

Podophyllotoxin, a medicinal agent of plant origin: past, present and future

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[ABSTRACT] The aryltetralin-type lignan podophyllotoxin, the main agent present in the resin extracted from the rhizomes of different podophyllum species, possesses important antineoplastic and antiviral properties and has been widely used in diverse cultures since ancient times for medicinal purposes as a cathartic and antihelminthic agent. Today, podophyllotoxin serves as the starting material for the preparation of the well-known cytostatic agents: etoposide and teniposide which are used in combination therapies with other drugs for the treatment of a variety of malignancies. The present review focuses on the lead compound, that is, podophyllotoxin and summarizes its structural characteristics, natural sources, biological activities, novel derivatives, medicinal applications and future perspectives.

[KEY WORDS] Podophyllotoxin; Lignans; Podophyllum; Antineoplastic; Tubulin; Etoposide; Teniposide

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1 Introduction

Lignans are an extremely vast class of natural products which are formed from two phenylpropane units. They are present in a wide range of plants. The attention of scientists for this family of natural products is due to the potent antiviral and antineoplastic properties of a large number of these compounds. This class has received much attention since the discovery of podophyllotoxin^[1].

Podophyllotoxin (C₂₂H₂₂O₈) occupies a very important place among the Lignans group because of its potent anti-

cancer properties^[2] and has been the subject of many investigations. Plants containing podophyllotoxin have been used for centuries as folk remedies by diverse cultures. This naturally occurring aryltetralin lignan is synthesized by different Podophyllum species and is nowadays used as the lead compound for the preparation of the well-known anticancer agents: etoposide, etopophos and teniposide which show good clinical effects against several types of neoplasms^[3-4] with fewer side-effects^[5]. In fact, many studies have reported that structural modifications on the skeleton of podophyllotoxin have led to the development of molecules with potent biological and pharmaceutical properties. Therefore, it is necessary to summarize the development and the progress that have been made in this field. The aim of the present review is to present the past and present status of podophyllotoxin, its distribution, applications, biosynthesis, natural and alternative sources, mechanism of action, novel derivatives and future perspectives.

2 Study of Podophyllotoxin: from Past to Present

2.1 Structural characteristics

The chemical structure of podophyllotoxin has been elucidated 80 years ago^[6]. This non-alkaloid toxin lignan ex-

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tracted from the roots and rhizomes of *Podophyllum* species has four chiral centers in a row – from C-1 to C-4. podophyllotoxin contains a five-ring system (A, B, C, D and E rings). The essential structural characteristics of most podophyllotoxin species are: (i) almost planar tetracyclic group ABCD going from the dioxole ring to the lactone ring; (ii) four ends with oxygen atoms at the functional groups dioxoles, methoxys, lactone, and secondary alcohol; (iii) an aromatic ring E located at position 1 with α -configuration and a bond with a certain degree of free rotation; (iv) the four adjacent asymmetrical centers; and (v) special stereo chemical properties at C4 which determine whether a given class of compounds has an affinity for tubulin or not [17-8] (see Fig. 1).

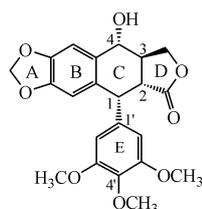


Fig. 1 Structure of podophyllotoxin

Derivatives of podophyllotoxin are synthesized as properties of the rings and carbons C-1 through C-4 are diversified. Earlier, it was reported that all the rings were essential for the podophyllotoxin activity, but now the statement has been modified. In fact, different studies have shown that only the A and E rings are essential for its activity [9].

Aromatization of ring C leads to loss of activity [10-11] while modifications at the C-4 position in ring C are mostly acceptable and bulky groups at this position enhance both anticancer and topoisomerase activities [12]. It has been found that modification of the A-ring give compounds with significant effect but less than that of etoposide, whereas modification of the B-ring resulted in the loss of activity. It has also been observed that free rotation of E-ring is necessary for the antitumor effect [11, 13].

