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Recent Progress on C-4-Modified Podophyllotoxin Analogs as Potent Antitumor Agents

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Abstract

Podophyllotoxin (PPT), as well as its congeners and derivatives, exhibits pronounced biological activities, especially antineoplastic effects. Its strong inhibitory effect on tumor cell growth led to the development of three of the most highly prescribed anticancer drugs in the world, etoposide, teniposide, and the water-soluble prodrug etoposide phosphate. Their clinical success as well as intriguing mechanism of action stimulated great interest in further modification of PPT for better antitumor activity. The C-4 position has been a major target for structural derivatization aimed at either producing more potent compounds or overcoming drug resistance. Accordingly, numerous PPT derivatives have been prepared via hemisynthesis and important structure–activity relationship (SAR) correlations have been identified. Several resulting compounds, including GL-331, TOP-53, and NK611, reached clinical trials. Some excellent reviews on the distribution, sources, applications, synthesis, and SAR of PPT have been published. This review focuses on a second generation of new etoposide-related drugs and provides detailed coverage of the current status and recent development of C-4-modified PPT analogs as anticancer clinical trial candidates.

Keywords

podophyllotoxin; cytotoxic agents; C-4 position; reviews

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1. INTRODUCTION

Podophyllotoxin (PPT, **1**, Fig. 1), a naturally occurring aryltetralin lignan, holds a unique position among natural products having been known for approximately 1000 years from its first application in folk medicines to its most recent developments in PPT-derived antitumor agents.[1–8] Interest in PPT was initiated by Kaplan,[9] who demonstrated its curative effect against tumor growth (*Condylomata acuminata*), and subsequently by King and Sullivan,[10,11] who found its antiproliferative effect to be similar to that of colchicine at the cellular level. However, the initially high expectation for clinical use of PPT declined rapidly due to its unacceptable side effects, including nausea, vomiting, and damage to normal tissues. Nevertheless, due to its remarkable biological activity and extensive use in traditional medicine, PPT has remained an important starting point in the development of new therapeutic agents.

During early chemical modification studies, stereotransformation of α to β (epipodophyllotoxin, EPPT, **2**) at the C-4 position, together with 4′-*O*-demethylation, gave 4′-demethylepipodophyllotoxin (DEPPT, **3**). Investigation of semisynthetic glucoconjugates based on DEPPT led to two anticancer drugs, etoposide (ETO, **4**) and teniposide (**5**), as well as etopophos (**6**), a water-soluble prodrug of ETO (Fig. 1).[12–22] These compounds are currently used as drugs, alone or in combination with other agents, in clinical cancer chemotherapy against small cell lung cancer, acute leukemia, lymphoma, testicular carcinoma, and Kaposi's sarcoma. Notably, the two structural modifications leading to DEPPT-type compounds also led to a different mechanism of action (MOA). While PPT acts as antimicrotubule agent, ETO and **5** function as topoisomerase II (topo II) inhibitors.[23–25] Their clinical success and intriguing MOA stimulated great interest in further exploration of DEPPT derivatives with better antitumor activity. However, during the almost 50 years since clinical trials on ETO began in 1967, intense research efforts have resulted in both enthusiasm and setbacks. Studies showed that some nonsugar substituted analogs, particularly *N*-linked congeners, such as NK-611 (**7**), GL-331 (**8**), and NPF (**9**), as well as *C*-linked congeners, such as TOP-53 (**10**), exhibited superior pharmacological properties compared to ETO.[26–32] These compounds were brought into clinical evaluations; however, they did not proceed further. Recently, tafluposide (F11782) (**10**), a novel catalytic inhibitor of topo I and II, has also been obtained (Fig. 1).[33] Overall, these variants displayed improved water solubility and cytotoxic activity, as well as drug resistance and antitumor profiles, indicating that rational C-4 modifications can further optimize the activity of DEPPT. In in silico studies, both a composite pharmacophore model and comparative molecular field analysis[34] further demonstrated that the C-4 molecular area could accommodate considerable structural diversity. These results suggested valuable directions for structural modification and hence, many researchers focused their studies on the design and synthesis of C-4 modified analogs.

Excellent reviews[35–42] on PPT derivatives from a historical point of view and recent reviews[43–49] on the distribution, sources, applications, total synthesis, and structure–activity relationship (SAR) correlations of PPT by us and other groups are available. However, an updated review focused on the important C-4 position is needed to facilitate the progress of future research for developing PPT-based new drugs. Herein, we describe PPT-related

analogs containing carbon-, sulfur-, selenium-, oxygen-, and nitrogen-linked substituents at the C-4 position, as well as analogs containing spin labels in the C-4 substituent and at other positions. Orientations at the C-4 position include both α (as in PPT) and β (as in EPPT, DEPPT, and ETO), and substitutions at C-4′ include both methoxy (as in PPT and EPPT) and hydroxyl (as in DEPPT and ETO).

2. BIOLOGICAL ACTIVITIES AND MEDICAL APPLICATIONS

PPT-containing extracts have been widely used as folk remedies in traditional oriental medicine. They were commonly used in China, Japan, and the Eastern world as purgatives and to treat snake bites, periodontitis, skin disorders, coughs, various intestinal worm diseases, venereal warts (*C. acuminata*), lymphadenopathy, and certain tumors.[1,8,50] Today, PPT is still an effective and comparatively safe drug choice in the treatment of venereal warts. Besides antitumor effects, PPT analogs exhibit diverse activities, including reverse transcriptase (RT) inhibition and anti-HIV activity; immunomodulatory activity; effects on the cardiovascular system; antileishmaniasis properties; 5-lipoxygenase inhibition; insecticidal activity; phytogrowth inhibitory activity; ichthyotoxic activity; antimelanocortin-4 receptor (MC4R) activity; and antirheumatic, antipsoriatic, antimalarial, and antiasthmatic properties.[40,42,51]

Among the plethora of physiological activities and agricultural applications, the anti-neoplastic and antiviral properties of PPT congeners are arguably the most eminent from a pharmacological perspective. An alcohol extract of podophyllin was first cited in 1942 as a topical treatment for venereal warts, an ailment caused by a papilloma virus.[9] This study was one of the first to report the antiviral activity of podophyllin. In the early 1980s, both Bedows and Hatfield[52] and Markkanen et al.[53] found that PPT and related lignans showed antiviral activity against measles and herpes simplex type I. Other researchers[54–58] surveyed the effects of PPT analogs against multiple viruses, including Sindbis virus (RNA virus), murine cytomegalovirus (herpes DNA virus), vesicular stomatitis virus, and HIV. The antiviral effects of PPT analogs appear due to their ability to bind tubulin, disrupt the cellular cytoskeleton, and interfere with viral replication. In addition to tubulin binding, synthetic PPT analogs also inhibit viral RT, which may be exploited to selectively combat RNA viruses, such as HIV.[58] PPT is also effective in the treatment of anogenital warts in children and against *Molluscum contagiosum*, generally a self-limiting benign skin disease that affects mostly children, young adults, and HIV patients.[59]

Antitumor activity is probably the most well-known effect of PPT analogs. PPT-derived anticancer drugs are widely used in the treatment of Wilms' tumor, various genital tumors, non-Hodgkin's and other lymphomas, as well as lung cancers. Their powerful anticancer properties result from either inhibition of microtubule assembly or inhibition of DNA-topo II enzymatic activity. Combination therapies of ETO with other chemotherapeutic agents or techniques are currently being implemented.[41,45,60]

Studies on penetration of PPT into human bioengineered skin have demonstrated that PPT analogs induce acantholysis and cytolysis in this skin-equivalent model.[61] We reported that PPT derivatives exhibit insecticidal activity against some economically important insects,

more promising and pronounced activity as compared with PPT.[62–64] Additionally, antifeedant and phytogrowth inhibitory activities of PPT have been described, which have application to pesticides.[65,66] Other biological activities of PPT analogs are receiving increased current interest, for example, antioxidative properties, prevention of carcinogen production from estrogens, and inhibition of aromatase enzymatic activity, which could contribute to the prevention of dependent cancers.[67] A benzylidated PPT glycoside **(**CPH82) induced clinical improvement in patients with rheumatoid arthritis and interfered with the cell cycle in rapidly proliferating cells with accumulation of bone marrow cells in mitosis.[1,68] In addition, PPT analogs possess immunosuppressive activity and are seen as candidates for use in organ transplantation.[69–71]

3. MECHANISM OF ACTION

Cytotoxic PPT derivatives can be divided into two main types, tubulin polymerization inhibitors for “PPT-like” compounds and topo II inhibitors for “ETO-like” compounds. In 1976, Loike and Horwitz[72] reported that “PPT-like” compounds exert cytotoxic activity by inhibiting tubulin polymerization that prevents microtubule formation and destabilizes microtubules, as well as arresting cell division in metaphase. More recent studies have further explored the MOA of PPT-derived cyclolignans. Gordaliza et al.[73] proposed that PPT cyclolignans might work as alkylating agents at the C-2 methylene, rather than as acylating agents. Schönbrunn et al.[74] cocrystallized PPT with a tubulin fragment in 1999 and described the effects of microtubule damaging agents, such as PPT and colchicine,[75] on DNA and the cell cycle.[43,76] López-Pérez et al.[77] further described the role of dipole moment in the activity of PPT-related cyclolignans.

In contrast to PPT, 4′-demethylation and introduction of a *β*-glycosidic moiety at the C-4 position (“ETO-like” compounds) converts the resulting DEPPT derivatives into potent irreversible inhibitors of DNA topo II. Their action is based on formation of a nucleic acid–drug–enzyme ternary complex that blocks DNA strand relegation, generates DNA breaks, and blocks cell cycle progression in late S and G2 phases.[78–81] The cytotoxic effects of DEPPT analogs have also been strongly linked to metabolic activation of the dimethoxyphenol ring (E-ring), producing metabolites that form chemical adducts with and inactivate DNA.[82–84] These investigations pointed out that an ETO *ortho*-quinone is relevant to the MOA. 3′,4′-Catechol derivatives of ETO formed in the presence of cytochrome P-450 were further oxidized to 3′,4′-*ortho*-quinones in the presence of oxygen or under the influence of horseradish peroxidase or prostaglandin E synthase. Both catechol and *ortho*-quinone bound strongly to purified calf thymus DNA through formation of free radicals or even through direct binding of the quinone to the DNA, which may contribute to ETO’s cytotoxic activity.[82–84]

During the period of 2001–2010, many investigators reported various effects of PPT and DEPPT derivatives on cellular proteins and their signaling pathways. Lin et al.[85] showed that GL-331 decreased extracellular signal-regulated kinase (ERK) phosphorylation and subsequently inhibited cyclin D1 transcription. In studies by Tseng et al.,[86] PPT induced *c*-jun *N*-terminal kinase (JNK) phosphorylation at the molecular level, while ETO activated ERK, JNK, and p38 in selected tumor cell lines, as shown by Boldt et al.[87] Qi et al. reported

that GP-7, a new spin-labeled derivative of PPT, activated the caspase signaling pathway by releasing cytochrome *c*.[88,89] In tumor necrosis factor α (TNF-α) induced human aortic smooth muscle cells, deoxy-PPT strongly inhibited matrix metalloproteinases (MMP-9) expression and migration, as well as mRNA transcription of MMP-9 gene expression and phosphorylation of ERK 1, ERK 2, p38, and JNK, as reported by Suh et al.[90] Furthermore, Yong et al. described the involvement of deoxy-PPT in inhibition of tubulin polymerization and dysregulation of cyclin A and cyclin B1 expression, resulting in mitotic cell cycle arrest and activation of caspase-3 and caspase-7 to promote apoptotic cell death.[91] The same studies also demonstrated that deoxy-PPT caused cell cycle arrest of HeLa cells at the G2/M phase, followed by induction of apoptosis. Shin et al.[92] found that deoxy-PPT-induced apoptosis could involve the activation of p53 and ataxia-telangiectasia mutated and checkpoint kinase 2, possibly through a mitochondria-mediated pathway. Lastly, PPT can induce *c*AMP response element-binding protein (CREB) activation and CRE-driven gene expression via protein kinase An activation by a *c*AMP-independent mechanism, as shown by Chen and Xie.[93]

4. HEMISYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS

A. C-4-C Derivatives

In an attempt to obtain topo II inhibitors with higher potency and greater distribution in lung tissue, scientists at Taiho Pharmaceutical Co. Ltd. synthesized modified 4-deoxy-DEPPT derivatives with various C-4β alkyl (**12–18**), amidomethyl (**19–25**), and aminoethyl (**26–38**) groups (Fig. 2, Table I).[31,94] The new compounds with carbon rather than oxygen at the C-4 position were screened for cytotoxic activity against P388 mouse leukemia in vitro. Although the 4β-alkyl derivatives (**12–18**) did not inhibit topo II, their cytotoxicity was equal to that of ETO. Compounds **19–25** with various 4β-amidomethyl groups showed decreased inhibition of topo II, but did exhibit cytotoxic effects. Remarkably, 4β-aminoethyl derivatives TOP53, **34**, **35**, and **38** exhibited significant cytotoxic activity against P-388 cells with IC_{50} values ranging from 0.001 to 0.0043 μM. In addition, these compounds also displayed potent inhibitory effects on topo II with either improved or similar potency compared with ETO as illustrated in Table I. Among this series, TOP-53 was selected for further evaluation.[95] Compared with ETO, TOP-53 displayed twofold greater topo II inhibitory activity and superior in vivo antitumor activity against several cancer types, especially metastatic lung tumors. In view of its high potency and good properties, TOP-53 progressed to phase II clinical trials, but did not reach clinical use.

Roulland et al.[96] used Takai olefination to introduce a methylene moiety at the C-4 position. The resulting compound **39** was hydrolyzed and further oxidized to give compounds **40–42** (Table II). Among these four compounds, the greatest cytotoxicity was shown by **39** (IC_{50} 35 nM). The cell cycle perturbation induced by these compounds was studied in the same L1210 cell line. Compounds **40** and **41** induced partial accumulation of cells in the G_2/M phase of the cell cycle (42–69% vs. 24% for untreated control cells) at relatively moderate concentrations.

4β-Cyano-4-deoxy-DEPPT was synthesized by reaction of DEPPT with trimethylsilyl cyanide in the presence of boron trifluoride etherate.[97] Hydrolysis of this intermediate in

acetic acid gave 4*β*-carboxy-4-deoxy-DEPPT (**43**), from which a series of 4*β*-alkoxylcarbonyl (**43–68**), thionylcarbonyl (**69–82**), and carbamoyl (**83–96**) derivatives (Fig. 3) were synthesized and evaluated for inhibitory activity against L-1210 and KB cell lines in vitro.[98,99] However, most of the compounds exhibited IC_{50} values ranging from 0.05 to 1 *μ*M and thus were less active than ETO. Compound **53** exhibited the highest potency.

Recently, pinacol PPT analogs **97–122** bearing different side chains and functional groups at C-4 were synthesized through reductive cross-coupling of PPT with different aldehydes and ketones (Table III).[100] In general, these compounds were fairly cytotoxic, and some of them (e.g., **115**, **117**) displayed better activity than the reference EPPT. Among all compounds, 4*β*-OH (epipodo) derivatives showed greater cytotoxicity than their corresponding 4*α*-OH (podo) analogs. Similarly, to PPT, the former compound series arrested the cellular cycle of A-549 cells at the G2/M phase, with differences only in potency. Significantly, 4-isopropyl-4-deoxypodophyllotoxin (**120**) was identified as a promising lead compound for a novel type of tubulin polymerization inhibitor.

B. C-4-S Derivatives

Showalterk et al.[101] reported compounds **123–126**, which are thioglucose-derived analogs of ETO (Fig. 4). They expected that substitution of sulfur in various oxidation states for the glycosidic oxygen would not only alter lipophilicity, but also vary the geometric disposition of the sugar moiety on the aglycone. Although this change should affect the biological activity remarkably, no biological data were reported.

Moreover, Wang et al.[102–104] developed two efficient methods employing the $BF_3{\cdot}Et_2O/H_2S$ and CH_3COSH/NH_3 reagent system to synthesize 4-sulfanyl-DEPPT. This compound then served as a valuable building block for the preparation of versatile 4*S*-substituted-DEPPT analogs, including various alkylthio (**127–135**) and 4-(2-aminoethylthio) (**136–154**) derivatives (Fig. 5). Most of the compounds showed comparable or better activity than ETO against L1210 and KB cells in vitro. A large activity range for **127–154** indicated that the substituents on 4*S*-derivatives can markedly affect the activity profiles of this compound class.

Many triazoles exhibit a wide range of biological activities, such as antifungal, antiviral, antiphlogistic, antitumor, and other effects. Therefore, Lu et al.[105] prepared seven DEPPT analogs linked through the sulfur of various 4-amino-5-alkyl-4*H*-1,2,4-triazole-3-thiol compounds. When screened in vitro against HL-60 and K562 cells, compounds **155–161** were more potent against the latter cell line (Table IV).

C. C-4-Se Derivatives

Se-derived PPT analogs have received very little attention, even though such molecules could be potential new pharmaceutical agents, as Se compounds are promising molecules in cancer prevention and have potential in cancer treatment.[106] Accordingly, Wang et al.[107] synthesized six 4-alkylselenyl-DEPPTs (**162–167**, Fig. 6) from 4-bromo-DEPPT and selenourea in the presence of Et_3N. All six compounds showed potent cytotoxic activity against L-1210 and KB cells. Miao et al.[108] found that 4-phenylselenyl-DEPPT (**168**, Fig. 6)

suppressed the proliferation of human hepatoma SMMC-7721 cells in a dose- and time-dependent manner and induced SMMC-7721 cell apoptosis by translocation of Bax, activating the mitochondrial pathway of apoptosis through release of proapoptotic factors, such as cytochrome *c*. Four related 4-selenyl-DEPPT derivatives (**169–171**, Fig. 6) synthesized by Zhang and Cao[109] showed cytotoxicity against HO-8910 cells.

D. C-4-O Derivatives

While ETO and **5** are *O*-glycosides of DEPPT, other nonglycosidic ether substituents have been incorporated into DEPPT in order to lower toxicity, while maintaining or enhancing activity. Terada et al.[110] synthesized various DEPPT ethers (**172–186**, Fig. 7) by reaction of protected 4′-*O*-benzyloxycarbonyl-DEPPT with alcohols in the presence of boron trifluoride etherate,followed by deprotection. The introduction of an aminoalkoxy group at the C-4 position increased both DNA topo II inhibitory and cytotoxic activities.[110]

Ma and co-workers[111,112] also reported a series of 4β-ether-DEPPT (**187–199**) and five 4,4′-*O*-bis(2-hydroxy-3-substituted-amino)-propyl-DEPPT (**200–204**) analogs that were screened for cytotoxic activity against L-1210 and KB cells (Fig. 8). Most compounds showed equivalent or greater potency compared to ETO, in particular, certain compounds with side chains containing a hydroxyl or an amino group.[111,112] Recently, Bathini et al.[113] synthesized 4β-*O*-propenylethers (**205–209**, Fig. 8) from 4-chloro-4-deoxy-PPT and allylic alcohols in the presence of barium carbonate. The cytotoxicity of these compounds against KB cells was comparable to that of ETO.

Because an ester group can be important to the cytotoxicity and antileukemic activity of compounds in certain classes, Lee and co-workers [114] prepared a series of PPT esters (**210–221**, Fig. 9) and examined their in vivo antileukemic activity against P-388 lymphocytic leukemia cell growth in mice. The biological data indicated that the introduction of an ester moiety into PPT did not enhance antileukemic activity, but instead generally caused a loss of activity.

In order to determine whether the presence of a glucosidic moiety on the C-4 position is essential, Gupta and Chenchaiah[115] synthesized different C-4 ester DEPPT analogs (**222–235**, Fig. 10) through the same general procedure described by Lee and co-workers[114] In biological studies, these analogs possessed similar biological activity to PPT, and none acted in the same manner as ETO. Treatment of CHO cells with these esters caused a large increase in the mitotic index of cells, and the SAR on these esters provided information regarding the role of substituents at the C-4 position on PPT-like activity.

More recently, López-Pérez et al.[116] synthesized various hydrophobic esters of PPT, including norbornene-carboxylate esters prepared through Diels-Alder cycloaddition by treating dienophilic acrylates of cyclolignans with cyclopentadiene. Compounds **236–241** (Fig. 11) were tested for in vitro cytotoxicity against four neoplastic cell lines (P-388, A-549, HT-29, MEL-28). The results showed that the introduction of a linear or aromatic C-4 acyl moiety in PPT analogs induced either loss or no effect on the cytotoxicity of the parent hydroxy derivatives, while C-4α-PPT norbornene-carboxylates **241a,b** showed improved potency compared with PPT. The introduction of one additional methylene unit

between the bicyclic system and the carboxyl group (**240**) generated more hydrophobic esters, but led to diminished activity. These results indicated that the cytotoxic activity is not primarily due to a lipophilic factor and the precise spatial arrangement of a bulky moiety may contribute to additional nonpolar interactions that can enhance binding to the target site.

Because TOP-53 showed promising potential in the treatment of several types of cancer compared with ETO, Duca et al.[117] introduced a carbamate substituted with a *N*-alkylamino or *N*-alkylazido group in the C-4β-position of DEPPT to give DEPPT-4-amino/azidoalkylcarbamates (**242–247**, Table V). In particular, compound **247** with an *N*-methyl-*N*-azidopropylcarbamate group displayed potent cytotoxicity against the L-1210 cellline (IC_{50}, 0.038 μM compared with 0.83 μM for ETO) and proved to be a more potent topo II poison than ETO.

Recently, a series of new 4β-carbamoyl-PPT analogs were prepared by Kamal et al.[118] (**248–257**, Table VI) and evaluated for cytotoxic activity against 11 cancer cell lines. As shown, most of the compounds exhibited better growth-inhibition activity than ETO against the tested cell lines. Compounds **254** and **256** were also evaluated for DNA topo II inhibitory activity and showed significant inhibition comparable to that of ETO.

In view of the significance of long-chain fatty acid (FA) in the treatment of cancer, Mustafa et al.[119] prepared a series of FA analogs of PPT (**258–267**, Table VII) by coupling unusual C-10 to C-20 FAs with the 4α-hydroxyl of PPT. These compounds were investigated for in vitro cytotoxic activity against a panel of human cancer cell lines, including SK-MEL, KB, BT-549, SK-OV-3, and HL-60 cells. All of the compounds showed significant cytotoxicity against all tumor cells tested. With IC_{50} values ranging from 0.07 μM for HL-60 cells to 0.4 μM for KB cells, compounds **258** and **263** were the most active analogs. Interestingly, they did not affect the growth of noncancerous mammalian cells (VERO cells) up to the highest concentration (15 μM) used in the assay, demonstrating promising selectivity toward tumor cells.

Based on these preliminary results, the same group further synthesized additional PPT-FA adducts (**268–277**, Table VIII).[120] These compounds were assayed in vitro against four human solid tumors (SK-MEL, KB, BT-549, SK-OV-3, and HL-60) and noncancerous VERO cell lines. The SAR indicated that a “12-hydroxy-*Z*-ene” system was probably important for activity against the human neoplastic cell line panel. The FA thioether analogs **276** and **277**, derived from **275**, showed promising activity against all five cancer cell lines, with one exemption; **277** was not active against ovary carcinoma (SK-OV-3) cells. Unlike PPT, none of these compounds were toxic toward normal mammalian cells, demonstrating selectivity for cancer cells over normal cells.

Recently, we reported[121] the synthesis of novel sulfonylamidine PPT derivatives (**278–285**, Table IX) via a Cu-catalyzed reaction along with their in vitro evaluation against a panel of human cancer cell lines, including K562, SGC, Hela, and HepG.[121] MOA studies were also investigated. Compound **282** displayed significant antiproliferative activity against all four cell lines and strong tubulin polymerization inhibitory effects. The results indicated that these compounds effectively interfered with tubulin dynamics and prevented mitosis in

cancer cells, thus leading to cell cycle arrest and, eventually, dose-dependent apoptosis. In addition, docking analysis and molecular dynamics showed that the binding of **282** to tubulin was mainly stabilized by hydrophobic interactions, together with hydrogen-bonding interactions with *β*-tubulin's Cys241 residue.

To overcome multidrug resistance (MDR) and lower the toxicity of PPT derivatives, Yu et al.[122] synthesized 4-*O*- and 4-*N*-indol-3-yl-glyoxyl-substituted derivatives of PPT and tested them against a panel of four human cancer cell lines, including HeLa (cervix), SKOV3 (ovary), K562 (leukemia), and K562ADR (adriamycin-resistant leukemia) in vitro. Generally, the *O*-linked derivatives (esters) of PPT showed greater potency than the corresponding *N*-linked congeners (amides). Compound **286** (L1EPO, Fig. 12) down-regulated the mdr-1 gene, reduced the expression of P-gp, and displayed dose-dependent cytotoxicity. Moreover, it was less cytotoxic against normal human cell lines (fibroblast, VEC, $GI_{50} > 10\ \mu M$) than cancer cell lines. L1EPO has the potential to overcome P-glycoprotein-mediated MDR in the K562/A02 cell line.[123]

As shown in Figure 13, Gupta et al.[124] prepared three DEPPT–lexitropsin conjugates (**287–289**) with the aim of conferring higher affinity for DNA and improving cellular uptake and metabolic stability. Investigation of the biological effects of these bifunctional hybrids demonstrated that conjugation with minor groove-binding moieties could alter or increase the number of topo II–induced cleavable sites.

Since nucleosides are biologically active moieties, Derry et al.[125] synthesized two novel derivatives of DEPPT and EPPT (**290** and **291**, respectively, Fig. 13), in which the nucleoside thymidine was conjugated with the parent compounds at the C-4 position using boron trifluoride etherate. The observed cross-resistance patterns of the thymidine derivatives suggested that these compounds display PPT-like activity without ETO-like activity. The two thymidine derivatives exhibited much lower activity in comparison with PPT and DEPPT, suggesting that the thymidine moiety interferes with the compounds' interaction with the receptor site on the tubulin molecule.

In order to improve the therapeutic efficacy of PPT, a novel PPT conjugate, 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimido-acetamidoglycinylglycine PPT ester (ETPA-gly-gly-PPT, **292**, Fig. 14), as well as its homo- and copolymer with acrylic acid were prepared by photopolymerization using 2,2-dimethoxy-2-phenylacetophenone as a photoinitiator.[126] Compared with PPT (IC_{50} 1.4–4.0 ng/mL), compound **292** showed decreased cytotoxicity (IC_{50} 13–100 ng/mL) against A375, KM12, PC3P, and CEM cancer cell lines.

Pharmacomodulation of biologically active compounds via conjugation approaches has become an appealing research area in different fields of medicinal chemistry. Also, the concept of a bivalent molecule is now accepted as an effective strategy for designing ligands, inhibitors, and drugs that influence biological systems. Thus, Passarella's group[127–130] recently reported a series of novel PPT-based hybrids. Some of these compounds exerted significant antiproliferative activity, having a marked ability to inhibit tubulin polymerization in vitro and to disrupt the microtubule network in vivo. Among the synthesized disulfide dimers of PPT, increased spacer length resulted in progressive

reduction of antiproliferative activity against the NCI-H460 human nonsmall cell lung carcinoma cell line. For example, the IC_{50} values of compounds **293** and **294** were 0.03 and 0.3 μg/mL, respectively, compared with 0.005 μg/mL for PPT.[130] Simultaneously, our group[131] also described disulfide dimers of PPT (**293–297**; Fig. 15), in which two PPT moieties were linked with different dithiodicarboxylic acid spacers.

E. C-4-N Derivatives

Allevi et al.[132] condensed 4-bromo-4-deoxy-DEPPT with 4,6-*O*-ethylidene-2,3-di-*O*-trimethylsilyl-β-D-glucopyranosylamine in the presence of $Hg(CN)_2$ to produce a 4-aminoglucose analog (**298**, Fig. 16) of ETO. However, no biological data were reported.

In recent years, C-4-*N*-substituted analogs have occupied a significant position in the development of DEPPT-derived antitumor agents. Lee and co-workers performed pioneering work in this field and have studied C4-*N*-substituted congeners of DEPPT for many years.[133–141] The various projects below illustrate several aspects of the development process. First, C-4α and C-4β–aliphatic amino derivatives (**299–306**, Fig. 17) were synthesized by direct nucleophilic substitution of 4-bromo-4-desoxy-DEPPT with appropriate alkylamines. Replacement of the glycosidic moiety of ETO with a 2″-hydroxyethylamino or 2″-methoxyethylamino moiety at the C-4β position resulted in potent inhibition of human DNA topo II, as well as strong ability to cause cellular protein-linked DNA strand breakage (compounds **299**, **303**, and **305**).[133] The C-4β isomers were more potent than the C-4α isomers, which indicated that the C-4 stereochemistry is quite important in determining the inhibitory potency.

In subsequent studies,[134] numerous substituted-4β-anilino side chains were introduced into the DEPPT structure by nucleophilic substitution reaction of 4β-bromo-4′-demethyl-4-desoxyPPT with various substituted arylamines (Table X). The modification resulted in substantially increased activity. Most of the 4β-arylamino-DEPPTs were as or more potent than ETO in topo II inhibition and/or cellular protein–DNA complex formation assays. In most cases, *para* substitution in the phenyl ring resulted in the greatest activity.[29,30,134] Compared with ETO, compounds **308**, **312**, and **324** were tenfold more active in inhibiting DNA topo II and caused two to three times more protein–DNA complex formation. As a highlight, GL331 (**329**) was selected as the optimal drug candidate. GL-331 functions as a highly potent topo II inhibitor, causing DNA double-strand breakage and G2 phase arrest. It could also induce cell death by stimulating protein tyrosine phosphatase activity and apoptotic DNA formation. GL-331 was also shown to be active in many MDR cancer cell lines. Because of its good stability and biocompatability, as well as favorable pharmacokinetic profiles, GL331 successfully reached clinical trials against several forms of cancers, especially ETO-resistant malignancies, but has not reached clinical status.

The synthesis and biological evaluation of a series of 4β-benzylamino (**330–343**) and 4β-benzoylamino (**344–355**) derivatives were then reported by the same group (Fig. 18).[135,136] These compounds were less potent than the 4β-arylamino derivatives, but most were as active or more active than ETO. In topo II inhibition and protein–DNA assays, the activity was observed in the order of 4β-arylamino > 4β-benzylamino > 4β-benzoylamino.

Subsequently, Lee and co-workers[137] synthesized another series of 4β-amino derivatives of DEPPT (Table XI) that could form water-soluble salts. Most compounds showed excellent activity in KB cell cytotoxicity and protein–DNA complex formation assays. In general, the salts showed comparable or greater activity than their parent-free bases. Compared with ETO, compounds **357** and **359–365** showed comparable or greater inhibition of human DNA topo II. In a dose–response study of protein–DNA complex formation, compound **359** was 20 times more active than ETO. Furthermore, both compound **359** and its free base **358** were highly active against ETO-resistant KB cell lines.

