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Synthetic and Application Perspectives of Azapodophyllotoxins: Alternative Scaffolds of Podophyllotoxin

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Abstract

Podophyllotoxin (**1**) has been known to possess anti-tumor activity and is still considered an important lead for research and development of antineoplastic agents. Derivatives of podophyllotoxin, namely etoposide (**2**), etopophos (**3**) and teniposide (**4**) have been developed and are currently used in clinic for the treatment of a variety of malignancies. These agents are also used in combination therapies with other drugs. Due to the drug resistance developed by cancer cells as well as side effects associated with the use of these agents in clinic, the search for new effective anticancer analogues of podophyllotoxin remains an intense area of research. The structural complexity of podophyllotoxin, arising from the presence of four stereogenic carbons in ring *C* has restricted most of the structural activity relationship (SAR) studied by derivatization of the parent natural product rather than by *de novo* multi-step chemical synthesis. These issues provide strong impetus to a search for analogues of **1** with simplified structures, which can be accessible *via* short synthetic sequences from simple starting materials. Even if such initial compounds might have diminished cytotoxic potencies compared with the parent cyclolignan, the ease of preparation of carefully designed libraries of analogues would lead to more informative SAR studies and expeditious structure optimization. In this regard, during the last two decades considerable efforts have been made to synthesize aza- analogs of podophyllotoxin, i. e. aza-podophyllotoxins, with hetero atoms at different positions of the podophyllotoxin skeleton, while keeping the basic podophyllotoxin structure. Recently, there have been significant efforts towards the convenient synthesis of aza-analogs of **1**. The use of multicomponent reactions (MCRs) and the synergies of ultrasound and microwave irradiations have increased the synthetic speed and variety of azapodophyllotoxins which are and will be available to be tested against a diverse population of carcinomas and other diseases. It has been reported that several aza-podophyllotoxins retain a great fraction of the cytotoxicity associated with the parent lignan. This review focuses on the strategies towards synthesis of various aza-podophyllotoxin analogues and their biological activities.

Keywords

Antineoplastic; azapodophyllotoxin; podophyllotoxin

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1. INTRODUCTION

Podophyllotoxin (**1**, Fig. 1), is an antimitotic cyclolignan isolated [1, 2] from plants of the genus *Podophyllum pelatum* L. and *P. emodi* L, studied extensively as an antitumor agent, but clinical results were disappointing due to severe gastrointestinal side effects [3]. However, continuous efforts towards the synthesis of its analogues led to the discovery of new anticancer drugs. For example its semisynthetic derivatives, etoposide (**2**) and etoposide phosphate (**3**) are currently used in the clinic for the treatment of a variety of malignancies including, lung and testicular cancers, lymphoma, nonlymphocytic leukemia, and glioblastoma multiforme [4]. Teniposide (**4**), also prepared by semisynthesis from **1**, is important for the treatment of childhood acute lymphocytic leukemia [4]. In contrast to the parent podophyllotoxin, which binds to tubulin and inhibits microtubule assembly, etoposide is not antimitotic. In fact, molecules such as etoposide, amsacrine, and mitoxantrone are topoisomerase II inhibitors that induce cell death by enhancing topoisomerase II-mediated DNA cleavage through stabilization of the transient DNA/topoisomerase II cleavage complex [5]. Recently, it has been suggested that etoposide- topoisomerase II interactions mediate cleavage complex stabilization, rather than etoposide-DNA interactions, as is the case with amsacrine, another potent topoisomerase II inhibitor [6]. Despite its extensive use, etoposide is not devoid of toxic side effects. Bone marrow depression is a frequent, dose-limiting, and toxic side effect encountered by patients receiving etoposide. The use of effective doses of the drug is also associated with an increased risk of secondary acute myelogenous leukemia [7, 8]. For this reason, the development of more potent analogues with less side toxicity remains a highly valuable objective. Extensive structural activity relationship (SAR) studies with etoposide analogues have suggested that the activity of the epipodophyllotoxin derivatives is related to three structurally distinct pharmacophoric domains: the DNA-intercalating moiety (central part of the molecule, see structure of **1**), the binding site (lower part), and a variable substituent region (glycoside moiety) [9–11].

Most of the SAR studies involving **1** have been restricted to the derivatization of the parent natural product rather than through novel chemical synthesis. This is due to its structural complexity, which arises due to the presence of four stereogenic carbons in ring C [12, 13]. Such approaches, however, are rather limited by the type of chemistry that **1** can undergo. For example, variations of the ring E are not easy to perform because of the three methoxy groups in the starting lignan. No matter how laborious, total synthesis efforts are indispensable in this regard. These issues provide strong impetus to a search for analogues of **1** with simplified structures, which can be prepared *via* short synthetic sequences from simple starting materials. Even if such initial compounds might have diminished cytotoxic potencies compared with the parent cyclolignan, the ease of preparation of carefully designed libraries of analogues would lead to more informative SAR studies and expeditious structure optimization. In this regard, during the last two decades considerable efforts have been made to synthesize aza- analogues of podophyllotoxin, i.e. azapodophyllotoxins, with heteroatoms at different positions of the podophyllotoxin skeleton, while keeping the basic podophyllotoxin structure [14]. It is worth mentioning that replacing carbon with nitrogen at specific positions, e.g. at the stereocenters at C-2 and C-3, could solve the problem of epimerization at C-2 that has plagued the clinical development of **1** and its stereochemically complex derivatives due to the formation of the significantly less potent *cis*-lactone metabolite [15]. Recently, significant efforts have been made towards the convenient synthesis of aza-analogues of **1**. The use multicomponent reactions (MCRs) with ultrasound and microwave irradiation has greatly increased the speed at which azapodophyllotoxins can be synthesized, making a greater variety available to test against a diverse population of carcinomas and other diseases. It has been reported that several azapodophyllotoxins retain a great fraction of the cytotoxicity associated with the parent lignan. This review focuses on the strategies towards the synthesis of various azapodophyllotoxin analogues and their

biological activities. What follows is a description of the various approaches taken towards the synthesis of aza-analogues of podophyllotoxin, i.e. azapodophyllotoxins, and their biological activities.

2. SYNTHESIS OF AZAPODOPHYLLOTOXIN DERIVATIVES

2.1. Classical Synthesis

The first synthesis of 2-azapodophyllotoxin was reported by Pearce *et al.* in 1989 [16]. This report details a stereo-controlled synthesis of 2-azapodophyllotoxin (**7**), a stable analogue of podophyllotoxin (**1**). Inversion of C4 stereochemistry in **5** was accomplished by first oxidizing **5** with pyridinium chlorochromate in dichloromethane at 23 °C to give 4-keto-2-azapodophyllotoxin (**6**), which on reaction with lithium tri-*t*-butyloxyaluminum hydride in THF at 23 °C afforded 2-azapodophyllotoxin (**7**) in 65% overall yield [16].

Tomioka and Koga *et al.* proposed several guidelines for the design of azapodophyllotoxin derivatives: the stereochemical structure should be similar to podophyllotoxin; the carbonyl oxygen should have enough electron density to form a hydrogen-bond; and the compounds should have minimal stereoisomers, shorter synthetic routes, and be available in an optically pure form. Based on these guidelines, synthesis of racemic and optically pure azadeoxypodophyllotoxin derivatives was reported. A cyclic urethane **9**, obtained from a known racemic amino acid **8**, on condensation with 3,4,5-trimethoxybenzaldehyde in the presence of H₂SO₄/CH₂Cl₂ provided a separable mixture of diastereomers **10** and **11**. The *trans*-isomer **10** was formed predominantly, while *cis*-isomer **11** was the minor product. A 4'-demethoxy derivative **12** was obtained from **10** or **11** by treating with HBr in 1,2-dichloroethane. (Scheme 1) [17, 18]. Optically pure cyclic urethanes (*R*)-(–)-**9** and (*S*)-(+)-**9** were also prepared starting from the corresponding optically active L- and D-amino acid **8**, respectively, which afforded optically pure (–)-**10**, (–)-**11** and (+)-**10**, (+)-**11**, respectively [18]. The same group proposed a direct introduction of oxygen functionality at the benzylic position, i.e. at C4 of azadeoxypodophyllotoxin, but the direct oxidation of **10** was unsuccessful [18, 19]. Finally, the desired oxygenated azapodophyllotoxin derivatives **15–18** were synthesized by reacting acetoxy urethane **13** and 3,4,5-trimethoxybenzaldehyde, possibly *via* intermediate **14** (Scheme 2) [18].

Vandewalle *et al.* synthesized the 1,3-*cis* and 1,3-*trans* isomer in a multistep synthesis [20]. The alkylation of hippuric acid ester **20** with piperonyl bromide led to an acyclic precursor **21**, which failed to cyclize under several reaction conditions due to the presence of the ester function. However, reduction of **21** and protection of the alcohol as acetate afforded **22**, which was cyclized by the Bischler-Napieralski reaction to give dihydroisoquinoline **23** in high yield. Methanolysis of acetate and aluminium hydride reduction efficiently afforded the 1,3-*cis*-isomer **24** as the sole product. In contrast, aluminium hydride reduction of the THP ether of **26** gave a 2:1 mixture of the *cis/trans* isomers **24/27**, which were separated by chromatography after the removal of a protecting group. Isomers **24** and **27** finally led to 4-desoxy-2-azapodophyllotoxins **25** and **28** (Scheme 3). The same report described an improved synthesis of optically active analogues starting from L- and D-DOPA (Scheme 4). The ester group of **29** was selectively reduced by following the Bischler-Napieralski approach after the formation of an ethylene dioxy unit (ring A), followed by acetylation to get **30**, which was then treated with POCl₃, giving optically pure dihydroisoquinoline **31**, which upon methanolysis and subsequent reduction afforded only the *cis* isomer, **32**. To obtain the *trans* isomer **36**, **31** was quaternized to give **34** followed by sodium borohydride reduction and hydrogenolysis. The same report also briefly described the synthesis of other analogues containing a modified heterocyclic ring D **38–41** [20].

