H pristimerin, R = CH <sub>3</sub>	<i>Reissantia buchananii</i> (Loes.) N. Hallé ( <i>Celastraceae</i> )	0.17 and 0.22 μM (1A9), respectively.	[107]
chlorojanerin		<i>Centaurothamnus maximus</i> (Forssk.) Wagenitz and Dittrich ( <i>Compositae</i> )	15 μM (SK-OV-3)	[108]
cycloviolacin O <sub>2</sub>	Cyclic peptide	<i>Viola odorata</i> L. ( <i>Violaceae</i> )	1.32 μM (OVCA)	[78]
methyl (10S)-hydroxypheophorbidea		<i>Clerodendrum calamitosum</i> L. ( <i>Lamiaceae</i> )	0.43 μM (1A9)	[109]

<sup>a</sup>IC<sub>50</sub> in other ovarian cancer cell lines rather than A2780. <sup>b</sup>very potent compounds with IC<sub>50</sub> less than 100 nM.

### 3. PHYTOCHEMICALS IN PRECLINICAL STUDY FOR THE TREATMENT OF OVARIAN CANCER

Despite the advances in the treatment of ovarian cancer such as the developments of camptothecins and taxanes, FDA has approved no new drug for ovarian cancer since 2006 [23]. Currently, several plant-derived products are in clinical trials.

#### Epipodophyllotoxin Derivatives Etoposide and Teniposide

Podophyllotoxin and epipodophyllotoxin (Figure 2) are lignans obtained from the roots of the North American May Apple, *Podophyllum peltatum* L. (family *Berberidaceae*). The compounds are used traditionally as a remedy for cough, skin diseases and have served as purgatives. The mechanism of action of podophyllotoxin lies in the formation of a complex with tubulin and prevention of the synthesis of microtubules. Surprisingly, two glycosidic derivatives etoposide and teniposide are inhibitors of topoisomerase II. Etoposide is currently in clinical use as antineoplastic agents in the treatment of kaposi sarcoma, lymphoma, testicular and lung cancer; while teniposide is used in the treatment of childhood acute lymphocytic leukaemia.

Clinical studies of etoposide for the treatment of ovarian cancer have been carried out. Single use of the compound showed activity in recurrent ovarian cancer after treatment with cisplatin, but response and survival periods are short [24]. A clinical trial that involved the use of alternating blotecan (a synthetic derivative of camptothecin) and oral etoposide in platinum-resistant ovarian cancer patients shows positive activity at the dose of 50 mg/day of etoposide [25]. A clinical study suggested that the combination chemotherapy with irinotecan plus oral etoposide has provided clinical benefit to patients with recurrent ovarian cancer previously treated with platinum and taxane agents [26]. However, a more recent study of the same drugs combination based on 60 patients suggested a moderate response rate but did not meet the primary endpoint [27, 28]. More clinical studies are needed to confirm their efficacy and safety.

#### Vinblastine Derivatives

The vinca alkaloids such as vinblastine and vincristine (Figure 2) were discovered from a Chinese medicinal plant *Catharanthus roseus* (L.) G. Don (family *Apocynaceae*) in the 1950s. They act on tubulin and prevent its formation of microtubule in a similar way as podophyllotoxin [29].

The vinca alkaloids were approved as antileukaemic agents. Vintafolide (EC145), a folic acid-desacetylvinblastine (folate receptor (FR)-targeted vinca alkaloid) conjugate is currently under development as a novel targeted agent for epithelial ovarian cancer [30]. The FR is overexpressed in ovarian cancer cells, which can be used as a target for anticancer agent as well as a diagnostic marker. The radiopharmaceutical, EC20, was therefore developed for non-invasive detection of functionally active FR. Vinofolate in companion with EC20 has shown promise in the trials in ovarian cancer [31]. In Phase I studies vintafolide demonstrated

an acceptable safety profile. In a Phase II study, a combination of vintafolide and pegylated liposomal doxorubicin showed a significant improvement in patients with platinum-resistant ovarian cancer. A Phase III study is currently underway [32].