To further address poor water solubility, a problem associated with ETO, Lee and coworkers[138] introduced protected α-amino acids into a 4β-[(*p*-benzamido)-amino] side chain of new DEPPT derivatives (Table XII). Compounds **369** and **370** exhibited better preclinical activity profiles, including cell growth inhibition, cell killing, and in vitro topo II inhibition, as compared to the prototype molecule ETO, while retaining the superior drug-resistance profile of GL-331. This observation indicated that introduction of bulky substituents at the *para* position of an anilino moiety would enhance topo II inhibition and still maintain superior cell growth inhibition and drug-resistance profiles. Other members in this series of 4β-[(*p*-benzamido)-amino]-DEPPT derivatives were also reported in detail by Lee and co-workers,[139] revealing important SAR information. The large activity range of compounds **369–381** (Table XII) indicated that the substituents on the α-carbon of the amino acids markedly affected the activity profiles of this compound class. The impressive ability of compounds **372**, **374**, and **376** to induce intracellular protein-linked DNA breaks suggested that a hydrophobic interaction might exist between the enzyme/DNA complex and this molecular area of the compounds. Inclusion of moieties containing nitrogen or oxygen atoms in the amino acid side chains (e.g., compounds **369**, **370**, **375**, **377**, and **380**) decreased protein-linked DNA breakage. Adding hydroxy groups on the phenyl ring sharply decreased the activity (e.g., compounds **369**, **376**, and **377**). Many factors could possibly contribute to this decrease. First, hydrogen bonding between the nitrogen or oxygen atom and the enzyme/DNA complex might cause the activity loss, presumably by preventing the molecules from assuming an optimal conformation. Second, it is also possible that relatively polar (e.g., hydroxyl in compounds **369** and **377**, imidazole in compound **375**) and sterically bulky (e.g., indolyl in compounds **370** and **380**) moieties might impede an important hydrophobic interaction between the molecules and enzyme/DNA complex. The orientation of the amino acid also showed a stereo preference (compare **372** vs. **373**; **381** vs. **370**). Hydrolysis of the methyl ester in **370** to give the free acid in **380** resulted in a dramatic reduction in cellular protein–DNA complex formation and cell growth inhibition. Interestingly, changing the amino acid to an alkylamine (i.e., deletion of the COOMe) significantly increased the inhibitory potency against KB cell growth; however, it also led to unfavorable drug-resistance profiles (compounds **378** vs. **369**; **379** vs. **376**). The unique activity profiles of compounds **378** and **379** implied that their action mode or cellular uptake mechanisms might be different from those of their amino acid congeners.[139]

Lee and co-workers also employed a conjugation strategy to overcome drug resistance or enhance cytotoxic activity.[140,141] They linked 4β-amino-DEPPT derivatives with camptothecin (a topo I inhibitor) or taxol derivatives (microtubule assembly promoters)

through aromatic bridges (Fig. 19). Surprisingly, the DEPPT-camptothecin hybrids **382** and **383** did not show cross-resistance in ETO-resistant cells. The two compounds inhibited topo I in a concentration-dependent manner, but only **383** was active against topo II. In a nude mouse model with human prostate DU-145 tumor cells, compound **383** showed better antitumor activity than ETO, camptothecin, and **382**. The cytotoxic potencies of the taxoid conjugates **384** and **385** were between those of ETO and the parent taxoid. Taxoid conjugate **386**, which has an additional DEPPT moiety at the taxoid 7-hydroxyl, was least active, suggesting that the 7-hydroxyl was important to the cytotoxic activity. Against paclitaxel-resistant cells, conjugates **384** and **385** showed enhanced activity. In contrast to the cytotoxic results, compound **386** was a better topo II inhibitor than **384** and **385**. The authors postulated a role for the 7-hydroxy group in drug transport.

The increased activity incurred by replacing the glycosidic moiety of ETO with 4-amino groups also led to the synthesis and testing of numerous 4-anilino-, 4-amido-, 4-arylamino-, 4-sulfonylamino-, and 4-alkylamino-DEPPT derivatives by other groups.[142–148] For example, Kamal et al.[149] synthesized 4β-amido or 4β-sulfonamido derivatives of DEPPT. All five 4β-amido-2-substituted benzophenone analogs (**387–391**) showed moderate cytotoxicity against the tested cell lines. Among the 4β-benzenesulfonamido derivatives, 4′-methylated analog **396** was highly cytotoxic, but the corresponding 4′-demethylated analog (**398**) was about 100-fold less potent. A methyl substituent on the phenyl ring at the *para*-position (**399**) further decreased activity by tenfold. Two (**392**, **394**) of the four 4β-nicotinylamido substituted analogs (**392–395**) showed high cytotoxicity. Overall, 4′-methylated derivatives were more cytotoxic than corresponding demethylated compounds (**397** vs. **399** and **392** vs. **393**; Fig. 20).[149]

As shown in Figure 21, Kamal et al.[150] also synthesized 4β-*N*-heteroaryl analogs (**400–404**) that exhibited better in vitro cytotoxic activity than ETO. Compound **402** with a fluoro substituent on the phenyl ring showed significant activity with GI_{50} values less than 0.010 μM against all six tested cancer cell lines, while the GI_{50} values of **401** without a fluoro group were 0.31, 0.02, 0.06, 0.03, 0.09, and 0.11 μM against DU145, HT29, MCF7, MCF7ADR, NCIH460, and U251 human cancer cell lines, respectively.

Current interest in dimeric analogs of lipophilic, neutral, DNA mono-intercalating agents as potential antitumor drugs prompted Kamal et al.[151] to prepare bis-4β-amino-PPT dimers by linking the amino groups through various aryl spacers (**405–418**; Fig. 22). Most of the analogs exhibited promising in vitro cytotoxic activity against different human tumor cell lines. Interestingly, compared with ETO, 4′-methylated analogs showed superior topo II poisoning activity. Dimer **409** linked through a biphenyl spacer was the most active tested compound with GI_{50} values of 0.2, 0.2, and 0.6 μM against DU145, HT29, and MCF7, respectively.

More recently, six series of 4-*N*-substituted PPT derivatives were synthesized by Kamal et al.[152–157] and evaluated for cytotoxicity against selected human cancer cell lines or for DNA topo II inhibitory activity. The first new compounds were a series of 4β-*N*-polyarylamino congeners (Table XIII) reported in 2010.[152] Cytotoxic activity was evaluated against several tumor cell lines (502713, HCT-15, HEP-2, IMR-32, A-549, DU-145, and PC-3). As shown

in Table XIII, most of the new compounds exhibited significant cytotoxic activity compared with ETO. Selected compounds **423**, **425**, and **426** also caused similar inhibition of DNA topo II catalytic activity compared with ETO. Cell cycle studies with **425** revealed that these compounds exert apoptosis-inducing activity.

In 2011, the same group designed and synthesized a series of benzothiazolo-4β-anilino-PPT derivatives through a one-pot iodination methodology.[153] These compounds exhibited significant cytotoxic activity against Colo205, HT1080, and DWD cell lines, and moderate activity against Hop-62 (Table XIV). In particular, compound **432** [IC_{50} 2.7 μM (Colo205), 2.3 μM (DWD)] was more potent than PPT (5.1 and 5.0 μM). All 16 compounds exhibited similar in vitro inhibition of topo II catalytic activity compared with m-AMSA.

As depicted in Table XV, Kamal et al.[154] obtained a series of new 4β-acrylamido-PPT derivatives (**447–461**) synthesized by coupling substituted stilbene moieties with 4β-amino-PPT. These compounds showed significant cytotoxic activity with GI_{50} values ranging from <0.1 to 0.29 μM. Compounds **456–458** caused G2/M cell cycle arrest, with **457** being slightly more effective. Interestingly, compound **457** also caused both single-strand (30%) and double-strand (70%) DNA damage, eventually leading to cancer cell death. The study results suggested that these compounds exhibit dual topo I and topo II inhibitory activities.

As outlined in Table XVI, a series of 4β-alkylamidochalcone as well as 4β-cinnamido linked PPTs were also synthesized by Kamal et al.[155] and evaluated for cytotoxic activity against five human cancer cell lines (A-549, A375, MCF-7, HT-29, and ACHN). From the screening results, chalcone–PPT conjugates **462–477** showed moderate activity against different cancer cell lines (IC_{50} 5.3–26.7 μM). The activity did not change with different lengths of the alkane chain spacer between the chalcone and PPT moieties. Comparatively, quinolino–chalcone linked PPTs **487–490** showed promising activity with IC_{50} values ranging from 2.2 to 15.4 μM. Finally, the cinnamido–PPT conjugates **478–486** exhibited IC_{50} values ranging from 2.1 to 9.5 μM against the A-549 cancer cell line, but generally were less active against the other tested cell lines. However, compounds **478** and **483** showed significant activity against A-549 (IC_{50} 2.7 and 2.1 μM), as well as HT-29 (IC_{50} 2.36 and 0.37 μM) and ACHN (IC_{50} 3.91 and 2.18 μM) cancer cell lines. The IC_{50} values of ETO in the respective cell lines were 2.34, 1.81, and 7.61 μM. Flow cytometric analysis showed that compounds **478** and **483** arrested the cell cycle in the G2/M phase leading to caspase-3 dependent apoptotic cell death. Furthermore, Hoechst 33258 staining and DNA fragmentation also suggested that **478** and **483** induced cell death by apoptosis.

A series of new 4β-sulfonamido and 4β-[(4′-sulfonamido)benzamide] derivatives of PPT (**491–500** and **501–507**) were synthesized and evaluated for cytotoxic activity against six human cancer cell lines (Table XVII).[156] All compounds exhibited significant cytotoxic activity with GI_{50} values ranging from 0.04 to 2.90 μM. Compounds **492**, **494**, and **495** showed more potent cytotoxic activity against Colo-205 cells than ETO. Flow cytometric analysis showed that the three compounds caused G2/M cell cycle arrest, and especially **494** caused both single- and double-strand DNA breaks. Compound **494** inhibited topo IIα as observed from Western blot analysis, as well as activated caspase-3, p21, p16, and NF-kB,

and down-regulated Bcl-2 protein. These findings suggested that **494** can induce apoptotic cell death, apart from acting as a topo IIα inhibitor.

More recently, Kamal et al.[157] synthesized three series of heteroaromatic linked 4β-carboxamido-PPT derivatives (**508–516**, **517–525**, and **526–529**, Table XVIII) and evaluated their cytotoxicity against five human cancer cell lines (A-549, HeLa, MCF-7, HT-29,and ACHN). Among all compounds, analog **523** with a (3,4-dichlorophenyl)-1*H*-pyrazolecarboxamide exhibited significant activity against A549 (IC_{50} 2.1 μM) and good activity against other cell lines, including HT-29 (7.1 μM), B-16 (9.3 μM) and HeLa (9.5 μM), whereas most of the other derivatives showed moderate to weak activity against the tested cancer cell lines. Flow cytometric analysis of **523**-treated cells showed cell cycle arrest in the G2/M phase. Hoechst 33258 staining and DNA fragmentation assays revealed that **523** induced cell death by apoptosis. Further, activation of caspase-3 also suggested that **523** produced apoptotic cell death.

During the period of 2002–2009, Tian and co-worker's laboratory[158–160] reported the synthesis and biological evaluation of many 4β-*N*-substituted-5-fluorouracil-DEPPT derivatives. Most of these analogs showed more significant cytotoxic activity against certain tumor cell lines compared with ETO and 5-fluorouracil (5-FU). Also, compounds **530–536** derived from DEPPT were evaluated as inhibitors of stromelysin-1 as well as collagenase-1 (Fig. 23). Among them, compounds **530** and **531** demonstrated superior inhibitory activity against stromelysin-1 compared with ETO and 5-FU.

Furthermore, 12 novel conjugates were synthesized by coupling DEPPT with 5-FU-N^1-alkyl amino acid esters (**537–548**).[161] When evaluated against four tumor cell lines (HL-60, K562, AGS, and A-549), most of the compounds showed more potent inhibition than ETO (Table XIX). Also, the new analogs showed superior water solubility, with lower log*P* values than ETO (Table XIX). In addition, the DNA conformation changed from B- to C-form in the presence of **548**, likely due to interaction of the compound with calf thymus DNA. Compound **548** was also relatively resistant to metabolism by human plasma.

Guianvarch et al.[162] synthesized a series of novel 4α-sulfonamide derivatives of DEPPT as depicted in Table XX. Their effects on human topo II and, in some cases, tubulin polymerization were evaluated. The alkyl side chain on the 4β-sulfonamide substituent had an important effect on the topo II inhibitory activity. Methyl sulfonamide analog **549** with the shortest side chain showed the strongest inhibitory activity that decreased sharply for butyl sulfonamide analog **550** with an increased chain length. Derivatives bearing an aromatic ring on the 4β-sulfonamide substituent displayed low topo II inhibition but comparable or slightly better cytotoxic potency (except **557** and **561**) than ETO. Substitution of the 4β-sulfonamide group with six different amino (**562–567**) rather than alkyl or aryl substituents preserved high topo II inhibition and cytotoxic activity (except for **564**). With T/C of 235 and 229% against P388 leukemia in vivo, the most active compounds contained morpholine (**566**) and piperazine (**567**) sulfonamides. However, these two compounds did not induce long-term survivors (LTS). Comparatively, ETO exhibited a T/C equal to 233%, with 6 of 42 LTS. Against an A-549 orthotopic model of lung carcinoma in vivo, **566** and **567** (T/C 155 and 159%, respectively) were more efficient than ETO (T/C 122–131%). The

activity correlated with the inhibitory activity against topo II and marked accumulation of cells in the G2/M phase.

Guo et al.[163] synthesized seven novel PPT and DEPPT derivatives (**568–574**, Table XXI) with 2-(1*H*-indol-2-yl)-2-oxoacetamides at the C-4β position and tested their cytotoxic activity against five human cancer cell lines, HeLa, KB, KBV, K562, and K562/AO2. Most of the compounds demonstrated improved in vitro antitumor activity and, most importantly, improvedanti-MDR activity compared with ETO. As shown in Table XXI, compounds **568**, **571**, and **572** exhibited stronger cytotoxic activity than ETO against HeLa cells, while derivatives **569–571**, **573**, and **574** were more effective than ETO against K562 and K562/AO2 cells.

Shang et al.[164] synthesized ten new 4β-imidazolyl PPT and DEPPT analogs (**575–584**, Fig. 24). All compounds were evaluated for cytotoxicity against three human cancer cell lines. Compound **580** exhibited the highest cytotoxicity with IC_{50} values of 8.12 ± 1.03, 7.43 ± 0.62, and 4.16 ± 0.54 μM. The latter activity against the K562/ADM drug resistant cell line was particularly notable. In comparison, the IC_{50} values of ETO were 2.99 ± 0.87, 6.0 ± 1.84, and 76.55 ± 8.36 μM, respectively.

As outlined in Table XXII, Wang et al.[165] synthesized three series of new 4β-anilino-DEPPT derivatives (**585–613**) for cytotoxicity evaluation against four human cancer cell lines, KB, KB/VCR, A549, and 95D. The IC_{50} values of **585–597** indicated that the alkylamino group attached to the 4β-anilino group was important to the in vitro cytotoxic activity. The ethyl pyrrolidine analog **585** exhibited the highest potency with IC_{50} values ranging from 0.036 to 1.57 μM against the tested cancer cell lines. Among compounds **598–601** with a hydrophilic alkoxy group on the end of the 4β-side chain, compound **598** with the shortest chain length showed the most potent cytotoxic activity. As the number of atoms between the benzene ring and the terminal hydroxy group increased (**599–601**), activity decreased (except for **599** against 95D cell line). Compared with ETO, compounds **602–613**, which have amide moieties on the *para*-position of the anilino ring, displayed comparable or slightly weaker cell growth inhibition against KB and A549 cell lines, but superior cytotoxic potency against KB/VCR and 95D cell lines (except **612** and **613**).

As depicted in Table XXIII, seven 4β-benzylamino PPT derivatives (**614–620**) were also synthesized by Wang et al.[166] The seven compounds displayed strong cytotoxic activity against A549, HCT-116, and HepG2 cancer cell lines. Among them, compound **615** possessed the highest cytotoxicity with an average IC_{50} value of 3.8 μM. The new compounds were generally more potent than ETO against the three tested cancer cell lines, indicating that PPT derivatives with a benzylamino structural modification possess potent cytotoxic activity.

Ren et al.[167] introduced 2-amino-1,3,4-oxadiazole moieties into the C-4 position to give nine novel 4β-*N*-substituted PPT derivatives (**621–629**) and evaluated their cytotoxicity against DU-145, SGC-7901, A549, SH-SY5Y, HepG2, and HeLa cell lines (Table XXIV). These derivatives displayed much lower cytotoxicity toward the tested normal cell lines (L929 and Vero) than PPT. Among them, compound **622** exhibited the highest potency

against HepG2 and HeLa with IC_{50} values of 1.29 and 2.68 μM, respectively. It also showed much better selectivity between the tumor and normal cells than ETO and PPT. Moreover, in MOA studies, **622** inhibited gene and protein expressions of DNA topo IIb, suspended the cell cycle at S-phase, and eventually caused apoptosis of the tumor cells.

Zhao et al.[168] reported 12 new aroylthiourea derivatives (**630–641**, Fig. 25) of 4β-amino-DEPPT. Compound **641** was the most potent (IC_{50} 4.4, 5.0, and 9.5 μM), although most of the derivatives displayed significant cytotoxic activity against HepG2, A549, and HCT-116 cancer cell lines. MOA studies indicated inhibition of the catalytic activity of DNA topo II, which caused HCT-116 cell cycle arrest at the G2/M phase.

A 1,2,3-triazole ring is widely present in various drugs and has also been incorporated by several research groups into PPT analogs. In 1999, Tao et al.[169] reported two novel 4β-[(5-substituted)-1,2,3-triazol-1-yl]-DEPPT derivatives (**642** and **643**, Fig. 26). In cytotoxicity testing against L1210 cells, compound **642** (ID_{50} 0.13 μM, 5-methyl) and ETO (ID_{50} 0.15 μM) exhibited almost equivalent activity, while compound **643** (ID_{50} 0.0030 μM, 5-phenyl) was 50-fold more active. Subsequently, Cao et al.[170] also reported a brief synthesis of 4-(1,2,3-triazol-1-yl)-PPT derivatives as a new class of antitumor compounds.

In 2008, Reddy's group[171,172] synthesized a library of 4β-[(4-substituted)-1,2,3-triazol-1-yl]-PPTs and DEPPTs using click chemistry protocols. The library focused on aniline-based (**644–653**, Table XXV), phenol-based (**654–667**, Table XXVI)-, and thiophenol-based (**668–673**, Table XXVI) 1,2,3-triazole derivatives. Many compounds showed promising antitumor activity, and certain analogs with aromatic substituents on the triazole moiety displayed excellent cytotoxicity. PPT analog **648** with a 3-nitroaniline moiety showed significant cytotoxicity against DU-145, PC-3, SF-295, HCT-15, and 502713 cell lines with highest potency against the HCT-15 cell line (IC_{50} 0.04 μM). PPT analogs **658** and **659** with 2-chloro- and 4-chlorophenoxy groups also showed significant cytotoxicity against the seven tested cancer cell lines with highest potency against HT-29 (IC_{50} 0.34 μM) and HCT-15 (IC_{50} 0.31 μM) cell lines, respectively.

In the same year, Reddy and co-workers[173] also generated a series of carbohydrate-based 1,2,3-triazole derivatives by condensing 1-*O*-propargyl monosaccharides with 4β-azido-PPT and 4β-azido-4′-*O*-demethyl-PPT (Table XXVII). The new compounds were screened for cytotoxicity against six human cancer cell lines, DU-145 (prostate), PC-3 (prostate), A-549 (lung), HOP-62 (lung), HCT-15 (colon), and SF-295 (CNS), but these generally were not as potent as ETO. Compound **675**, in which the sugar moiety was per-acylated, was less potent than the corresponding free glycoside (**674**). The presence of a hydroxy moiety on ring E was essential to the activity; for example, compounds containing dimethoxy substitution (**679–682**) were more active than those having trimethoxy substitution (**674–678**). Thus, compound **679**, which contained dimethoxy rather than trimethoxy substitution on ring E and a glucose moiety on the triazolyl ring, exhibited the best activity among the nine tested compounds.

Information on docking studies prompted Reddy et al.[174] to synthesize additional 4β-[(4-alkyl)-1,2,3-triazol-1-yl] derivatives of PPT and DEPPT using click chemistry (Table

XXVIII). Most of the derivatives exhibited better cytotoxicity than ETO. In particular, three compounds, **683**, **691**, and **692**, showed notable potency against PC-3 (IC_{50} 0.03, 0.06, 0.06 μM, respectively) and HEP-2 (IC_{50} 0.06, 0.06, 0.05 μM, respectively) cell lines. Compounds **691** and **692** also displayed excellent IC_{50} values (0.01 and 0.04 μM, respectively) against MCF-7. The cytotoxic activity generally decreased as the alkyl chain length increased, so that analogs with ethyl (**683**, **692**) or hydroxymethyl (**691**) groups on the triazole moiety were the most potent. Moreover, DNA fragmentation and flow cytometric results revealed that these derivatives induced dose-dependent apoptosis. Docking experiments showed a good correlation between their calculated interaction energies with observed IC_{50} values and topo II inhibitory effects of all compounds.

In addition, Chen's group[175] synthesized and evaluated nine different 4β-[(4-substituted)-1,2,3-triazol-1-yl]-PPT derivatives (**701–709**, Fig. 27) for cytotoxicity against human cancer cell lines (HeLa, K562, K562/A02). Most of the compounds demonstrated significant cytotoxic activity. Some derivatives exhibited high cytotoxicity toward the drug resistant K562/A02 leukemic cell line, whereas ETO was not active. Among them, compound **707** showed excellent potency, with IC_{50} values of 0.082, 0.053, and 0.059 μM against HeLa, K562, and K562/A02, respectively, more than 40 times more cytotoxic than ETO.

The same synthetic route was also used by Chen et al.[176] to prepare several additional series of 4β-[(4-substituted)-1,2,3-triazol-1-yl]-PPT congeners (**710–734**, Table XXIX). When evaluated against four tumor cell lines (HepG2, MKN-45, NCI-H1993, and B16), seven compounds (**717–721**, **728**, and **730**) were notably more potent compared with ETO. Among phenyoxymethyl and phenylthiomethyl substituted compounds (**710–723**), analogs with an electron-donating group (e.g., **712**, **714**, **717**, **719**, and **720**) at the *meta* or *para* position of the aromatic ring tended to be more active than those with an electron-withdrawing group (e.g., **710**, 7**11**, **715**, and **716**). Methoxy groups at both *meta* and *para* positions of the phenyl ring were also tolerated, for example, compound **720** displayed significant activity with an average IC_{50} value of 1.58 μM. The two methyl benzamide substituted compounds **724** and **725** were less active. Significantly, the presence of a fluorine atom on the phenyl moiety (e.g., **730**) was optimal for chalcone-containing analogs (**726**–**734**).

5. PPT DERIVATIVES SPIN-LABELED AT C-4 POSITION

Our prior review on PPT[46] included rationale for introduction of a stable nitroxide radical into PPT derivatives. Free radical compounds of this type have also been introduced into many antitumor drugs, such as thiotepa, 5-fluorouracil, nitrosourea, rubomycin and 6-mercaptopurine,[177] camptothecin,[178] rotenone,[179] and glycyrrhetinic acid.[180] The resulting new compounds often showed superior pharmacological properties compared with the parent compounds. Nitroxyl radicals can serve as transporter vehicles through cell membranes, regulate levels of oxidized cytochrome P-450 that have been brought down by lethal doses of cytostatic agents, and generally improve antitumor and antioxidant properties of drugs.

In view of the above advantages, our laboratory introduced stable radicals as spin labels at different positions of the PPT skeleton, particularly the C- or D-ring, over the past 27 years.[181–194] Such analogs frequently exhibited significant antitumor activity against several mouse transplantable tumors together with remarkably decreased toxicity.

Both antitumor and antioxidant activities of D-ring spin-labeled PPT derivative GP–1 (**735**) and two congeners GP-1-OH (**736**) and GP-1-H (**737**) were determined (Fig. 28).[187] The former testing used mice with transplanted S180 and HepA tumors. The rank order of potency was GP-1 > GP-OH > GP-1-H, which was attributed to the influence of partition coefficients and ionization constants on the compounds' properties. Moreover, the related GP-11 (**738**, Fig. 28) was reported as a low immunosuppressive antitumor agent;[188] it increased the mitotic index and arrested cells at the G2/M rather than S phase. In addition, the 4β-amino DEPPT-modified compound, GP-7 (**739**, Fig. 28), which is spin-labeled on the C-ring, showed lower toxicity but equivalent activity both in vivo (mouse solid tumors S180 and HePA) and in vitro (mouse leukemia L1210 and human stomach carcinoma SGC-7901) in comparison with ETO.[189] Additional spin-labeled PPT derivatives included 4β-amido[190] and 4β-ester[191] compounds. These modifications resulted in substantially increased activity relative to ETO, as exemplified in three spin-labeled 4β-amido derivatives (**740–742**, Fig. 28).[190] Compounds **740** and **741** were as or more potent than ETO against human nasopharyngeal carcinoma KB, lung cancer A-549, stomach carcinoma SGC-7901, and mouse leukemia L1210 and P388 cells.[191]

Most spin-labeled PPT esters (**743–754**, Table XXX) displayed greater cytotoxicity than ETO against P-388 and A-549 cell lines.[191] Compound **750** showed the highest potency with IC_{50} values less than 0.01 μM against P-388 and 0.13 μM against A-549. The cytotoxic and antioxidative activities of 4α-substituted compounds (**747–754**) generally were superior to those of 4α-substituted compounds (**743–746**).[191] The studies also found a correlation between cytotoxicity against tumor cells and antioxidant activity, suggesting that these compounds may act on tumor cells through an antioxidative mechanism. No distinct correlation was found between cytotoxic activity and the size or degree of saturation of the ring system in the nitroxide moiety.

Because of their good water solubility, L-amino acids are often used as carriers for drugs. Therefore, we linked *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-amino acids with PPT to give five novel spin-labeled amino acid-linked PPT derivatives (**755–759**, Table XXXI).[192,193] All five compounds showed significant activity against K562, L-1210, and HL-60 cell lines, with compounds **757–759** exhibiting the highest cytotoxicity against HL-60 cells (IC_{50} 0.108, 0.552, and 0.171 μM, respectively).

As outlined in Table XXXII, we subsequently prepared similar spin-labeled derivatives linked through a 4β-amide rather than 4α-ester bond by reaction of 4β-amino-DEPPT with *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-amino acids.[194] Compounds **760–766** were tested for in vitro cytotoxicity against three tumor cell lines as well as for antioxidative activity in tissues of SD rats. Most of the new compounds showed superior or comparable activity to ETO against A-549, HL-60, and RPMI-8226. Notably, compounds **762–764** and **766** exhibited excellent inhibitory activity against RPMI-8226 with IC_{50}

values ranging from 0.06 to 0.09 μM. Furthermore, all target compounds displayed three- to sixfold more potent antioxidative activity (IC_{50} 5.89–9.63 μM) in homogenate tissues of rat liver, heart, and kidney compared with ETO. Thus, introduction of L-amino acids with a stable nitroxyl radical into PPT could lead to improved biological activity.

Overall, novel spin-labeled PPTs provide a promising direction in antitumor chemotherapy, not only because they exhibit superior activity, but also because they can be monitored by electron spin resonance in pharmacological experiments.[46] As a whole, the introduction of a stable nitroxyl radical into the PPT molecule led to potentiated antitumor and antioxidative effects, proving that the design and synthesis of these compounds should be beneficial.

6. CONCLUSION

In summary, the successful history of PPT and related lignans confirms the importance of natural products as a major source of lead compounds for drug research. Since PPT's isolation in 1880 but unsuccessful clinical usage, the dynamics of PPT research has remained very high, resulting in hundreds of publications every year. The tremendous efforts in PPT research have mainly focused on the semisynthetic construction of novel PPT derivatives, prodrugs, and new forms of administration. These efforts have resulted in PPT-derived drugs, including approval for clinical use of ETO and teniposide, as well as many other drug candidates, such as NK611, TOP53, and GL-331, with C-4 modifications. However, the development of safe, economical, and site-specific anticancer drugs still faces challenges. Undoubtedly, continued studies on PPT analogs and their interaction with topo II will expand our understanding of the detailed MOA, which in turn will suggest new synthetic directions toward more effective anticancer drugs. The expectations and value of PPT as a lead compound go beyond the development of anticancer agents, based on the numerous biological activities continually being discovered for it and its derivatives. In conclusion, although the conversion of PPT-derived compounds to clinically effective drugs has been slow and unpredictable, the application of rational drug design technologies should promote the process at a faster pace.