2.2 Natural and alternative sources of podophyllotoxin

Traditionally, podophyllotoxin is isolated from podophyllin which is the resin presents in plants of the genus *Podophyllum* (Berberidaceae). This genus has two species that are the most commercially exploited sources of podophyllotoxin; *P. peltatum* L. and *P. emodi* L. [14-15]. *P. emodi* Wall (syn. *P. hexandrum* Royle) is a good source of podophyllotoxin because it gives more resin than *P. peltatum* [16]. In fact, podophyllotoxin is present at concentrations of 0.3 to 1.0% by mass in the rhizome of *P. peltatum* [5, 17] while the content in podophyllotoxin is about 4.3% of dry weight in *P. emodi* [18-19]. More recently, podophyllotoxin was isolated from species belonging to the sections *Linum* [20], *Dasylinum* and *Linopsis* which represent another alternative source of podophyllotoxin [21]. *Podophyllum* species, which grow very slowly, are becoming increasingly scarce due to intensive collection, lack of cultivation and to their own biological

characteristics. This limits the supply of podophyllotoxin and thus necessitates the search for alternative production methods.

Due to the fact that it is the starting compound for the synthesis of a large number of cytotoxic agents, the worldwide demand for podophyllotoxin is becoming more and more important. This explains the urgency to find alternative sources since the supply from *Podophyllum* species is becoming limited. Deoxypodophyllotoxin (DPT), the main lignan isolated from rhizomes of *Anthriscus sylvestris* (L.) Hoffm (Apiaceae) [22-23], is structurally closely related to podophyllotoxin. The dried roots of *A. sylvestris* have traditionally been used in Asian countries, including China, Korea and Japan, as an antipyretic, an analgesic and a cough remedy [24]. DPT, in turn, possesses potent antiproliferative, antitumor, antiviral, anti-inflammatory, antiplatelet aggregation, and antiallergic properties [25-27]. This aryltetralin-lignan is much more abundant in the plant kingdom than podophyllotoxin and can be used as a biosynthetic precursor for the production of podophyllotoxin [18, 28]. Feeding experiments with cultures of undifferentiated plant cells (*Linum album*) or fungi (*Penicillium F-0543* and *Aspergillus niger*) have shown that DPT can be converted into podophyllotoxin [28-29]. A better insight into the occurrence of deoxypodophyllotoxin combined with detailed knowledge of its biosynthetic pathway(s) may help to develop alternative sources for podophyllotoxin.

Besides plant sources, recent studies have reported that endophytic fungi, both strains of *Phialocephala fortinii*, isolated from the rhizomes of *P. peltatum* as well as *Fusarium oxysporum* isolated from *Juniperus recurva* produce considerable amounts of podophyllotoxin [30-31]. Although optimization studies to increase the production by the cultured fungal endophytes are in progress, these organisms can be promising candidates for large scale production of podophyllotoxin.

2.3 Biosynthesis of podophyllotoxin

Traditionally, podophyllotoxin serves as precursor for the production of important anticancer drugs like etoposide and teniposide which have been widely applied in therapies for cancers and venereal wart [32]. Due to the scarcity of the natural supply, researchers sought to explore new sources of podophyllotoxin, like cultivation, plant cell or organ culture, and chemical synthesis. Therefore, a search for an alternative approach is imperative. Biosynthesis seems to be one attractive option which may help to develop alternative sources and provide sustainable production of podophyllotoxin in an economical way. Indeed, understanding the biosynthesis of podophyllotoxin is one of the basic necessary steps for standard cultivation of medicinal plants and metabolite engineering.

Although the biosynthetic pathway to podophyllotoxin and analogs has been elucidated, it is still a matter of debate and general conclusions cannot be drawn as yet. It is only

possible to speculate about major parts of the podophyllotoxin biosynthesis.