The 1,2-*cis*-2,3-*trans* configuration of ring C in the azapodophyllotoxin structure has been shown to be crucial for the compound's antitumor activity. This structural feature was a serious obstacle for the total synthesis of α and β hydroxyl at C-4 in podophyllotoxin derivatives. Vandewalle *et al.* reported a multistep synthesis of α - and β -4-hydroxy azapodophyllotoxins by following Schollkopf's method (Schemes 5 and 6) [21]. Ethyl isocyanoacetate, upon reaction with **42** under thermodynamic conditions followed by reduction, led to *trans* **43**, which upon hydrolytic cleavage of oxazoline gave **44**; the diol was protected as acetonide. The Bischler-Napieralski reaction on the *threo* isomer **46** under Lewis acid conditions led to isoquinoline **47**, which was reduced to *cis*- and *trans*-tetrahydroisoquinolines **48** and **49**. Finally, the acetonide groups were hydrolysed and treated with phosgene to obtain **51** and **53** (Scheme 5). The epidophyllotoxin analogue **53** was also transformed into podophyllotoxin analogue **54** via oxidation-reduction [21]. For the *erythro* series, β -keto-ester **51** was prepared by reacting **50** with the lithium-enolate anion of ethyl *N*-(3,4,5-trimethoxybenzoyl)-glycinate. Ketalization of **52**, followed by a Bischler-Napieralski reaction, gave **54**, which was stereoselectively reduced to 1,3-*cis* isomer **55**, then treated with phosgene, leading to a mixture of the desired product **57** and the side product **56** (Scheme 6) [21].

Hitotsuyanagi *et al.* speculated that substitution of the methylene group at the C-4 position by oxygen would not alter the whole stereochemistry of **1** due to similarities between the C-O and C-CH₂ bond lengths and the C-O-C and C-CH₂-C bond angles. However, it could have an effect on biological activity due to changes in polarity [22]. Based on this hypothesis, they reported the synthesis of 4-oxa-2-azapodophyllotoxin **63**. Benzylation and bromination of sesamol afforded the O-benzyl bromide **58**, which upon lithiation through a metal-halogen exchange reaction, followed by treatment with 3,4,5-trimethoxybenzaldehyde, led to benzhydrol **59** in good yield. The reaction of **59** with 2,4-oxazolidinedione under Mitsunobu conditions gave **60**, which was reduced and then reoxidized using the Dess-Martin reaction to give aldehyde **61**. Careful debenylation of **61** gave labile hemiaminal **62**, which was treated with acetic acid to obtain 4-oxa-2-azapodophyllotoxin **63** (Scheme 7) [22]. Using a similar hypothesis, this group has also synthesized 2,4-diaza-4-deoxypodophyllotoxins **69a-d** and **70a-d**, where the carbons at the C2 and C4 positions were substituted by nitrogen atoms (Scheme 8) [23, 24]. The objective here was to determine the effect of changes in the whole stereochemical structure on biological properties. The synthesis started with *N*-benzylanilines **64** and **65**, which were reacted with boron trichloride and triethylamine followed by methoxybenzaldehydes to give boracyclic intermediates, which upon hydrolysis led to benzhydryl alcohols **66a-d**. The alcohols **66a-d** were converted to urethanes **67a-d**, which upon reaction with glyoxylic acid, followed by reduction, produced alcohols **68a-d**. The cyclization of **68a-d** was done by using sodium methoxide to give oxazolones, which were debenzylated to yield the *cis* analogues **69a-d**. The *cis* analogues, upon treatment with trifluoroacetic acid, were converted into their corresponding *trans* isomers **70a-d**. The *N*-amino analogues **71** and **72** were also prepared by reacting **69a** and **70a**, respectively, with *O*-(2,4-dinitrophenyl)hydroxylamine. Oxidation of **70a** was also carried out by reacting it with lead tetraacetate to give the dehydro derivative **73** (Scheme 8) [23, 24]. Later, to evaluate the effect of replacing the methylene group of **1** with nitrogen on biological activity, the same group reported the synthesis of (-)-4-aza-4-deoxypodophyllotoxin (**74**) in an optically pure form from natural (-)-podophyllotoxin (**1**) through C-ring cleavage followed by a Curtius rearrangement and intramolecular *N*-alkylation [25].

In 1997, the first convenient and simple synthetic method for the synthesis of azapodophyllotoxins was reported based on condensation reactions of three components: anilines, tetronic acid and benzaldehydes was reported [26]. In this approach, substituted anilines were treated with an equimolar amount of tetronic acid in dioxane at room

temperature, leading to the production of anilinolactones, which were reacted with substituted aldehydes in the presence of *p*-chloranil in trifluoroacetic acid at room temperature to produce 4-aza-analogues of 1-arylnaphthalene lignans **75a–o** in high yield. These lignans were later reduced by an excess amount of sodium cyanoborohydride in acetic acid to the corresponding 4-aza-2,3-dehydro-4-deoxypodophyllotoxins **76a–o** (Scheme 9) [14, 26]. Although this method [26] was convenient, its scope was limited, as it required three steps to afford the required azapodophyllotoxins. This method also failed to give *N*-substituted derivatives, as the alkylation of **76** was unsuccessful. To address this issue, Giorgi-Renault *et al.* reported the first one-pot MCR for the synthesis of azapodophyllotoxins and their *N*-alkylated derivatives (**77**) (Scheme 10) [27].

Magedov *et al.* implemented a bioisosteric replacement of the methylenedioxybenzene subunit of azapodophyllotoxin with a pyrazole moiety to produce tetracyclic dihydropyridopyrazoles **78–110**. Libraries of these analogues were prepared using a simple one-step MCR approach involving the condensation of substituted 5-amino-pyrazoles, tetronic acid, and substituted aldehydes (Scheme 11) [28]. The resulting dihydropyridopyrazoles precipitate as the reaction mixtures are allowed to cool to room temperature, are isolated by simple filtration and, in most cases, no further purification is required. Shi *et al.* reported the synthesis of similar compounds **110–126** using the MCR approach in the presence of organocatalysts in ethanol [29]. The reaction of 5-amino-1-phenyl-3-methylpyrazole, 4-methylbenzaldehyde, and tetronic acid was studied in EtOH in the presence of 10 mol% L-proline at 80 °C (Scheme 12). L-proline is soluble in EtOH and the solubilities of the desired products in EtOH are low. Therefore the products were easily separated by cooling the reaction to room temperature and filtering it after the reaction was complete. A possible mechanism for the L-proline-catalyzed synthesis of dihydropyridopyrazole derivatives was also proposed in the same report [29].

Madec *et al.* reported the synthesis of 4-aza-2,3-didehydropodophyllotoxins, starting with a reaction between *N*-benzyl tetronamide or *N*-phenyl tetronamide with the functionalized benzhydrylic alcohol **127** to produce the benzhydrylated *N*-benzyl tetronamide intermediate **128**. The latter was arylated using a copper-mediated Ullmann-type *N*-arylation. Thus, treatment of **128** with CuI and CsCO₃ in dimethylformamide (DMF) (according to Fukuyama's protocol [26]) afforded the expected 4-aza-2,3-didehydropodophyllotoxins (**129**) in a quantitative yield (Scheme 13) [30].

Giorgi-Renault *et al.* reported a rigid aminologue of 4-aza-2,3-didehydropodophyllotoxins, where the rotation between the tetracycle and the aryl ring was blocked by the creation of an additional pseudocycle [31]. The quinoline-lactone **130** was prepared using the MCR route, followed by oxidation of the dihydroquinoline intermediate in hot DMSO. The 4-chloroquinoline-lactone **131** was then prepared by reacting POCl₃ with the *N*-oxide intermediate obtained by *m*-CPBA oxidation of **130**. This was followed by a nucleophilic substitution by aniline to give **132**, which was quaternized with trifluoromethyl sulfonate, leading to the quinolinium salt **133**. Deprotonation of **133**, followed by reduction or hydrogenation of imine **134**, led to the desired aminologue **135** (Scheme 14) [31].

Recently, Shi *et al.* extended their earlier reported MCR synthesis of dihydropyridopyrazoles [21] using L-proline as an organo-catalyst for the synthesis of 4-azapodophyllotoxin derivatives **136a–x** (Scheme 15) [32]. This method has the advantages of high yields, high regioselectivity, extensive adaptability, easy operation, and environmental friendliness. The reaction was carried out with 10 mol% L-proline in refluxing ethanol, resulting in the isolation of product in over 90% yields. The products could be separated by cooling the reaction mixture to room temperature followed by simple filtration. The filtrate containing L-proline can be directly recovered and recycled. Shi *et al.*

found that, even after seven uses, the catalytic efficiency of the L-proline reaction solution was unchanged. The cytotoxic activities of these compounds were also investigated, and some appeared to have good cytotoxic activity against tumor cells [32].

The synthesis of a new series of azapodophyllotoxin analogues and their antivasular activity was recently reported by Giorgi-Renault *et al.* [33] In this study, a linker was inserted between the trimethoxyphenyl ring *E* and the tetracyclic ABCD moiety of the 4-aza-1,2-didehydropodophyllotoxins (Scheme 16). Most of these derivatives were *N*-methylated for synthetic and stability purposes, as *N*-substitution prevents aromatization of the 1,4-dihydro-quinoline moiety. This linker enables free rotation between the two moieties. The synthesis strategy for **137–140** was straightforward, involving the MCR approach, where substituted aniline, tetronic acid, and substituted aldehyde were reacted to obtain the desired products. The two-carbon homologated analogue **141** was obtained in good yield by catalytic hydrogenation of the exocyclic double bond of vinylogue **139** without reduction of the lactone. For the synthesis of ethynologue **143**, direct dehydrogenation of **140** using manganese dioxide, selenium dioxide, or dichlorodicyanoquinone was unsuccessful.

An addition-elimination approach was applied for the synthesis of **143**, where **140** was first treated with osmium tetroxide followed by mesylation, bromination, and elimination using potassium tertbutoxide (Scheme 16) [33].

Recently, we reported the synthesis of novel arylamino alcohols **144–146**, which we used to prepare novel *N*-(2-hydroxy-ethyl)-2,3-didehydroazapodophyllotoxins **147–149** by simple reflux in ethanol [34]. The 4-aza-2,3-didehydropodophyllotoxin core was functionalized at C4 of the *C* ring in a fashion similar to that which occurs in podophyllotoxin derivatives (Scheme 17). These *N*-(2-hydroxyethyl)-2,3-didehydro-azapodophyllotoxins have the potential to be conjugated to tissue-targeting carriers. This series was recently expanded to include three different types of hydroxyazapodophyllotoxin derivatives: those containing (i) a five-membered methylenedioxy ring (**147a–f**), (ii) a five-membered ring with no heteroatom (**148a–f**), and (iii) a six-membered ethylenedioxy ring (**149a–f**) as ring *A* [34]. Each series has a different substitution pattern of methoxy groups on ring *E*. The screening of these azapodophyllotoxin derivatives for their antitumor activity is in progress.