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REFERENCES

1. Ayres, DC.; Loike, JD. Lignans, chemical, biological and clinical properties. Cambridge University Press; Cambridge: 1990. Chapters 3 and 4
2. Jardine, I. Podophyllotoxin, in anticancer agents based on natural products models. Academic Press, Inc.; New York: 1980. p. 319-351.
3. King J. Discovery of podophyllin. Coll J M Sci. 1857; 2:557–559.
4. Podwyssotzki V. The active constituent of podophyllin. Pharm J Trans. 1881; 12:217–218.
5. Podwyssotzki V. On the active constituents of podophyllin. Am J Pharm. 1882; 12:102–115.
6. Hartwell JL, Schrecker AW. Chemistry of podophyllum. Fortshr Chem Org Naturst. 1958; 15:83–166.
7. Kelly MG, Hartwell JL. The biological effects and the chemical composition of podophyllin: A review. J Natl Cancer Inst. 1954; 14:967–1010. [PubMed: 13233838]
8. MacRae DW, Towers GH. Biological activity of lignans. Phytochemistry. 1984; 23:1207–1220.
9. Kaplan IW. Condylomata acuminata. N Orleans Med Surg J. 1942; 94:388–390.
10. King ML, Sullivan MM. The similarity of the effect of podophyllin and colchicine and their use in the treatment of *Condylomata acuminata*. Science. 1946; 104:244–245.
11. Sullivan BJ, Wechsler HJ. The cytological effects of podophyllin. Science. 1947; 105:433. [PubMed: 17752486]
12. Stähelin H, von Wartburg A. From podophyllotoxin glucoside to etoposide. Prog Drug Res. 1989; 33:169–266. [PubMed: 2687938]
13. Canetta R, Hilgard P, Florentine S, Bedogni P, Lenaz L. Current development of podophyllotoxins. Cancer Chemother Pharmacol. 1982; 7:93–98. [PubMed: 7044596]
14. Kuhn M, von Wartburg A. Concerning a new glycoside preparation method. Synthesis of epipodophyllotoxin-β-D-glucopyranoside. Helv Chim Acta. 1968; 51:1631–1641. [PubMed: 5688036]
15. Kuhn M, von Wartburg A. On a new glycoside synthesis process. Glycosides of 4′-demethylepipodophyllotoxin. Helv Chim Acta. 1969; 52:948–955. [PubMed: 5798941]

16. Keller-Juslén C, Kuhn M, Stähelin H, von Wartburg A. Synthesis and antimitotic activity of glycosidic lignan derivatives related to podophyllotoxin. J Med Chem. 1971; 14:936–940. [PubMed: 5165570]
17. Joel S. The clinical pharmacology of etoposide: An update. Cancer Treatment Rev. 1996; 22:179–221.
18. Stähelin HF, von Wartburg A. The chemical and biological route from podophyllotoxin glucoside to etoposide: Ninth Cain Memorial Award Lecture. Cancer Res. 1991; 51:5–15. [PubMed: 1988106]
19. Ross W, Rowe T, Glisson B, Yalowich J, Liu L. Role of topoisomerase II in mediating epipodophyllotoxin-induced DNA cleavage. Cancer Res. 1984; 44:5857–5860. [PubMed: 6094001]
20. Long, BH.; Brattain, MG. The activity of etoposide and teniposide against human lung tumor *in vitro*: Cytotoxicity and DNA breakage. In: Issell, BF.; Muggia, FM.; Carter, SA., editors. Etoposide: Current Status and New Developments. Academic Press; New York: 1984. p. 223
21. Budman DR. Early studies of etoposide phosphate, a water-soluble prodrug. Semin Oncol. 1996; 23:8–14. [PubMed: 8996570]
22. Greco FA, Hainsworth JD. Clinical studies with etoposide phosphate. Semin Oncol. 1996; 23:45–50. [PubMed: 8996575]
23. Long BH, Minocha A. Inhibition of topoisomerase II by VP-16 (etoposide), VM-26 (teniposide) and structure congeners as explanation for *in vivo* DNA breakage and cytotoxicity. Proc Am Assoc Cancer Res. 1983; 24:1271–1277.
24. Hande KR. Etoposide: Four decades of development of a topoisomerase II inhibitor. Eur J Cancer. 1998; 34:1514–1521. [PubMed: 9893622]
25. Long BH, Musial ST, Brattain MG. Comparison of cytotoxicity and DNA breakage activity of congeners of podophyllotoxin including VP16–213 and VM26: A quantitative structure-activity relationship. Biochemistry. 1984; 23:1183–1188. [PubMed: 6712942]
26. Saito H, Yoshikawa H, Nishimura Y, Kondo S, Takeuchi T, Umezawa H. Studies on lignan lactone antitumor agents. II. Synthesis of *N*-alkylamino- and 2,6-dideoxy-2-aminoglycosidic lignan variants related to podophyllotoxin. Chem Pharm Bull. 1986; 34:3741–3746. [PubMed: 3815596]
27. Pagani O, Zucchetti M, Sessa C, de Jong J, D'Incalci M, De Fusco M, Kaeser-Fröhlich A, Hanauske A, Cavalli F. Clinical and pharmacokinetic study of oral NK611, a new podophyllotoxin derivative. Cancer Chemother Pharmacol. 1996; 38:541–547. [PubMed: 8823496]
28. Lee KH. Novel antitumor agents from higher plants. Med Res Rev. 1999; 19:569–596. [PubMed: 10557371]
29. Lee KH, Beers SA, Mori M, Wang ZQ, Kuo YH, Li L, Liu SY, Chang JY, Han FS, Cheng YC. Antitumor agents. 111. New 4-hydroxylated and 4-halogenated anilino derivatives of 4′-demethylepipodophyllotoxin as potent inhibitors of human DNA topoisomerase II. J Med Chem. 1990; 33:1364–1368. [PubMed: 2158562]
30. Wang ZQ, Kuo YH, Schnur D, Bowen JP, Liu SY, Han FS, Chang JY, Cheng YC, Lee KH. Anti-tumor agents. 113. New 4β-arylamino derivatives of 4′-*O*-demethyl epipodophyllotoxin and related compounds as potent inhibitors of human DNA topoisomerase II. J Med Chem. 1990; 33:2660–2666. [PubMed: 2167985]
31. Terada T, Fujimoto K, Nomura M, Yamashita J, Wierzba K, Yamazaki R, Shibata J, Sugimoto Y, Yamada Y, Kobunai T. Antitumor agents. 3. Synthesis and biological activity of 4β-alkyl derivatives containing hydroxy, amino, and amido groups of 4′-*O*-demethyl-4-desoxypodophyllotoxin as antitumor agents. J Med Chem. 1993; 36:1689–1699. [PubMed: 8389875]
32. Cragg GM, Newman DJ. A Tale of two tumor targets: Topoisomerase I and tubulin. The Wall and Wani contribution to cancer chemotherapy. J Nat Prod. 2004; 67:232–244. [PubMed: 14987065]
33. Barret JM, Etiévant C, Baudouin C, Skov K, Charvéron M, Hill BT. F11782, a novel catalytic inhibitor of topoisomerases I and II, induces atypical, yet cytotoxic DNA double-strand breaks in CHO-K1 cells. Anticancer Res. 2002; 22:187–192. [PubMed: 12017285]

34. Cho SJ, Tropsha A, Suffness M, Cheng YC, Lee KH. Antitumor agents. 163. Three-dimensional quantitative structure-activity relationship study of 4′-*O*-demethyl-epipodophyllotoxin analogs using the modified CoMFA/q2-GRS approach. J Med Chem. 1996; 39:1383–1395. [PubMed: 8691468]
35. Imbert TF. Discovery of podophyllotoxins. Biochimie. 1998; 80:207–222. [PubMed: 9615861]
36. Bohlin L, Rosen B. Podophyllotoxin derivatives: Drug discovery and development. Drug Discov Today. 1996; 1:343–351.
37. Hande KR. Etoposide: Four decades of development of a topoisomerase II inhibitor. Eur J Cancer. 1998; 34:1514–1521. [PubMed: 9893622]
38. Ward RS. Synthesis of podophyllotoxin and related compounds. Synthesis. 1992; 1:719–730.
39. Damayanthi Y, Lown JW. Podophyllotoxins: Current status and recent developments. Curr Med Chem. 1998; 5:205–252. [PubMed: 9562603]
40. Gordaliza M, Castro MA, del Corral JM, Feliciano AS. Antitumor properties of podophyllotoxin and related compounds. Curr Pharm Des. 2000; 6:1811–1839. [PubMed: 11102564]
41. Sackett DL. Podophyllotoxin, steganacin and combretastatin: Natural products that bind at the colchicine site of tubulin. Pharmacol Ther. 1993; 59:163–228. [PubMed: 8278462]
42. Gordaliza M, García PA, del Corral JM, Castro MA, Gomez-Zurita MA. Podophyllotoxin: Distribution, sources, applications and new cytotoxic derivatives. Toxicon. 2004; 44:441–459. [PubMed: 15302526]
43. Desbène S, Giorgi-Renault S. Drugs that inhibit tubulin polymerization: The particular case of podophyllotoxin and analogues. Curr Med Chem Anticancer Agents. 2002; 2:71–90. [PubMed: 12678752]
44. You Y. Podophyllotoxin derivatives: Current synthetic approaches for new anticancer agents. Curr Pharm Des. 2005; 11:1695–1717. [PubMed: 15892669]
45. Xu H, Lv M, Tian X. A review on hemisynthesis, biosynthesis, biological activities, mode of action, and structure-activity relationship of podophyllotoxins: 2003–2007. Curr Med Chem. 2009; 16:327–349. [PubMed: 19149581]
46. Liu YQ, Yang L, Tian X. Podophyllotoxin: Current perspectives. Curr Bioact Compd. 2007; 3:37–66.
47. Lee KH. Current developments in the discovery and design of new drug candidates from plant natural product leads. J Nat Prod. 2004; 67:273–283. [PubMed: 14987069]
48. Lee, KH.; Xiao, Z. Antitumor agents 277. Podophyllotoxins and analogs. In: Cragg, GM.; Kingston, DGI.; Newman, DJ., editors. Anticancer Agents from Natural Products. Taylor & Francis; New York: 2011. p. 95-122.
49. Lv M, Xu H. Recent advances in semisynthesis, biosynthesis, biological activities, mode of action, and structure-activity relationship of podophyllotoxins: An update (2008–2010). Mini Rev Med Chem. 2011; 11:901–909. [PubMed: 21781026]
50. Liu CJ, Hou SS. Current research status of podophyllotoxin lignans. Nat Prod Res Dev. 1997; 9:81–89.
51. Pujol MD, Romero M, Sánchez I. Synthesis and biological activity of new class of dioxygenated anticancer agents. Anticancer Agents Med Chem. 2005; 5:215–237.
52. Bedows E, Hatfield GM. An investigation of the antiviral activity of *Podophyllum peltatum*. J Nat Prod. 1982; 45:725–729. [PubMed: 6298369]
53. Markkanen T, Makinen ML, Maunuksela E, Himanen P. Podophyllotoxin lignans under experimental antiviral research. Drug Exp Clin Res. 1981; 7:711–718.
54. Gordaliza M, Castro MA, Garcia-Gracalos MD, Ruiz P, Miguel del Corral JM, Feliciano AS. Antineoplastic and antiviral activities of podophyllotoxin. Arch Pharm (Weinheim). 1994; 327:175–179. [PubMed: 8179476]
55. Hammonds TR, Denyer SP, Jackson DE, Irving WL. Studies to show that with podophyllotoxin the early replicative stages of herpes simplex virus type 1 depend upon functional cytoplasmic microtubules. J Med Microbiol. 1996; 45:167–172. [PubMed: 8810942]
56. Canel C, Moraes RM, Dayan FE, Ferreira D. Podophyllotoxin. Phytochemistry. 2000; 54:115–120. [PubMed: 10872202]

57. Charlton JL. Antiviral activity of lignans. J Nat Prod. 1998; 61:1447–1451. [PubMed: 9834179]
58. Hara H, Fujihashi T, Sakata T, Kaji A, Kaji H. Tetrahydronaphthalene lignan compounds as potent anti-HIV type 1 agents. AIDS Res Hum Retroviruses. 1997; 13:695–705. [PubMed: 9168238]
59. Markos AR. The successful treatment of *Molluscum contagiosum* with podophyllotoxin (0.5%) self-application. Int J STD AIDS. 2001; 12:833–834. [PubMed: 11791521]
60. Mukherjee AK, Basu S, Sarkar N, Ghosh AC. Advances in cancer therapy with plant based natural products. Curr Med Chem. 2001; 8:1467–1486. [PubMed: 11562277]
61. Hermanns-Lê T, Arrese JE, Goffin V, Piérard GE. Podophyllotoxin-induced acantholysis and cytolysis in a skin equivalent model. Eur J Morphol. 1998; 36:183–187. [PubMed: 9845264]
62. Liu YQ, Xiao H, Gao R, Tian X. Synthesis and insecticidal activities of novel derivatives of podophyllotoxin: Part XII. Pest Biochem Physiol. 2008; 91:116–121.
63. Liu YQ, Zhao CY, Yang L, Zhao YL, Liu TT. Evaluation of insecticidal activity of podophyllotoxin derivatives against *Brontispa longissima*. Pest Biochem Physiol. 2011; 99:39–44.
64. Liu YQ, Zhao YL, Yang L, Zhou XW, Feng G. Design, semisynthesis and insecticidal activity of novel podophyllotoxin derivatives against *Brontispa longissima in vivo*. Pest Biochem Physiol. 2012; 102:11–18.
65. Oliva A, Moraes RM, Watson SB, Duke SO, Dayan FE. Aryltetralin lignans inhibit plant growth by affecting the formation of mitotic microtubular organizing centers. Pest Biochem Physiol. 2002; 72:45–54.
66. Garcia ES, Azambuja P. Lignoids in insects: Chemical probes for the study of ecdysis, excretion and *Trypanosoma cruzi*-triatomine interactions. Toxicon. 2004; 44:431–440. [PubMed: 15302525]
67. Botta B, Delle Monache G, Misiti D, Vitali A, Zappia G. Aryltetralin lignans: Chemistry, pharmacology and biotransformations. Curr Med Chem. 2001; 8:1363–1381. [PubMed: 11562272]
68. AIDS International. CPH 82. BioDrugs. 2003; 17:373–374. [PubMed: 14498767]
69. Pugh N, Khan IA, Moraes RM, Pasco DS. Podophyllotoxin lignans enhance IL-1β but suppress TNF-α mRNA expression in LPS-treated monocytes. Immunopharmacol Immunotoxicol. 2001; 23:83–95. [PubMed: 11322652]
70. Gordaliza M, Faircloth GT, Castro MA, Miguel del Corral JM, López-Vázquez ML, San Feliciano A. Immunosuppressive cyclolignans. J Med Chem. 1996; 39:2865–2868. [PubMed: 8709118]
71. Gordaliza M, Castro MA, del Corral JMM, López-Vázquez ML, San Feliciano A, Faircloth GT. *In vivo* immunosuppressive activity of some cyclolignans. Bioorg Med Chem Lett. 1997; 21:2781–2786.
72. Loike JD, Horwitz SB. Effects of podophyllotoxin and VP-16–213 on microtubule assembly *in vitro* and nucleoside transport in HeLa cells. Biochemistry. 1976; 15:5435–5443. [PubMed: 999818]
73. Gordaliza M, del Corral JM, Castro MA, López-Vázquez ML, San Feliciano A, García-Grávalos MD, Carpy A. Synthesis and evaluation of pyrazolignans. A new class of cytotoxic agents. Bioorg Med Chem. 1995; 3:1203–1210. [PubMed: 8564411]
74. Schönbrunn E, Phlippen W, Trinczek B, Sack S, Eschenburg S, Mandelkow EM, Mandelkow E. Crystallization of a macromolecular ring assembly of tubulin liganded with the anti-mitotic drug podophyllotoxin. J Struct Biol. 1999; 128:211–215. [PubMed: 10600574]
75. Cowan CR, Cande WZ. Meiotic telomere clustering is inhibited by colchicine but does not require cytoplasmic microtubules. J Cell Sci. 2002; 115:3747–3756. [PubMed: 12235285]
76. Tseng CJ, Wang YJ, Liang YC, Jeng JH, Lee WS, Lin JK, Chen CH, Liu IC, Ho YS. Microtubule damaging agents induce apoptosis in HL 60 cells and G2/M cell cycle arrest in HT 29 cells. Toxicol. 2002; 175:123–142.
77. López-Pérez JL, del Olmo E, de Pascual Teresa B, Merino M, Barajas M, San Feliciano A. A role for dipole moment in the activity of cyclolignans. J Mol Struct (Theochem). 2000; 504:51–57.
78. van Maanen JM, Retèl J, de Vries J, Pinedo HM. Mechanism of action of antitumor drug etoposide: A review. J Natl Cancer Inst. 1988; 80:1526–1533. [PubMed: 2848132]
79. Champoux JJ. DNA topoisomerases: Structure, function, and mechanism. Annu Rev Biochem. 2001; 70:369–413. [PubMed: 11395412]

80. Burden DA, Osheroff N. Mechanism of action of eukaryotic topoisomerase II and drug targeted to the enzyme. Biochim Biophys Acta. 1998; 1400:139–154. [PubMed: 9748545]
81. Berger JM, Gambin SJ, Harrison SC, Wang JC. Structure and mechanism of DNA topoisomerase II. Nature. 1996; 379:225–232. [PubMed: 8538787]
82. Haim N, Nemec J, Roman J, Sinha BK. Peroxidative free radical formation and *O*-demethylation of etoposide (VP-16) and teniposide (VM-26). Biochem Biophys Res Commun. 1986; 135:215–220. [PubMed: 3006680]
83. Haim N, Nemec J, Roman J, Sinha BK. Peroxidase-catalyzed metabolism of etoposide (VP-16–213) and covalent binding of reactive intermediates to cellular macromolecules. Cancer Res. 1987; 47:5835–5840. [PubMed: 3117357]
84. Sinha BK. Free radicals in anticancer drug pharmacology. Chem Biol Interact. 1989; 69:293–317. [PubMed: 2659197]
85. Lin S, Huang HC, Chen LL, Lee CC, Huang TS. GL331 induces down-regulation of cyclin D1 expression via enhanced proteolysis and repressed transcription. Mol Pharmacol. 2001; 60:768–775. [PubMed: 11562439]
86. Tseng CJ, Wang YJ, Liang YC, Jeng JH, Lee WS, Lin JK, Chen CH, Liu IC, Ho YS. Microtubule damaging agents induce apoptosis in HL 60 cells and G2/M cell cycle arrest in HT 29 cells. Toxicology. 2002; 175:123–142. [PubMed: 12049842]
87. Boldt S, Weidle UH, Kolch W. The role of MAPK pathways in the action of chemotherapeutic drugs. Carcinogenesis. 2002; 23:1831–1838. [PubMed: 12419831]
88. Qi SN, Yoshida A, Wang ZR, Ueda T. GP7 can induce apoptotic DNA fragmentation of human leukemia cells through caspase-3-dependent and -independent pathways. Int J Mol Med. 2004; 13:163–167. [PubMed: 14654989]
89. Qi SN, Jing YX, Dong GX, Chen Y, Yoshida A, Ueda T. GP7 induces internucleosomal DNA fragmentation independent of caspase activation and DNA fragmentation factor in NB4 cells. Oncol Rep. 2007; 18:273–277. [PubMed: 17549379]
90. Suh SJ, Kim JR, Jin UH, Choi HS, Chang YC, Lee YC, Kim SH, Lee IS, Moon TC, Chang HW, Kim CH. Deoxypodophyllotoxin, flavolignan, from *Anthriscus sylvestris* Hoffm inhibits migration and MMP-9 via MAPK pathways in TNF-α-induced HASMC. Vasc Pharmacol. 2009; 51:13–20.
91. Yong Y, Shin SY, Lee YH, Lim Y. Antitumor activity of deoxypodophyllotoxin isolated from *Anthriscus sylvestris*: Induction of G2/M cell cycle arrest and caspase-dependent apoptosis. Bioorg Med Chem Lett. 2009; 19:4367–4371. [PubMed: 19501508]
92. Shin SY, Yong Y, Kim CG, Lee YH, Lim Y. Deoxypodophyllotoxin induces G2/M cell cycle arrest and apoptosis in HeLa cells. Cancer Lett. 2010; 287:231–239. [PubMed: 19616373]
93. Chen YQ, Xie X. Podophyllotoxin induces CREB phosphorylation and CRE-driven gene expression via PKA but not MAPKs. Mol Cells. 2010; 29:41–50. [PubMed: 20033853]
94. Tadafumi T, Katsuhiko F, Makoto N. Antitumor agents II: Regio- and stereospecific synthesis of 1β-alkyl-1-desoxypodophyllotoxin derivatives and biological activity. Chem Pharm Bull. 1993; 41:907–912. [PubMed: 8393386]
95. Tadafumi T, Katsuhiko F, Makoto. TOP-53. Drugs Future. 1996; 21:1136–1139.
96. Roulland E, Magiatis P, Arimondo P, Bertounesque E, Monneret C. Hemi-synthesis and biological activity of new analogues of podophyllotoxin. Bioorg Med Chem. 2002; 10:3463–3471. [PubMed: 12213460]
97. Chen ZX, Ma WY, Zhang CN. Stereoselective synthesis and antitumor activities of new podophyllotoxin derivatives: 4β-Cyano-4-deoxy-4′-demethylepipodophyllotoxin and 4β-carboxyl-4-deoxy-4′-demethylepipodophyllotoxin. Chin Chem Lett. 2000; 11:505–508.
98. Chen ZX, Ma WY, Zhang CN. Synthesis and antitumor activity of 4-β-alkylthiocarbonyl-4-deoxy-4′-demethylepipodophyllotoxin. Chin J Med Chem. 2000; 10:84–87.
99. Chen ZX, Ma WY, Chen XH, Wang JD, Zhang CN. Synthesis and antitumor activity of 4-β-substituted carbamoyl-4-deoxy-4′-demethylepipodophyllotoxin. Chin J Pharm. 2000; 30:265–267.
100. Abad A, López-Pérez JL, del Olmo E, Garcia-Fernández LF, Francesch A, Trigili C, Barasoain I, Andreu JM, Diaz JF, San Feliciano A. Synthesis and antimitotic and tubulin interaction profiles of novel pinacol derivatives of podophyllotoxins. J Med Chem. 2012; 55:6724–67377. [PubMed: 22607205]

101. Showalterk HD, Winters RT, Sercel AD, Michel A. Facile synthesis of thioglucose analogues of the anticancer agent etoposide. Tetrahedron Lett. 1991; 32:2849–2852.
102. Wang ZG, Ma WY, Zhang CN. Stereocontrolled synthesis of 4-sulfanyl-4-deoxy-4-demethylepipodophyllotoxin. Acta Chim Sin. 1992; 50:696–701.
103. Wang ZG, Yin SF, Zhuang W, Li BS, Ma WY, Zhang CN. Synthesis and antitumor activities of 4*β*-alkylthio-4-deoxy-4′-demethylepipodophyllotoxin and its derivatives. Acta Pharm Sin. 1992; 50:696–701.
104. Wang ZG, Yin SF, Zhuang W, Li BS, Ma WY, Zhang CN. Synthesis and antitumor activities of 4*β*-(2-aminoethylthio)-4-deoxy-4′-demethylepipodophyllotoxin and its derivatives. Chin J Pharm. 1992; 23:159–167.
105. Lu KK, Liu FM, Chen YZ. Synthesis and antitumor activity of podophyllotoxin analogues. J Chin Pharm Sci. 1998; 7:182–185.
106. Sanmartin C, Plano D, Sharma AK, Palop JA. Selenium compounds, apoptosis and other types of cell death: An overview for cancer chemotherapy. Int J Mol Sci. 2012; 13:9649–9672. [PubMed: 22949823]
107. Wang ZG, Ma WY, Zhang CN. Synthesis and antitumor activities of 4-alkylselenyl-4-deoxy-4′-demethylepipodophyllotoxin. Acta Chim Sin. 1993; 51:933–936.
108. Miao RD, Han Y, An LZ, Yang JB, Wang Q. Seleno-podophyllotoxin derivatives induce hepatoma SMMC-7721 cell apoptosis through Bax pathway. Cell Biol Int. 2008; 32:217–223. [PubMed: 17993285]
109. Zhang YZ, Cao CN. Cytotoxicity determination and synthesis of selenoyl-4-deoxy-4′-demethylepipodophyllotoxin. J Qinghai Norm Univ. 2004; 3:76–79.
110. Terada T, Fujimoto K, Nomura M, Yamashita J, Kobunai T, Takeda S, Wierzba K, Yamada Y, Yamaguchi H. Antitumor agents. I. DNA topoisomerase II inhibitory activity and the structural relationship of podophyllotoxin derivatives as antitumor agents. Chem Pharm Bull. 1992; 40:2720–2727. [PubMed: 1334447]
111. Zhuang W, Ma WY, Zhang CN. Synthesis and anticancer activity of 4*β*-ether-4′-demethylepipodophyllotoxin derivatives. Chin J Pharm. 1991; 22:446–448.
112. Yin SF, Ma WY, Zhang CN. Synthesis and antitumor activity of 4,4′-*O*-bis(2-hydroxy-3-substituted-amino)propyl-4′-demethylepipodophyllotoxins. Chin J Pharm. 1993; 24:486–488.
113. Bathini Y, Scholte A, Kelly S, Micetich RG, Daneshtalab M. New podophyllotoxin derivatives as potential anticancer agents: Synthesis and cytotoxicity of 4*β*-*O*-propenylpodophyllotoxin ethers. Syn Commun. 1999; 29:379–385.
114. Levy RK, Hall IH, Lee KH. Antitumor Agents LXII: Synthesis and biological evaluation of podophyllotoxin esters and related derivatives. J Pharm Sci. 1983; 72:1158–1161. [PubMed: 6644563]
115. Gupta RS, Chenchaiah PC. Synthesis and biological activities of the C-4 esters of 4′-demethylepipodophyllotoxin. Anticancer Drug Des. 1987; 2:13–23. [PubMed: 3449082]
116. López-Pérez JL, del Olmo E, de Pascual-Teresa B, Abad A, San Feliciano A. Synthesis and cytotoxicity of hydrophobic esters of podophyllotoxins. Bioorg Med Chem Lett. 2004; 14:1283–1286. [PubMed: 14980682]
117. Duca M, Guianvarch D, Meresse P, Bertounesque E, Dauzonne D, Kraus-Berthier L, Thirot S, Léonce S, Pierré A, Pfeiffer B, Renard P, Arimondo PB, Monneret C. Synthesis and biological study of a new series of 4′-demethylepipodophyllotoxin derivatives. J Med Chem. 2005; 48:593–603. [PubMed: 15658872]
118. Kamal A, Kumar BA, Suresh P, Juvekar A, Zingde S. Synthesis of 4*β*-carbamoyl epipodophyllotoxins as potential antitumour agents. Bioorg Med Chem. 2011; 19:2975–2979. [PubMed: 21489802]
119. Mustafa J, Khan SI, Ma G, Walker LA, Khan IA. Synthesis and anticancer activities of fatty acid analogs of podophyllotoxin. Lipids. 2004; 39:167–172. [PubMed: 15134144]
120. Mustafa J, Khan SI, Ma G, Walker LA, Khan IA. Synthesis and *in vitro* cytotoxic activity of *N*-, *F*-, and *S*-ether derivatives of podophyllotoxin fatty acid adducts. Lipids. 2005; 40:375–381. [PubMed: 16028720]

121. Liu YQ, Wei DF, Zhao YL, Cheng WD, Lu Y, Ma YQ, Li X, Han C, Wei YX, Cao HM, Zhao CY. Synthesis and biological evaluation of a series of podophyllotoxin derivatives as a class of potent antitubulin agents. Bioorg Med Chem. 2012; 20:6285–6295. [PubMed: 23022053]
122. Yu PF, Chen H, Wang J, He CX, Cao B, Li M, Yang N, Lei ZY, Cheng MS. Design, synthesis and cytotoxicity of novel podophyllotoxin derivatives. Chem Pharm Bull. 2008; 56:831–834. [PubMed: 18520089]
123. Chen H, Wang J, Zhang J, Wang Y, Cao B, Bai S, Yu PF, Bi W, Xie W. L1EPO, a novel podophyllotoxin derivative overcomes P-glycoprotein-mediated multidrug resistance in K562/A02 cell line. Biol Pharm Bull. 2009; 32:609–613. [PubMed: 19336892]
124. Gupta R, Al-said NH, Oreski B, Lown JW. Design, synthesis and topoisomerase II inhibition activity of 4′-demethylepipodophyllotoxin-lexitropsin conjugates. Anticancer Drug Des. 1996; 11:325–328. [PubMed: 8679055]
125. Derry WB, Pamidi CC, Gupta RS. Synthesis and biological activity of novel thymidine derivatives of podophyllotoxin and 4′-demethylepipodophyllotoxin. Anticancer Drug Des. 1993; 8:203–221. [PubMed: 8517914]
126. Lee NJ, Jeong IC, Cho MY, Jeon CW, Yun BC, Kim YO, Kim SH, Chung I. Synthesis and *in vitro* antitumor activity of phthalimide polymers containing podophyllotoxin. Eur Polym J. 2006; 42:3352–3359.
127. Passarella D, Giardini A, Peretto B, Fontana G, Sacchetti A, Silvani A, Ronchi C, Cappelletti G, Cartelli D, Borlake J, Danieli B. Inhibitors of tubulin polymerization: Synthesis and biological evaluation of hybrids of vindoline, anhydrovinblastine and vinorelbine with thiocolchicine, podophyllotoxin and baccatin III. Bioorg Med Chem. 2008; 16:6269–6285. [PubMed: 18468444]
128. Passarella D, Peretto B, Yepes RB, Cappelletti G, Cartelli D, Ronchi C, Snaith J, Fontana G, Danieli B, Borlak J. Synthesis and biological evaluation of novel thiocolchicine–podophyllotoxin conjugates. Eur J Med Chem. 2010; 45:219–226. [PubMed: 19880222]
129. Cappelletti G, Cartelli D, Peretto B, Ventura M, Riccioli M, Colombo F, Snaith JS, Borrelli S, Passarella D. Tubulin-guided dynamic combinatorial library of thiocolchicine-podophyllotoxin conjugates. Tetrahedron. 2011; 67:7354–7357.
130. Danieli B, Giardini A, Lesma G, Passarella D, Peretto B, Sacchetti A, Silvani A, Pratesi G, Zunino F. Thiocolchicine-podophyllotoxin conjugates: Dynamic libraries based on disulfide exchange reaction. J Org Chem. 2006; 71:2848–2853. [PubMed: 16555841]
131. Liu YQ, Liu YQ, Tian X, Yang L. Synthesis of novel derivatives of bispodophyllotoxin as potential antineoplastic agents—part XVI. Nat Prod Res. 2008; 22:285–291. [PubMed: 18322843]
132. Allevi P, Anastasia M, Ciuffreda P. The first synthesis of the *N*-glucosyl analogue of the antitumor agent etoposide. Tetrahedron Lett. 1993; 34:7313–7316.
133. KH, Lee; Imakura, Y.; Haruna, M.; Beers, SA.; Thurston, LS.; Dai, HJ.; Chen, CH.; Cheng, YC. Antitumor agents. 107: New cytotoxic 4-alkylamino analogues of 4′-demethyl-epipodophyllotoxin as inhibitors of human DNA topoisomerase II. J Nat Prod. 1989; 52:606–613. [PubMed: 2550587]
134. Zhu XK, Guan J, Tachibana Y, Bastow KF, Cho SJ, Cheng HH, Cheng YC, Gurwith M, Lee KH. Antitumor agents. 194. Synthesis and biological evaluations of 4*β*-mono-, -di-, and -trisubstituted aniline-4′-demethyl-podophyllotoxin and related compounds with improved pharmacological profiles. J Med Chem. 1999; 42:2441–2446. [PubMed: 10395485]
135. Zhou XM, Wang ZQ, Chang JY, Chen HX, Cheng YC, Lee KH. Antitumor agents. 120. New 4-substituted benzylamine and benzyl ether derivatives of 4′-*O*-demethylepipodophyllotoxin as potent inhibitors of human DNA topoisomerase II. J Med Chem. 1991; 34:3346–3350. [PubMed: 1662724]
136. Zhou XM, Wang ZQ, Chen HX, Cheng YC, Lee KH. Antitumor agents. 125. New 4*β*-benzoylamino derivatives of 4′-*O*-demethyl-4-desoxypodophyllotoxin and 4*β*-benzoyl derivatives of 4′-*O*-demethylpodophyllotoxin as potent inhibitors of human DNA topoisomerase II. Pharm Res. 1993; 10:214–219. [PubMed: 8384366]

137. Zhang YL, Guo X, Cheng YC, Lee KH. Antitumor agents. 148. Synthesis and biological evaluation of novel 4*β*-amino derivatives of etoposide with better pharmacological profiles. J Med Chem. 1994; 37:446–452. [PubMed: 8120864]

138. Xiao Z, Bastow KF, Vance JR, Lee KH. Antitumor agents. Part 227: Studies on novel 4′-*O*-demethyl-epipodophyllotoxins as antitumor agents targeting topoisomerase II. Bioorg Med Chem. 2004; 12:3339–3344. [PubMed: 15158802]

139. Xiao Z, Bastow KF, Vance JR, Sidwell RS, Wang HK, Chen MS, Shi Q, Lee KH. Antitumor agents. 234. Design, synthesis, and biological evaluation of novel 4*β*-[(4″-benzamido)-amino]-4′-*O*-demethyl-epipodophyllotoxin derivatives. J Med Chem. 2004; 47:5140–5148. [PubMed: 15456257]

140. Bastow KF, Wang HK, Cheng YC, Lee KH. Antitumor agents–CLXXIII. Synthesis and evaluation of camptothecin-4*β*-amino-4′-*O*-demethylepipodophyllotoxin conjugates as inhibitors of mammalian DNA topoisomerases and as cytotoxic agents. Bioorg Med Chem. 1997; 5:1481–1488. [PubMed: 9313854]

141. Shi Q, Wang HK, Bastow KF, Tachibana Y, Chen K, Lee FK, Lee KH. Antitumor agents. 210. Synthesis and evaluation of taxoid-epipodophyllotoxin conjugates as novel cytotoxic agents. Bioorg Med Chem. 2001; 9:2999–3004. [PubMed: 11597482]

142. Wang ZG, Yin SF, Ma WY, Li BS, Zhang CN. Synthesis and antitumor activities of 4-acylamino-4-deoxy-4′-demethylepipodophyllotoxin analogues. Acta Pharm Sin. 1993; 28:422–427.