Lignans are defined as dimerization products of two phenylpropane units linked by β -carbon atoms of their side chains^[33]. These compounds originate from phenylpropanoid biosynthesis route via shikimic acid pathway. Several studies have investigated the biosynthetic route of podophyllotoxin and have suggested a common pathway starting from coniferyl alcohol being converted to (+)-pinoresinol in the presence of a one-electron oxidant^[34] through dimerization

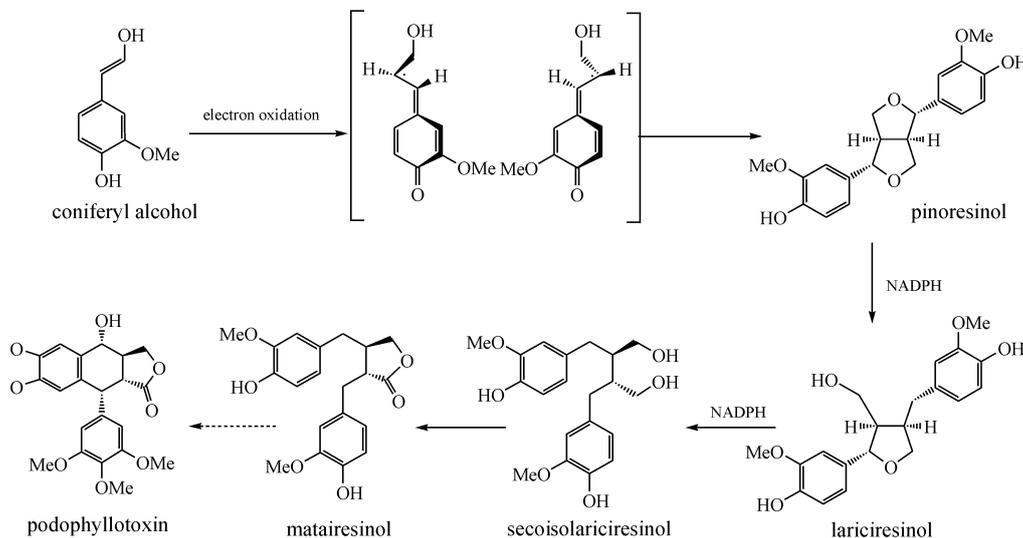


Fig. 2 Biosynthetic pathway of podophyllotoxin

2.4 Mechanism of action

Antineoplastic and antiviral properties are the most pronounced and eminent pharmacological effects of podophyllotoxin. Primary molecular mechanisms responsible for the antineoplastic activities of podophyllotoxin include preventing the assembly of tubulin into microtubules and inducing apoptosis. Different studies have examined the interaction of podophyllotoxin with tubulin and its effect on microtubule assembly *in vitro*. The conclusion was that podophyllotoxin inhibits the formation of the mitotic-spindles microtubules by preventing the polymerization of tubulin which induces cell cycle arrest at mitosis. This mode of action is comparable to that of the alkaloid colchicine.

Microtubules — key components of the cytoskeleton — are directly involved in many cell functions, such as mitosis. They are highly dynamic structures with tubulin monomers constantly added at one end, and dissociated from the other. Podophyllotoxin reversibly binds to tubulin, disturbs the dynamic equilibrium between the assembly and disassembly of microtubules, and eventually causes mitotic arrest^[5]. However, semi-synthetic epipodophyllotoxin derivatives of podophyllotoxin, such as etoposide, teniposide and etopophos, have potent inhibitory activity of DNA topoisomerase II which prevent the re-ligation of DNA^[34-35] and are not inhibitors of microtubule due to the presence of the bulky glucoside moiety^[35].

of stereospecific radical intermediate (Fig. 2). (+)-pinoresinol is then selectively reduced in the presence of co-factor NADPH and generates the first (+)-lariciresinol followed by (-)-secoisolariciresinol. Selective dehydrogenation of (-)-secoisolariciresinol gives rise to (-)-matairesinol which is considered to be the precursor of podophyllotoxin (Fig. 2). (-)-matairesinol is assumed to be converted to yatein via appropriate quinomethane intermediates, leading to podophyllotoxin^[34].