2.2. Microwave-irradiated Synthesis

It is well documented in the literature that microwave irradiation accelerates organic reactions and has been used as a tool for the efficient synthesis of a variety of organic compounds. Also, as described earlier, MCRs offer a wide range of possibilities for the efficient, single-step construction of azapodophyllotoxins. Tu *et al.* have utilized the combination of these two tools extensively in their study of azapodophyllotoxin synthesis, providing diversified libraries of these compounds. They first reported a microwave-irradiated MCR consisting of aldehyde, aromatic amine, and tetronic acid in water, without a catalyst, yielding a new series of 4-azapodophyllotoxin derivatives (**150**) (Scheme 18) [35]. Several solvents were examined *viz.*: water, glycol, DMF, glacial acetic acid, and ethanol at 80 to 120 °C, and the microwave irradiation power was optimized. The highest yield was obtained when water was used as the solvent in the reaction of equimolar amounts of 4-bromophenyl aldehyde, *p*-toluidine, and tetronic acid at 100 °C with a microwave power of 150 W. For comparison, the synthesis was performed under both microwave irradiation and classical heating conditions at 100 °C. Microwave irradiation promoted the reaction more efficiently: The reaction time was shorted from six hours (under the traditional heating conditions) to only four minutes. Additionally, the yield was increased from 72% to 97%. [35]. The same group also reported the synthesis of a new series of azapodophyllotoxin analogues containing a furo[3,4-*b*]quinoline motif (**151**) using a facile MCR consisting of an

aldehyde, tetronic acid, and an enamino in glacial acetic acid, using microwave irradiation. Optimization of reaction conditions by screening different solvents, reaction temperatures, and microwave power levels revealed that the best yields were obtained at 300 W and 100 °C using acetic acid as a solvent and no catalyst (Scheme 19). Comparing microwave conditions with conventional heating showed that microwave-irradiated reactions proceed much faster and have higher yields [36].

Using a similar approach, Tu *et al.* later reported the synthesis of *N*-substituted furo[3,4-*b*]indeno[2,1-*e*]pyridine analogues of azapodophyllotoxin **152** through an MCR of aldehyde, 2H-indene-1,3-dione, and 4-(arylamino)-furan-2(5*H*)-one using ethylene glycol as solvent at 100 °C under microwave irradiation at 200 W without a catalyst (Scheme 20). Although the detailed mechanism of the reaction remains to be fully clarified, the authors speculate that the formation of *N*-substituted furo[3,4-*b*]indeno[2,1-*e*]pyridine analogues of azapodophyllotoxin is due to a reaction sequence of condensation, addition, cyclization, and dehydration [37].

A series of new polycyclic-fused isoxazolo[5,4-*b*]pyridines was obtained by a one-pot tandem reaction under microwave irradiation in water, without the use of additional reagents or catalysts [38]. The three-component equimolar reaction of 4-fluorobenzaldehyde, 3-methylisoxazol-5-amine, and tetronic acid was investigated to establish the feasibility of the strategy and optimize the reaction conditions. Reaction temperatures and different solvents (including ethylene glycol, ethanol, acetic acid and water) were screened using the model reaction and the optimum yields were obtained in water at 120 °C with 200 W of microwave power (Scheme 21). A range of novel structures (**153**) was reported, with good to excellent yields. The reaction of aromatic aldehydes bearing electron-withdrawing or electron-donating groups proceeded smoothly; however, aliphatic aldehydes were unable to react under similar conditions. Another active methylenic compound, 1,3-indanedione, was examined as a replacement for tetronic acid in the reaction with aromatic aldehydes and 3-methylisoxazol-5-amine and a new series of isoxazolo[5,4-*b*]pyridines (**154**) was successfully synthesized (Scheme 21) [38].

Most of the reported modifications on azapodophyllotoxins have been performed on rings *B* and *C*; the modifications on ring *A* were not well documented. Recently, Tu *et al.* reported a microwave-assisted, four-component reaction of dimedone, aromatic aldehydes, and tetronic acid with excess ammonia water. Instead of the suspected product, **156**, the presence of aromatic aldehydes with electron-withdrawing groups led to product **155** in high yields. In contrast, the same reaction with electron-donating aldehydes gave the product **157**, which suggested that tetronic acid did not take part in the reaction (Scheme 22) [39].

2.3. Ultrasound-irradiated Synthesis

The application of ultrasound in organic synthesis has increased over the last couple of decades. Ultrasound-mediated synthesis is usually more convenient and easily controlled compared to traditional methods and has often resulted in shorter reaction times, utilizing milder reaction conditions. Tu *et al.* reported the synthesis of furo[3',4':5,6]pyrido[2,3-*d*]pyrimidine **158** and indeno[2',1':5,6]pyrido[2,3-*d*]pyrimidine **159** derivatives *via* a three-component condensation of aldehydes, 2,6-diaminopyrimidine-4(3*H*)-one, and tetronic acid or 1,3-indanedione in ethylene glycol, using ultrasonic irradiation without a catalyst (Scheme 23). The reactions using ethylene glycol as the solvent resulted in higher yields and shorter reaction times than those using AcOH, DMF, or EtOH [40].

3. BIOLOGICAL ACTIVITY OF AZAPODOPHYLLOTOXINS

Podophyllotoxin (**1**) has been shown to isomerize into inactive picropodophyllotoxin *via* epimerization at the C2 center under physiological conditions. Tomioka *et al.* replaced the sp^3 C2 center with sp^2 nitrogen, which is incapable of epimerizing at the C2 center, and studied the antitumor activity of the resulting racemic and optically pure azapodophyllotoxin derivatives [17–19]. *In vitro* growth inhibition data of these compounds for KB cells is provided in Table 1, and indicate promising growth inhibition by the racemic compounds. Some of these compounds also exhibited promising *in vivo* activity against P-388 mouse (T/C 145 (**10**) and 170 (**11**)). It is also important to note that the data indicate that the cytotoxicity of azadeoxypodophyllotoxin relies primarily on the absolute configuration at the C1 position, not that at the C3 position [18].

Hitotsuyanagi *et al.* replaced the C4 methylene group of **1** with oxygen, which would not alter the stereochemistry of the molecule, but could change its polarity. The resulting compound **63** showed significant activity (IC₅₀ 0.031 μ g/mL) against adriamycin-resistant P-388 leukemia cells [22]. Later, the same group synthesized diazapodophyllotoxin analogues and studied their cytotoxicity and antitumor activity. All analogues of **69–73** were assayed for *in vitro* cytotoxicity against L1210 murine leukemia cells and for antitumor activity against P-388 leukemia cells in mice; the results were compared with podophyllotoxin (**1**) as control. Podophyllotoxin (**1**) was found to be the most cytotoxic *in vitro* against L1210 leukemia cells; however, it showed no *in vivo* activity against P-388 leukemia cells. In contrast, both the *cis* analogue **69a** and the *trans* analogue **70a** showed potent antitumor activity. Analogues **69b–d** and **70b–d** also showed significant activity (Table 2). These results suggest that the ethylenedioxy group on ring *B* and the 3,5-dimethoxy group on ring *E* are important for activity. The *N*-amino analogues **71** and **72** retained activity both *in vitro* and *in vivo*. In general, the *trans* analogues were more cytotoxic than the corresponding *cis* analogues. This could be attributed to the former being topologically more similar to podophyllotoxin (**1**) than the latter, which would also explain the reduced activity of the dehydrated analogue **73**. However, it is still not clear whether the improved antitumor activity of analogues *in vivo* is due to pharmacokinetics or other factors.

The activities of analogues **69a** and **70a**, which showed the most promising activity in terms of their *T/C* values, were further investigated using vincristine-resistant P388 leukemia (P388/VCR) and B-16 melanoma cells (Table 3). Interestingly, **1** showed only marginal (*T/C* = 125%) activity against the P388/VCR cells and was inactive against the B16 melanoma cells, while **69a** and **70a** showed significant activity against both P388/VCR and B16 melanoma cells. The mode of action of **69a** and **70a** was examined by assessing the effects of these compounds on an assembly of microtubules prepared from bovine brain. The concentrations of **1**, **69a**, and **70a** necessary to inhibit microtubule assembly by 50% were 0.13, 1.7, and 0.42 μ g/mL, respectively, which correlated to their *in vitro* cytotoxicity against L1210 leukemia cells (Table 2). These results suggest that incorporation of heteroatoms within the podophyllotoxin core structure could be a possible approach for producing more promising analogues [22].