143. Tian X, Yang MG, Chen YZ. Synthesis, antitumor activity and QSAR study of derivatives of 4*β*-halogenated arylacylamido-4-deoxy-4′-demethylepipodophyllotoxin. J Lanzhou Univ. 1998; 34:114–119.

144. Hansen HF, Jensen RB, Willumsen AM, Nørskov-Lauritsen N, Ebbesen P, Nielsen PE, Buchardt O. New compounds related to podophyllotoxin and congeners: Synthesis, structure elucidation and biological testing. Acta Chim Scand. 1993; 47:1190–1200.

145. Kamal A, Gayatri NL, Rao NV. Facile and improved synthesis of 4*β*-aminopodophyllotoxin congeners. Bioorg Med Chem Lett. 1998; 8:3097–3100. [PubMed: 9873683]

146. He Y, Ma WY, Chen XH, Zhang CN. Synthesis and activities of 4-deoxy-4*β*-arylmethylenesulfonylamino-4′-demethylpodophyllotoxins. Acta Pharm Sin. 2001; 36:105–107.

147. Ma WY, Li Y, Zhang CN. Synthesis and activities of 4-deoxy-4*β*-sulfonylamino-4′-demethylepipodophyllotoxins. Chin J Pharm. 1993; 24:198–201.

148. Kamal A, Gayatri NL, Reddy DR, Mohan Reddy PS, Arifuddin M, Dastidar SG, Kondapi AK, Rajkumar M. Synthesis and biological evaluation of new 4*β*-anilino- and 4*β*-imido-substituted podophyllotoxin congeners. Bioorg Med Chem. 2005; 13:6218–6225. [PubMed: 16061385]

149. Kamal A, Ashwini Kumar B, Arifuddin M, Dastidar SG. Synthesis of 4*β*-amido and 4*β*-sulphonamido analogues of podophyllotoxin as potent antitumor agents. Bioorg Med Chem. 2003; 11:5135–5142. [PubMed: 14604676]

150. Kamal A, Kumar BA, Arifuddin M, Dastidar SG. Synthesis and biological activity of new 4*β*-*N*-heteroaryl analogues of podophyllotoxin. Lett Drug Des Dis. 2006; 3:205–213.

151. Kamal A, Laxman E, Khanna GB, Reddy PS, Rehana T, Arifuddin M, Neelima K, Kondapi AK, Dastidar SG. Design, synthesis, biological evaluation and QSAR studies of novel bisepipodophyllotoxins as cytotoxic agents. Bioorg Med Chem. 2004; 12:4197–4209. [PubMed: 15246096]

152. Kamal A, Kumar BA, Suresh P, Agrawal SK, Chashoo G, Singh SK, Saxena AK. Synthesis of 4*β*-*N*-polyaromatic substituted podophyllotoxins: DNA topoisomerase inhibition, anticancer and apoptosis-inducing activities. Bioorg Med Chem. 2010; 18:8493–8500. [PubMed: 21074444]

153. Kamal A, Kumar BA, Suresh P, Shankaraiah N, Kumar MS. An efficient one-pot synthesis of benzothiazolo-4*β*-anilino-podophyllotoxin congeners: DNA topoisomerase-II inhibition and anti-cancer activity. Bioorg Med Chem Lett. 2011; 21:350–353. [PubMed: 21144748]

154. Kamal A, Suresh P, Janaki Ramaiah M, Mallareddy A, Kumar BA, Raju P, Vinay Gopal J, Pushpavalli SN, Lavanya A, Sarma P, Pai-Bhadra M. Synthesis and biological evaluation of 4*β*-acrylamidopodophyllotoxin congeners as DNA damaging agents. Bioorg Med Chem. 2011; 19:4589–4600. [PubMed: 21737288]

155. Kamal A, Mallareddy A, Suresh P, Lakshma Nayak V, Shetti RV, Sankara Rao N, Tamboli JR, Shaik TB, Vishnuvardhan MV, Ramakrishna S. Synthesis and anticancer activity of 4β-alkylamidochalcone and 4β-cinnamido linked podophyllotoxins as apoptotic inducing agents. Eur J Med Chem. 2012; 47:530–545. [PubMed: 22136907]
156. Kamal A, Suresh P, Ramaiah MJ, Mallareddy A, Imthiajali S, Pushpavalli SN, Lavanya A, Pai-Bhadra M. Synthesis and biological evaluation of 4β-sulphonamido and 4β-[(4′-sulphonamido)benzamide]podophyllotoxins as DNA topoisomerase-IIα and apoptosis inducing agents. Bioorg Med Chem. 2012; 20:2054–2066. [PubMed: 22364746]
157. Kamal A, Tamboli JR, Vishnuvardhan MV, Adil SF, Nayak VL, Ramakrishna S. Synthesis and anticancer activity of heteroaromatic linked 4β-amido podophyllotoxins as apoptotic inducing agents. Bioorg Med Chem Lett. 2013; 23:273–280. [PubMed: 23182091]
158. Zhang FM, Tian X. Synthesis and anticancer activity of novel derivatives of 4′-demethylepipodophyllotoxin. Acta Chim Sin. 2002; 60:720–724.
159. Liu YQ, Yang H, Tian X. Design, synthesis and biological evaluation of novel etoposide analogue as cytotoxic agents. Chin J Chem. 2006; 24:785–790.
160. Chen SW, Tian X, Tu YQ. Synthesis and cytotoxic activity of novel derivatives of 4′-demethylepipodophyllotoxin. Bioorg Med Chem Lett. 2004; 14:5063–5066. [PubMed: 15380199]
161. Chen SW, Xiang R, Liu J, Tian X. Synthesis and biological evaluation of novel conjugates of podophyllotoxin and 5-FU as antineoplastic agents. Bioorg Med Chem. 2009; 17:3111–3117. [PubMed: 19324555]
162. Guianvarch D, Duca M, Boukarim C, Kraus-Berthier L, Léonce S, Pierré A, Pfeiffer B, Renard P, Arimondo PB, Monneret C, Dauzonne D. Synthesis and biological activity of sulfonamide derivatives of epipodophyllotoxin. J Med Chem. 2004; 47:2365–2374. [PubMed: 15084135]
163. Guo YE, Chen H, Zuo S, Liu DL, Lu YL, Lv JJ, Wen SP, Zhang TC. Synthesis and antitumor activity of novel podophyllotoxin derivatives against multidrug-resistant cancer cells. J Asian Nat Prod Res. 2011; 13:417–424. [PubMed: 21534039]
164. Shang H, Chen H, Zhao DM, Tang XW, Liu YF, Pan L, Cheng MS. Synthesis and biological evaluation of 4α/4β-imidazolylpodophyllotoxin analogues as antitumor agents. Arch Pharm Chem Life Sci. 2012; 345:43–48.
165. Wang L, Yang FY, Yang XC, Guan XH, Hu CQ, Liu T, He QJ, Yang B, Hu YZ. Synthesis and biological evaluation of new 4β-anilino-4′-*O*-demethyl-4-desoxypodophyllotoxin derivatives as potential antitumor agents. Eur J Med Chem. 2011; 46:285–296. [PubMed: 21145139]
166. Wang CN, Wu ZH, Zhao Y, Ni CY, Zhao XD, Zhu L. Synthesis and cytotoxicity evaluation of novel podophyllotoxin derivatives. Arch Pharm Chem Life Sci. 2011; 344:735–740.
167. Ren J, Wu L, Xin WQ, Chen X, Hu K. Synthesis and biological evaluation of novel 4β-(1,3,4-oxadiazole-2-amino)-podophyllotoxin derivatives. Bioorg Med Chem Lett. 2012; 22:4778–4782. [PubMed: 22687745]
168. Zhao Y, Ge CW, Wu ZH, Wang CN, Fang JH, Zhu L. Synthesis and evaluation of aroylthiourea derivatives of 4β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin as novel topoisomerase II inhibitors. Eur J Med Chem. 2011; 46:901–906. [PubMed: 21277655]
169. Tao L, Wang YG, Ma C, Zheng B, Chen YZ. Synthesis of 4β-(1,2,3-triazol-1-yl) podophyllotoxins as potential antitumor drugs. Syn Commun. 1999; 29:2053–2059.
170. Cao CN, Chen YY, Wang YG, Chen YZ. Synthesis and antitumor activity of 4-(1,2,3-triazol-1-yl) podophyllotoxins. Chin Chem Lett. 1999; 10:1003–1006.
171. Bhat BA, Reddy PB, Agrawal SK, Saxena AK, Kumar HM, Qazi GN. Studies on novel 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxins as potential anticancer agents. Eur J Med Chem. 2008; 43:2067–2072. [PubMed: 17988764]
172. Reddy PB, Agrawal SK, Singh S, Bhat BA, Saxena AK, Kumar HM, Qazi GN. Synthesis and biological evaluation of 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxins as potential anticancer agents. Chem Biodiver. 2008; 5:1792–1802.
173. Reddy PB, Paul DV, Agrawal SK, Saxena AK, Kumar M, Qazi GN. Design, synthesis, and biological testing of 4β-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxin analogues as antitumor agents. Arch Pharm (Weinheim). 2008; 341:126–131. [PubMed: 18214838]

174. Reddy DM, Srinivas J, Chashoo G, Saxena AK, Sampath Kumar HM. 4β-[(4-Alkyl)-1,2,3-triazol-1-yl]podophyllotoxins as anticancer compounds: Design, synthesis and biological evaluation. Eur J Med Chem. 2011; 46:1983–1991. [PubMed: 21477899]
175. Chen H, Zuo S, Wang X, Tang X, Zhao M, Lu Y, Chen L, Liu J, Liu Y, Liu D, Zhang S, Li T. Synthesis of 4β-triazole-podophyllotoxin derivatives by azide-alkyne cycloaddition and biological evaluation as potential antitumor agents. Eur J Med Chem. 2011; 46:4709–4714. [PubMed: 21821321]
176. Chen J, Ma L, Zhang R, Tang J, Lai H, Wang J, Wang G, Xu Q, Chen T, Peng F, Qiu J, Liang X, Cao D, Ran Y, Peng A, Wei Y, Chen L. Semi-synthesis and biological evaluation of 1,2,3-triazole-based podophyllotoxin congeners as potent antitumor agents inducing apoptosis in HepG2 cells. Arch Pharm (Weinheim). 2012; 345:945–956. [PubMed: 22949330]
177. Wang YG, Tian X, Chen YZ. Recent advances in spin labeled anticancer drugs. Acta Pharm Sin. 1988; 23:792–798.
178. Liu YQ, Tian X, Yang L, Zhan ZC. First synthesis of novel spin-labeled derivatives of camptothecin as potential antineoplastic agents. Eur J Med Chem. 2008; 43:2610–2614. [PubMed: 18313176]
179. Liu YQ, Ohkoshi E, Li LH, Yang L, Lee KH. Design, synthesis and cytotoxic activity of novel spin-labeled rotenone derivatives. Bioorg Med Chem Lett. 2012; 22:920–923. [PubMed: 22204911]
180. Liu Y, Qian K, Wang CY, Chen CH, Yang XM, Lee KH. Synthesis and biological evaluation of novel spin labeled 18β-glycyrrhetinic acid derivatives. Bioorg Med Chem Lett. 2012; 22:7530–7533. [PubMed: 23122524]
181. Chen YZ, Zhang CJ, Tian X. Spin-labeled antitumor derivatives of podophyllotoxin. Sci Sin B. 1987; 30:1070–1079. [PubMed: 2832936]
182. Tian X, Yan ZQ, Li JX, Chen YZ. Anticancer drugs (V)—Synthesis of etoposide derivatives. Chem Res Chin Univ. 1995; 11:79–83.
183. Tian X, Yang MG, Chen YZ. Anticancer drugs (VI)—Synthesis and activity of spin labeled derivatives of 4-amino-4-deoxy-4′-demethylepipodophyllotoxin. Chem Res Chin Univ. 1996; 12:304–308.
184. Lu KK, Wang YG, Chen YZ. Synthesis of novel spin labeled analogues of podophyllotoxin glycoside. Syn Commun. 1997; 27:1963–1968.
185. Wang YG, Pan JL, Shi JF, Chen YZ. New spin labeled analogues of podophyllotoxin as potential antitumor agents. Life Sci. 1997; 61:537–542. [PubMed: 9247323]
186. Pan JL, Wang YG, Shi JF, Chen YZ. Synthesis of new spin labeled derivatives of podophyllotoxin as potential anticancer agents. Chin Chem Lett. 1997; 8:207–208.
187. Tian X, Zhang FM, Li WG. Antitumor and antioxidant activity of spin labeled derivatives of podophyllotoxin (GP-1) and congeners. Life Sci. 2002; 70:2433–2443. [PubMed: 12150207]
188. Wang JZ, Tian X, Tsumura H, Shimura K, Ito H. Antitumor activity of a new low immunosuppressive derivative of podophyllotoxin (GP-11) and its mechanisms. Anticancer Drug Des. 1993; 8:193–202. [PubMed: 8517913]
189. Chen YZ, Wang YG, Li JX, Tian X, Jia ZP, Zhang PY. Anticancer drugs. II. Synthesis and biological evaluation of spin labeled derivatives of podophyllotoxin. Life Sci. 1989; 45:2569–2575. [PubMed: 2615556]
190. Tian X, Wang YG, Yang MG, Chen YZ. Synthesis and antitumor activity of spin labeled derivatives of podophyllotoxin. Life Sci. 1997; 60:511–517. [PubMed: 9042385]
191. Jin Y, Chen SW, Tian X. Synthesis and biological evaluation of new spin-labeled derivatives of podophyllotoxin. Bioorg Med Chem. 2006; 14:3062–3068. [PubMed: 16406792]
192. Liu YQ, Tian X. Synthesis of novel spin-labeled derivatives of podophyllotoxin as potential anti-neoplastic agents. Syn Commun. 2005; 35:2479–2758.
193. Yang L, Liu YQ, Tan H, Li WG, Tian X. Design, synthesis, and biological evaluation of novel spin-labeled derivatives of podophyllotoxin as potential antineoplastic agents. Part XII. Nat Prod Res. 2007; 21:998–1008. [PubMed: 17691049]

194. Zhang JQ, Zhang ZW, Hui L, Chen SW, Tian X. Novel semisynthetic spin-labeled derivatives of podophyllotoxin with cytotoxic and antioxidative activity. Bioorg Med Chem Lett. 2010; 20:983–986. [PubMed: 20053564]

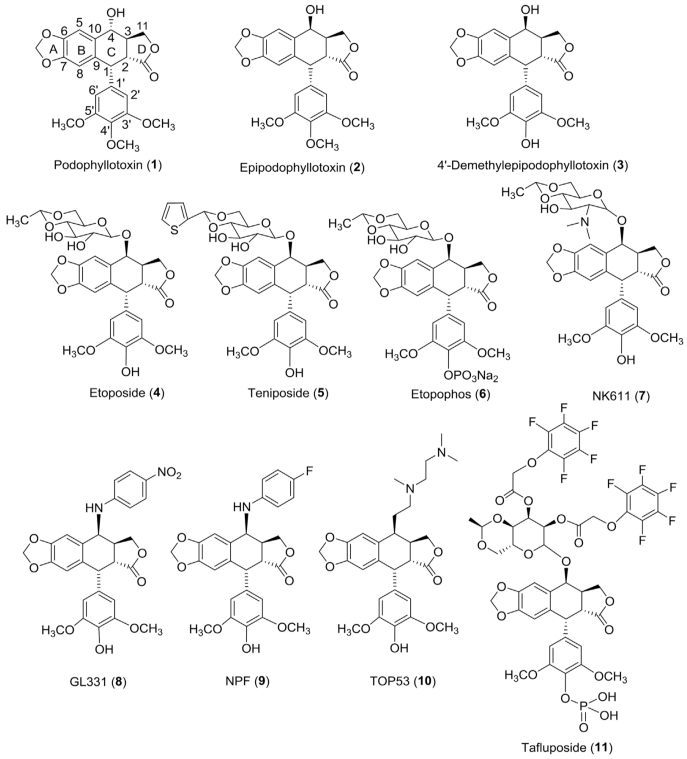

Figure 1.

Structures of podophyllotoxin (**1**, PPT), epipodophyllotoxin (**2**, EPPT), 4′-demethylepipodophyllotoxin (**3**, DEPPT), etoposide (**4**, ETO), teniposide (**5**), etopophos (**6**), NK-611(**7**), GL-331(**8**), NPF (**9**), TOP-53 (**10**), and tafluposide (**11**).

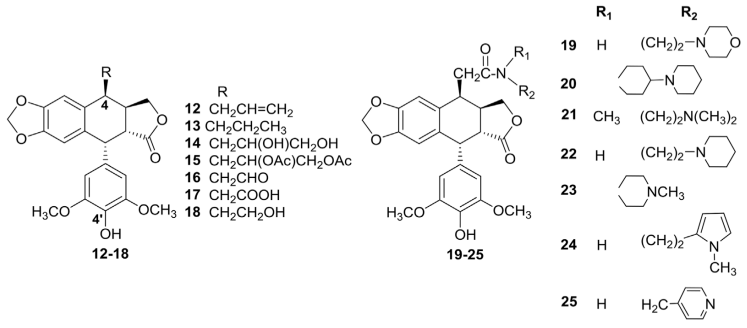

Figure 2.
Structures of alkyl and amidomethyl analogs **12–25**.

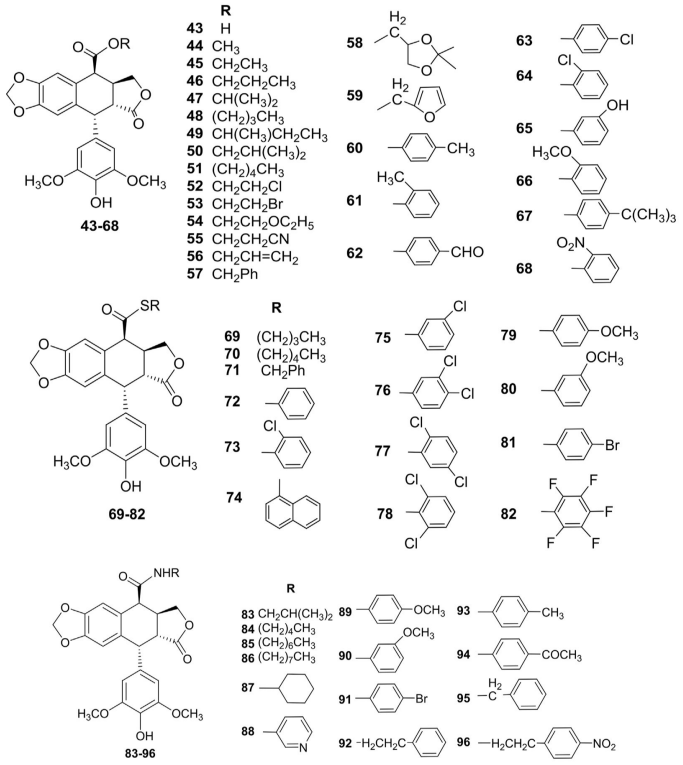

Figure 3.
Structures of compounds **43–96**.

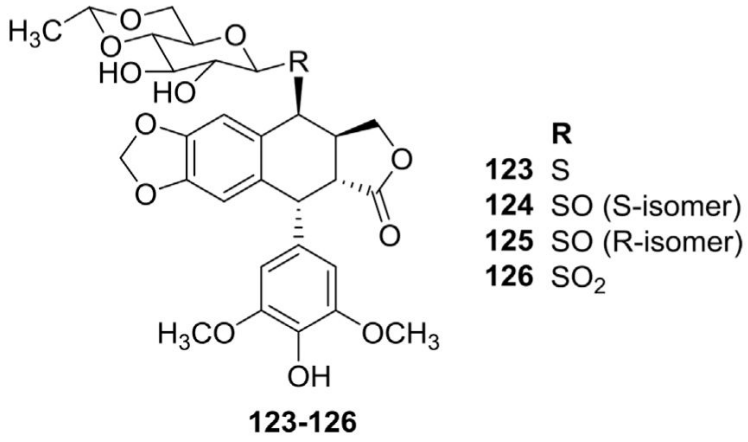

Figure 4.
Structures of thioglucose ETO analogs **123–126**.

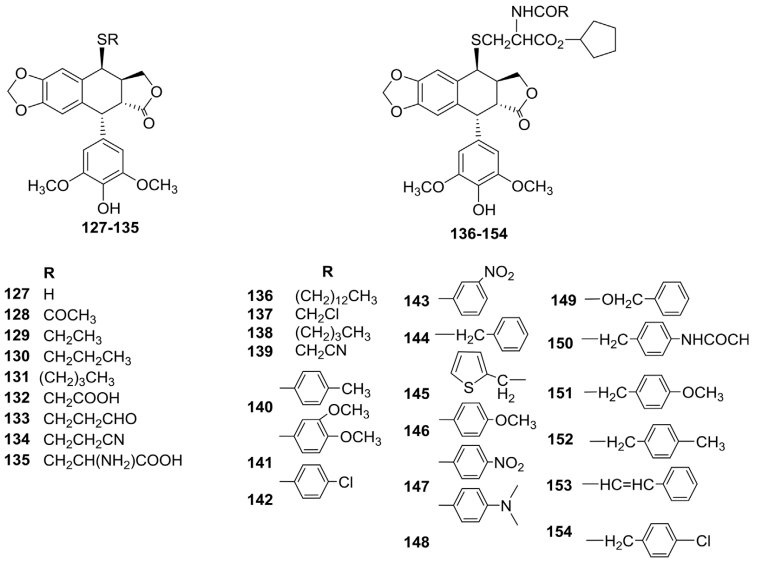

Figure 5.

Structures of thioether analogs **127–154**.

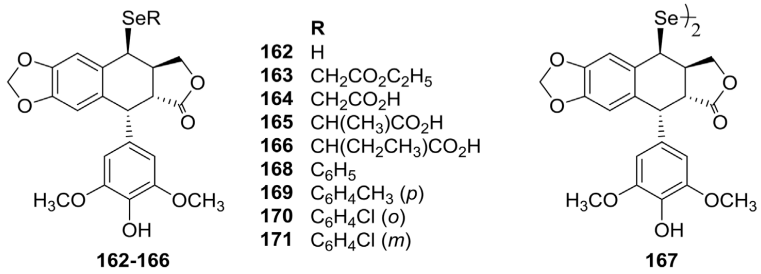

Figure 6.
Structures of Se-linked analogs **162–171**.

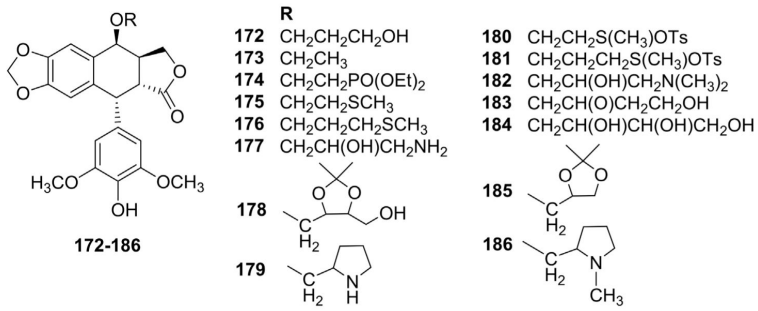

Figure 7.

Structures of ether analogs **172–186**.

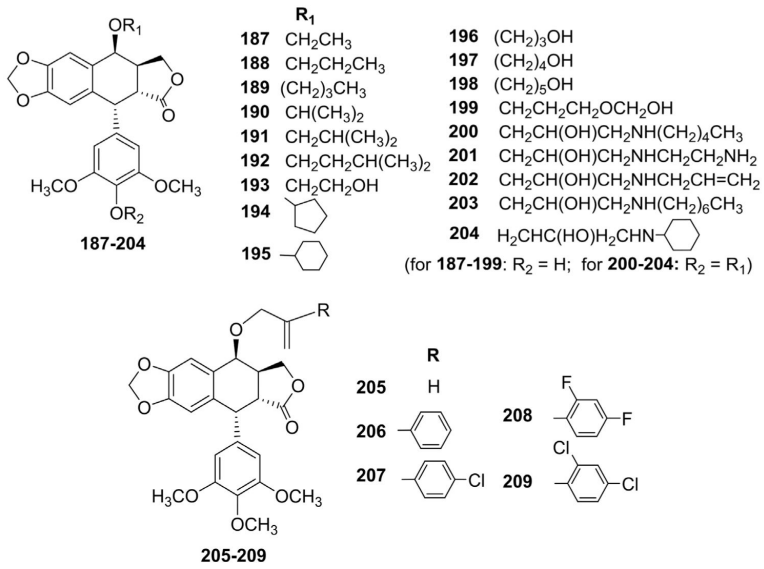

Figure 8.
Structures of ether analogs **187–209**.

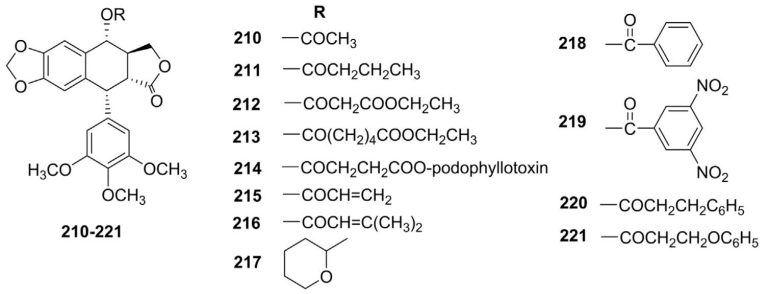

Figure 9.
Structures of ester analogs **210–221**.

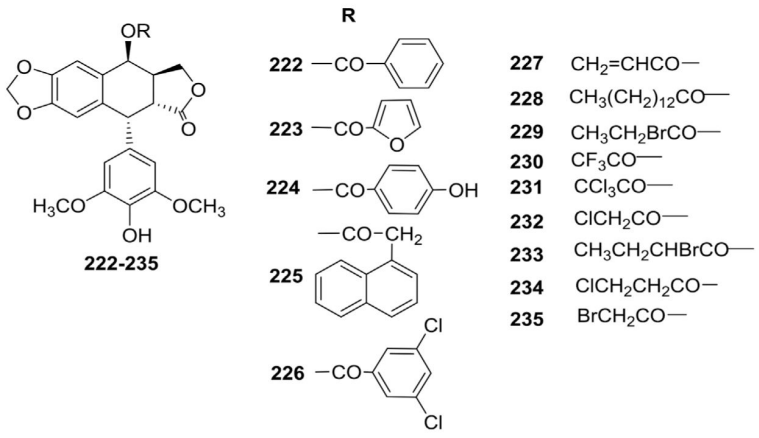

Figure 10.
Structures of ester analogs **222–235**.

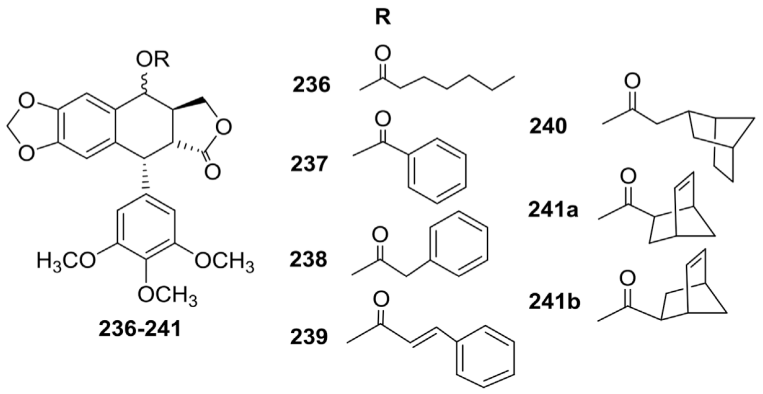

Figure 11.
Structures of ester analogs 236–241.

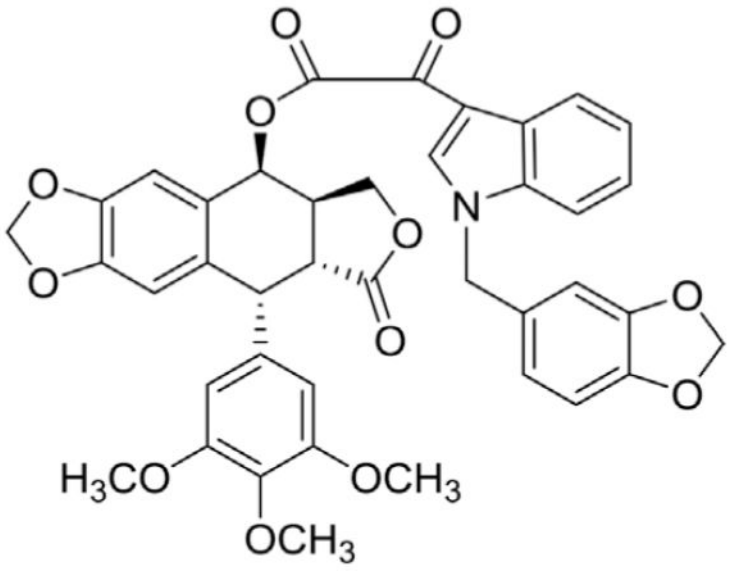

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Figure 12.
Structure of L1EPO (**286**).