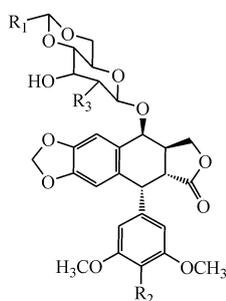
DNA topoisomerases are ubiquitous enzymes responsible for controlling the topological state of DNA in cells^[36]. They are classified into two main classes: type I enzymes, which cleave a single strand of DNA during the course of the reaction, and type II enzymes, which cleave both strands. The mechanism of action of podophyllotoxin analogs is based on the formation of a nucleic acid–drug–enzyme complex, which induces single- and double-strand DNA breaks as the initial step in a series of biochemical transformations that eventually lead to cell death^[3, 37-38].

Podophyllotoxin is also widely used as an antiviral agent for the treatment of *Condyloma acuminatum* caused by human papilloma virus (HPV), and is regarded as the most effective cure for venereal, perianal, and other general warts^[34]. This antiviral effect is associated with disparate mechanisms including disruption of the cellular cytoskeleton and inhibition of integrase which interfere with viral replication. It was also reported that podophyllotoxin binds to a hinge domain of E2 in human papillomavirus (HPV) and inhibited the E2/E7 interaction *in vitro*^[39]. In addition to this mode of action, synthetic podophyllotoxin analogs show inhibition of reverse transcriptase, which may be exploited to selectively combat RNA viruses such as the human immunodeficiency virus (HIV)^[5, 40].

2.5 Analogs of podophyllotoxin: etoposide, etopophos and teniposide

Because of the severe toxicity associated with the use of

podophyllin especially its gastrointestinal side effects [2, 41], scientists began an investigation with the aim to find clinically applicable drugs which retain the antineoplastic properties with less toxicity. It has been shown that modifications at the C-4 position of ring-C and in the ring-E of podophyllotoxin led to the generation of analogs with more potent activity and less toxicity. Two main derivatives were thus synthesized: etoposide (VP-16) in 1966 and teniposide (VM-26) in 1967, which are currently used in frontline cancer chemotherapy against various cancer types [42] (Fig. 3).



etoposide (VP 16-213)	$R_1 = -CH_3$; $R_2 = -OH$; $R_3 = -OH$
teniposide (VM 26)	$R_1 = -S$; $R_2 = -OH$; $R_3 = -OH$
etopophos	$R_1 = -CH_3$; $R_2 = -P(=O)(OH)_2$; $R_3 = -OH$

Fig. 3 Structures of etoposide, teniposide and etopophos

From podophyllotoxin to etoposide/teniposide, the chemical modifications which have been made led to a change in the mechanism of action, from the inhibitor of microtubule formation by the parent compound podophyllotoxin to DNA topoisomerase II inhibitor by etoposide and congeners. In fact, unlike podophyllotoxin, etoposide prevents the entry of cells into mitosis rather than trapping cells in metaphase. Different *in vitro* studies showed that it acts in late S or G2 phase of the cell cycle [43], which is the checkpoint for DNA damage or DNA replication, rather than spindle assembly. Indeed, etoposide shows little effect on tubulin polymerization. However, fragmentation of DNA in HeLa cells was observed upon treatment with etoposide [35, 44]. Until the 1980's, it had not been recognized that the ability of etoposide to induce DNA breaks was mediated by DNA topoisomerase II [1, 45].