The screening results for *in vivo* antitumor activity against vincristine-resistant P388 leukemia cells showed that 2,4-diaza-4-deoxypodophyllotoxin exhibit better activity than podophyllotoxin (**1**) and 2-aza-4-deoxypodophyllotoxin. This suggests that substitution of the carbon atom at position 4 by a nitrogen atom is important for antitumor activity. Hitotsuyanagi *et al.* synthesized (–)-4-aza-4-deoxypodophyllotoxin (**74**) in an optically pure form from natural (–)-podophyllotoxin (**1**) and the biological activity evaluation revealed that both **1** and its 4-aza analogue **74** possess same the antitumor activity against P-388 leukemia cells (IC₅₀ = 0.0050 μ g/mL for both) [25]. The same group synthesized the 4-

aza-2,3-dehydro-4-deoxypodophyllotoxin analogues **76a–n** through quinolines **75a–n** and studied their cytotoxicity using P-388 leukemia cells (Table 4) [14]. In general, the quinoline analogues **75a–n** showed very weak or no activity. However, 4-aza-justicidin B (**75e**) showed moderate activity ($IC_{50} = 2.0 \mu\text{g/mL}$), which is greater than the reported data for justicidin B, a cytotoxic principal from several plants ($IC_{50} = 3.3 \mu\text{g/mL}$ against same cell line). In contrast, dihydroquinoline analogues **75a–n** showed significant antitumor activity. The screening results revealed the substituent effects of the ring *B* on the activity of these compounds. Analogues **76a** (which has the same 1,3-benzodioxole structure as **1**), **76b**, and **76g** (both of which have a cyclic ring *A* structure consisting of 1,4-benzo-ioxane and indan, respectively) showed higher activity than the analogues that contain a dimethoxyphenyl (**76c**), a trimethoxyphenyl (**76f**), or a fluorene (**76h**) unit. Analogue **76c**, with its two free rotatable methoxy groups, was more than 2700 times less toxic than analogues **76a** and **76b** with a cyclic ether structure, although the electronic effects of the methylenedioxy, ethylenedioxy, and *o*-dimethoxy groups are quite similar. Also, while sterically very similar but electronically dissimilar, the indan analogue **76g** was only 2.3 times less toxic than analogue **76a**. These results suggest that the steric effects are more influential than the electronic effects. The reduced activity of fluorene analogue **76h** compared to that of indan analogue **76g** also supports this possibility. The same tendency was also seen between **76d** and **76i**, and between **76e** and **76m**. A comparison of the **76a–m** analogues illustrates some of the substituent effects of ring *E*. Interestingly, **76l**, with an unsubstituted phenyl group as the ring *E*, was only 2.9 times less toxic than analogue **76a**. The results also suggest that an *m*-methoxy group may either enhance or not influence the activity, while a *p*-methoxy group reduces the activity. The potency of the analogues was found to be in the order of **76a**>**76j**=**76l**>**76i**>**76k**. The cyclic ketone analogue **76n** was about 15 times less toxic than the lactone analogue **76a**, indicating that an oxygen atom is more effective than a methylene group at position 12 (Table 4) [14].

It is important to note that podophyllotoxin (**1**) possesses four chiral centers on the ring *C* carbon atoms, its 1,2-*cis*-2,3-*trans* system provides the two aromatic rings with an appropriate topology, and that the absolute configuration at the C-1 center is critical for activity expression [18]. On the contrary, analogues **76a** and **76b**, with one chiral center, are more than twice as cytotoxic as **1**, despite being racemic. A comparison of the crystal structure of podophyllotoxin (**1**) and the MM2*10 energy-minimized structure of analogue **76a** also revealed that the two aromatic rings in analogue **76a** well mimic the topology of those in **1**, which could account for its potent activity [14].

Magedov et al. implemented a bioisosteric replacement of the methylenedioxybenzene subunit of podophyllotoxin with a pyrazole moiety to synthesize tetracyclic dihydropyridopyrazoles **78–110** and evaluated their antiproliferative activity against three cancer cell lines: HeLa (human cervical), MCF-7/AZ (breast adenocarcinomas), and Jurkat (T-cell leukemia) (Table 5) [28]. Podophyllotoxin **1** and its 4-aza-2,3-didehydro analogues **5** and **7** were also tested for comparison. The pyrazole moiety of the dihydropyridopyrazole was optimized by putting different substituents on this ring (compounds **78–83**). Screening results showed that **83** exhibited potency and activity profiles similar to **76a** and **76c**, indicating that the alkoxybenzene ring can be replaced by the pyrazole moiety without loss of potency. However, the potency of podophyllotoxin (**1**) remained superior to all of them. The analogues with larger substituents on their pyrazole rings (**78–81**) and the methylated pyrazole **82** exhibited more limited activity than **83**. Optimization of ring *E* revealed that, in addition to the significant antiproliferative and apoptosis-inducing activity of **83** (which possesses a trimethoxy ring *E* just like podophyllotoxin), analogues possessing the *m*-bromo substitution (**91–96**) were the most potent. Comparing the profiles of analogues **91–96** reveals that the potency remains unchanged, regardless of whether there are other substituents at the 4' or 5' position. However, *m*-chloro, *m*-fluoro, and *o*- or *p*-bromo–

substituted analogues were comparatively less active. Also, the heterocyclic analogues **104–110** were found to be either inactive or much less potent (Table 5). In general, the level of apoptosis induction in Jurkat cells showed a trend similar to that of the analogue's antiproliferative potency, with the meta-bromo analogues **91–96** having the highest level, at 50–58%. The GI₅₀ (concentration required for 50% of the effect) values of selected analogues obtained by dose-dependent studies against a HeLa cell line revealed that dihydropyridopyrazoles (**83, 91–96**) and Itokawa/Takeya's analogues (**76a** and **76c**) have similar potencies, possessing GI₅₀ values in the low micromolar range, while analogue **96** showed submicromolar activity (Table 6). All meta-bromo analogues exhibited activity 35–400 times higher than that of podophyllotoxin (**1**) [28].

Shi *et al.* recently screened 4-azapodophyllotoxin analogues **136** for anticancer activity using the sulforhodamine B (SRB) method against the human liver cancer cell line HepG₂ (Table 7) and found a strong inhibitory effect for most of the analogues [32].

Podophyllotoxin (**1**) works by acting on the colchicine binding site of tubulin, resulting in microtubule depolymerization. Colchicine and podophyllotoxin bind to β -tubulin at its interface with α -tubulin and the trimethoxyphenyl nucleus being hidden into the β -subunit. Dynamic NMR and X-ray studies show that, in solution, **1** adopts a single conformation, with its trimethoxyphenyl ring in a quasi-axial position nearly perpendicular to the tricyclic moiety. Giorgi-Renault *et al.* investigated this point for 4-aza-2,3-didehydropodophyllotoxins by synthesizing two series of novel azapodophyllotoxin derivatives and studying their tubulin polymerization inhibition activity. In the first series, a linker was inserted between the tetracyclic moiety and ring *E* of 4-aza-1,2-didehydropodophyllotoxins to allow free rotation between the two parts (compounds **137–141** and **143**) [33]. The second series involved a conformational restriction of the ring *E* nucleus *via* a rigid analogue in which the rotation between the tetracycle and the aryl ring was blocked by the creation of an additional pseudocycle (compounds **132, 134, 135**) (Table 8) [31, 33]. The activities of the parent compound **76a** and reference compound combretastatin A-4 (**159**) were also tested for comparison. The reference compounds **76a** (ITP = 0.47) and **159** (ITP = 0.72) both showed strong anti-microtubule activity, with IC₅₀ values lower than that of colchicine.

Among the carbon homologue series, only the *N*-unsubstituted vinylogue **139** was found to be significantly active (ITP = 0.75). This shows that the insertion of a double bond between the tetraline moiety and the trimethoxyphenyl ring of **76a** is tolerable. As the trimethoxyphenyl group of podophyllotoxin is buried within a hydrophobic pocket of β -tubulin, the additional double bond may reinforce the Van der Waals interactions between vinylazapodophyllotoxin **139** and tubulin. The compounds **137, 138, 140, 141, and 143**, containing both a spacer group and *N*-methylation, were inactive for tubulin polymerization inhibition as indicated by their higher ITP values (>40). In contrast, *N*-methylation was well tolerated with an amino spacer, as aminologue **135** showed significant ITP activity (ITP = 2.89). The imine **134** was found to be inactive compared to aminologue **135** and quinoline **132**, which suggests that the hydrogen bond between the amino linker and the lactone carbonyl plays a crucial role for tubulin inhibition activity (Table 8) [33]. The cytotoxicity of these compounds has also been evaluated against a solid-tumor-derived murine B16 melanoma cell line and the EA.hy 926 cell line (an immortalized HUVE line). The results were compared to the compounds' cytotoxicity against a murine fibroblast NIH 3T3 cell line as a normal cell control. The reference compounds **76a** and **159** showed significant toxicity, with IC₅₀'s in the nanomolar range compared to linker analogues **139, 140, 132, and 135**. Similar activity profiles were observed in all three cell lines. Vinylogue **139** and quinoline **132** showed 40-fold lower anti-proliferative effects against B16 cells than those of **159**, whereas their anti-microtubule activities were similar. Aminologue **135** displayed high ITP

activity associated with poor B16 cytotoxicity, which is considered a good indicator for antivasular activity [41, 42].

4. CONCLUSIONS AND FUTURE PERSPECTIVES

Even after drastically reducing the natural structural complexity found in podophyllotoxin (**1**), many of the azapodophyllotoxin analogues described above expressed more pronounced antitumor activity than that of **1**. Thus, the incorporation of heteroatoms within the podophyllotoxin core structure should constitute a convenient approach for the production of even more promising analogues. Moreover, some of the new compounds are racemic, and thus the separation and testing of individual enantiomers will undoubtedly lead to the discovery of more potent compounds.

The use of MCRs in combination with ultrasound and microwave irradiation has increased the speed of synthesis and the variety of azapodophyllotoxins available to test against a diverse population of carcinomas and other diseases.

Dihydropyridopyrazolyl azapodophyllotoxins are able to induce apoptosis in Jurkat cells on a scale similar to that of podophyllotoxin, while showing virtually no apoptotic activity against noncancerous, nucleated white blood cells. This opens new avenues for the synthesis of cancer-selective azapodophyllotoxins. How azapodophyllotoxin compounds exert their cytotoxic effect, whether through a podophyllotoxin-like antitubulin mechanism, by targeting topoisomerase II, or have a totally independent mode of action, constitutes the subject of further investigation.

The structural complexity of **1**, arising from the presence of four stereogenic carbons in ring *C* has restricted most of the structural activity relationship (SAR) studies to those using derivatives of the parent natural product rather than compounds obtained through *de novo* chemical synthesis. Such approaches, however, are rather limited by the type of chemistry that **1** can undergo. These issues provide a strong impetus to search for analogues of **1** with simplified structures, which can be prepared *via* short, synthetic sequences from simple starting materials, such as many of the azapodophyllotoxin derivatives described here. Even if such initial compounds might have diminished cytotoxic potencies compared with the parent cyclolignan, having an easy method to produce carefully designed analogue libraries would lead to more informative SAR studies and expeditious structure optimization.