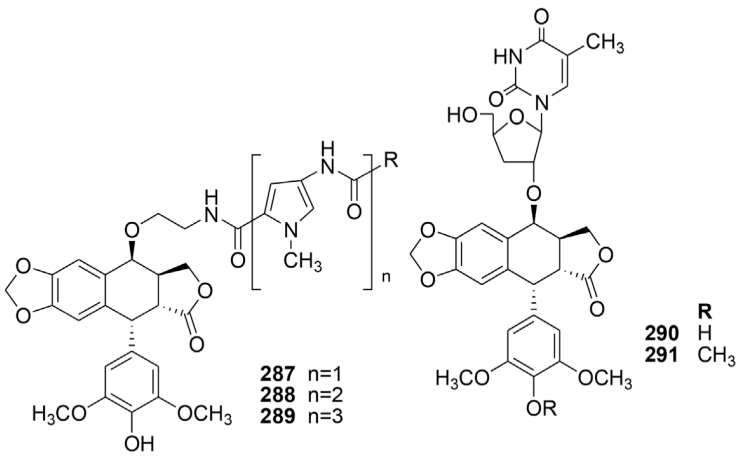
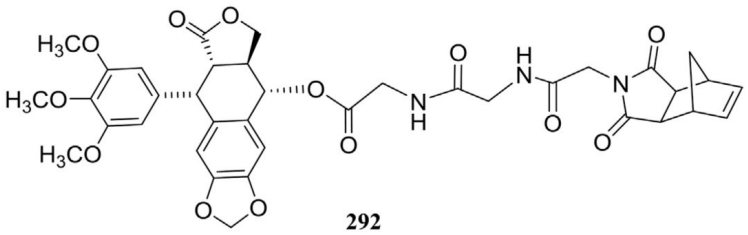


Figure 13.
Structures of compounds **287–291**.



292

Figure 14.
Structure of compound **292**.

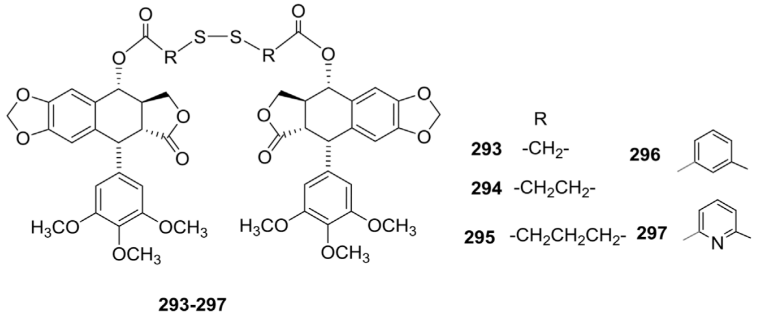


Figure 15.
Structures of novel bis-ester-linked analogs **293–297**.

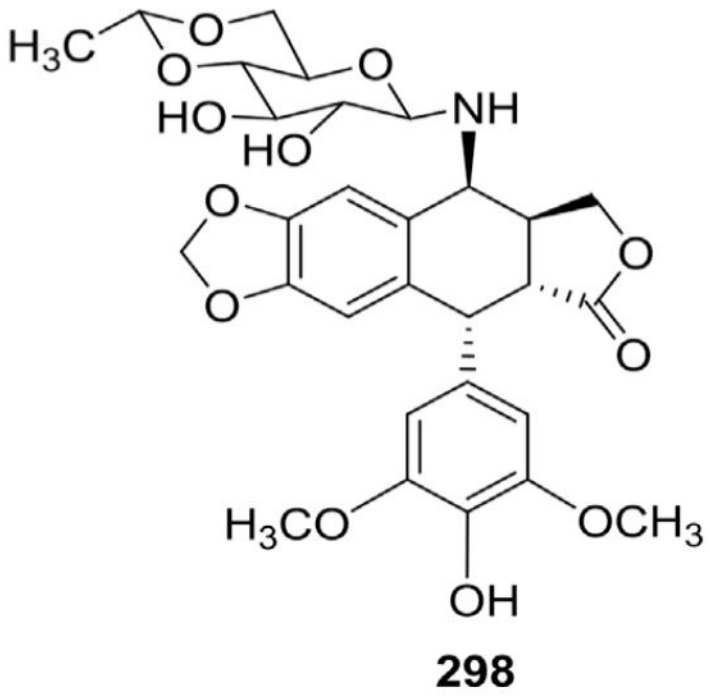


Figure 16.
Structure of 4-aminoglucose ETO analog **298**.

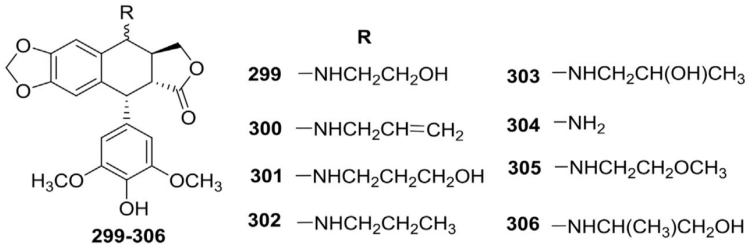


Figure 17.
Structures of alkylamino analogs **299–306**.

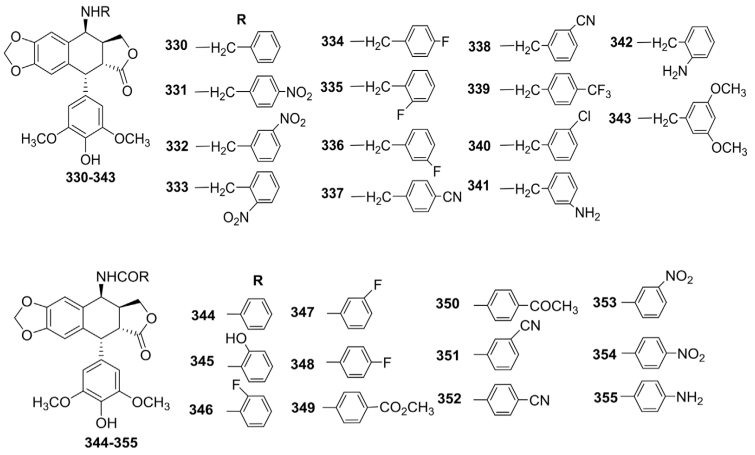


Figure 18.
Structures of benzyl- and benzoyl-amino analogs **330–355**.

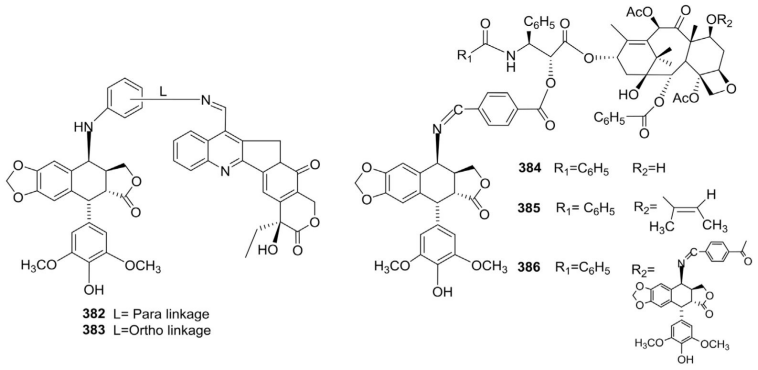
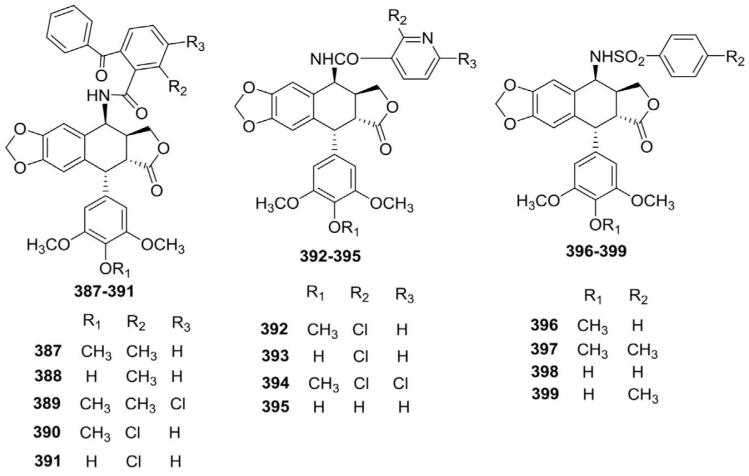


Figure 19.
Structures of amino conjugates (**382–386**) with camptothecin or taxoids.



	R_1	R_2	R_3
387	CH_3	CH_3	H
388	H	CH_3	H
389	CH_3	CH_3	Cl
390	CH_3	Cl	H
391	H	Cl	H

	R_1	R_2	R_3
392	CH_3	Cl	H
393	H	Cl	H
394	CH_3	Cl	Cl
395	H	H	H

	R_1	R_2
396	CH_3	H
397	CH_3	CH_3
398	H	H
399	H	CH_3

Figure 20.
Structures of compounds **387–399**.

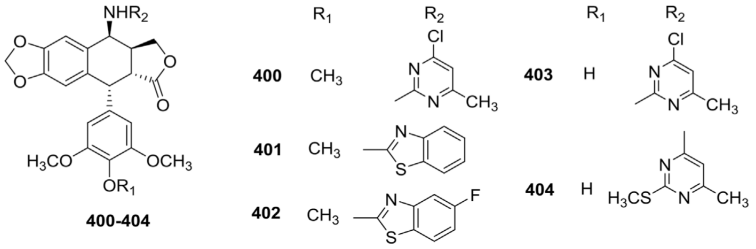


Figure 21.
Structures of heteroarylamino analogs **400–404**.

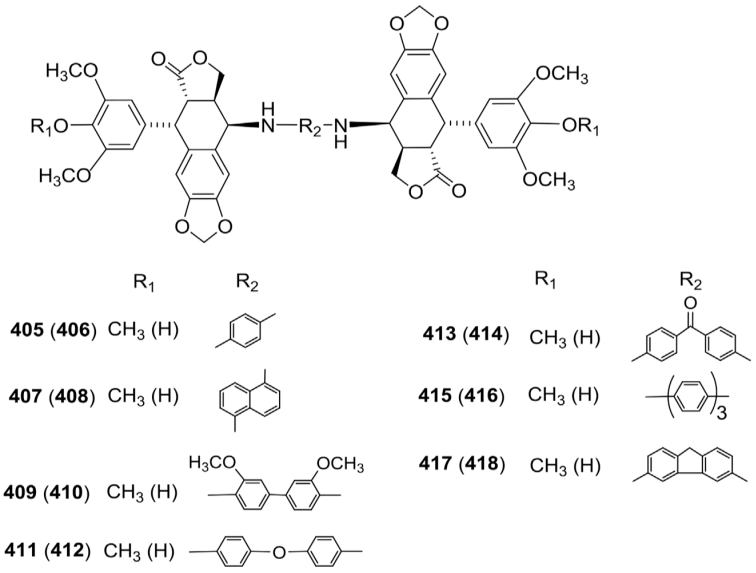


Figure 22.
Structures of bis-*N*-linked dimers **405–418**.

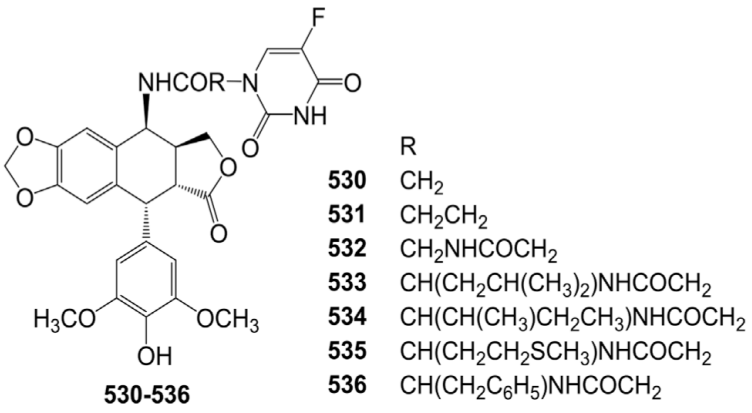
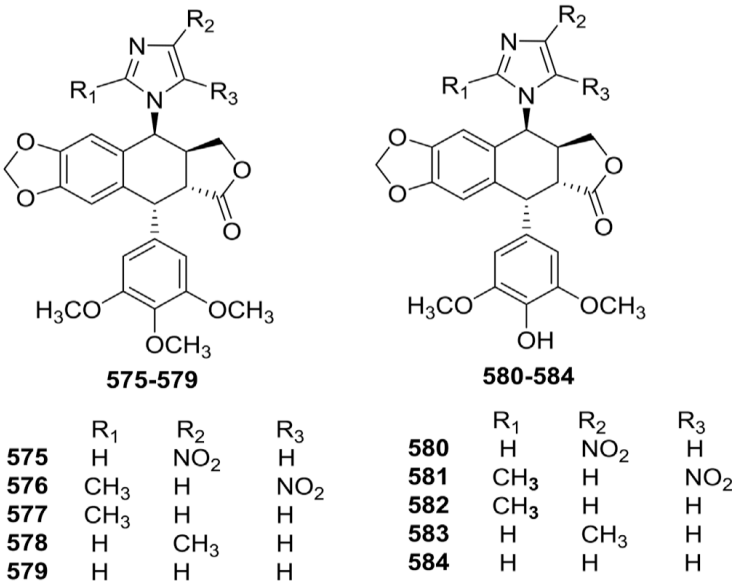


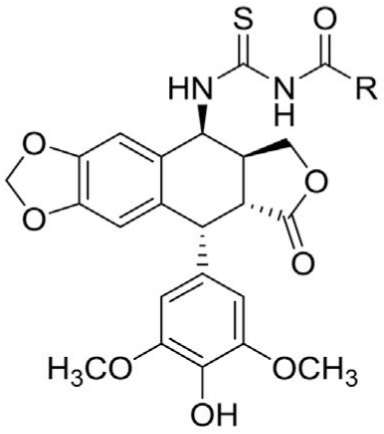
Figure 23.
Structures of 5-FU–DEPPT analogs **530–536**.



	R_1	R_2	R_3
575	H	NO_2	H
576	CH_3	H	NO_2
577	CH_3	H	H
578	H	CH_3	H
579	H	H	H

	R_1	R_2	R_3
580	H	NO_2	H
581	CH_3	H	NO_2
582	CH_3	H	H
583	H	CH_3	H
584	H	H	H

Figure 24.
Structures of imidazolyl analogs **575–584**.



630-641

	R
630	Ph
631	2-Cl-Ph
632	3-Cl-Ph
633	4-Cl-Ph
634	2-Br-Ph
635	2-I-Ph
636	4-F-Ph
637	α-naphthyl
638	2-Me-Ph
639	3-Me-Ph
640	4-Me-Ph
641	thiophene

Figure 25.
Structures of aroylthiourea-amino analogs **630–641**.

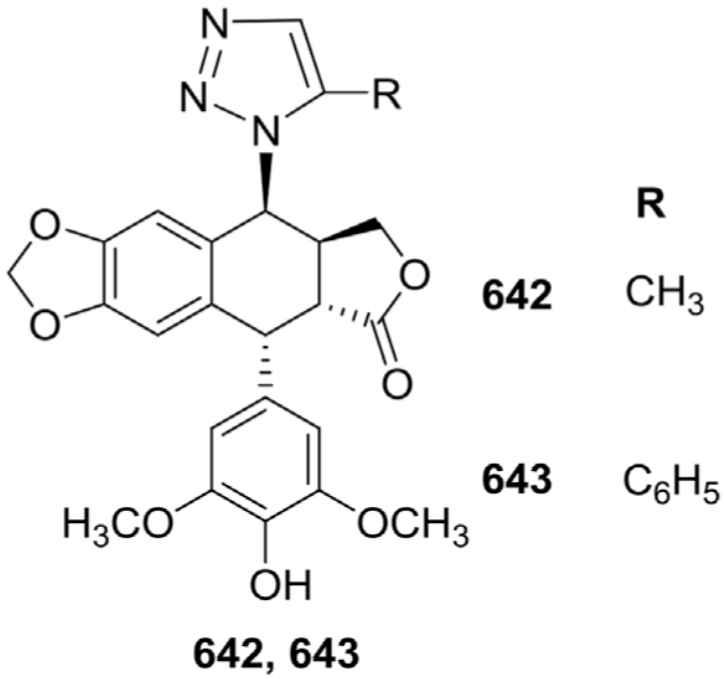


Figure 26.
Structures of triazole analogs **642–643**.

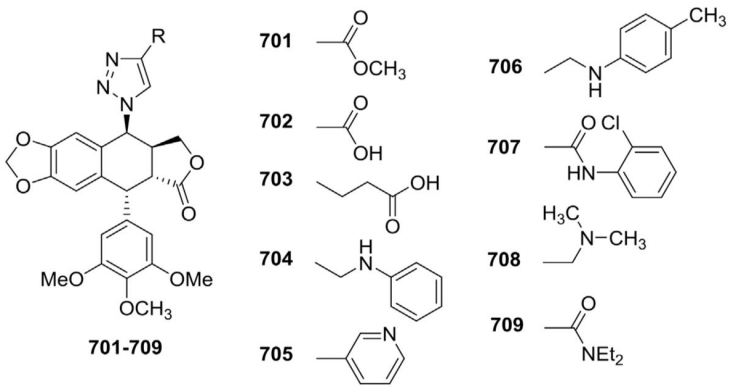


Figure 27.
Structures of compounds **701–709**.

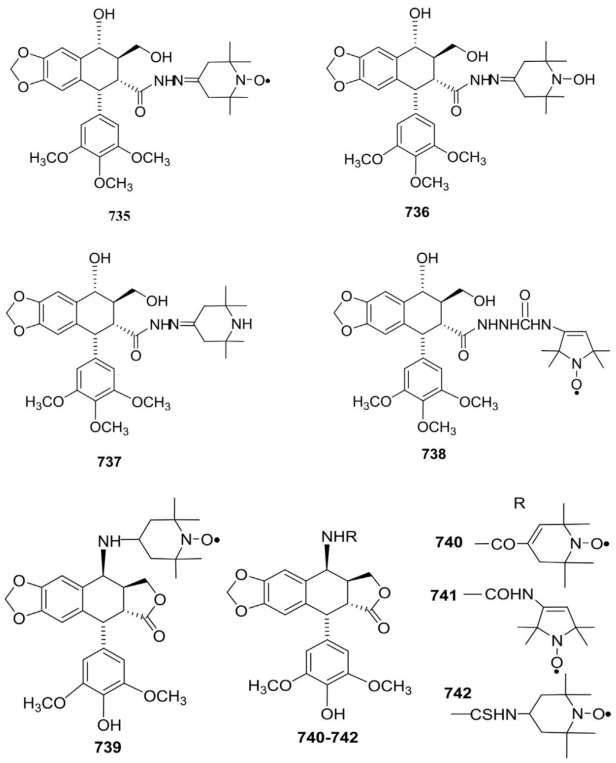
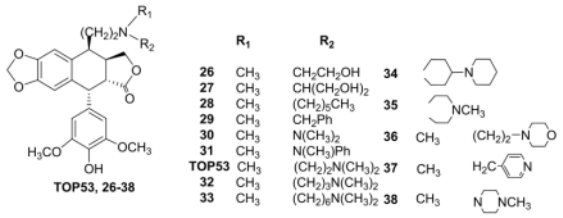


Figure 28.
Structures of spin-labeled compounds **735–742**.

Table I

Biological Data for Aminoethyl Analogs **TOP53** and **26–38**



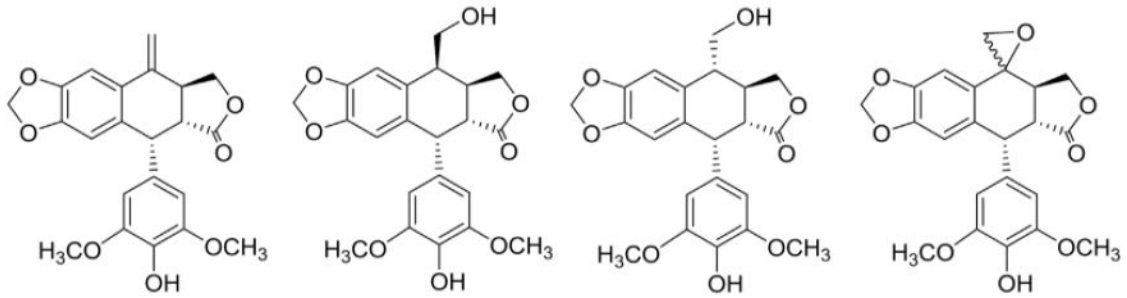
Compound	Cytotoxicity P388 (IC_{50}, μM)	Topo II (IC_{50}, μM)
ETO	0.01	59.2 (1.0)[a]
26	0.07	17.2 (0.29)
27	0.66	25.1 (0.42)
28	0.019	61.4 (1.03)
29	0.15	97.3 (1.64)
30	0.02	58.3 (0.98)
31	1.0	NT[b]
TOP53	0.001	32.5 (0.54)
32	0.055	26.9 (0.45)
33	0.037	30.0 (0.50)
34	0.0033	29.8 (0.53)
35	0.0030	33.6 (0.56)
36	0.26	115.7 (1.95)
37	0.10	31.3 (0.52)
38	0.0043	32.3 (0.54)

[a] The value in parentheses is the ratio of IC_{50} of compound/IC_{50} of ETO.

[b] NT, not tested.

Table II

Biological Data for New Compounds **39–42**



39 **40** **41** **42**

Compound	L1210 (IC_{50}, μM)	Cell cycle[a]			Inhibition of microtubule assembly[b] (IC_{50}, μM)
		Percent of G2/M	Percent of 8N	Concentration (μM)	
39	0.035	60	27	0.1	2.3
40	0.24	42	44	0.25	NT[c]
41	0.10	69	15	0.5	7.3
42	1.30	69	20	5	8.3

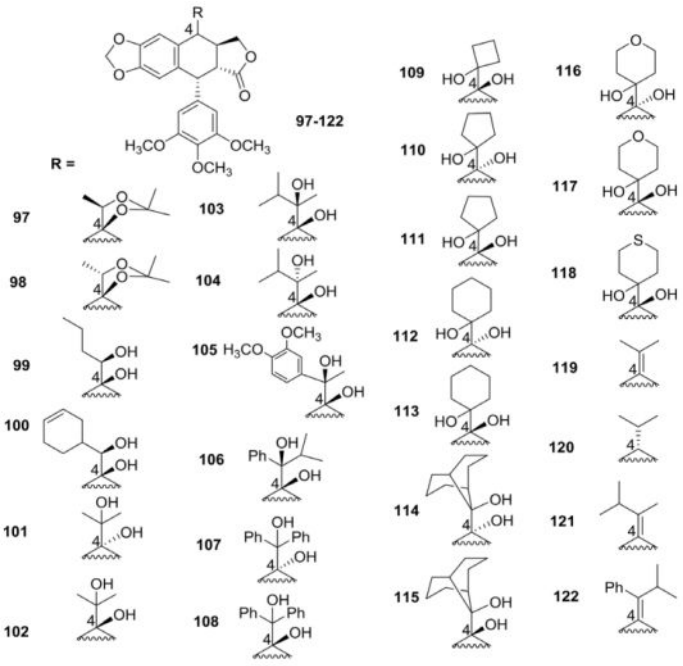
[a] Untreated control cells: 24% G2/M, 1% 8N.

[b] 4-Deoxypodophyllotoxin IC_{50} = 1.1 μM.

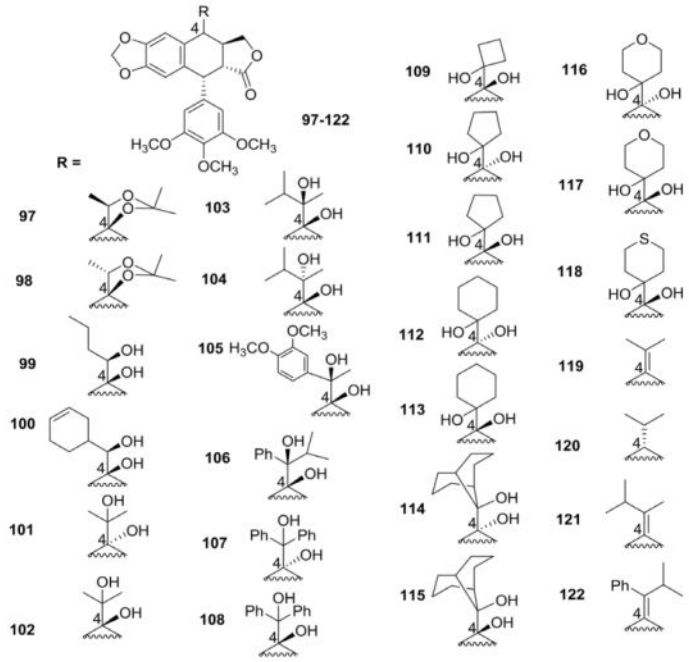
[c] NT, not tested.

Table III

Cytotoxicity Data for Pinacol, Alkylidene, and Alkyl Analogs **97–122**



	GI_{50} ± SD (nM)[a]		
Compound	**A-549**	**HT-29**	**SK-BR3**
EPPT	60	60	NT
97	6220 ± 562	5230 ± 443	5870 ± 126
98	6020 ± 526	6560 ± 456	6140 ± 786
99	279 ± 12	404 ± 75	288 ± 8
100	2030 ± 15	2510 ± 413	2430 ± 9
101	1480 ± 564	659 ± 43	511 ± 65
102	577 ± 61	598 ± 83	457 ± 24
103	254 ± 6	204 ± 2	181 ± 7
104	1150 ± 27	1730 ± 87	1030 ± 70
105	2620 ± 65	4750 ± 126	4990 ± 345
106	139 ± 5	159 ± 16	148 ± 15
107	874 ± 68	528 ± 20	524 ± 36
108	376 ± 25	281 ± 32	313 ± 57
109	191 ± 18	198 ± 18	219 ± 40
110	1690 ± 356	2720 ± 315	2420 ± 154
111	444 ± 12	451 ± 8	439 ± 29
112	547 ± 71	859 ± 13	610 ± 24
113	90.8 ± 1.3	97.5 ± 4.6	64.9 ± 2.8
114	5680 ± 6	6420 ± 85	6720 ± 41

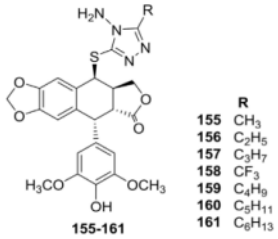


Compound	GI_{50} ± SD (nM)[a]		
	A-549	HT-29	SK-BR3
115	6.39 ± 0.15	5.14 ± 0.50	7.39 ± 0.6
116	8920 ± 317	15440 ± 491	8010 ± 140
117	29.0 ± 1.1	26.8 ± 0.3	25.9 ± 0.4
118	3320 ± 213	2620 ± 53	1780 ± 187
119	6440 ± 477	8280 ± 386	3780 ± 82
120	79.5 ± 9.0	83.1 ± 1.4	84.1 ± 3.0
121	7700 ± 137	10200 ± 221	4530 ± 141
122	6210 ± 326	11400 ± 582	14300 ± 589

[a] Cytotoxicity results are expressed as GI_{50} values, the compound concentration producing a 50% cell growth inhibition and represent the mean ± SD of three independent experiments. Values under 100 nM are highlighted for easier comparison.

Table IV

Biological Data for *S*-Linked Analogs **155–161**

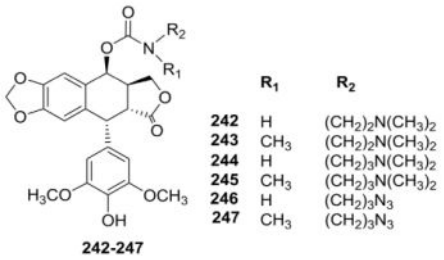


Compound	Concentration (mol/L)	Inhibition rate (%)[a]	
		HL-60	K562
155	10^{-6}	37.9	68.76
	10^{-5}	48.2	77.24
	10^{-4}	58.6	80.24
156	10^{-6}	48.2	76.15
	10^{-5}	55.1	83.10
	10^{-4}	58.6	86.83
157	10^{-6}	24.1	68.32
	10^{-5}	55.1	73.95
	10^{-4}	55.1	86.32
158	10^{-6}	10.3	52.30
	10^{-5}	55.1	62.61
	10^{-4}	62.0	80.90
159	10^{-6}	22.3	60.71
	10^{-5}	49.6	72.86
	10^{-4}	58.6	79.07
160	10^{-6}	40.0	NT
	10^{-5}	65.0	NT
	10^{-4}	67.5	NT
161	10^{-6}	20.6	NT
	10^{-5}	58.6	NT
	10^{-4}	62.0	66.86

[a]Results obtained after 72 hr.

Table V

Biological Data for Carbamoyl Analogs **242–247**



	R_1	R_2
242	H	$(CH_2)_2N(CH_3)_2$
243	CH_3	$(CH_2)_2N(CH_3)_2$
244	H	$(CH_2)_3N(CH_3)_2$
245	CH_3	$(CH_2)_3N(CH_3)_2$
246	H	$(CH_2)_3N_3$
247	CH_3	$(CH_2)_3N_3$

242-247

Compound	Topo IIα inhibition (% linear DNA)[a]	Cytotoxicity IC_{50} (μM)[b]	Cell cycle effect[c]
ETO	50	0.83	76% (2.5 μM)
242	56	0.038	67% (0.1 μM)
243	27	1.7	77% (2.5 μM)
244	58	0.34	72% (1 μM)
245	17	0.96	NT[d]
246	38	1.3	NT
247	8	0.24	NT

[a] Average value of at least three independent experiments at 20 μM of drug.

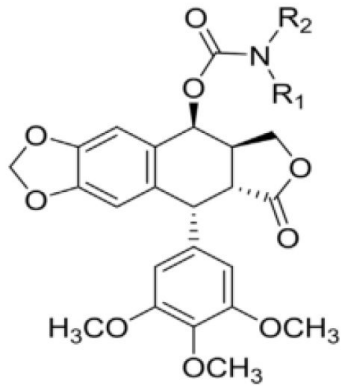
[b] IC_{50}, concentration of drug required to cause 50% reduction in L1210 cell growth.

[c] Percentage of L1210 cells in the G2/M phase at the specified drug concentration.

[d] NT, not tested.