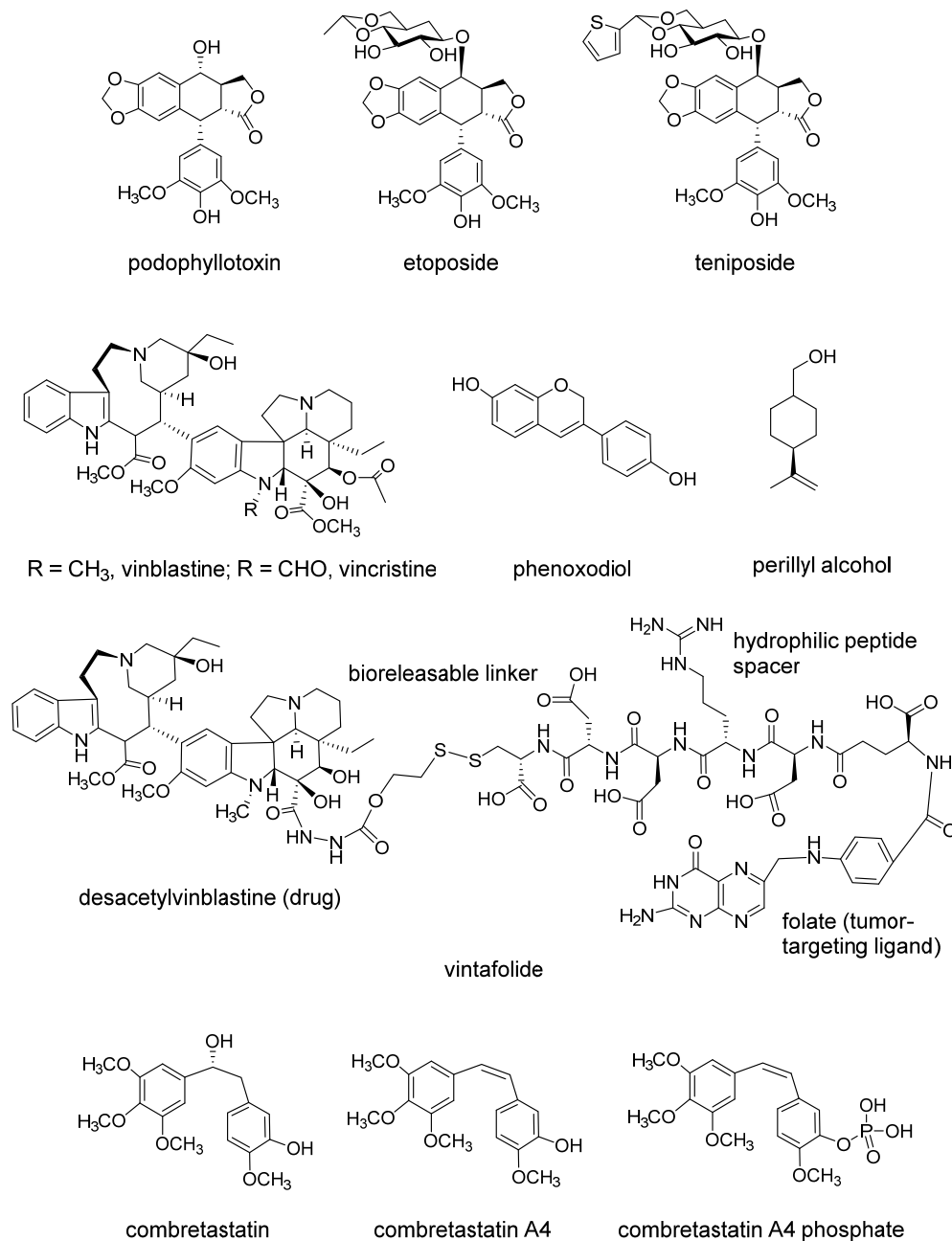


Figure 2. Phytochemicals and their derivatives currently in clinical trials for the treatment of ovarian cancer.