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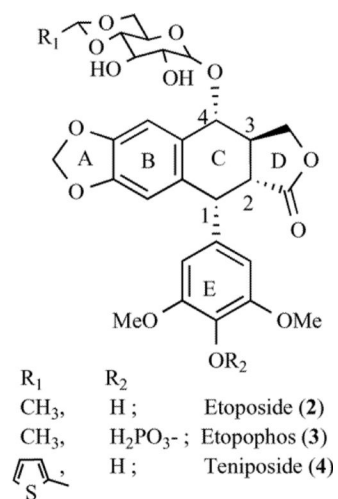
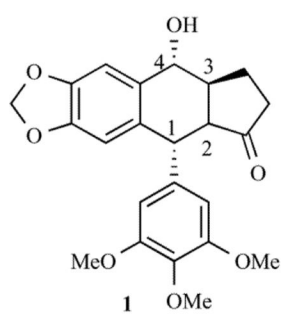


Fig. (1).
Podophyllotoxins.

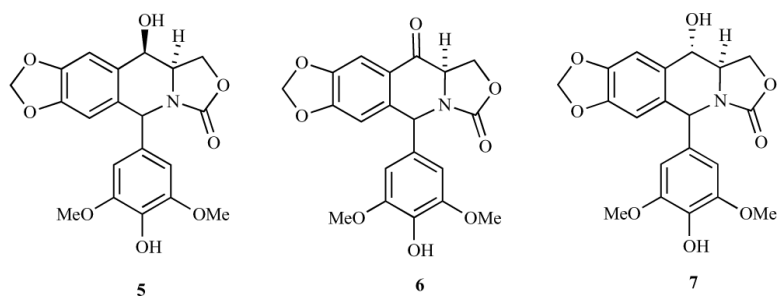


Fig. (2).
Chiral analogues of podophyllotoxins.

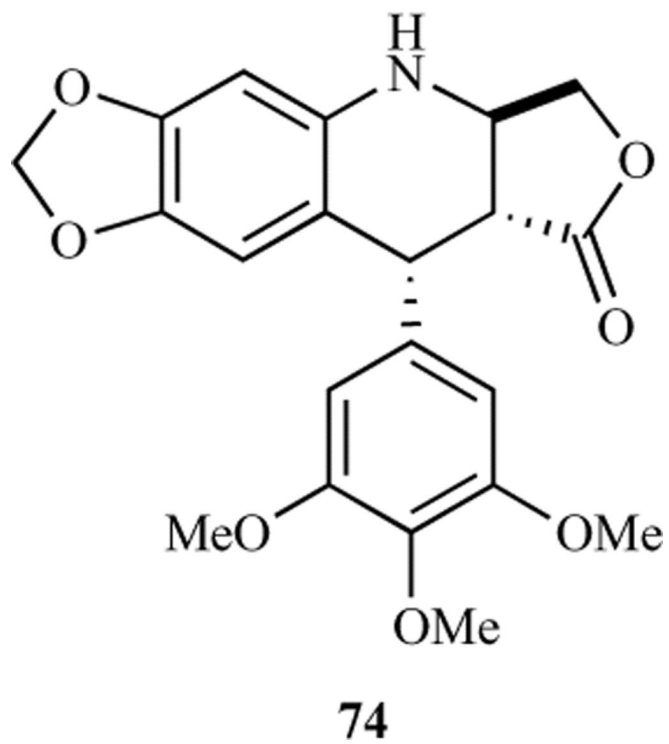


Fig. (3).
(-)-4-aza-4-deoxypodophyllotoxin **74**.

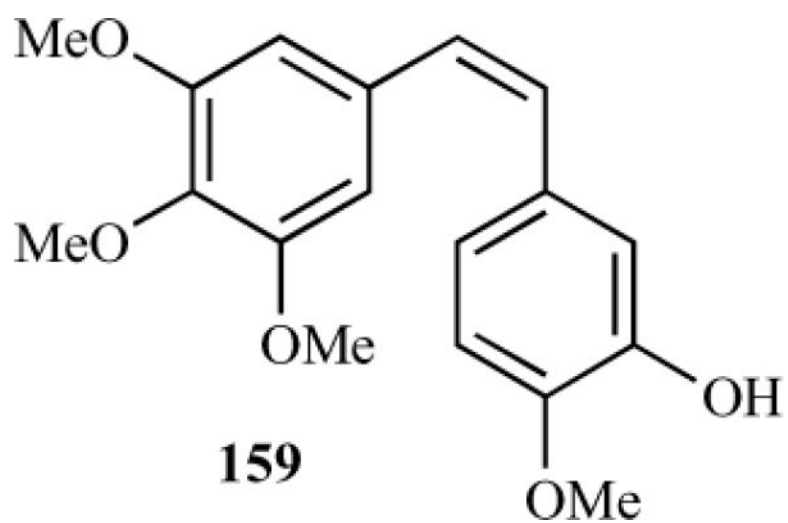
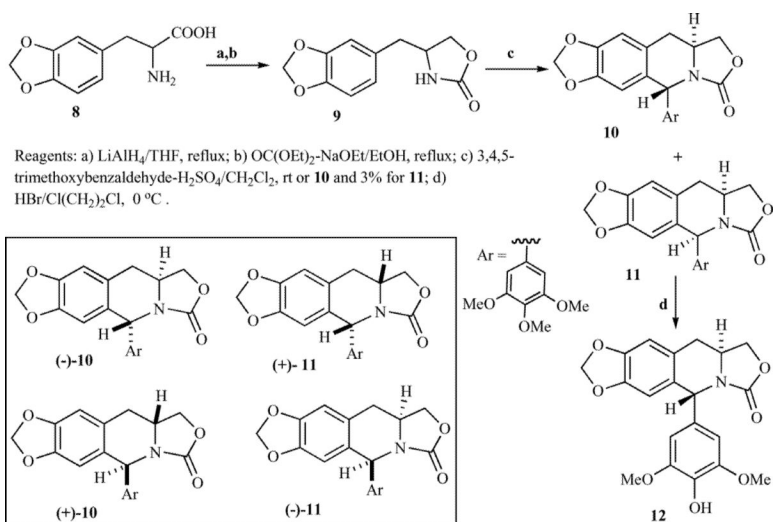
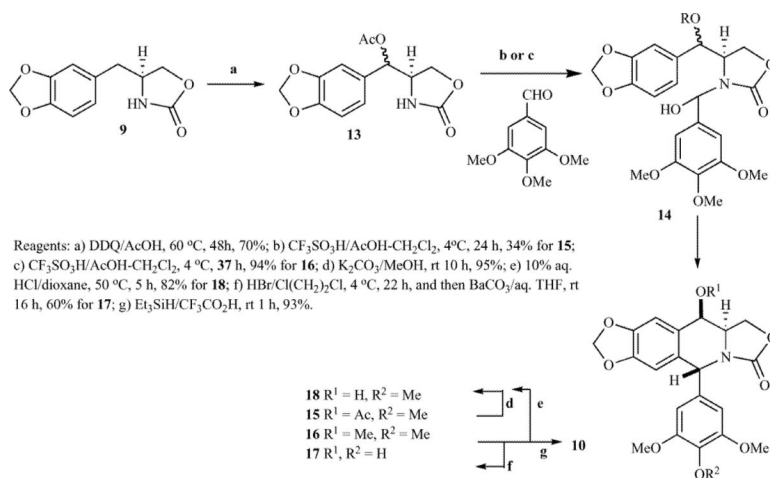


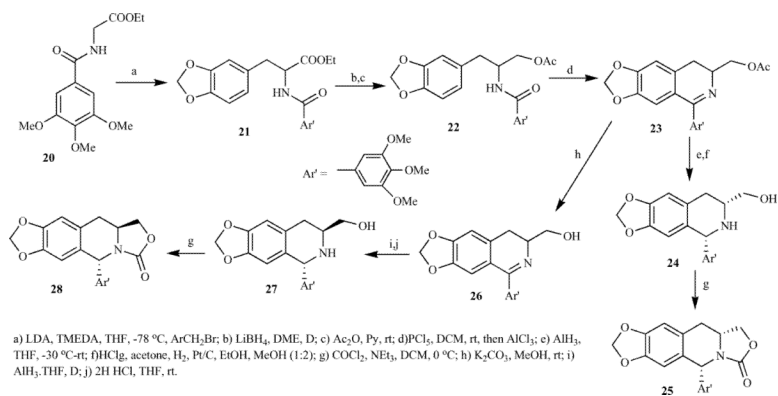
Fig. (4).
Combretastatin A-4.



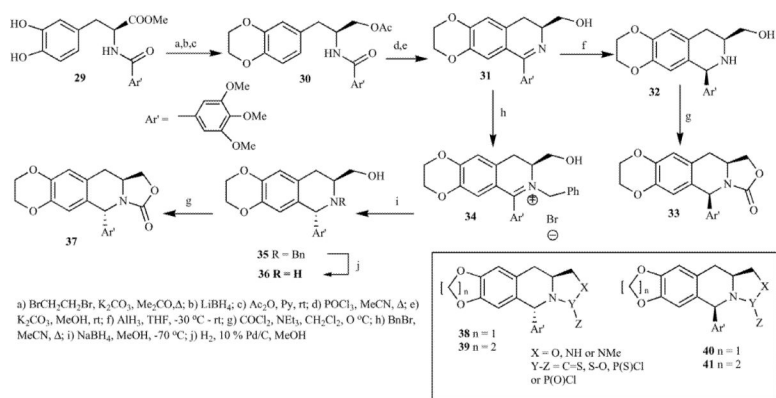
Scheme 1.



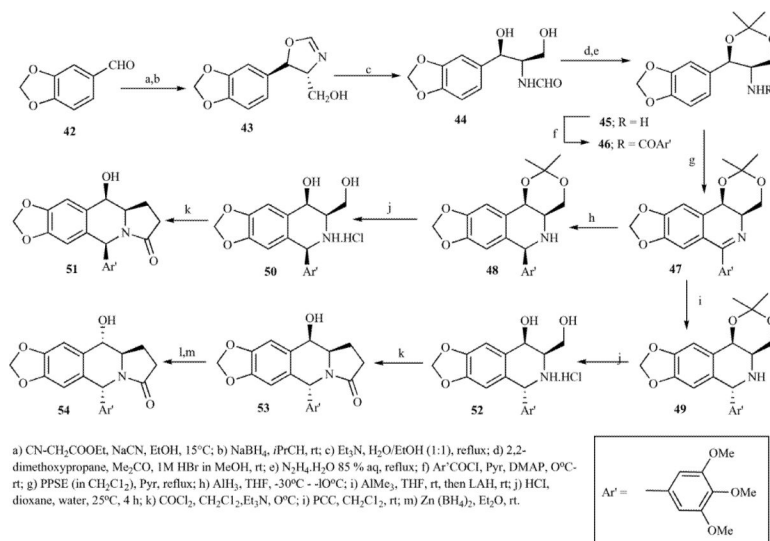
Scheme 2.