Table VI

Cytotoxic Data for Carbamoyl Analogs **248–257**



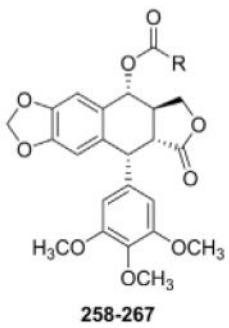
248-257

	R_1	R_2
248	H	propyl
249	H	isopropyl
250	H	cyclopropylmethyl
251	CH_3	CH_3
252	H	2-methoxyethyl
253	H	prop-2-yn-1-yl
254	NR_1R_2 = morpholinyl	
255	H	4-trifluoromethylbenzyl
256	H	3-ethyl-1H-indole
257	H	2-dimethylaminoethyl

	GI_{50} (μM)										
Compound	**Zr-75–1**	**MCF7**	**KB**	**Gurav**	**DWD**	**Colo 205**	**A549**	**Hop62**	**PC3**	**SiHa**	**A2780**
ETO	0.2	2.1	0.3	0.5	0.6	0.13	3.08	0.80	2.6	3.1	1.3
248	0.19	2.0	2.1	0.17	2.0	2.7	<0.1	0.16	01.7	1.2	0.19
249	0.15	0.19	0.18	0.16	<0.1	2.7	<0.1	2.1	0.19	0.19	0.14
250	0.19	2.3	2.4	0.18	2.0	2.5	<0.1	2.5	2.3	2.3	2.0
251	0.12	0.16	0.18	0.17	<0.1	2.1	<0.1	0.13	0.15	2.1	0.18
252	<0.1	0.15	0.15	0.14	<0.1	2.2	<0.1	0.12	0.16	2.4	0.15
253	0.14	0.19	0.17	0.14	<0.1	2.7	<0.1	0.14	0.16	1.9	0.16
254	0.14	0.16	0.12	0.14	<0.1	2.7	<0.1	0.13	0.16	1.4	0.13
255	01.6	0.18	0.18	0.19	2.0	0.15	<0.1	2.3	2.1	2.0	0.18
256	0.13	0.16	0.18	0.12	<0.1	0.12	<0.1	2.1	0.16	1.8	0.11
257	<0.1	0.11	0.16	0.15	<0.1	2.1	<0.1	0.12	0.15	2.1	0.12

Table VII

Cytotoxicity Data for Ester Analogs **258–267**



258-267

R

258 $(CH_2)_9$-OH

259 $(CH_2)_{11}$-OH

260 $(CH_2)_{14}$-OH

261 $(CH_2)_{15}$-OH

262 $(CH_2)_7$-CH=(Z)CH-CH_2-CH(OH)-$(CH_2)_5CH_3$

263 $(CH_2)_3$-CH=(Z)CH-CH_2-CH=(Z)CH-CH_2-CH=(Z)CH-CH_2-CH=(Z)CH-$(CH_2)_4$-CH_3

264 $(CH_2)_6$-CH=(Z)CH-CH_2-CH=(Z)CH-CH_2-CH=(Z)CH-$(CH_2)_4$-CH_3

265 $(CH_2)_9$-CH=(Z)CH-CH_2-CH=(Z)CH-$(CH_2)_4$-CH_3

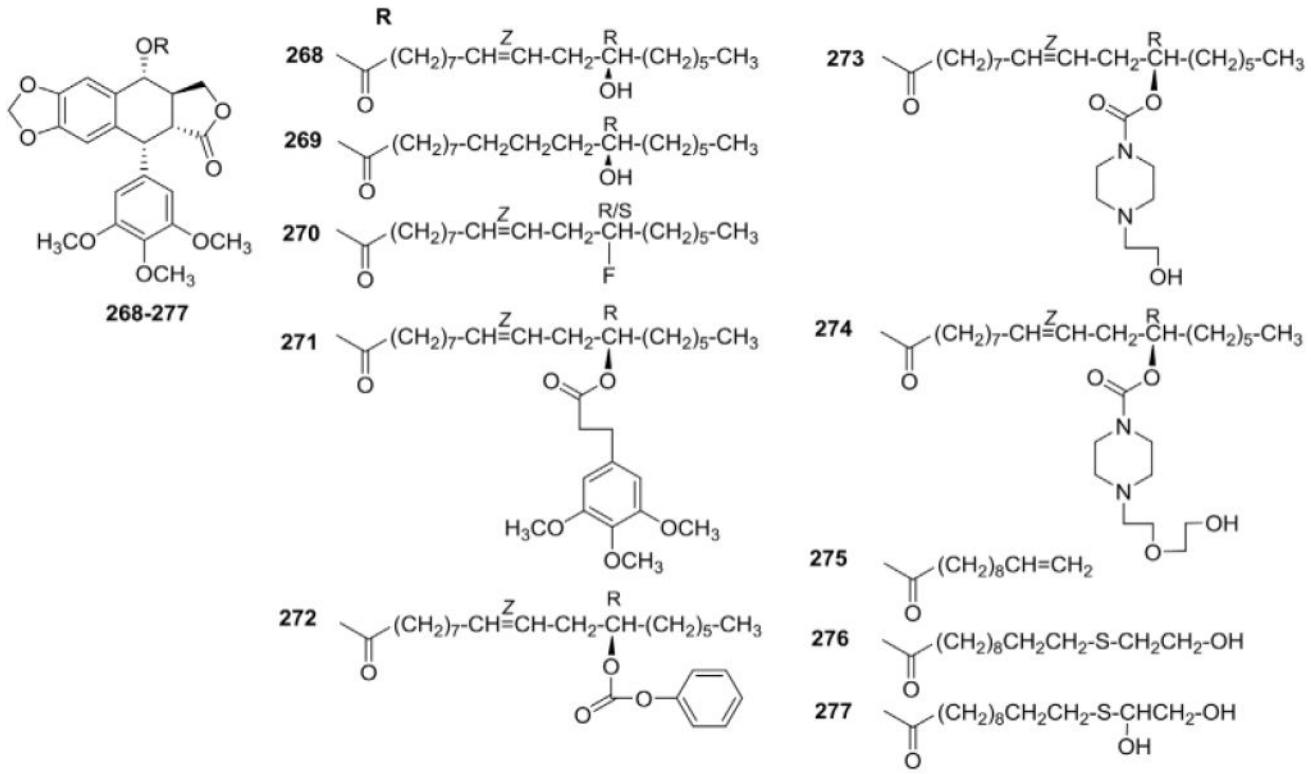
266 $(CH_2)_9$-CH=(Z)CH-$(CH_2)_7$-CH_3

267 $(CH_2)_{18}CH_3$

Compound	IC_{50} (μM)					Kidney fibroblast VERO cells
	SK-MEL	KB	BT-549	SK-OV-3	HL-60	
PPT	0.22	0.24	0.36	0.19	0.01	0.55
258	0.21	0.31	0.22	0.26	0.07	NA
259	0.41	1.19	0.29	0.44	0.19	NA
260	0.89	NA	1.19	0.76	0.24	NA
261	0.90	2.39	0.85	0.81	0.18	NA
262	4.0	4.30	2.59	3.75	0.47	NA
263	0.34	0.40	0.27	0.27	0.07	NA
264	1.17	1.35	1.08	1.08	0.24	NA
265	1.42	1.42	0.89	0.79	0.85	NA
266	NA	NA	NA	NA	NA	NA
267	NA	NA	NA	NA	NA	NA

Table VIII

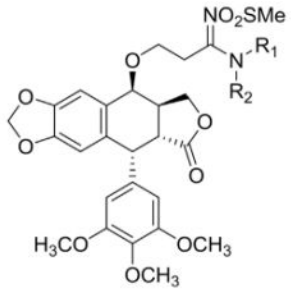
Cytotoxicity Data for Ester Analogs **268–277**



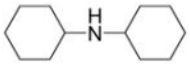
	IC_{50} (μM)					
Compound	**SK-MEL**	**KB**	**BT-549**	**SK-OV-3**	**HL-60**	**VERO**
PPT	0.22	0.24	0.36	0.19	0.01	0.55
268	0.45	1.26	4.76	0.17	0.66	NA
269	8.12	NA	7.50	10.64	0.22	NA
270	NA	NA	NA	NA	0.73	NA
271	NA	NA	NA	NA	NA	NA
272	NA	NA	NA	NA	NA	NA
273	0.72	2.4	1.2	0.84	0.58	NA
274	1.87	2.2	2.53	0.77	0.55	NA
275	NA	0.93	1.33	NA	0.08	NA
276	0.30	0.45	0.30	0.30	0.10	NA
277	2.9	0.35	0.81	NA	0.07	NA

Table IX

Cytotoxicity Data for Analogs **278–285**



278-285

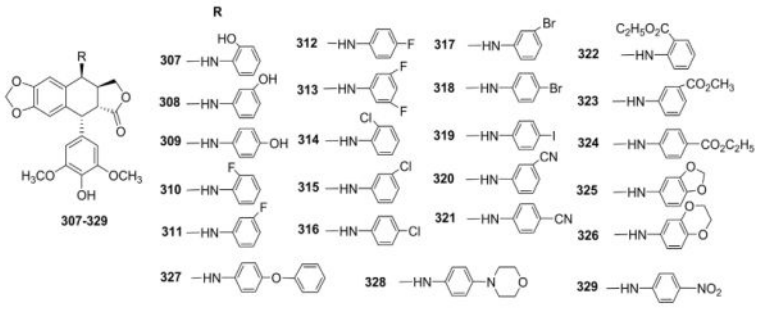
	HNR_1R_2		
278	$(n\text{-}Pr)_2NH$	**282**	
279	$PhCH_2NH_2$	**283**	
280		**284**	NH_3
281		**285**	Me_2NH

	IC_{50} (μM)[a]			
Compound	**K562**	**SGC-7901**	**HeLa**	**HepG2**
Colchicine	0.55 ± 0.02	0.05 ± 0.02	0.10 ± 0.02	0.02 ± 0.03
PPT	4.51 ± 0.03	0.34 ± 0.02	0.14 ± 0.04	0.51 ± 0.02
278	2.96 ± 0.03	2.87 ± 0.03	2.63 ± 0.03	2.14 ± 0.04
279	12.07 ± 0.04	19.16 ± 0.02	11.23 ± 0.02	17.88 ± 0.02
280	9.01 ± 0.03	18.20 ± 0.03	10.37 ± 0.02	57.14 ± 0.01
281	8.34 ± 0.02	11.21 ± 0.04	5.99 ± 0.03	20.79 ± 0.02
282	1.01 ± 0.02	1.36 ± 0.01	0.75 ± 0.03	0.79 ± 0.01
283	2.61 ± 0.02	3.21 ± 0.02	4.33 ± 0.01	1.95 ± 0.02
284	3.48 ± 0.03	4.31 ± 0.01	3.75 ± 0.02	2.87 ± 0.03
285	5.18 ± 0.02	3.71 ± 0.03	2.58 ± 0.02	4.17 ± 0.02

[a]Cytotoxicity results are expressed as IC_{50} values, the compound concentration producing a 50% cell growth inhibition and represent the mean ± SD of three independent experiments.

Table X

Biological Data for Arylamino Analogs **307–329**



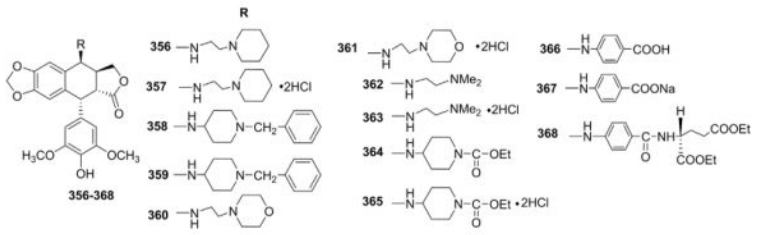
Compound	Cytotoxicity[a] ID_{50} KB (μM)	Inhibition of DNA topo II activity ID_{50},[b] μM	Cellular protein–DNA complex formation % (10 μM)
ETO	0.2	50	100
307	4.54	25	151
308	0.45	5	290
309	2.26	25	211
310	0.25	–	121
311	0.23	50	158
312	0.24	10	213
313	1.08	50	115
314	2.34	–	32
315	2.29	–	51
316	0.22	50	99
317	2.36	–	62
318	0.22	100	179
319	0.34	–	64
320	0.69	25	137
321	0.64	10	211
322	1.0	>100	4
323	2.7	50	249
324	0.84	5	207
325	<1.0	50	164
326	0.68	10	279
327	1.0	25	97
328	0.66	10	140
329	0.49	10	323

[a] ID_{50}, concentration of drug that afforded 50% reduction in cell number after a 3-day incubation.

[b] Each compound was examined at 25, 50, and 100 μM. The ID_{50} value was established on the basis of the degree of inhibition at these three concentrations.

Table XI

Biological Data for Amino Analogs **356–368**



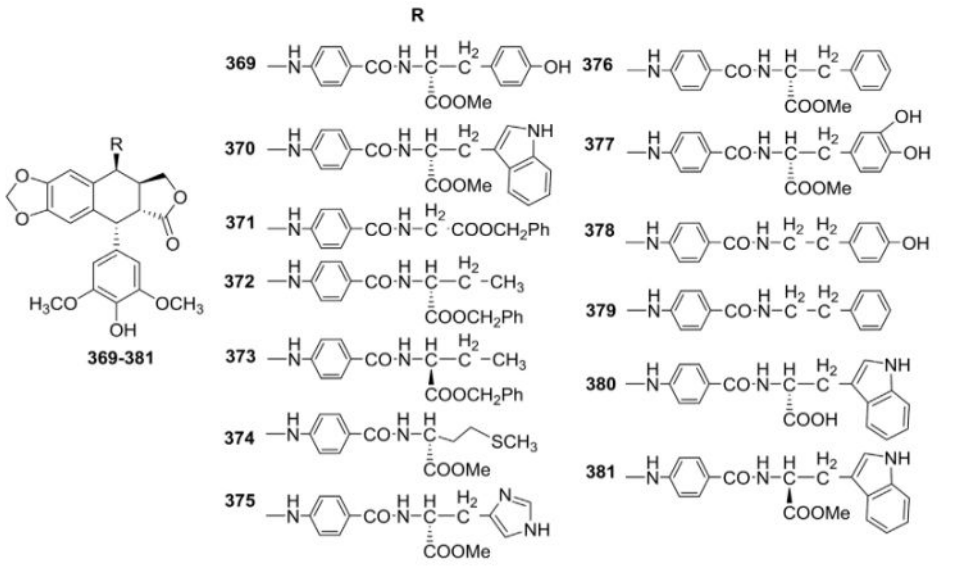
Compound	Cytotoxicity[a] ID_{50} KB, μM	Inhibition of DNA topo II activity ID_{50},[b] μM	Cellular protein–DNA complex formation % (20 μM)
ETO	0.20	50	100
356	1.4	25	190
357	1.6	50	183
358	0.027	100	83
359	0.021	50	172
360	2.0	>100	77
361	4.0	50	140
362	0.4	25	203
363	–	25	183
364	0.74	25	17
365	1.13	25	138
366	>4.0	>100	1.9
367	>4.0	>100	6.9
368	<0.4	100	83

[a] ID_{50}, concentration of drug that afforded 50% reduction in cell number after a 3-day incubation.

[b] Each compound was examined at 25, 50, and 100 μM. The ID_{50} was established on the basis of the degree of inhibition at these three concentrations.

Table XII

Biological Data for 4″-Benzamido-Amino Analogs **369–381**



Compound	Percent PLDB formation[a]	KB ED_{50}[b] (μg/mL)	KB-7d ED_{50}[b] (μg/mL)	Relative resistance[c] (fold)
ETO	100 (100)	0.5	>10	>20
369	76	1.9	5	2.6
370	58	0.5	2.5	5
371	178	0.5	0.3	0.6
372	210	0.5	0.25	0.5
373	133	0.1	0.25	2.5
374	295	0.8	2.4	3
375	122	0.23	2.2	9.6
376	226	0.55	1.0	1.8
377	33	1	5.5	5.5
378	368 (275)	0.025	0.8	32
379	(227)	0.035	0.5	14
380	7	4	8	2
381	121	0.065	0.4	6.2

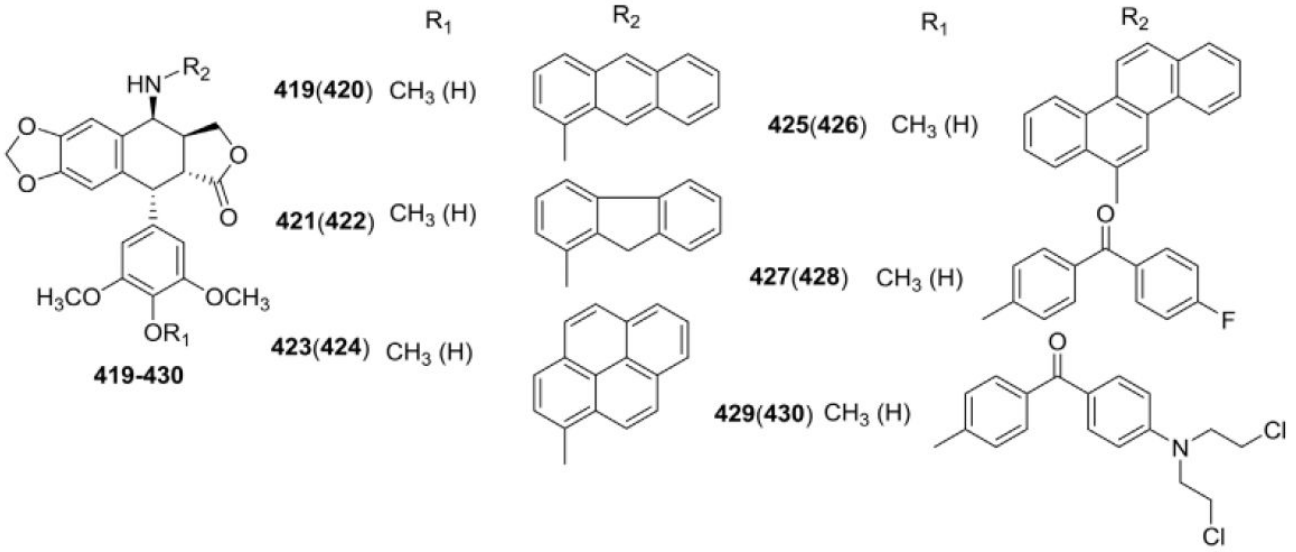
[a] Percent protein-linked DNA breaks (PLDB) formation was determined by the SDS/potassium precipitation method. Percentage values are levels of protein-linked DNA breaks induced by drug treatment relative to the ETO control set arbitrarily at 100%. Values in parentheses reflect effects at a concentration of 5 μg/mL. Other values reflect effects at a concentration of 10 μg/mL.

[b] ED_{50} is the concentration of drug that afforded 50% reduction in cell number after a 3-day incubation.

[c] Relative resistance (fold) values are the ED_{50} values against KB-7d over those against KB cells.

Table XIII

Cytotoxicity Data for Polyaromatic Amino Analogs **419–430**

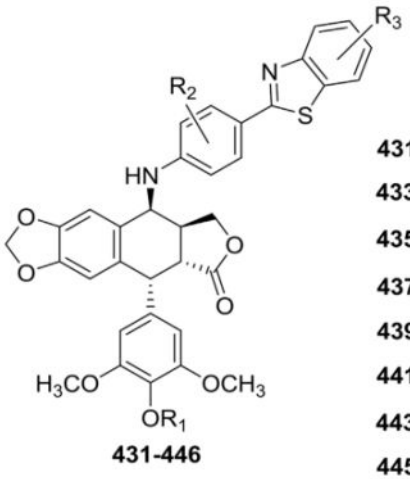


	IC_{50} (μM)						
Compound	**Colo502713**	**HCT-15**	**HEP-2**	**IMR-32**	**A549**	**DU145**	**PC-3**
ETO	0.1	0.9	0.9	6.3	3.08	3.7	2.6
419	9.4	6.3	6.7	6.7	8.8	21	7.9
420	–	8.2	8.6	2.3	8.3	18	–
421	5.5	0.09	3	5.5	7.1	6.6	4.3
422	0.001	0.0001	1	10	1	2.7	0.5
423	0.002	0.0001	–	–	0.0009	–	0.0001
424	0.002	0.0001	0.0002	0.2	1.7	2.2	–
425	0.1	0.002	0.002	0.003	0.04	2.2	0.1
426	0.1	0.1	0.01	0.001	0.0003	0.0003	0.0003
427	4.1	6.2	7.1	19	17	–	6.7
428	1.4	0.08	1.3	1.3	0.2	0.3	6.0
429	0.1	0.9	0.9	6.3	3.08	3.7	2.6
430	NT[a]	NT	NT	NT	NT	NT	NT

[a] NT, not tested.

Table XIV

Cytotoxicity Data for Arylamino Analogs **431–446**



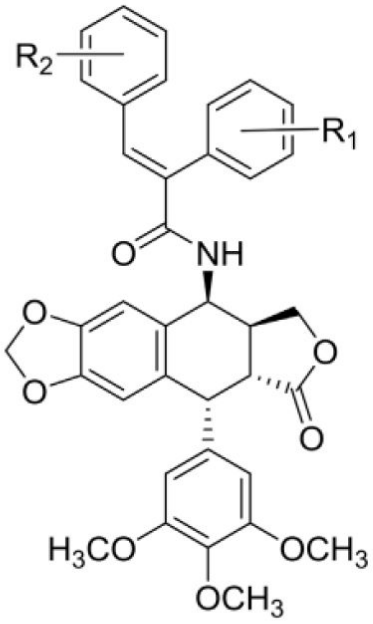
431-446

	R_1	R_2	R_3
431(432)	CH_3 (H)	H	H
433(434)	CH_3 (H)	2'-Cl	H
435(436)	CH_3 (H)	3'-CH_3	H
437(438)	CH_3 (H)	3'-Br	H
439(440)	CH_3 (H)	H	6-OCH_3
441(442)	CH_3 (H)	H	6-F
443(444)	CH_3 (H)	H	4-Cl
445(446)	CH_3 (H)	H	4,6-dichloro

	IC_{50} (μM)			
Compound	**Colo 205**	**Hop62**	**HT1080**	**DWD**
PPT	5.0	13.1	8.1	6.1
431	8.1	>80	8.2	22
432	2.7	30.1	9.7	2.3
434	8.3	8.6	>80	6.5
437	7.0	15.2	9.5	5.2
441	7.1	60.3	9.1	7.3
442	8.3	10.1	>80	9.1

Table XV

Cytotoxicity Data for Acrylamido Analogs **447–461**



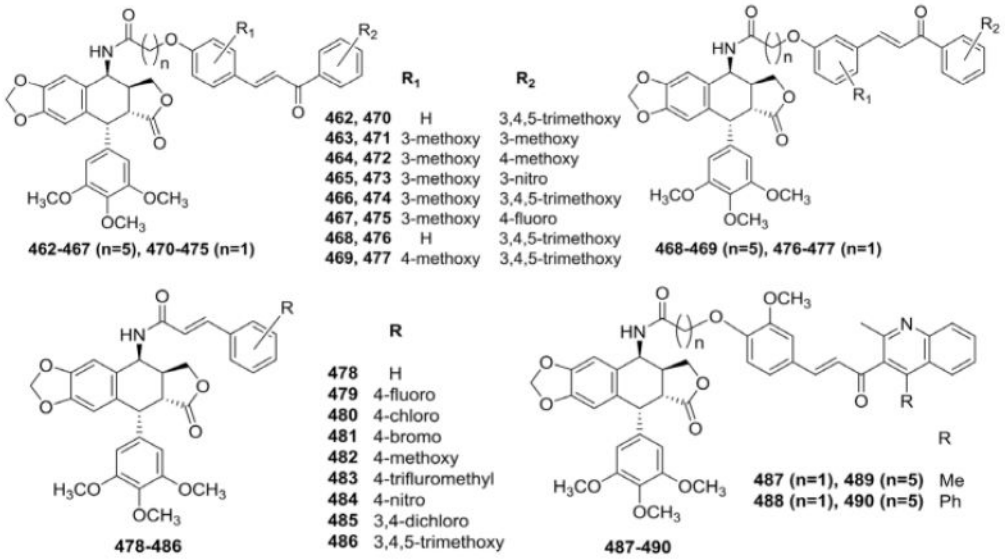
447-461

	R_1	R_2
447	3,4,5-trimethoxy	4-fluoro-3-methoxy
448	3,4,5-trimethoxy	3-fluoro-4-methoxy
449	3,4,5-trimethoxy	2-fluoro-5-methoxy
450	3,4,5-trimethoxy	2-fluoro-4-methoxy
451	3,4,5-trimethoxy	3-nitro-4-methoxy
452	3,4,5-trimethoxy	4-hydroxy-3-nitro
453	3,4,5-trimethoxy	4-nitro
454	3,4,5-trimethoxy	3-nitro
455	3,4,5-trimethoxy	4-methoxy
456	3,4,5-trimethoxy	3-methoxy
457	2-methoxy	4-nitro
458	2-methoxy	2-nitro
459	3,4,5-trimethoxy	4-hydroxy-3-methoxy
460	3,4,5-trimethoxy	3-hydroxy-4-methoxy
461	3,4,5-trimethoxy	4-hydroxy

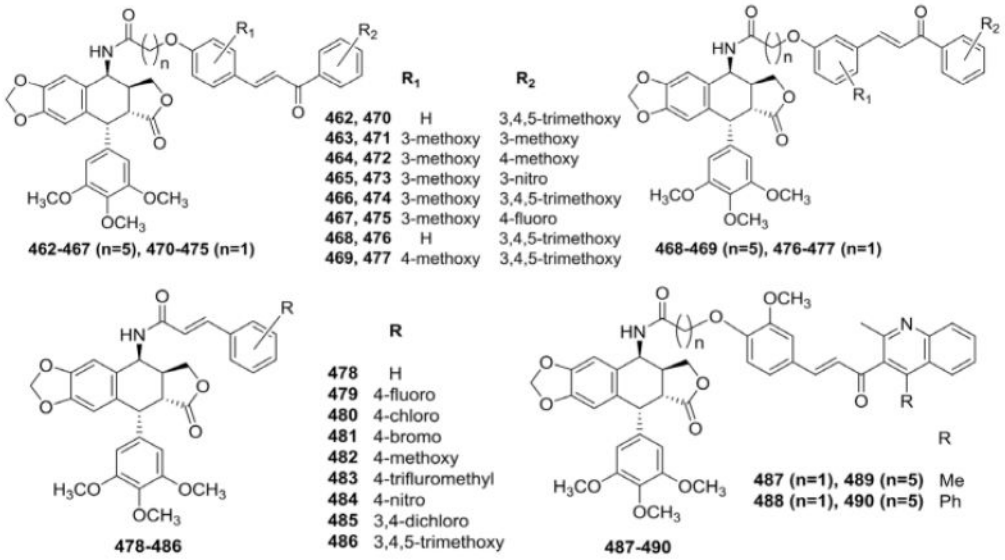
	GI_{50} (μM)							
Compound	**Zr-75–1**	**MCF7**	**Gurav**	**DWD**	**Colo 205**	**A549**	**Hop62**	**A2780**
ETO	0.2	2.1	0.5	0.6	0.13	3.08	0.8	1.3
447	2.2	–	2.4	–	–	−2	2.5	–
448	–	2.7	–	–	2.4	–	–	–
449	2.7	–	2.5	–	–	–	–	–
450	2.5	2.4	0.18	0.19	–	2.1	0.17	2.7
451	2.2	–	2.6	2.9	2.7	–	2.3	2.5
452	0.18	2.7	0.19	<0.1	2.0	–	0.19	2.2
453	2.2	–	2.1	2.4	2.1	–	2.7	2.6
454	–	2.7	2.1	<0.1	–	2.4	0.17	2.5
455	2.9	2.9	2.2	2.3	0.17	–	–	2.5
456	0.18	<0.1	2.1	2.2	–	2.7	0.18	<0.1
457	2.1	<0.1	2.1	2.3	–	2.7	2.0	2.3
458	2.2	<0.1	2.2	2.3	–	2.8	2.1	2.2
459	2.9	–	–	–	–	2.4	2.5	–
460	–	–	–	–	–	2.9	2.9	–
461	2.7	–	2.7	2.7	–	2.3	2.6	–

Table XVI

Cytotoxicity Data for Alkylamido Chalcone and Cinnamido Analogs **462–490**



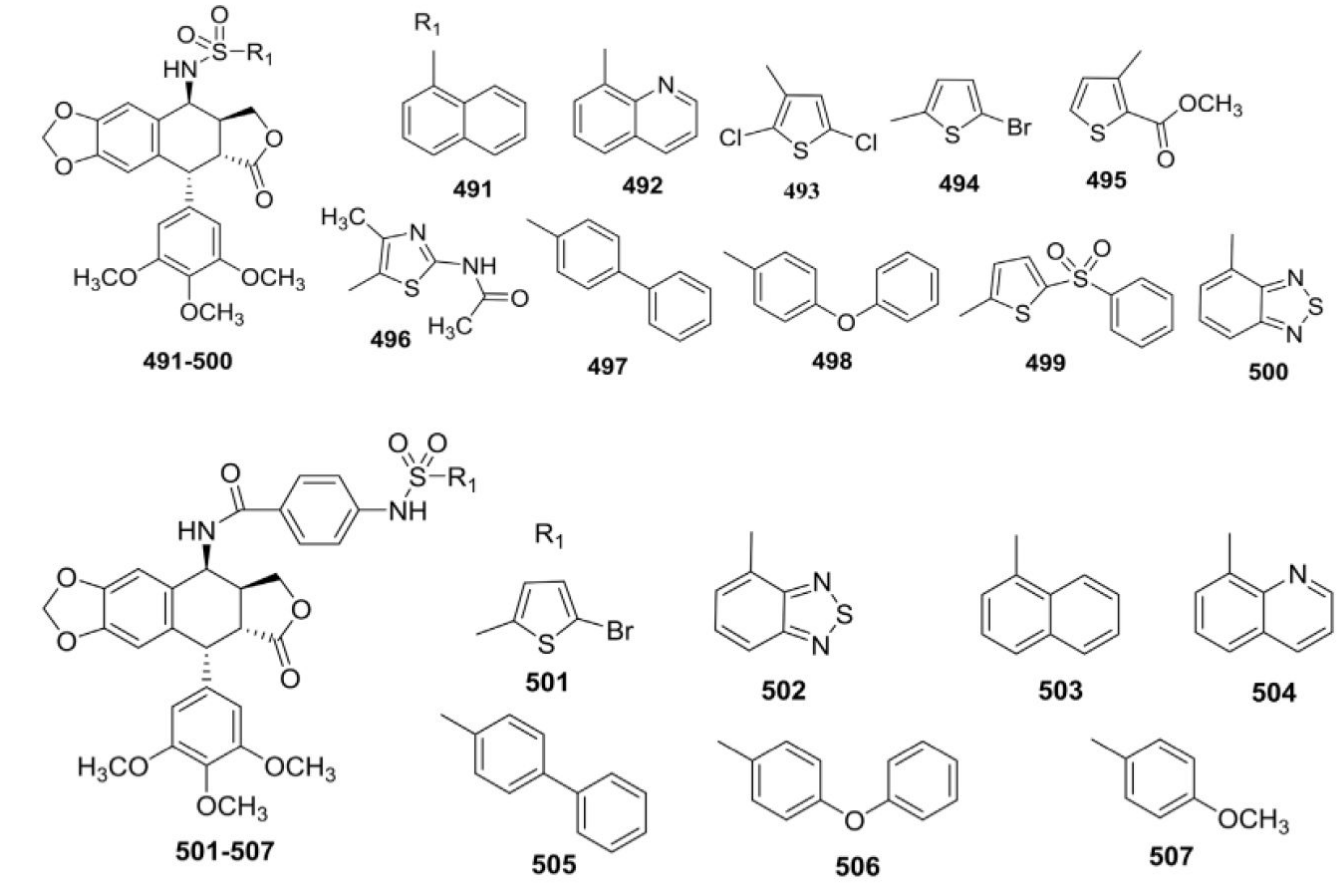
Compound	GI_{50} (μM)				
	A549	A375	MCF-7	HT-29	ACHN
ETO	2.34	1.39	0.68	1.81	7.61
PPT	3.75	2.62	1.18	0.78	2.12
462	2.15	18.4	24.8	15.3	16.5
463	14.4	20.1	17.5	11.9	13.7
464	19	13	12	16	10
465	10.7	10.8	18.1	8.75	11.9
466	13.6	9.8	10.2	12.1	19.5
467	5.9	11.4	15.8	11.7	9.3
468	14.7	11.5	8.3	21.1	20.8
469	16.7	10.3	12.6	6.47	13.4
470	19.1	25.8	24.2	12.4	10.18
471	9.1	13.8	21.8	8.1	13.8
472	8.3	9.3	16.2	16.9	16.1
473	9	5.3	6.7	8.41	13.2
474	10.7	7.8	14.9	9.54	8.51
475	26.4	12.2	19.1	21.6	26.7
476	16.8	18.2	24.4	15.6	9.1
477	11.7	11.5	15.4	5.98	11.1
478	2.7	13.4	19.7	2.36	391
479	6.8	9.5	14.6	7.1	18.6
480	8.8	5.7	13.7	11.4	7.54
481	9.5	17.8	11.2	19.2	14.3