## Phenoxodiol

Phenoxodiol (2H-1-benzopyran-7-0,1,3-[4-hydroxyphenyl]) is a derivative of plant isoflavone genistein (Figure 2) found in soybean [*Glycine max* (L.) Merr.; family *Fabaceae*]. Phenoxodiol is a multiple signal transduction regulator, enhancing mitotic arrest and apoptosis of tumor cells through multiple mechanisms that include decreasing the level of antiapoptotic proteins and inhibiting the activity of DNA topoisomerase II similar as etoposide and teniposide. Further research suggested that phenoxodiol may have dual effects on the proliferation of the cancer cells as well as the angiogenic stimulation [33]. Preclinical studies indicated its promise in the treatment of both ovarian cancer and hematologic cancers [34]. Phenoxodiol has also been evaluated in advanced clinical trial for development as a “chemosensitizer” when combined with platinum drugs for the treatment of ovarian cancer [35] and as a monotherapy for cervical and prostate cancers [36]. A further Phase II study in 2011 showed that a combination of phenoxodiol and cisplatin or paclitaxel was active within patients with platinum/taxane-refractory/resistant ovarian cancers [37]. In contrast, a recent report indicated that orally delivered phenoxodiol showed no evidence of clinical activity, when combined with weekly AUC2-carboplatin in platinum-resistant ovarian cancer [38]. Thus, more clinical investigations on phenoxodiol or its combination with other drugs are necessary.

## Combretastatins

Combretastatins (Figure 2) are stilbenoid phenols isolated from *Combretum caffrum* (Eckl. and Zeyh) Kuntze (family *Combretaceae*) [39]. The compounds were reported to be active against colon, lung and leukemia cancers. One of its analogues - combretastatin A-4 was suspected to be the most cytotoxic phytomolecule ( $IC_{50} = 1$  nM) among all isolated compounds [39, 40].

The mechanism of action of combretastatin A-4 is to bind to beta-tubulin at the colchicine-binding site and to inhibit tubulin polymerization [41]. Its poor solubility in water has necessitated the synthesis of several pro-drugs. Combretastatin A-4 phosphate, fosbretabulin (CA4P, Zybrestat) as a pro-drug has undergone phase II clinical trial for the treatment of relapsed ovarian cancer, NSCL and thyroid cancers [42]. A Phase II clinical trial of a combination of combretastatin A4 phosphate, paclitaxel, and carboplatin resulted in an improved response in the patients with platinum-resistant ovarian cancer than when the chemotherapeutic agents were administered without combretastatin A4 phosphate [43].

## Perillyl Alcohol

Perillyl alcohol (Figure 2) is a plant-derived monoterpene from lavender, peppermint, spearmint, cherries, celery seed and other plants [44]. Its anticancer mechanism of action might involve blocking signal transduction by inhibition of post-translational isoprenylation of small G proteins [45]. In a phase II study of perillyl alcohol, orally administered this compound did not show effect on extending the time-to-progression in patients with advanced ovarian cancer [46]. Recent development of inhalation chemotherapy with perillyl alcohol for

the treatment of recurrent malignant glioma seems to be effective and a safe strategy [47] and has been applied in clinical trials of patients with malignant brain tumors in Brazil [48].

#### **4. PHYTOCHEMICALS IN PRECLINICAL STUDY FOR THE TREATMENT OF OVARIAN CANCER**

##### **Quercetin**

Quercetin (Figure 3) is a naturally occurring flavonoid found in foods like capers, dill, watercress, red onion and the leaves of fennel and radish. The compound has anti-allergic, anti-inflammatory and potentially anti-tumor activities. Quercetin exhibited a dose-dependent growth inhibition in the ovarian cancer cell line OVCA433 and also showed synergic anti-proliferative activity with cisplatin [49]. A recent study on the anti-cancer activities of quercetin in A2780S ovarian cancer cells showed the induction of apoptosis and the activation of caspase 3 and 9.

The anticancer activities of quercetin on a xenograft A2780 ovarian tumor was studied by the administration of an intravenous solution of a nano-formulation of quercetin encapsulated in mono-methoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) particles. The researchers observed a significant retardation in tumor growth [50]. Studies have shown that quercetin has low toxicity and is able to enhance the therapeutic effects of cisplatin and paclitaxel in the treatment of ovarian cancer at low doses. The compound's ability to reverse multi-drug resistance of chemotherapeutic drugs has been established in *in vitro* experiments [51]. In a xenograft mouse model of ovarian cancer, quercetin has also been shown to enhance the antitumor effect of cisplatin recently [52].