The main function of DNA topoisomerases is to solve the topological problems arising in cells during DNA replication, transcription, recombination, chromatin assembly and chromosome segregation [46]. *In vivo*, DNA topoisomerase II catalyzes the double-stranded DNA cleavage, allowing passage of a second DNA duplex through the break. Etoposide stabilizes the covalent DNA-enzyme cleavable complex, inhibits the catalytic activity of topoisomerase II, and induces topoisomerase II-mediate DNA breakage. These actions convert the essential enzyme into a cellular poison, trigger cascade reactions, and eventually lead to cell death [3, 34, 38]. This to-

poisomerase II inhibition mechanism is shared by etoposide, teniposide, and other derivatives.

Etoposide, which received FDA approval in 1983, is the most commonly used analog of podophyllotoxin due to its potent antineoplastic properties [47]. It is used in combination therapy for the treatment of lymphomas, acute leukemia, testicular cancers, small cell lung cancer, ovarian, bladder, brain cancers, etc [3, 48]. However, Etoposide was associated with some problems especially those related to the development of drug resistance, cytotoxicity toward normal cells and poor bioavailability [49-51].

In order to overcome these limitations especially the poor water solubility, a phosphate analog, etopophos, was launched by Bristol-Myers Squib Co. in 1996 [48]. This prodrug can be administered in higher doses than etoposide as a short intravenous injection, whereafter it is rapidly converted to the parent compound by plasma phosphatases, and thus constitutes an improved formulation of etoposide.

Teniposide is another derivative [52], which is less frequently used for chemotherapy in comparison with etoposide [53] but has shown more potent *in vitro* inhibitory action of topoisomerase II than the etoposide one. It is mainly used in the treatment of refractory childhood acute lymphocytic leukemia [54] and in glioma therapy.

In addition to these three semi-synthetic analogs, numerous new podophyllotoxin derivatives are currently under development and evaluation as topoisomerase inhibitors and potential anticancer drugs.

2.6 Novel derivatives of podophyllotoxin

Despite their extensive use alone or in combination with other chemotherapies to treat cancer, Etoposide and Teniposide use is not free of toxicity. Besides to some limitations such as metabolic inactivation, low aqueous solubility and development of resistance, these drugs have been reported to induce stomatitis, nausea, vomiting, irritation of skin and mucous membranes, diarrhea, hair loss, teratogenic effects and myelosuppression which increases the risk of infections and leads to anemia due to a lack of erythrocytes. To overcome these severe side-effects, the development of more potent agents with less toxicity remains a highly valuable objective for researchers. Diverse structural modifications on the skeleton of podophyllotoxin have been performed to achieve this purpose. This has led to the development of new agents such as Tafluposide, TOP53, NK611, GL-331 and Azatoxin.

NK611 carries a dimethylamino group at the D-glucose moiety which improves the bioavailability properties [55]. In addition to its water solubility, the antineoplastic activity of this compound proved to be comparable or superior to etoposide in various *in vitro* and *in vivo* investigations [56-57].

GL-331 contains a *p*-nitroanilino group at the 4 β -position instead of a glycoside of etoposide [58]. It has shown a topoisomerase II inhibition activity [59-61] and caused cell cycle stops at G2/M phase [55]. It was reported that GL-331 could induce cell death by stimulating protein tyrosine phos-

phatase activity and apoptotic DNA formation. Due to its good biocompatibility and pharmacokinetic properties, GL-331 has completed phase I clinical trials [55] and has undergone phase II clinical trials for the treatment of various cancers [62].

With increasing information about the structure-activity relationships of podophyllotoxin, wide investigations have generated a range of new chemical compounds such as azatoxin, a hybrid drug that fuses chemical structures from etoposide and ellipticine [63]. This novel cytotoxic agent has shown a potent antitumor effect through the inhibition of topoisomerase II activity and/or tubulin polymerization [64].

TOP-53, a 4 β aminoalkyl derivative of 4'-*O*-demethyl-4-desoxypodophyllotoxin, has been synthesized at Taiho Pharmaceutical Co., Ltd. [65] through the substitution of the glucopyranoside group by an aminoalkyl moiety that is directly connected to 4 β position through carbon—carbon bond. The elaboration of this analog was aiming to improve the cell

penetration of compounds as well as the interaction with intracellular molecules [66]. TOP-53 has shown a higher antitumor activity than the etoposide one and is still in phase II clinical trials [55].