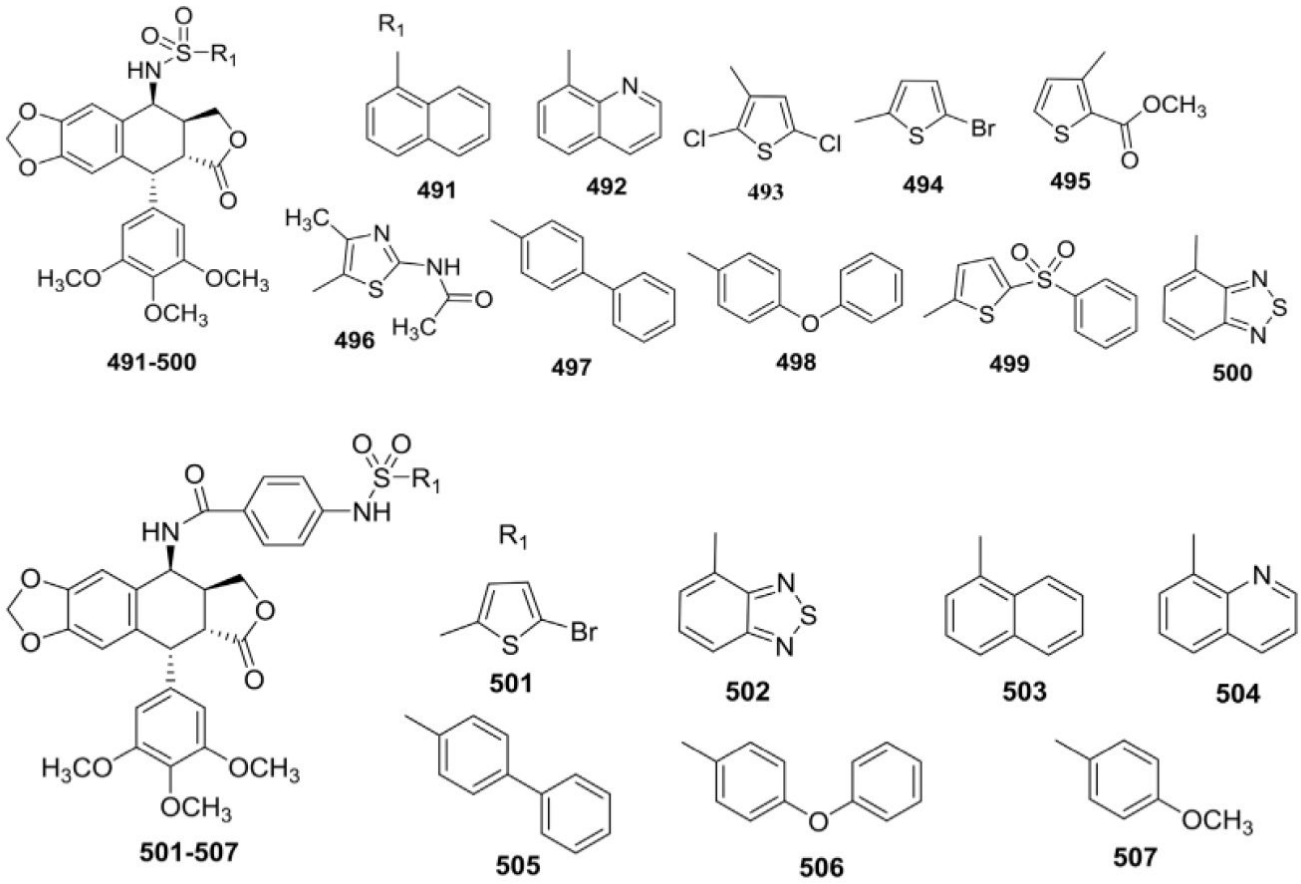
	GI_{50} (μM)				
Compound	**A549**	**A375**	**MCF-7**	**HT-29**	**ACHN**
482	7.7	19.1	15.7	17.6	17.8
483	2.1	18	19.2	0.37	2.18
484	8.2	14.5	10.1	18.1	12.8
485	8.4	13.2	20.1	21	17.7
486	7.9	14	17.7	8.34	13.1
487	15.4	14.5	13.8	12.3	10.8
488	13.4	7.7	11.2	7.75	15.7
489	10.6	10.3	8.6	11.8	10.7
490	7.7	6.8	2.2	8.9	9.46

Table XVII

Cytotoxicity Data for Sulfonamido Analogs **491–507**



	GI_{50} (μM)[a]							
Compound	**Zr-75–1**	**MCF7**	**KB**	**DWD**	**Colo 205**	**A549**	**Hop62**	**A2780**
ETO	0.20	2.11	0.31	0.62	0.13	3.08	0.80	1.31
491	2.10	0.09	2.30	2.00	2.10	0.16	2.40	1.91
492	0.08	0.12	0.06	0.17	0.04	0.04	0.06	0.08
493	2.50	0.11	2.10	0.15	0.15	0.14	2.00	0.16
494	0.18	0.13	0.04	0.05	0.04	0.12	0.04	0.06
495	0.06	0.18	0.04	0.04	0.04	0.15	0.04	0.05
496	0.17	0.15	0.17	0.14	0.11	0.12	0.16	0.15
497	2.30	2.61	NT[b]	2.21	2.71	2.00	2.01	2.11
498	2.31	2.21	2.31	2.91	NT	2.11	NT	2.40
499	2.11	0.14	2.11	2.61	NT	NT	NT	2.30
500	2.51	2.21	NT	NT	NT	2.71	2.21	2.311
501	2.90	2.71	NT	2.50	NT	NT	2.01	2.11
502	2.23	0.17	NT	2.80	NT	NT	2.91	2.11



	GI_{50} (μM)[a]							
Compound	**Zr-75–1**	**MCF7**	**KB**	**DWD**	**Colo 205**	**A549**	**Hop62**	**A2780**
503	2.81	2.32	2.00	NT	2.12	2.60	2.22	2.00
504	NT	2.90	NT	0.14	2.31	2.51	2.61	2.42
505	2.11	2.30	2.50	2.88	2.80	NT	NT	2.51
506	2.90	NT	2.01	2.10	NT	2.70	2.00	NT
507	2.12	0.16	–	2.02	2.51	–	2.71	–

[a] 50% Growth inhibition; values are means of three determinations.

[b] NT, not tested.

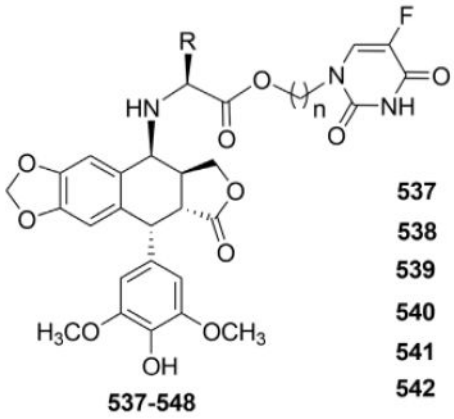
Table XVIII

Cytotoxicity Data for Compounds **508–529**

	IC_{50} (μM)				
Compound	**A549**	**HT-29**	**ACHN**	**B-16**	**HELA**
ETO	2.8	1.81	7.61	1.39	1.52
PPT	3.6	1.02	2.3	2.5	2.8
508	13.6	19.5	17.5	16.3	5.9
509	17.9	22.6	26.4	16.8	7.5
510	24.7	27.9	28.5	20.3	5.26
511	38.1	32.1	30.3	22.5	11.2
512	23.1	38.4	23.1	12.7	13.3
513	19.8	25.1	34.2	23.4	18.4
514	31.07	11.2	16.8	22.7	12.6
515	12.7	14.1	16.4	27.5	21.2
516	20.6	19.9	15.6	23.5	16.6
517	28.9	26.5	30.2	16.3	7.5
518	19.4	20.6	23.5	NA	14.6
519	22.3	26.5	25.8	16.6	16.9
520	5.3	8.4	11.6	9.5	9.4
521	39.5	36.3	31.6	45.3	25.1
522	15.2	16.8	27.1	18.3	11.5
523	2.1	7.1	15.9	9.5	9.3
524	15.8	10.8	32.5	25.6	14.9
525	21.2	26.3	18.3	26.1	15.2
526	10.6	10.9	17.7	18.4	12.1
527	11.2	5.7	17.3	18.3	8.9
528	17.3	14.5	19.3	10.7	14.4
529	9.5	9.2	13.8	12.7	13.7

Table XIX

Biological Data for 5-FU–DEPPT Conjugates **537–548**



537-548

	n	R		n	R
537	3	Me	**543**	3	$CH_2C_6H_5$
538	3	$CHMe_2$	**544**	3	$CH_2(p\text{-}OH\text{-}C_6H_4)$
539	3	CH(OH)Me	**545**	4	CH_2CHMe_2
540	3	CH_2CHMe_2	**546**	4	$CH_2C_6H_5$
541	3	$CH(Me)CH_2Me$	**547**	5	CH_2CHMe_2
542	3	CH_2CH_2SMe	**548**	5	$CH_2C_6H_5$

	Cytotoxic activity (IC_{50}, μM)[a]				
Compound	**K562**[b]	**AGS**[c]	**HL-60**[b]	**A-549**[c]	**log *P***
ETO	>100	50.8	2.75	7.38	0.69
5-FU	>100	>100	65.3	50.5	–
537	19.5	40.4	22.3	2.59	−0.07
538	13.5	62.5	5.80	1.92	0.34
539	30.8	140.8	23.2	6.95	−0.12
540	9.5	59.2	0.13	0.01	0.33
541	5.1	56.2	0.24	0.18	0.59
542	8.8	24.8	0.99	0.29	0.23
543	5.8	23.6	0.31	0.48	0.65
544	28.6	114.7	26.6	21.7	0.07
545	5.3	34.5	0.73	<0.01	0.35
546	9.2	23.7	0.42	0.30	0.43
547	6.0	23.5	0.31	0.53	0.51
548	13.2	38.8	0.04	<0.01	0.29

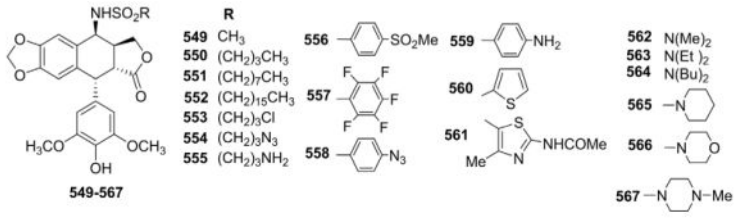
[a] Average of triplicate experiments.

[b] The microculture [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay method, 48 hr drug exposure.

[c] the sulforhodamine B (SRB) colorimetric assay method, 72 hr drug exposure.

Table XX

Biological Data for Sulfonamide Analogs **549–567**



Compound	Topo II[a] (% linear DNA)	IC_{50} (μM)[b]	Cell cycle effect[c]
ETO	50	0.83	80% (2.5 μM)
549	50	0.07	77% (0.5 μM)
550	10	0.033	75 (0.2 μM)
551	35	0.25	76 (1 μM)
552	0	5.5	73 (25 μM)
553	14	0.045	66 (0.25 μM)
554	32	0.035	65 (0.25 μM)
555	45	2.5	75 (50 μM)
556	10	0.55	75 (2.5 μM)
557	0	1	52 (5 μM)
558	9	0.21	69 (0.5 μM)
559	0	0.34	75 (0.2 μM)
560	9	0.17	71 (0.5 μM)
561	7	3.6	68 (10 μM)
562	44	0.037	66 (0.25 μM)
563	24	0.083	86 (0.25 μM)
564	0	0.51	60 (2.5 μM)
565	34	0.12	77 (0.5 μM)
566	31	0.1	76 (0.25 μM)
567	45	0.048	84 (0.1 μM)

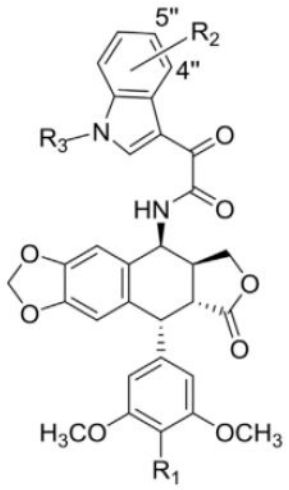
[a] Average of three independent experiments in the presence of drug at 50 μM.

[b] IC_{50} is the concentration of drug required to reduce L1210 cell growth by 50%.

[c] Percent of L1210 cells in the G2 + M phases at the indicated concentration.

Table XXI

Cytotoxicity Data for Indole-Substituted Analogs **568–574**



568-574

	R_1	R_2	R_3
568	OCH_3	4"-carbomethoxy	H
569	OCH_3	4"-carbomethoxy	2'''-fluorobenzyl
570	OH	4"-carbomethoxy	4'''-methylbenzyl
571	OH	5"-fluoro	2'''-chlorobenzyl
572	OCH_3	5"-fluoro	2'''-chlorobenzyl
573	OCH_3	4"-carbomethoxy	benzoyl
574	OCH_3	4"-carbomethoxy	acetyl

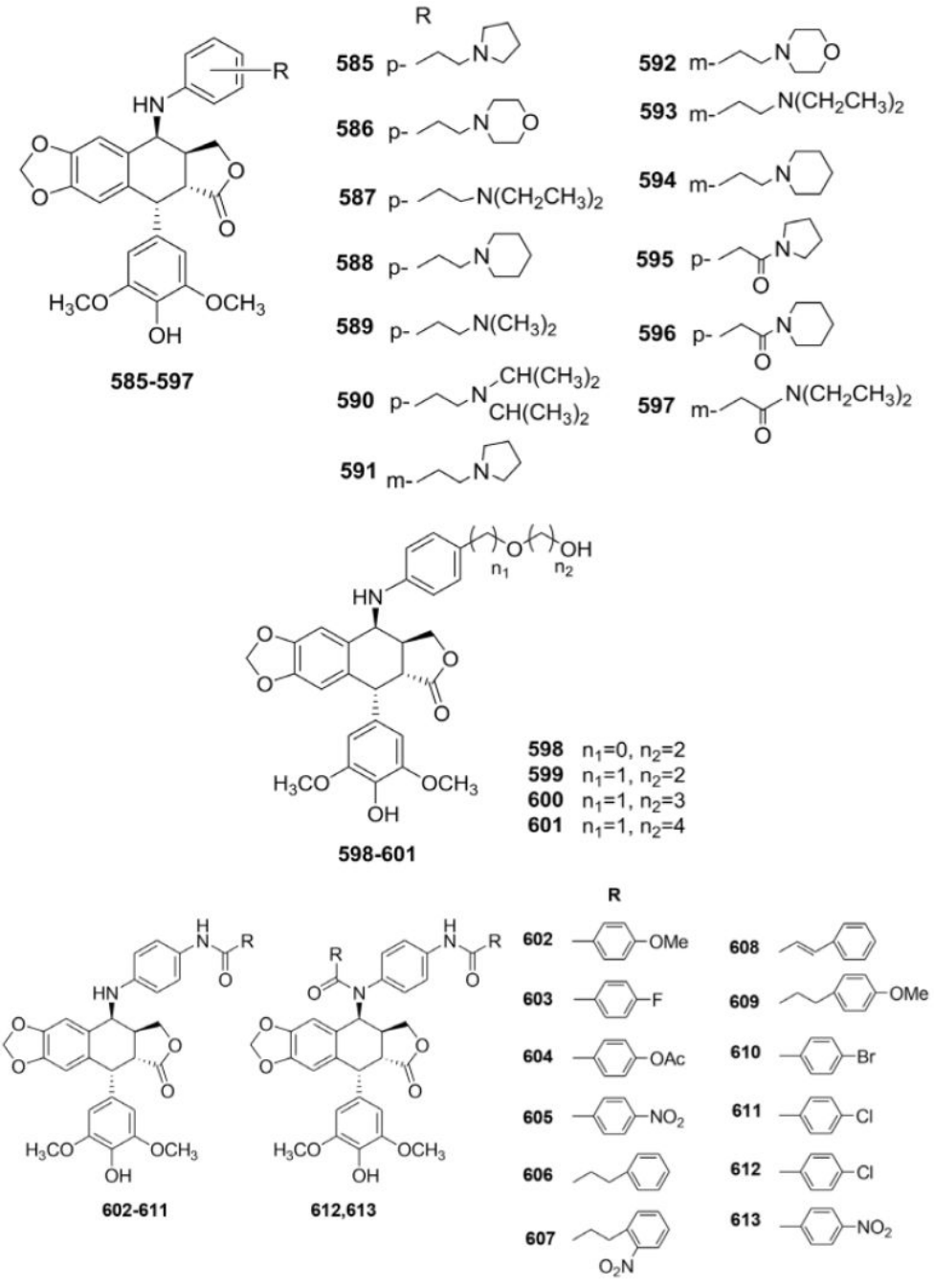
	IC_{50} (μM)				
Compound	**HeLa**	**K562**	**K562/AO2**	**KB**	**KBV**
ETO	2.11 ± 1.17	0.9 ± 0.08	7.05 ± 5.08	3.36 ± 1.05	55.47 ± 4.36
568	1.05	0.99 ± 0.60	4.04 ± 0.97	3.99 ± 1.28	6.28 ± 2.05
569	5.237	0.29 ± 0.19	0.12 ± 00.6	2.49 ± 0.94	3.49 ± 1.05
570	3.805	0.23 ± 0.16	0.70 ± 0.02	0.88 ± 0.23	3.62 ± 2.17
571	0.15	0.40 ± 0.14	0.29 ± 0.26	1.00 ± 0.78	3.16 ± 1.46
572	0.73	1.22 ± 0.35	0.62 ± 0.28	4.99 ± 1.36	5.46 ± 3.65
573	10.67	0.31 ± 0.18	0.22 ± 0.20	1.03 ± 0.38	2.59 ± 0.28
574	11.11	0.46 ± 0.37	0.10 ± 0.01	1.62 ± 1.25	2.30 ± 2.17

[a] Cytotoxicity against HeLa, KB, and KBV cell lines and against K562 and K562/AO2 cell lines measured by standard MTT and SRB assay methods, respectively.

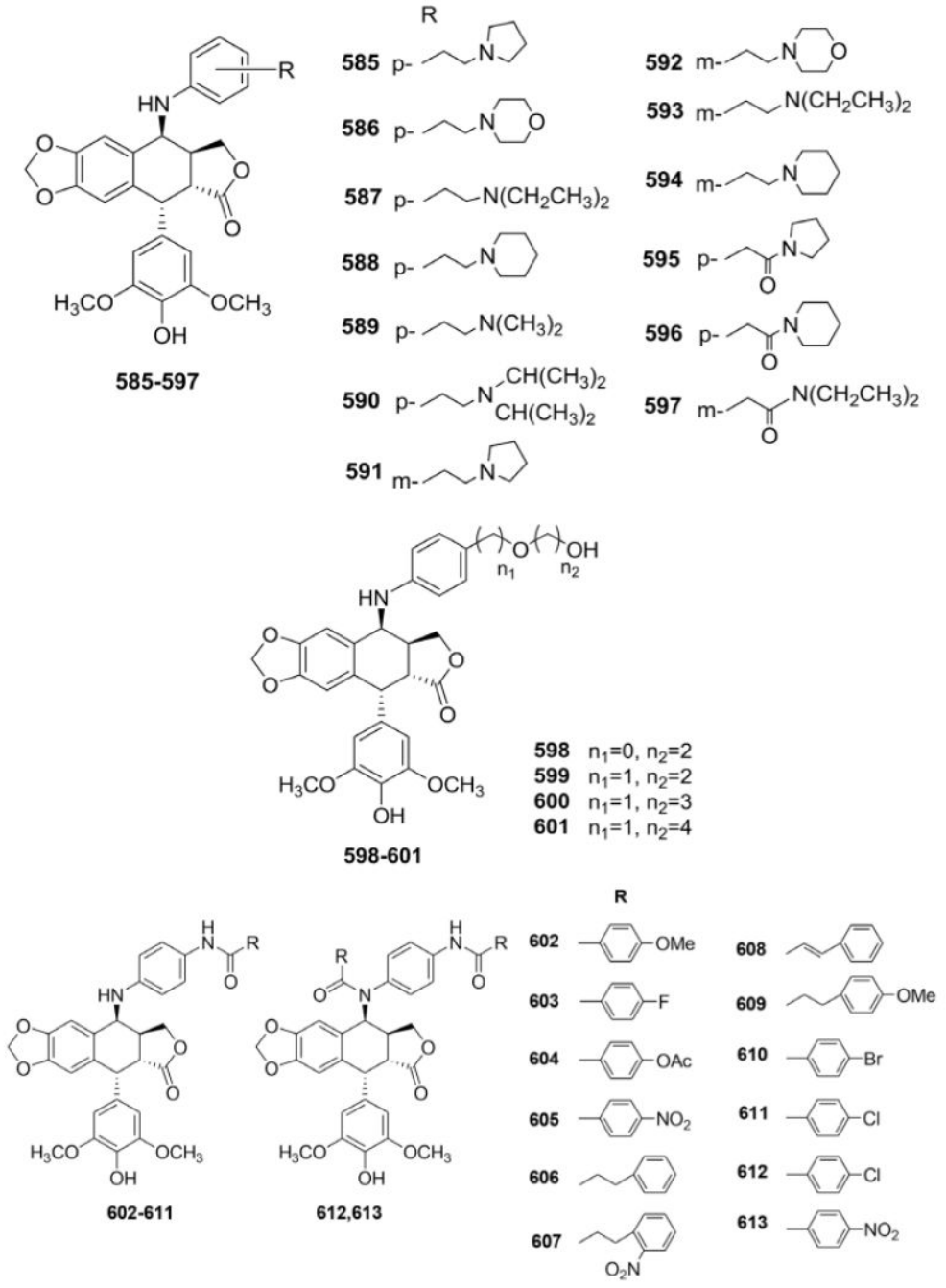
[b] Each value represents mean ± SD of three independent experiments.

Table XXII

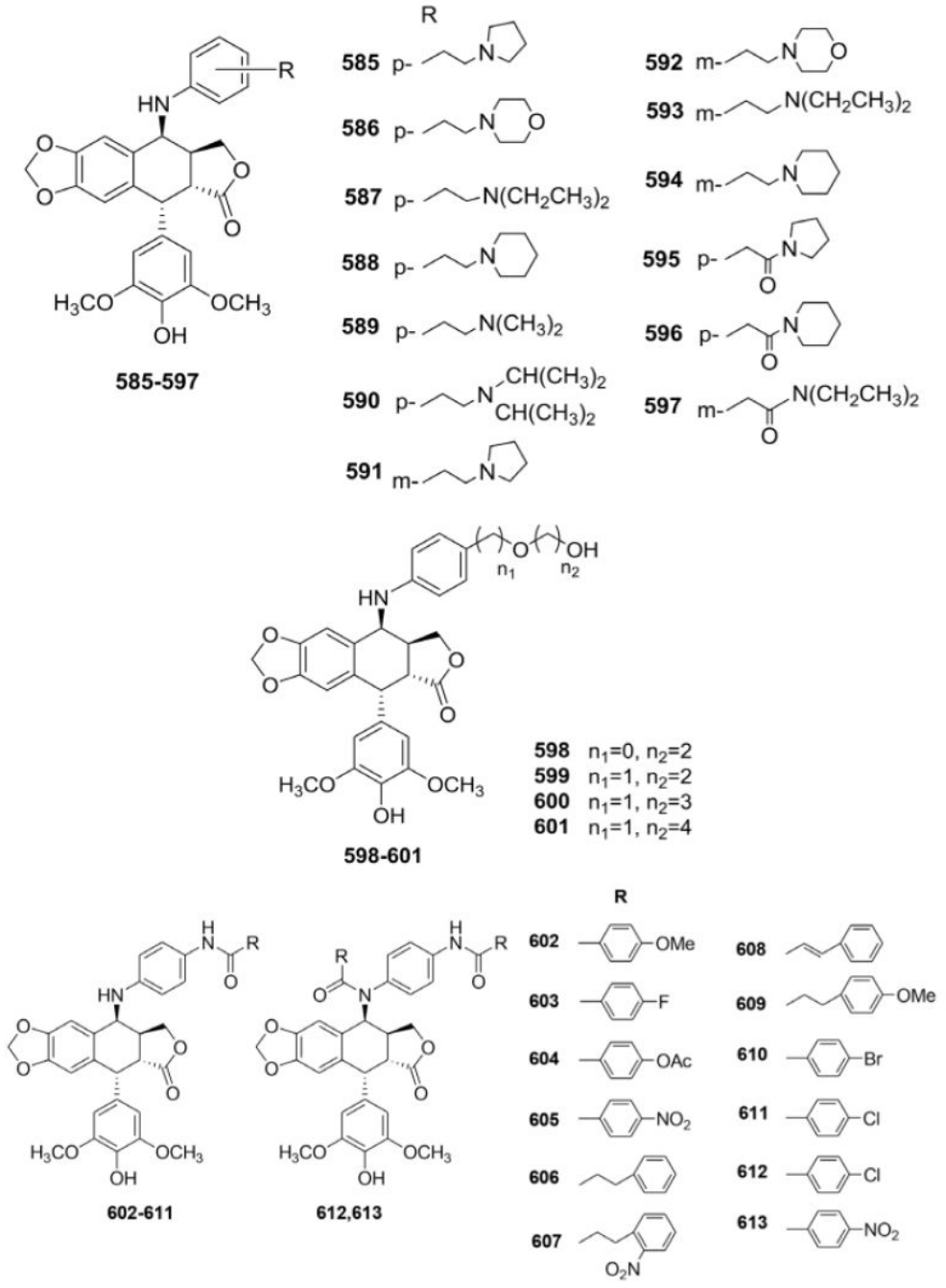
Cytotoxicity Data for Anilino Analogs **585–613**



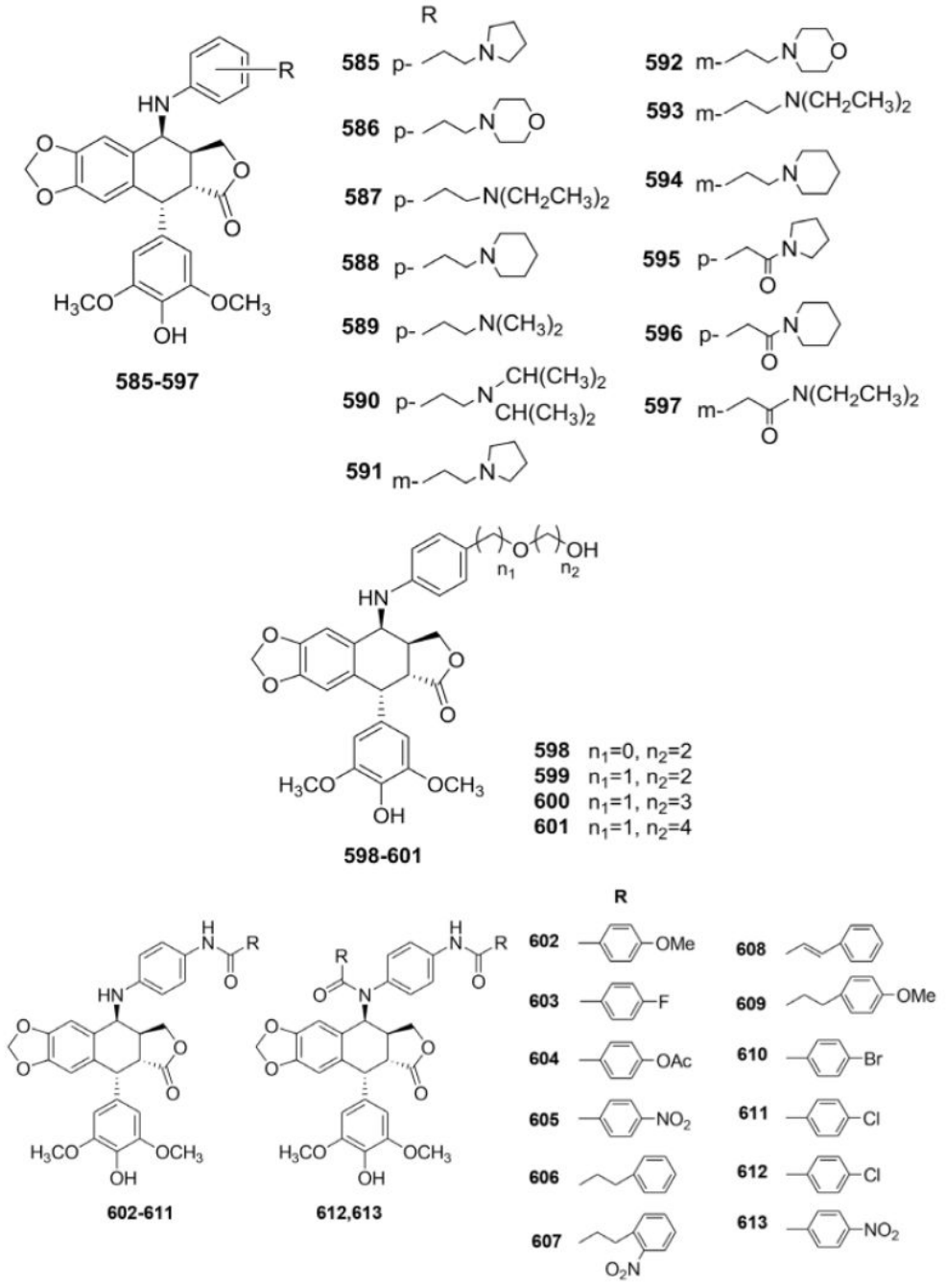
	IC$_{50}$ (μM)				
Compound	**KB**	**KB/VCR**	**A549**	**95D**	**RF**[a]
ETO	4.61	83.4	2.56	20.2	18.09