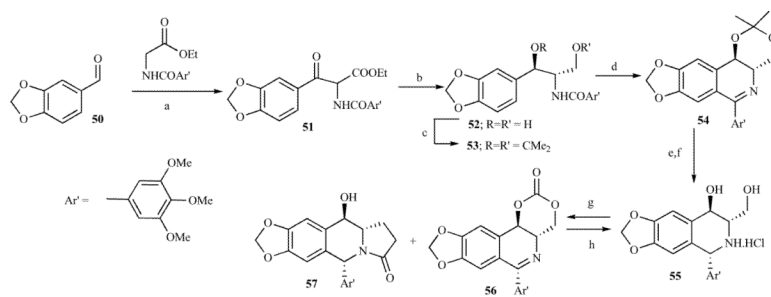
Scheme 3.



Scheme 4.

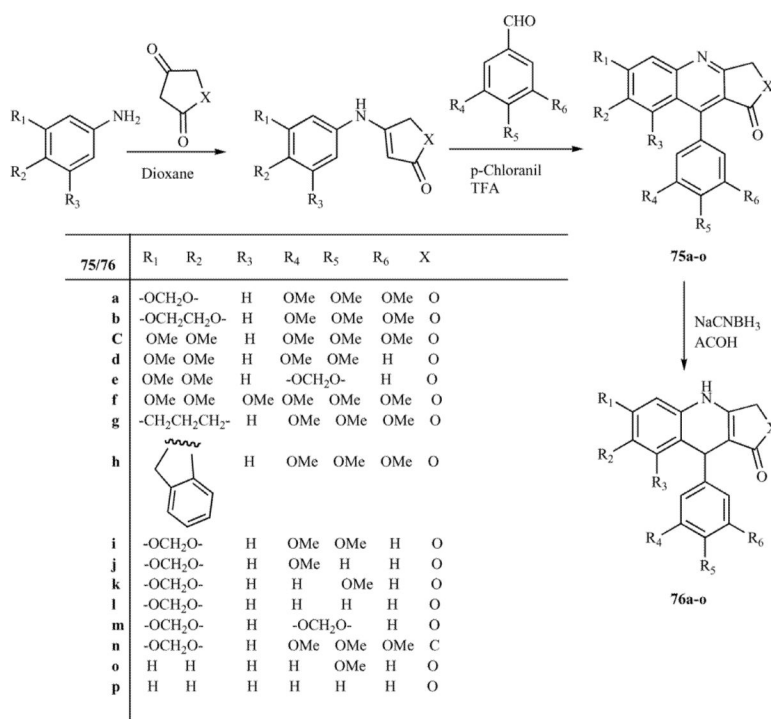


Scheme 5.

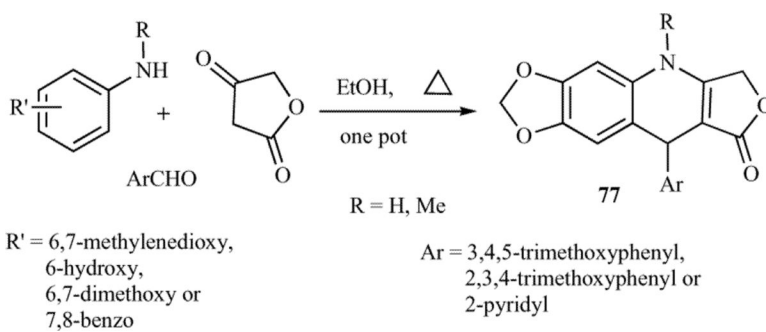


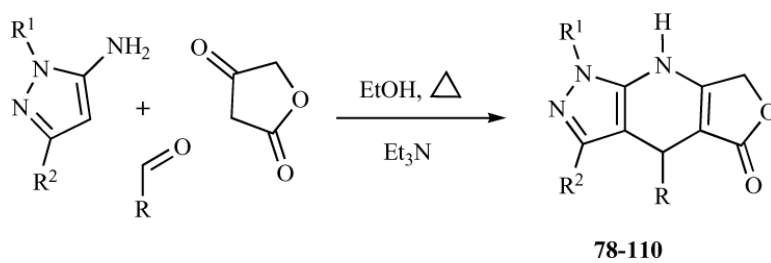
a) LDA, HMPA, -70°C ; b) 3,4,5-trimethoxybenzoyl chloride, NEt_3 , DCM, 0°C -rt; c) Me_2O , DMP, pTSA, rt; d) P_2O_5 , Pyr, 110°C ; e) AlH_3 , THF, -30°C - -10°C ;
 f) 1H HCl , THF, rt; g) COCl_2 , CH_2Cl_2 , Et_3N , 0°C ; h) 50% NaOH, H_2O , rt.

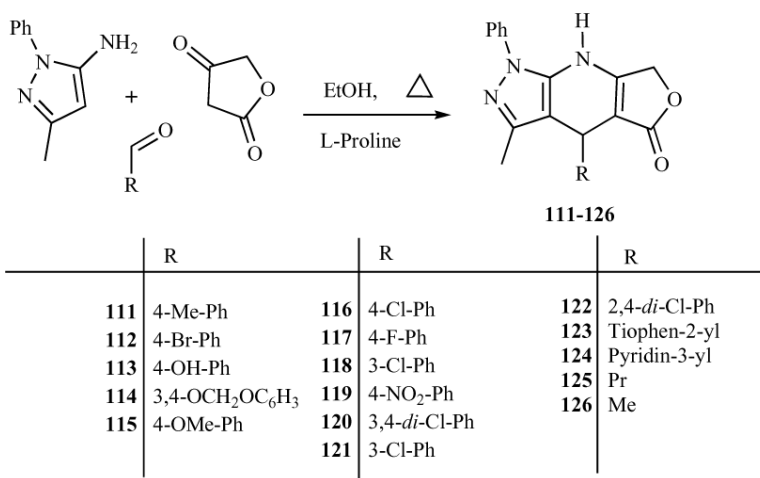
Scheme 6.



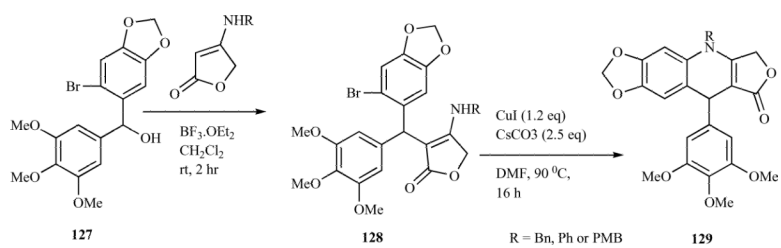
Scheme 9.

**Scheme 10.**

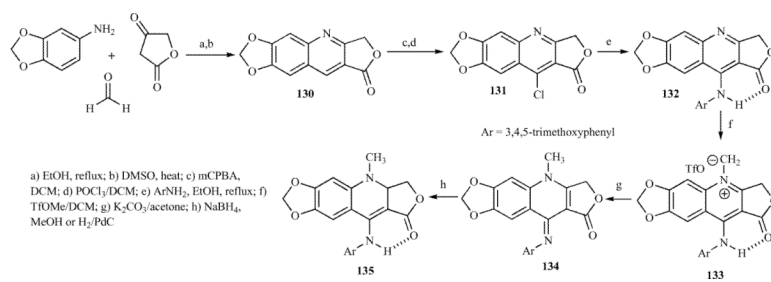
**Scheme 11.**



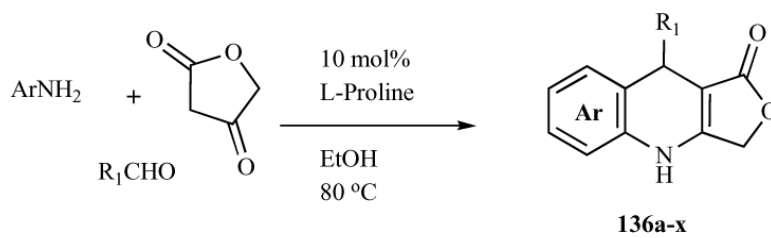
Scheme 12.



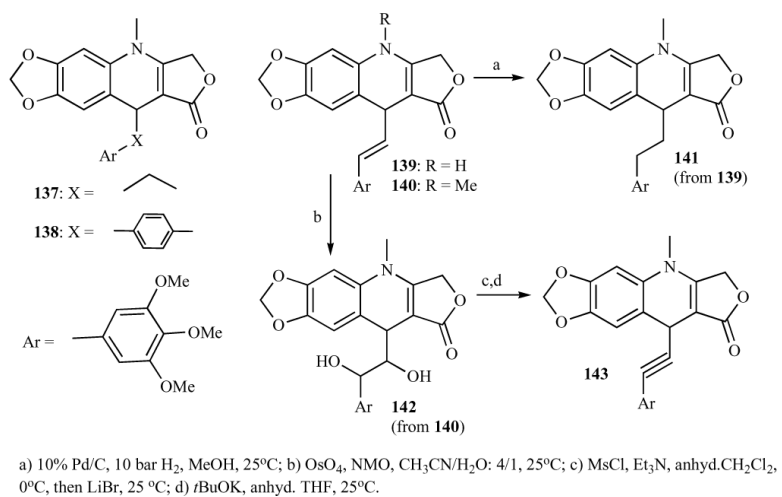
Scheme 13.