More recently, Tafluposide (F 11782), a lipophilic fluorinated etoposide phosphate derivative, has been synthesized [67]. The highly lipophilic properties of this molecule have improved the cellular penetration as well as the bio-distribution with a potent cytotoxic action via the inhibition of both topoisomerase I and II activities. F14512, another interesting candidate, is a topoisomerase II poison that combines an epipodophyllotoxin core with a spermine moiety introduced as a cell delivery vector [68]. It exploits the polyamines transport system (PTS) to target preferentially tumor cells [69]. The structures of all these new derivatives are shown in Fig. 4.

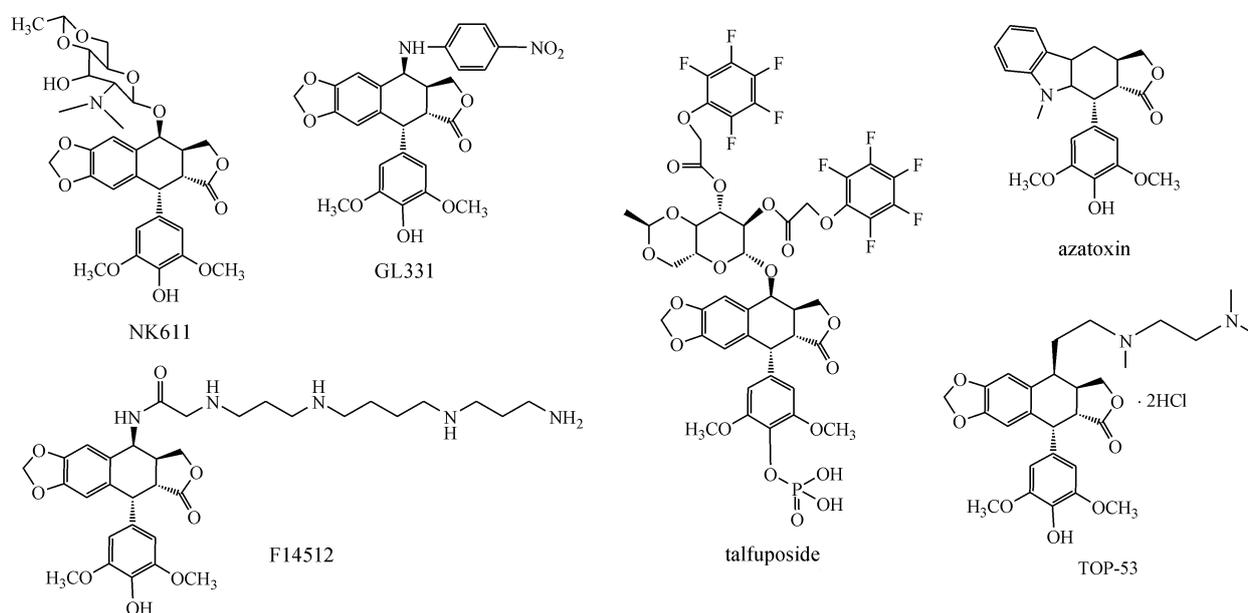


Fig. 4 Structures of novel podophyllotoxin cytotoxic derivatives

The impressive antineoplastic potency and clinical efficacy of podophyllotoxin derivatives are due to the extensive structure-activity investigations. In fact, it was essential for researchers to understand how systemic variations on the skeleton of podophyllotoxin may enhance or suppress the effect of the resulting molecule in a biological context. Initial structure-activity relationship studies were mainly based on the antimetabolic properties of podophyllotoxin analogs. However, after the elucidation of the mechanism of action of etoposide and teniposide, structural modifications have been aimed to enhance topoisomerase II inhibition property, which is the principal mechanism of action of therapeutically useful analogs.