##### **Baicalin and Baicalein**

Baicalin and its aglycone, baicalein (Figure 3), are flavones found in several Chinese medicinal plants, such as *Scutellaria baicalensis* Georgi (family *Lamiaceae*) and *Scutellariae radix*. These compounds recently gained scientific focus and showed anti-inflammatory, neuroprotective and anti-cancer activities [53].

The study reported significant cytotoxic activities in OVCAR3 and CP-70 ovarian cancer cell lines, with minimized cytotoxicity in normal ovarian cells. Baicalein with  $IC_{50}$  of 25-40  $\mu$ M had a slightly better inhibitory effect than baicalin with  $IC_{50}$  of 45-55  $\mu$ M in ovarian cancer cells [54]. Recently, baicalein was shown to inhibit the matrix metalloproteinase-2 expression and possibly involve the p38 MAPK-dependent NF-kappaB signalling pathway [55].

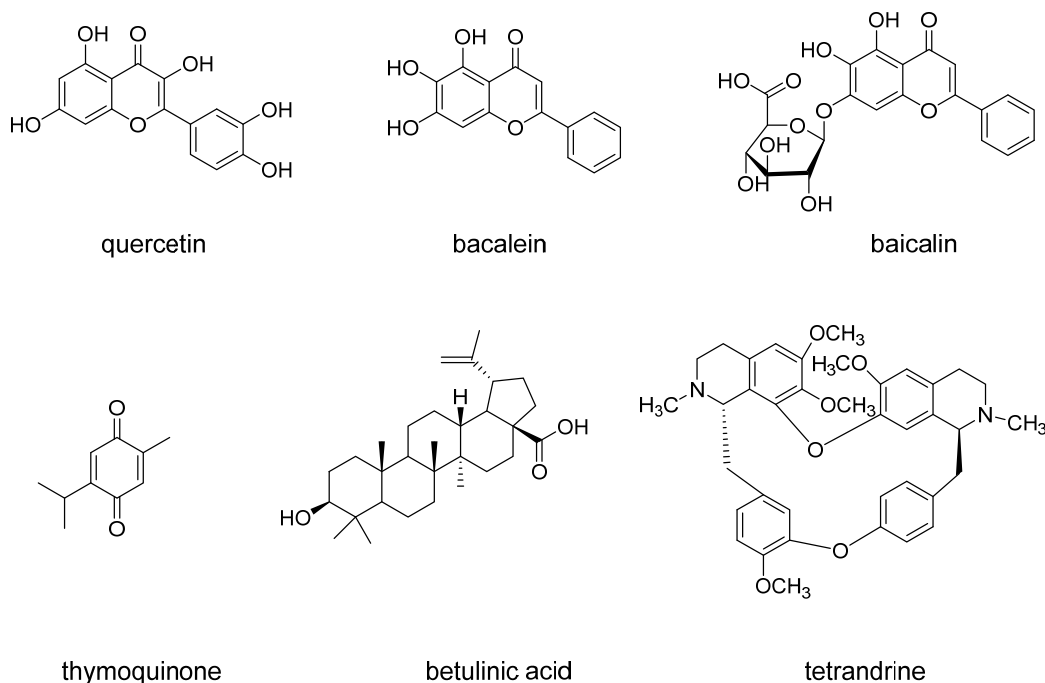


Figure 3. Structures of quercetin, baicalein, baicalin, thymoquinone, betulinic acid and tetrandrine.

## Thymoquinone

Thymoquinone (Figure 3), a promising anticancer agent is one of the compounds isolated from the volatile oil of *Nigella Sativa* L. (family *Ranunculaceae*) [56, 57]. Thymoquinone has demonstrated cytotoxicity against a wide range of cancers such as breast adenocarcinoma, colorectal cancer, leukaemia, lung cancer, pancreatic cancer, prostate cancer, osteosarcoma and ovarian cancer [57-59]. It has been shown to exhibit cytotoxicity to human ovarian adenocarcinoma cell line via induction of apoptosis but more resistance in non-cancerous cells [60]. Studies on the mechanism of action of thymoquinone in breast cancer cells showed that thymoquinone induced apoptosis, and activated caspases 8, 9 and 7 in a dose-dependent manner. It was found that progression of MDA-MB-231 cells was reduced in its presence. Also thymoquinone increased peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ) activity and down-regulated the expression of the genes for Bcl-2, Bcl-xL and survivin in breast cancer cells [61].