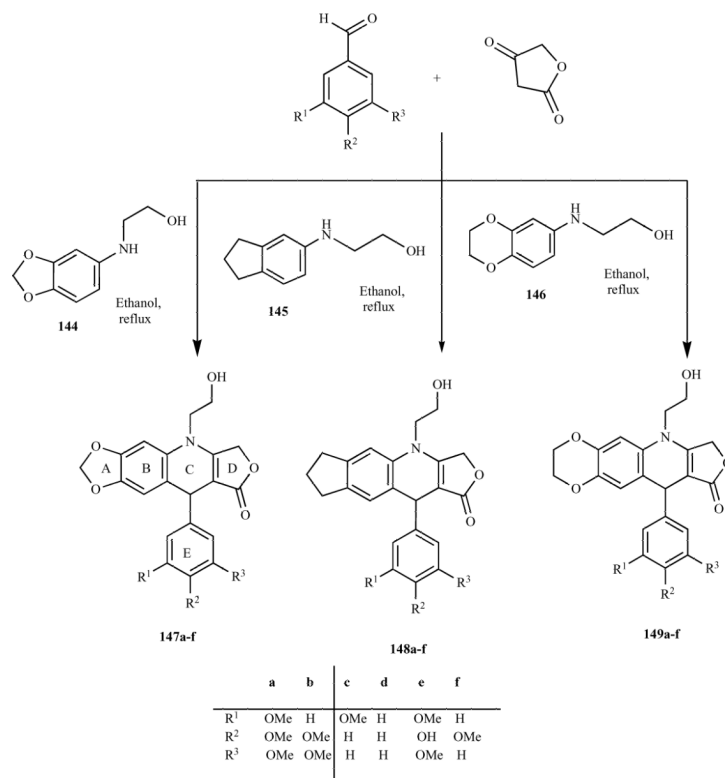
Scheme 14.



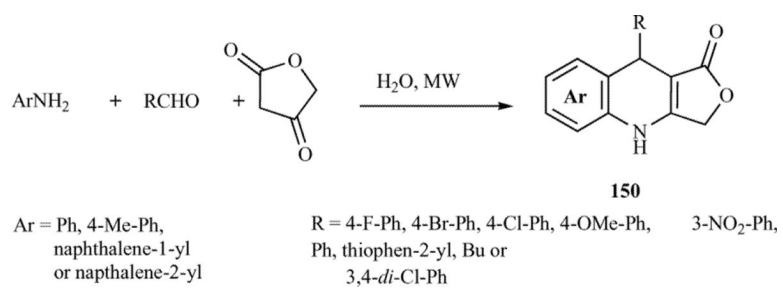
Scheme 15.

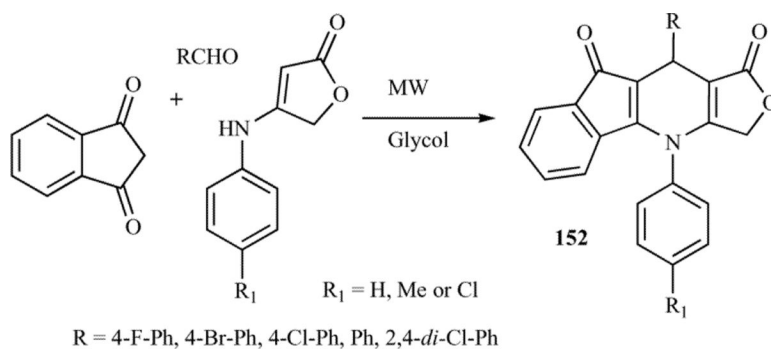


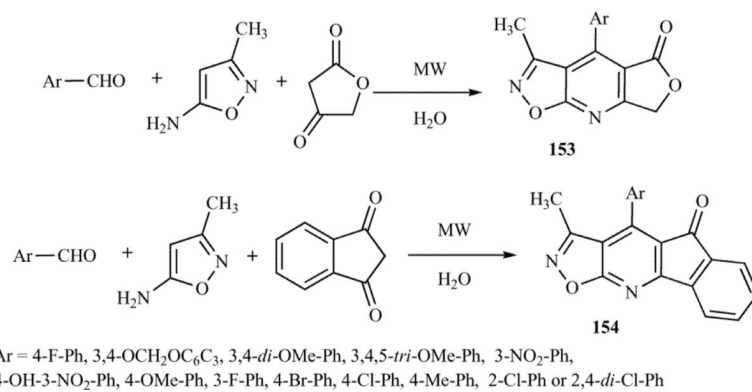
Scheme 16.

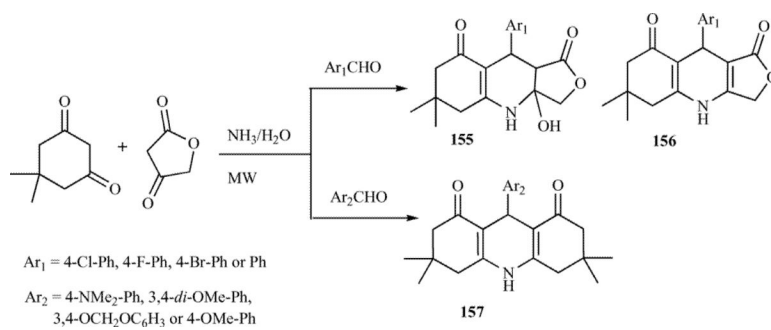


Scheme 17.

**Scheme 18.**

**Scheme 20.**

**Scheme 21.**

**Scheme 22.**

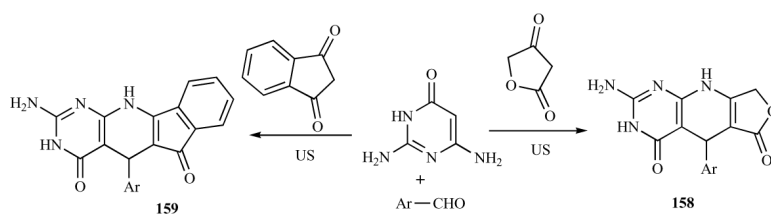
**Scheme 23.**

Table 1

Growth Inhibition of KB Cells by Azapodophyllotoxin Derivatives

Entry	Compound	ED ₅₀ (µg/mL)
1	<i>rac</i> 10	<0.3
2	<i>rac</i> 12	<0.3
3	<i>rac</i> 28	4.55
4	<i>rac</i> 29	<0.3
5	<i>rac</i> 26	0.62
6	<i>rac</i> 27	2.75
7	(-)- 10	<0.3
8	(+)- 11	<0.3
10	(-)- 11	38.5

Table 2

Antitumor and Cytotoxicity Activity of Diazapodophyllotoxin Analogues 69–73

Compds	Antitumor activity against P-388 leukemia T/C (%) @ different doses (mg/kg/injection) ^a										Cytotoxicity L1210 IC ₅₀ (µg/mL)
	2.5	5	10	25	50	100	250				
1	111		109	111	<85						0.0036
69a		117	144	161	239						0.42
69b			123	137	159	214					0.22
69c		119	131	153	165	233	<85				0.052
69d		117	133	150	172	94	<85				0.080
70a	111	124	128	150	161	172	>326				0.050
70b			110	139	156	206	206				0.16
70c		139	144	156	177	<85					0.055
70d		114	131	147	199	<85					0.028
71			111	122	147	147	161				0.75
72		104	109	114	134	153	<85				0.090
73		104	109	109	111	109	144				0.54

^a single i.p. treatment on day 1. T/C, median survival time of test animals/median survival time of control animals; 125% or above considered active; <85% considered toxic.

Table 3

Antitumor Activity of Podophyllotoxin and its Diaza-analogues Against P-388/VCR Leukemia and B16 Melanoma Cells

Compsds	P-388/VCR <i>T/C</i> (%) ^a @ different doses (mg/kg/injection)					B16 melanoma <i>T/C</i> (%) ^b @ different doses (mg/kg/injection)		
	1	2.5	5	10	25	25	50	100
1	122	125	<85%			110		
69a			137	<85		163	190	
70a		113	129	152	152		153	197

^a Dose given i.p on days 1–5. *T/C*: median survival time of test animals/median survival time of control animals; 125% or above considered active; <85% considered toxic.^b Dose given i.p. on days 1, 5, and 9.

Table 4

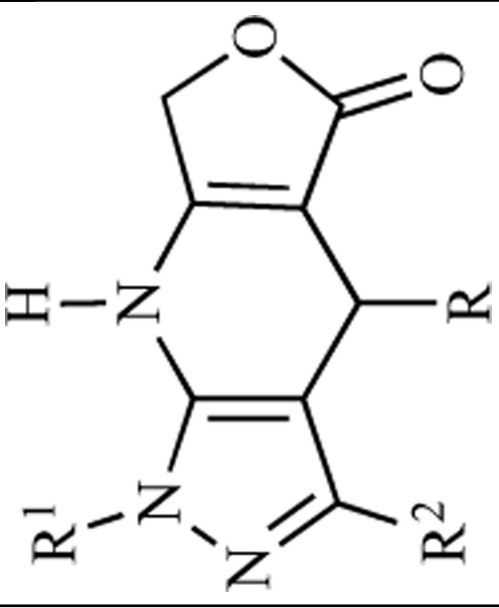
Cytotoxicity of Aza-Lignans 75a-n and 76a-n Against P-388 Leukemia Cells

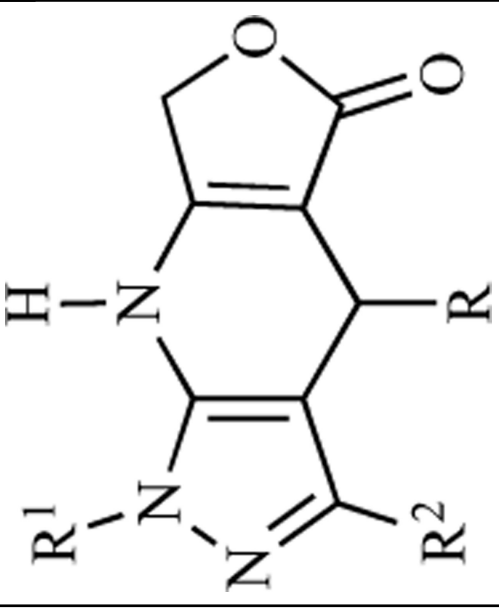
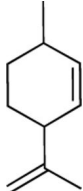
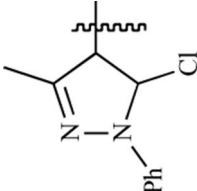
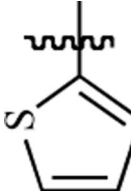
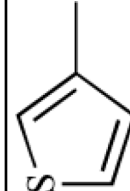
Compd	IC ₅₀ (µg/mL)	Compd	IC ₅₀ (µg/mL)
1	0.0043		
75a	>100	76a	0.0018
75b	80	76b	0.0017
75c	>100	76c	4.9000
75d	39	76d	0.7600
75e	2	76e	0.7700
75f	29	76f	2.6000
75g	>100	76g	0.0041
75h	63	76h	0.9200
75i	40	76i	0.0480
75j	>100	76j	0.0053
75k	>100	76k	0.1300
75l	60	76l	0.0053
75m	>100	76m	0.0300
75n	71	76n	0.0280