The design of novel derivatives is based on few working assumptions which have revealed important structural features essential for their biological activity. For example, the structural preferences of topoisomerase II inhibitors over antimetabolic agents have been roughly identified as: (i) 4'-demethylation, (ii) 4 β -configuration at C-4 position, and (iii) 4 β -bulky substitution [52, 70]. Additionally, free hydroxy at C-4' position, dioxolane A ring, free rotation of ring E and trans-lactone D ring with 2 α , 3 β configuration are all required to obtain new molecules with potent activity [55]. The main features that are critical for the cytotoxic activity of podophyllotoxin analogs are schematized in Fig. 5.

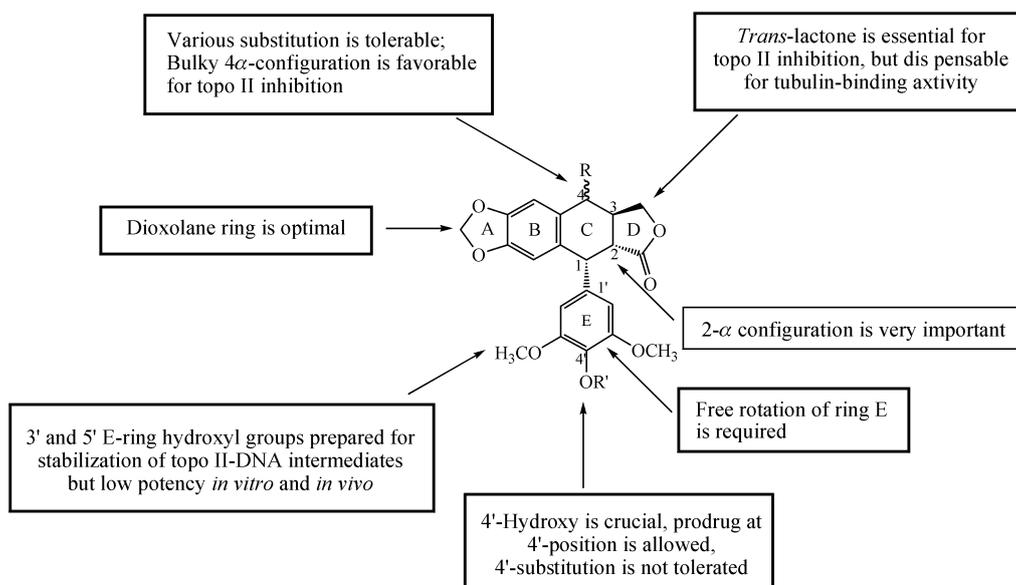


Fig. 5 Key features for podophyllotoxin derivatives' antitumor activity

2.7 Medicinal applications

Since remote times, podophyllin, a resin obtained by ethanolic extraction of the *Podophyllum* roots and rhizomes, have been used as an anthelmintic, a purgative and an antidote against poisons and toxic [3]. In 1820, podophyllin was included in the first U.S. Pharmacopoeia for its cathartic and cholagogue properties. A century later, the drug was removed from the 12th edition of this Pharmacopoeia because of its severe toxicity [3, 44]. However, in 1942, it was reported that venereal warts could be selectively destroyed by the topical application of podophyllin. A crude extract of *Podophyllum peltatum* was observed to reduce the cytopathic effect of herpes simplex type II, influenza A and vaccinia viruses. Subsequently podophyllotoxin, β -peltatin, deoxypodophyllotoxin, picropodophyllotoxin and α -peltatin were tested and found to be active against measles and herpes simplex type I [71]. Different studies have reported the use of *Podophyllum hexandrum* in several traditional systems of medicine, including Ayurveda, for treatment of a number of ailments such as constipation, cold, bacterial infections, biliary fever, septic wounds, burning sensation, erysipelas, insect bite, mental disorders, rheumatism and plague [72]. It has also been used to provide symptomatic relief in some of the allergic and inflammatory conditions [73-74]. Nowadays, podophyllin is still in use [75], and pure podophyllotoxin is also applied [76].