Thymoquinone not only killed pancreatic cancer cells but also prevented their occurrence in a study by Kimmel Cancer Centre in Philadelphia [62]. It was shown to affect histone deacetylases (HDAC) more effectively than trichostatin A - a proven anticancer drug as a HDAC inhibitor. However, from a crystallographic study, it has been discovered that thymoquinone displaces phosphopeptides in a non-covalent binding mode to bind to the phosphoserine/ phosphothreonine recognition site in the polo-box domain of polo-like kinase 1, a major mitotic regulator which is responsible for carcinogenesis when overexpressed [63]. An analogue of thymoquinone, poloxin also as an inhibitor of polo-like kinase is at the early

clinical trial [64]. Thymoquinone showed synergic effect when used in combination with cisplatin and oxaliplatin in ovarian cancer cells [65-67]. Some synthetic analogues of thymoquinones have been made in our laboratory and tested their anti-ovarian cancer activity in vitro, as a result few of them such as a nitrogen-substituted thymoquinone showed more potent activity and higher water solubility [68].

### **Betulinic Acid**

Betulinic acid (Figure 3), a lupine-type pentacyclic triterpene is widely distributed in plants such as *Ziziphus mauritiana* Lam. (family *Rhamnaceae*), *Diospros Leucomelas* (family *Ebenaceae*), and *Crossopteryx febrifuga* (family *Rubiaceae*) [69, 70]. Betullinic acid has demonstrated cytotoxicity in neuroectodermal and malignant brain tumor cell lines. It also selectively inhibits the growth of human melanoma cell lines [69]. The cytotoxicity of betulinic acid in ovarian cancer cells has been reported and the compound demonstrated increased cytotoxic activities when administered with 5-fluorouracil in ovarian cancer cells [71].

### **Tetrandrine**

Tetrandrine, a bisbenzylisoquinoline alkaloid (Figure 3) isolated from the dried root of *Stephania tetrandra* S Moore (family *Menispermaceae*), exhibits very broad pharmacological actions, including anti-cancer activity [72]. In one study, tetrandrine could reduce the paclitaxel concentration required to achieve 50% inhibition of cell growth to HCT15 (P-gp-positive) cells about 3100-fold, while they could not affect the accumulation and residual rate of rhodamine 123 in SK-OV-3 (P-gp-negative) cells. Therefore, tetrandrine was concluded to enhance the cytotoxicity of drugs via modulation of P-glycoprotein (P-gp) in the resistant cancer cells [73]. Tetrandrine could suppress the cancer cell growth by causing redistribution of the cell cycle in ovarian cancer. *In vivo* a combination of tetrandrine and cisplatin exhibits the synergic effect, which supports the application of tetrandrine as an adjunct to cisplatin in the chemotherapy of ovarian cancer [74]. Tetrandrine in combination with doxorubicin showed a significant synergistic effect in multidrug resistant Caco-2 and CEM/ADR5000 cancer cells. It could also reverse the multidrug resistance by reducing the expression of P-glycoprotein [75].

### **Novel Phytochemicals Active against Ovarian Cancer**

Searching for novel anti-ovarian cancer compounds from medicinal plants is on-going all over the world. A complete list of novel and potent plant natural products with IC<sub>50</sub>s less than 10  $\mu$ M in different ovarian cancer cell lines discovered from 2001-2014 has been compiled in Table 1.