Table 5

Antiproliferative Activities of Dihydropyridopyrazoles 78–110

Compd	R			% cell viability			% apoptosis Jurkat
	R ₁	R ₂	R	HeLa	MCF-7/AZ	Jurkat	
1				19 ± 5	55 ± 3	18 ± 5	54 ± 2
2				91 ± 2	76 ± 2	75 ± 5	4 ± 3
76a				53 ± 5	58 ± 4	35 ± 6	49 ± 4
76c				54 ± 6	52 ± 3	54 ± 1	55 ± 4
78	Ph	4-Br-Ph	3,4,5-tri-OMe-Ph	73 ± 5	90 ± 3	99 ± 1	2 ± 1
79	4-Me-Ph	Me	3,4,5-tri-OMe-Ph	77 ± 4	71 ± 5	77 ± 2	23 ± 1
80	H	4-Br-Ph	3,4,5-tri-OMe-Ph	67 ± 4	98 ± 3	78 ± 7	5 ± 1
81	iPr	Me	3,4,5-tri-OMe-Ph	83 ± 5	99 ± 0	77 ± 9	4 ± 0
82	Me	Me	3,4,5-tri-OMe-Ph	55 ± 3	98 ± 1	79 ± 4	5 ± 1
83	H	Me	3,4,5-tri-OMe-Ph	50 ± 2	58 ± 5	47 ± 3	55 ± 4
84	H	Me	3,4-di-OMe-Ph	73 ± 3	95 ± 4	88 ± 2	5 ± 1
85	H	Me	4-OMe-Ph	73 ± 7	82 ± 3	94 ± 4	11 ± 1
86	H	Me	4-OCF ₃ -Ph	97 ± 2	84 ± 3	97 ± 6	9 ± 2
87	H	Me	4-SMe-Ph	45 ± 2	95 ± 4	87 ± 4	14 ± 6

Compd				% cell viability			% apoptosis Jurkat
	R ₁	R ₂	R	HeLa	MCF-7/AZ	Jurkat	
88	H	Me	Ph	74 ± 4	84 ± 3	87 ± 5	10 ± 2
89	H	Me	2-NO ₂ -Ph	71 ± 4	99 ± 2	64 ± 1	6 ± 1
90	H	Me	4-OH-3-OMe-5-NO ₂ -Ph	78 ± 3	89 ± 5	90 ± 2	2 ± 1
91	H	Me	3-Br-Ph	51 ± 3	63 ± 4	68 ± 3	58 ± 2
92	H	Me	3,5-di-Br-4-OH-Ph	58 ± 7	60 ± 4	58 ± 3	53 ± 0
93	H	Me	3-Br-4-OEt-5-OMe-Ph	52 ± 2	49 ± 4	28 ± 5	49 ± 1
94	H	Me	4-OAc-3-Br-5-OMe-Ph	47 ± 2	46 ± 2	36 ± 6	58 ± 1
95	H	Me	3-Br-4-NMe ₂ -Ph	55 ± 3	59 ± 3	34 ± 5	41 ± 1
96	H	Me	3-Br-4,5-di-OMe-Ph	16 ± 3	47 ± 1	29 ± 3	56 ± 1
97	H	Me	3-Cl-Ph	43 ± 4	54 ± 3	76 ± 4	28 ± 4
98	H	Me	3,4-di-Cl-Ph	88 ± 1	56 ± 3	71 ± 4	33 ± 4
99	H	Me	3-F-Ph	99 ± 1	76 ± 2	84 ± 2	28 ± 1
100	H	Me	2-Br-Ph	57 ± 4	100 ± 3	90 ± 1	8 ± 2
101	H	Me	4-Br-Ph	62 ± 3	92 ± 2	90 ± 1	10 ± 2
102	H	Me	Me	70 ± 4	97 ± 2	78 ± 5	5 ± 1

Compd				% cell viability				% apoptosis Jurkat
	R ₁	R ₂	R	HeLa	MCF-7/AZ	Jurkat		
103	H	Me		95 ± 5	91 ± 4	89 ± 6	14 ± 2	
104	H	Me		58 ± 2	100 ± 1	94 ± 2	6 ± 1	
105	H	Me		82 ± 4	98 ± 3	74 ± 4	18 ± 1	
106	H	Me		98 ± 2	100 ± 2	82 ± 5	6 ± 4	

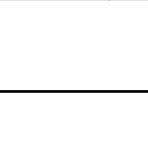

Compd	Chemical Structure			% cell viability				% apoptosis Jurkat
	R ₁	R ₂	R	HeLa	MCF-7/AZ	Jurkat		
107	H	Me		94 ± 1	100 ± 1	92 ± 5	4 ± 2	
108	H	Me		69 ± 4	96 ± 2	88 ± 9	11 ± 3	
109	H	Me		84 ± 4	100 ± 3	85 ± 6	7 ± 2	
110	H	Me		72 ± 6	100 ± 4	97 ± 3	11 ± 2	

Table 6Comparative Antiproliferative Potencies Expressed as GI₅₀

Compd	GI ₅₀ (μM) ^a HeLa	Compd	GI ₅₀ (μM) ^a HeLa	Compd	GI ₅₀ (μM) ^a HeLa
1	0.02	83	5 ± 1	94	4 ± 1
2	8 ± 2	91	5 ± 1	95	6 ± 1
76a	6 ± 1	92	8 ± 2	96	0.75 ± 0.1
76c	6 ± 1	93	5 ± 1	97	10 ± 2

^aConcentration required to reduce the viability of HeLa cells by 50%.

Table 7

Inhibition Rates of 4-Azapodophyllotoxins 136a-w on HepG2 Cells

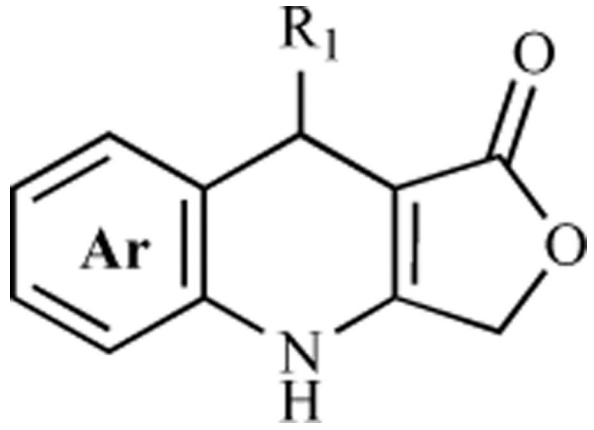
Compd			Inhibition rate (%)
	Ar	R ₁	
136a	4-Me-Ph	4-Br-Ph	74.63
136b	4-Me-Ph	4-Me-Ph	80.22
136c	4-Me-Ph	4-Cl-Ph	72.39
136d	4-Me-Ph	4-NO ₂ -Ph	77.61
136e	4-Me-Ph	3,4- <i>di</i> -Cl-Ph	79.97
136f	3-Me-Ph	4-Me-Ph	82.64
136g	3,4-OCH ₂ OC ₆ C ₃	3,4,5- <i>tri</i> -OMe-Ph	78.73
136h	3,4-OCH ₂ OC ₆ C ₃	4-F-Ph	76.49
136i	3,4-OCH ₂ OC ₆ C ₃	4-OMe-Ph	80.97
136j	3,4-OCH ₂ OC ₆ C ₃	4-Br-Ph	80.60
136k	3,4-OCH ₂ OC ₆ C ₃	4-Cl-Ph	80.22
136l	naphthalene -1-yl	4-OMe-Ph	64.06
136m	naphthalene -1-yl	4-Br-Ph	73.74
136n	naphthalene -1-yl	4-Cl-Ph	78.63
136o	naphthalene -1-yl	3,4-OCH ₂ OC ₆ C ₃	76.41
136p	naphthalene -1-yl	4-Me-Ph	82.19
136q	naphthalene -1-yl	2,4- <i>di</i> -Cl-Ph	76.41
136r	naphthalene -1-yl	thiophen-2-yl	71.51
136t	quinolin-6-yl	4-Cl-Ph	65.30
136u	3-Cl-4-MeC ₆ H ₃	4-Cl-Ph	74.63
136v	3-Cl-4-MeC ₆ H ₃	4-Br-Ph	85.76
136w	3-Cl-4-MeC ₆ H ₃	3,4- <i>di</i> -Cl-Ph	87.98

Table 8

Inhibition of Tubulin Polymerization, Cytotoxicity, and Morphological Effects on EA.hy 926 Endothelial Cells Caused by Azapodophyllotoxin Analogues

Compd	ITP ^a	Cytotoxicity: IC ₅₀ [uM] ^b		
		B16	NIH 3T3	EA.hy926
76a	0.47	0.016 ± 0.001	0.15 ± 0.07	0.15 ± 0.05
132	1.17	0.40 ± 0.01	0.5 ± 0.1	2.8 ± 1.5
134	>40	>30	>30	>30
135	2.89	38.4 ± 10.1	2.1 ± 0.8	8.6 ± 2.4
137	>40	>30	>30	>30
138	>40	>10	>10	>10
139	0.75	0.41 ± 0.21	0.08 ± 0.02	0.77 ± 0.05
140	>40	14.1 ± 2.9	7.5 ± 2.1	19.1 ± 1.8
141	>40	>30	>30	>30
143	>40	>30	>30	>30
159	0.72	0.010 ± 0.003	0.020 ± 0.005	0.03 ± 0.01

^a Inhibition of tubulin polymerization (ITP) is expressed as the ratio of IC₅₀ compd/IC₅₀ colchicine. IC₅₀ compd is the concentration of compound required to inhibit 50% of the rate of microtubule assembly, and the average IC₅₀ value for colchicine was 0.36 mM under the given conditions.

^b Concentration of compound corresponding to 50% growth inhibition after a 48-hour incubation.