The use of pure podophyllotoxin, in creams and gels, is nowadays recommended instead of podophyllin due to its higher efficacy and lower side-effects [77-78]. Podophyllotoxin has also been used as an antiviral agent in the treatment of *Condyloma acuminatum* caused by human papilloma virus (HPV) [79] and other venereal and perianal warts [3, 80]. Therefore, it seems to be an important weapon in the fight against sexually transmitted diseases. It is also a useful agent for the

treatment of *Psoriasis vulgaris* [81]. This substance serves other purposes as a purgative, vesicant and anti-rheumatic agent.

Antitumor activity is another outstanding property of podophyllotoxin. In fact, podophyllotoxin and related cyclo lignans are widely used in the clinical treatment of malignant neoplasms such as nephroblastoma, lymphomas, genital tumors [3], and lung carcinoma [82]. Furthermore, podophyllotoxin-related analogs have been found to possess immunosuppressive activity and are seen as candidates for use in organ transplantation [83-84]. Combination therapies are currently being implemented with other chemotherapeutic agents or with other techniques useful in the fight against viral infections and cancer. Podophyllotoxin holds the promise to be a good candidates for the design and synthesis of more potent, less toxic and more selective compounds [4].

3 Future Prospects

Recently, the interest of international pharmaceutical industries has been directed more and more to plant-based anticancer compounds. Podophyllotoxin is still used as adduct for the synthesis of potent antineoplastic agents which are of importance for therapy, and there is a high demand for the commercial drug and its precursors for the treatment of cancer now and in the future. However, toxicity and drug resistance associated with the clinical use of some of these compounds have pushed researchers to produce better therapeutic agents with improved pharmacological and pharmacokinetic profiles thanks to the major chemical modification efforts which were the key elements to achieve this purpose. Podophyllotoxin definitely is and continues to be a promising molecule for future investigations. The coming years are likely to witness clinical trials on formulations developed from this lignan, particularly for the design of new drugs with

several other medical applications.

4 Conclusion

Since ancient times, various plants have been used to cure different types of diseases. Podophyllotoxin is a potent plant-derived antiviral and antitumor agent. Critical modifications on the skeleton of this natural compound led to the development of a number of therapeutically useful analogs that have shown potent cytotoxic activity with fewer side-effects. Many years after its first clinical use, this aryltetralin lignan continues to be the subject of extensive research with the challenge to design novel derivatives with superior pharmacological profiles and broader therapeutic scope, and several strategies have been adopted to increase its production since it is the starting compound for the production of potent antineoplastic drugs. Although cytotoxic activity will undoubtedly continue to be the most clinically relevant property of podophyllotoxin derivatives; their antiviral, anti-inflammatory, and immunosuppressive properties are nowadays attracting more and more attention and the future perspective seems to be the discovery of new analogs with therapeutic utility other than cancer chemotherapy based on extended biological and structural explorations.

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天然来源的鬼臼毒素研究进展

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【摘要】 芳基萘满木脂素鬼臼毒素, 是从不同鬼臼属植物根茎中提取的树脂中的主要成分, 具有显著的抗肿瘤和抗病毒活性, 自古以来被多个国家广泛用于相关疾病的治疗。目前, 鬼臼毒素主要作为细胞毒类药物合成的母体结构而受到广泛关注。例如依托泊苷和替尼泊苷, 这些药物常与其他药物合并使用治疗一系列肿瘤疾病。本文对鬼臼毒素予以综述, 对其结构特征、药用来源、生物活性、衍生物和应用方面进行了总结。

【关键词】 鬼臼毒素; 抗肿瘤活性; 微管蛋白; 依托泊苷; 替尼泊苷

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