In particular, Kingston's group at Virginia Polytechnic Institute, US has discovered a number of potent compounds including very promising and potent compounds such as bufatrienolides and ipomoeassin D from plants collected from Madagascar Rainforest (Table 1). Lee's group at University of North Carolina, US, also discovered many anti-ovarian



cancer compounds such as 2'-(R)-O-acetylglauucarubinone and molvizarin (Table 1). Bufatrienolides, ipomoeassin D, 2'-(R)-O-acetylglauucarubinone, and molvizarin showed very low IC<sub>50</sub>s (less than 100 nM), which might possess different mechanisms of action from those of platinum/paclitaxel due to their unique and diverse chemical structures, and therefore providing a potential new therapy to attack multidrug resistance in ovarian cancer.

In our laboratory we are also focusing on the discovery of natural products from medicinal plants. Cycleanine, a bisbenzylisoquinoline alkaloid from *Trichlisia subcordata* Oliv. (family *Menispermaceae*), was found to be an anti-ovarian cancer agent with IC<sub>50</sub> of 7.6 μM in A2780 cells [76]. Securinine and gallic acid have been identified from *M. discoidea*, which showed IC<sub>50</sub> ranging from 5 to 16 μM in OVCAR8, A2780 and cisplatin resistant A2780cis ovarian cancer cell lines (Table 1) [77]. Semi-synthetic analogs of these compounds are being made to understand their structure-activity relationship and obtain more potent compounds for testing their toxicity in animals. Cyclotides as exceptionally stable disulphide-rich plant circular proteins have been reported to exert cytotoxic or lethal effects on ovarian cancer cell lines [78] which are probably due to its ability to target and bind to the cell membrane [79]. Varv A from *Viola yedeensis* Makino (family *Violaceae*) showed IC<sub>50</sub>s ranging from 4 to 10 μM in A2780, Igrov1 and OVCAR8 cells [80].

## 5. PROSPECTS AND OUTLOOK

Plant-derived natural products continue to provide anti-cancer medicines for human health. In particular, paclitaxel and camptothecin have already been developed and widely used for ovarian cancer patients. During the last fifteen years a lot of phytochemicals with high potency have been discovered through *in vitro* bioassay-guided isolation and identification. Such approach should be continued for many untapped resources of medicinal plants. However, most of the ovarian cancer cell lines used in the previous bioassays were A2780 and/or SKOV3 cells (Table 1) which were categorized under being unlikely high-grade serious, therefore more serious and drug-resistant cell lines such as OVSAHO and SNU119 cell lines [110] should be used to evaluate the potency of plant extracts and pure compounds. Recently, plant endophytes that reside in the plant tissues have been found to produce phytochemicals including paclitaxel [111], camptothecin and vinblastine found in their host original plants, which provides an alternative production method for the minor components and a source of novel chemicals [11]. This is a promising area that needs more attention and intensive research. Furthermore, many natural products when isolated in pure form like camptothecin, paclitaxel and podophyllotoxin often have poor water solubility or high toxicity. Therefore the more water soluble and potent analogues must be found to overcome their shortcomings through semisynthesis and chemical modification of parent molecules. Following this approach, there have been plenty of successful examples, e.g., the making of topotecan and irinotecan based on podophyllotoxin.

In order to specifically target cancer cells without harming normal cells, drug (phytochemicals)-biologicals (antibody, biological agents, etc.) conjugates are promising candidates and are highly demanded for preclinical and clinical studies [112]. Therefore novel conjugates such as folate-paclitaxel and folate-camptothecin conjugates might be designed and prepared as vintafolide for targeting cancer cells. Metabolism of phytochemicals *in vivo* can also provide possible novel metabolites as potential drugs, which needs to be

investigated. For example, SN-38 is an active metabolite of irinotecan via hydrolysis by carboxyesterase and metabolized through glucuronidation by UGT1A1, which is more bioactive and in current clinical trial [113]. Another approach would be to investigate the combination of multiple drugs including the phytochemicals and approved drugs such as paclitaxel and platinum derivatives by targeting multiple targets and different pathways in order to inhibit the growth of cancer cells and to avoid drug resistance more effectively.

In summary, a multi-disciplinary and collaborative approach must be utilized by natural product chemists, synthetic chemists, chemical biologists, pharmacologist and clinicians in order to generate novel, safe and effective anti-ovarian cancer drugs based on the lead compounds discovered from plants. New drugs for the treatment of ovarian cancer can be expected and will emerge in future.

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