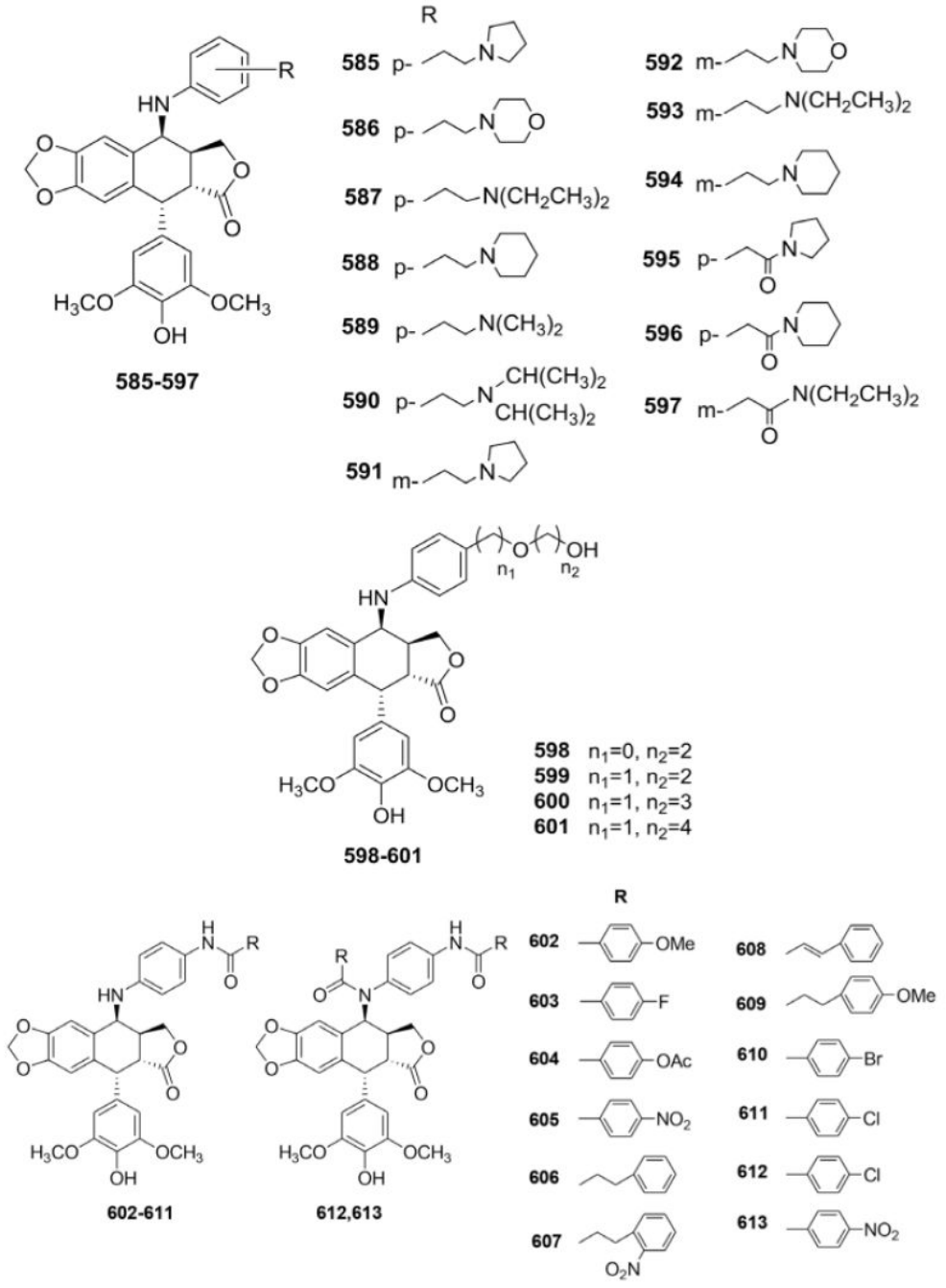
Compound	IC50 (μM) KB	KB/VCR	A549	95D	RF[a]
585	0.21	1.57	0.24	0.036	7.84
586	0.78	1.27	1.94	11.5	1.63
587	0.45	1.53	0.40	9.84	3.4
588	4.21	19.1	13.4	17.2	4.54



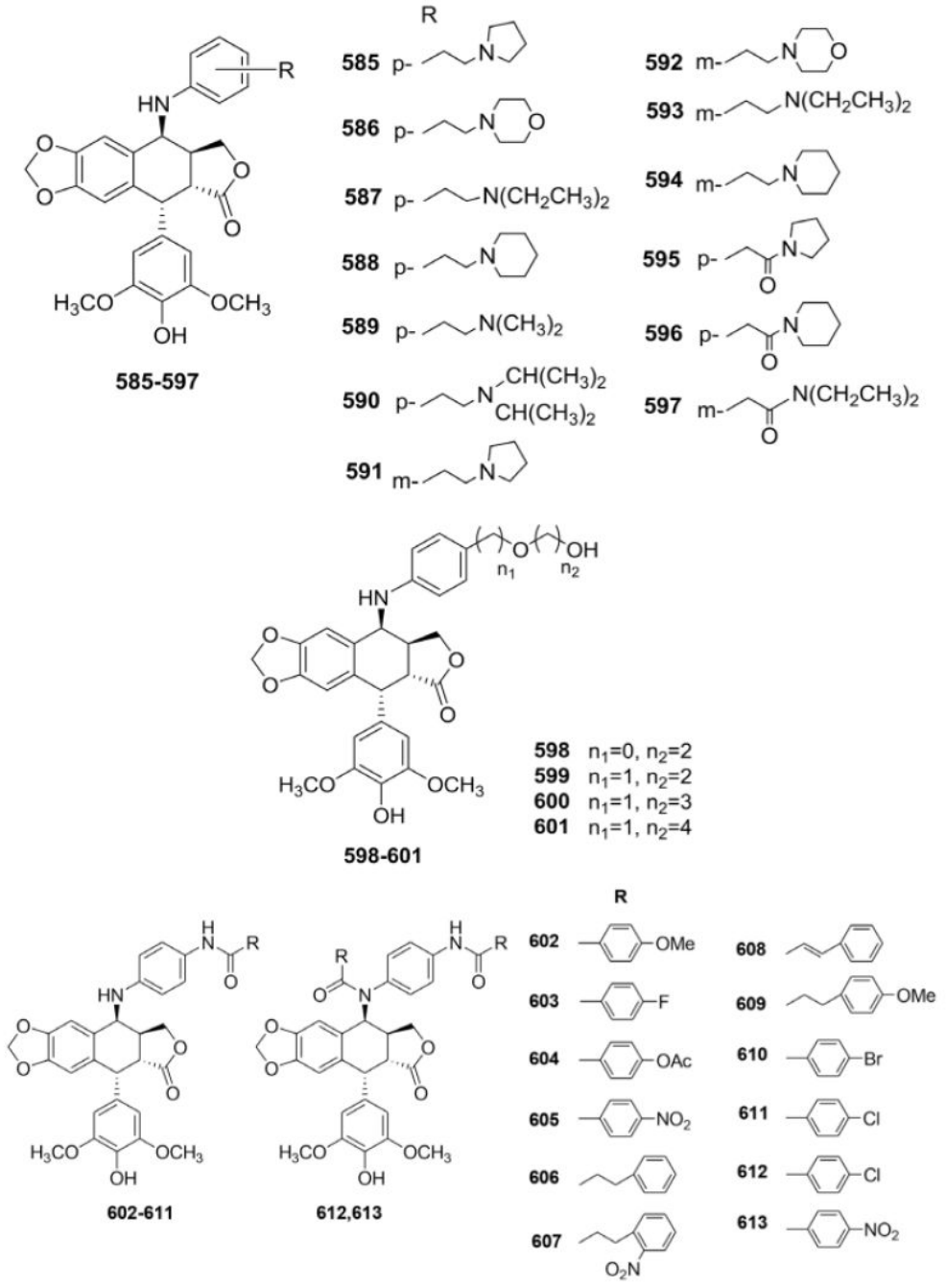
Compound	IC50 (μM) KB	KB/VCR	A549	95D	RF[a]
589	2.42	3.95	19.0	17.1	1.63
590	0.44	2.20	0.49	10.0	5
591	0.39	1.85	3.91	1.17	4.74
592	0.48	2.09	5.10	14.8	4.35



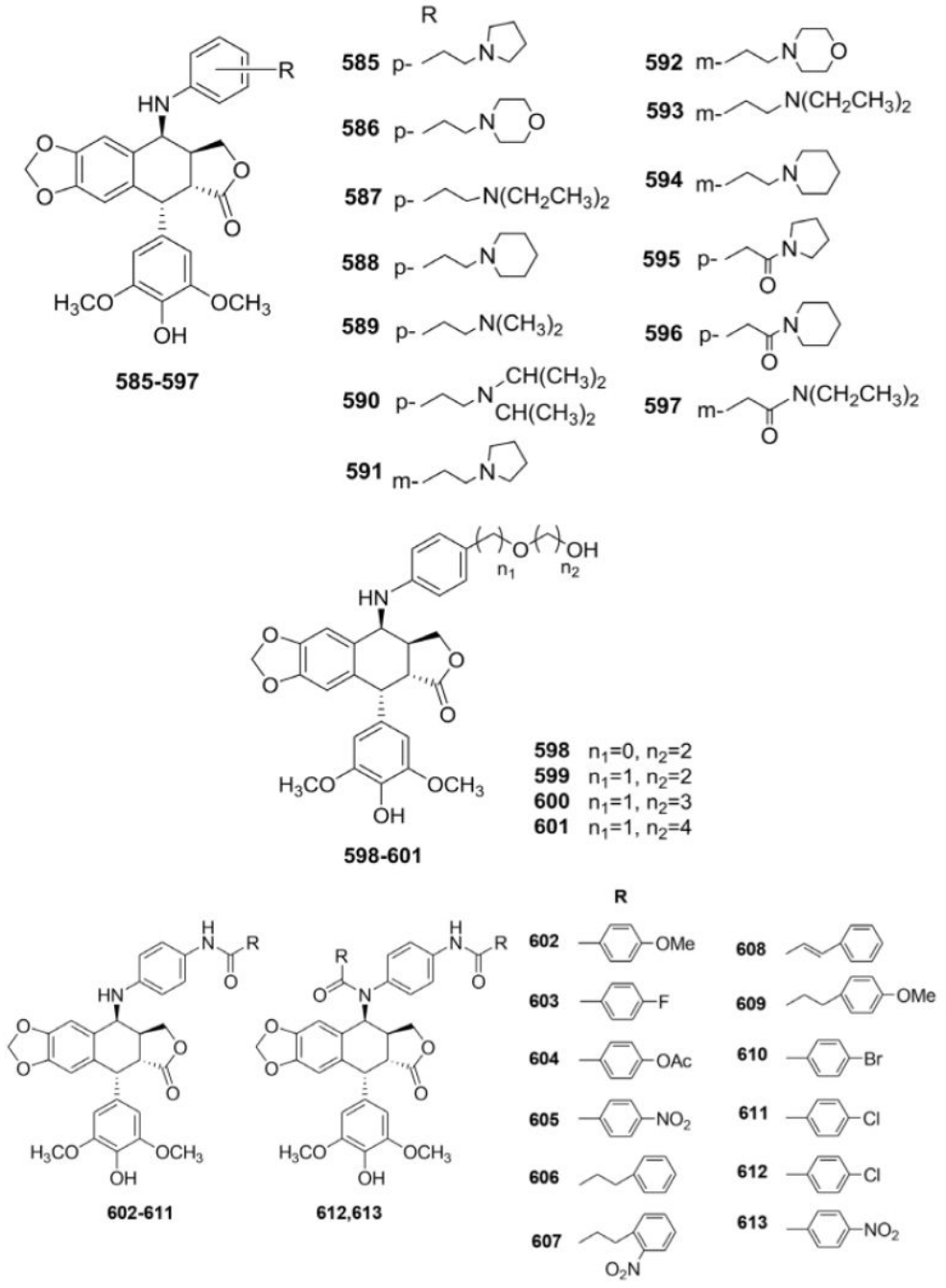
	IC$_{50}$ (μM)				
Compound	**KB**	**KB/VCR**	**A549**	**95D**	**RF**[a]
593	0.77	3.87	7.49	14.6	5.03
594	0.43	1.78	2.75	7.40	4.14
595	1.52	2.20	15.9	>50	1.45
596	0.40	2.33	5.20	25.8	5.83



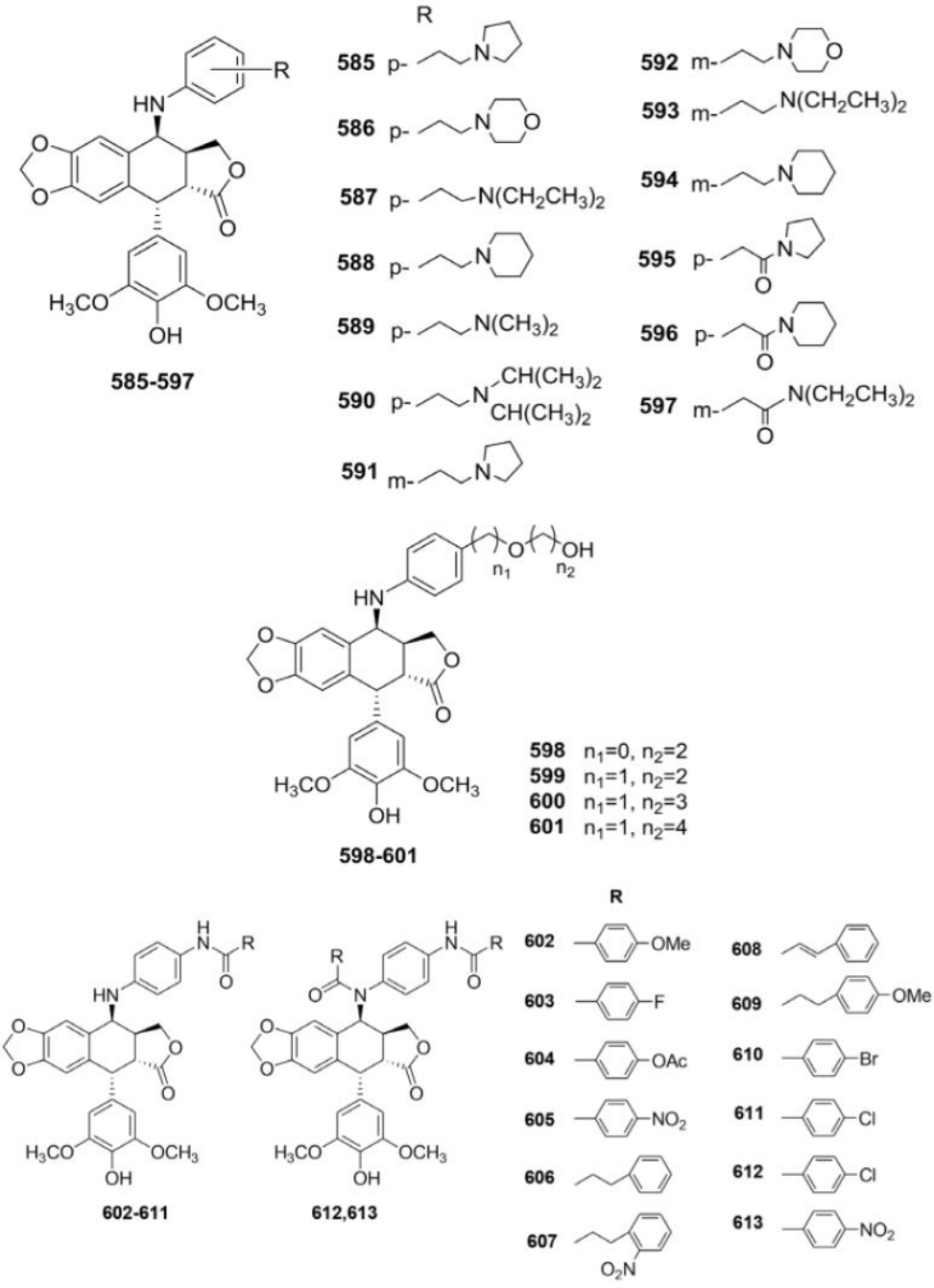
Compound	IC50 (μM) KB	KB/VCR	A549	95D	RF[a]
597	0.42	27.2	4.78	>50	64.76
598	2.88	6.51	4.54	4.13	2.26
599	3.12	7.39	5.11	0.55	2.37
600	6.00	5.08	7.45	12.04	0.85



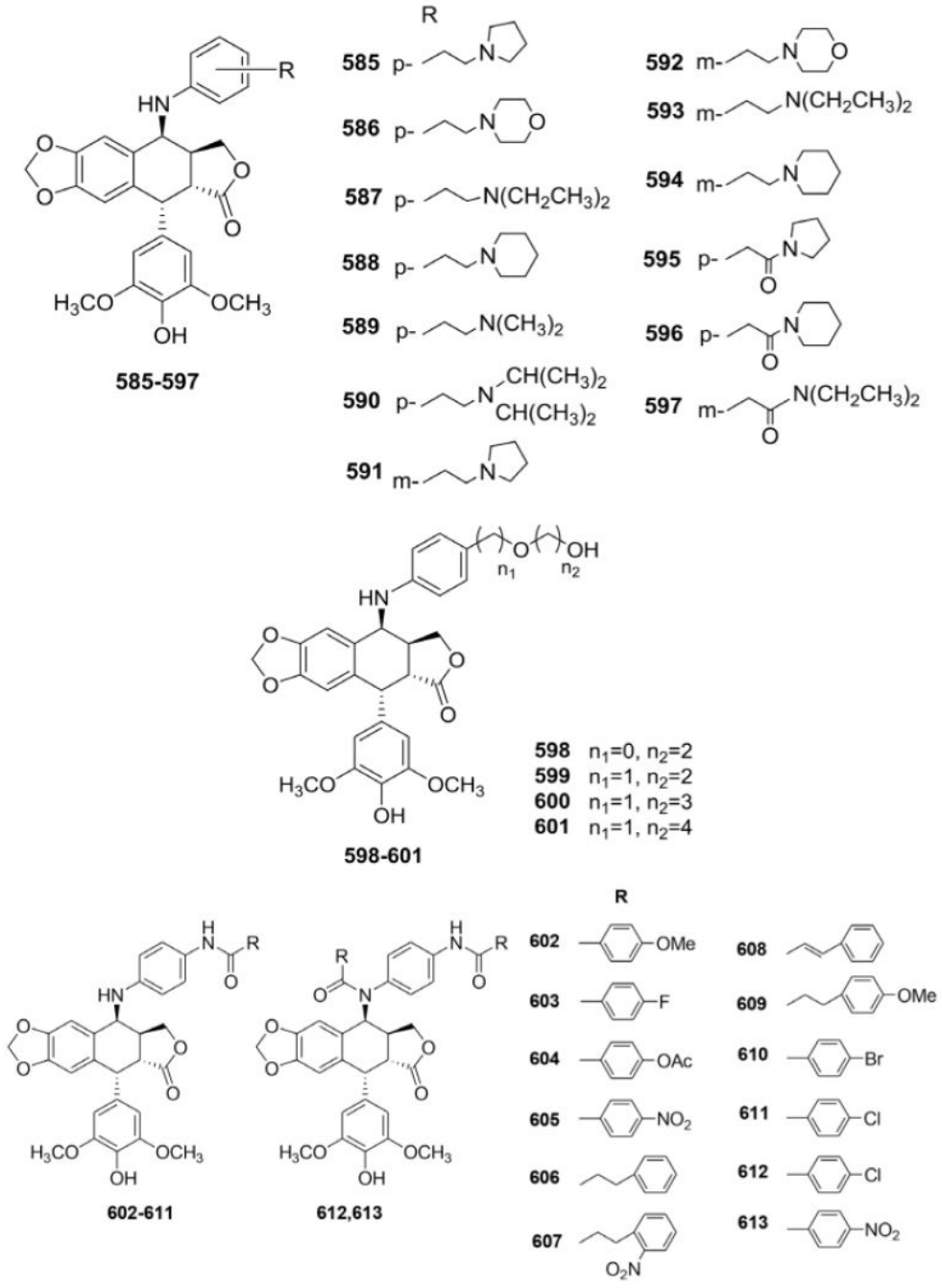
Compound	IC50 (μM) KB	KB/VCR	A549	95D	RF[a]
601	6.60	9.55	8.5	20.30	1.45
602	6.76	9.28	3.41	0.44	1.37
603	2.91	5.31	1.56	1.08	1.82
604	1.91	8.48	2.68	0.42	4.44



	IC$_{50}$ (μM)				
Compound	**KB**	**KB/VCR**	**A549**	**95D**	**RF**[a]
605	>50	13.7	11.0	3.41	<0.27
606	6.88	3.70	3.31	1.15	0.54
607	6.69	5.30	8.43	0.41	0.79
608	6.53	2.80	>50	1.25	0.43



	IC$_{50}$ (μM)				
Compound	**KB**	**KB/VCR**	**A549**	**95D**	**RF**[a]
609	>50	3.08	>50	1.23	<0.06
610	8.75	5.72	18.9	1.66	0.65
611	22.1	13.06	19.5	6.56	0.59



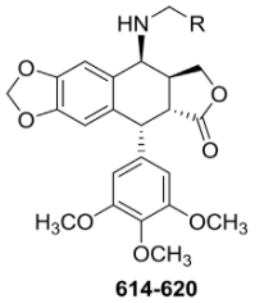
	IC$_{50}$ (μM)				
Compound	**KB**	**KB/VCR**	**A549**	**95D**	**RF**[a]
612	>50	>50	>50	15.7	NT[b]
613	>50	14.21	>50	19.6	<0.28

[a] Resistance factor calculated as ratio of IC$_{50}$ against MDR cells and IC$_{50}$ against corresponding drug-sensitive parental cells.

[b] NT, not tested.

Table XXIII

Cytotoxicity Data for Benzylamino Analogs **614–620**



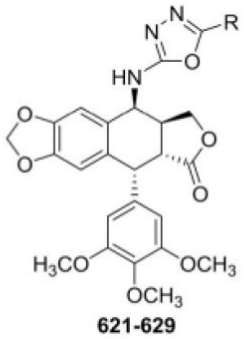
614-620

	R
614	4-Br-Ph
615	4-Cl-Ph
616	4-F-Ph
617	2-Cl-Ph
618	4-Me-Ph
619	4-OMe-Ph
620	Ph

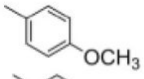
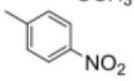
	IC_{50} (μM)			
Compound	**A549**	**HCT-116**	**HepG2**	**Average**
ETO	21.4	13.5	4.9	13.3
614	4.9	6.3	5.8	5.7
615	2.9	5.6	3.0	3.8
616	4.0	5.1	5.0	4.7
617	5.5	6.4	6.3	6.1
618	3.2	5.2	5.8	4.7
619	9.5	8.7	8.1	8.8
620	4.8	6.1	5.1	5.3

Table XXIV

Cytotoxicity Data for Oxadiazole-Amino Analogs **621–629**



621-629

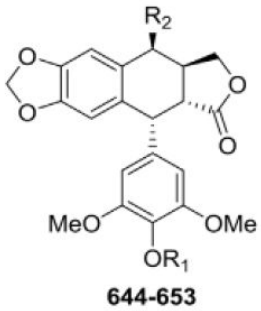
	R						
621	-H	**624**		**627**			
622	$-CH_3$	**625**		**628**			
623		**626**		**629**			

	IC_{50} (μM)							
Compound	**DU-145**	**SGC-7901**	**A549**	**SH-SY5Y**	**HepG2**	**HeLa**	**L929**	**Vero**
ETO	64.26	12.09	10.12	5.37	5.48	96.26	1.00	31.14
PPT	>100	2.95	13.62	2.54	2.27	7.58	1.37	1.78
621	11.34	>100	>100	25.81	11.85	>100	43.29	15.37
622	>100	38.11	>100	14.66	1.51	2.68	19.62	5.49
623	>100	>100	NT[a]	26.88	74.20	85.34	>100	>100
624	76.12	>100	53.66	7.39	27.95	17.66	18.15	7.43
625	>100	>100	NT	16.58	>100	>100	>100	NT
626	>100	56.58	>100	20.02	38.82	42.53	70.85	>100
627	35.42	NT	69.22	29.48	38.81	58.85	>100	>100
628	>100	>100	>100	22.47	72.77	>100	54.56	>100
629	>100	>100	>100	41.53	96.56	>100	20.47	>100

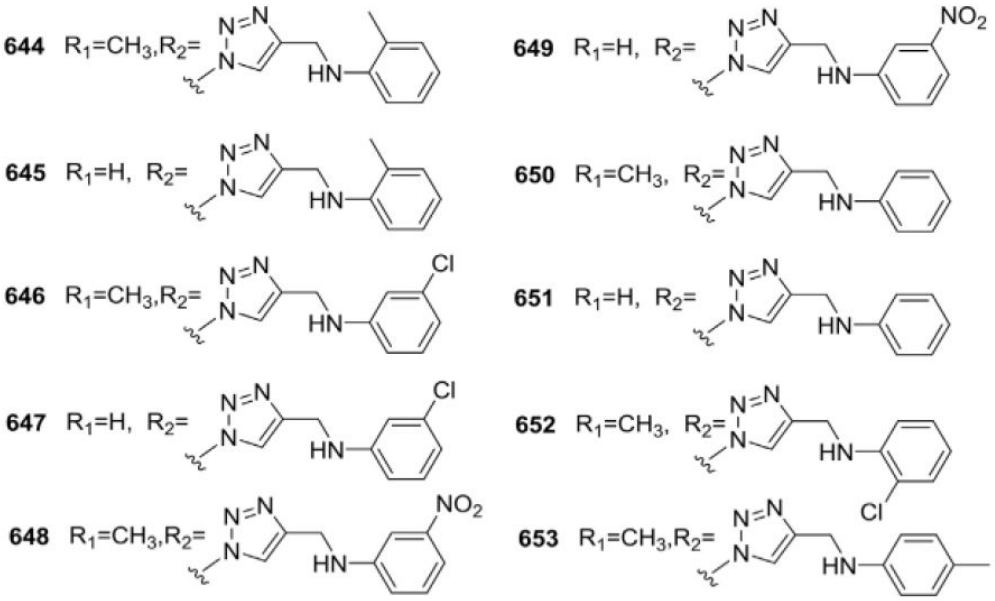
[a] NT, not tested.

Table XXV

Cytotoxicity Data for Triazole Analogs **644–653**



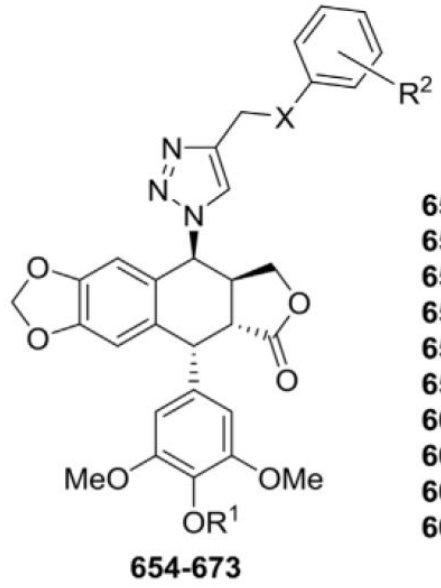
644-653



	IC_{50} (μM)						
	Prostate		CNS	Colon		Liver	Lung
Compound	DU-145	PC-3	SF-295	HCT-15	502713	HEP-2	A-549
PPT	2.97	18.9	5.69	1.00	3.88	1.42	7.63
ETO	3.85	17.4	13.3	1.48	2.42	2.15	5.62
644	1.06	1.09	1.94	0.36	0.78	9.14	7.88
645	1.19	1.53	0.96	0.14	0.62	1.50	5.17
646	0.93	1.18	0.84	0.34	0.71	1.37	4.99
647	4.16	1.37	3.11	4.22	0.98	1.22	8.63
648	0.65	0.74	0.68	0.04	0.56	3.65	5.78
649	4.37	6.63	4.20	1.55	1.19	4.10	7.43
650	1.12	0.88	0.78	0.14	0.61	4.64	6.10
651	1.09	1.03	0.70	0.40	0.68	2.96	7.46
652	3.57	3.45	1.00	1.43	1.88	1.26	9.24
653	2.75	4.49	6.03	1.69	1.34	3.27	7.41

Table XXVI

Cytotoxicity Data for Triazole Analogs **654–673**



654-673

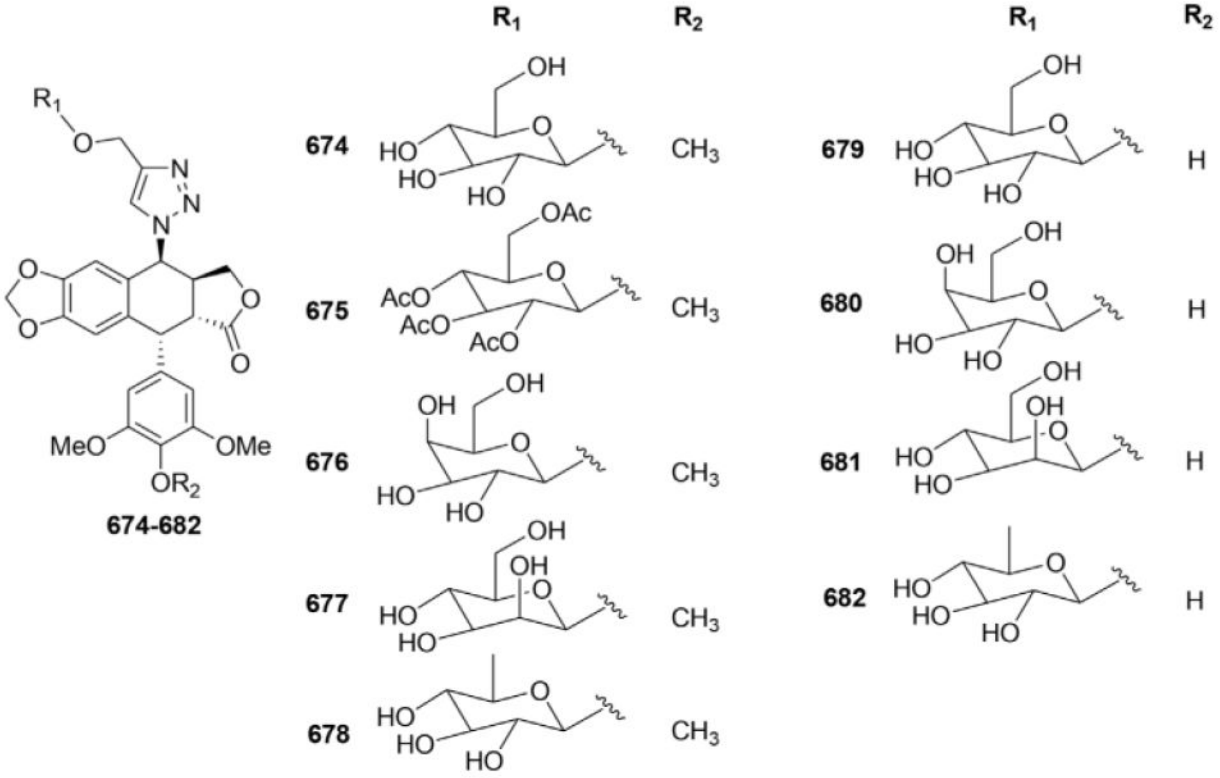
	R_1	R_2	X
654	Me	H	O
655	Me	o-Me	O
656	Me	m-Me	O
657	Me	p-Me	O
658	Me	o-Cl	O
659	Me	p-Cl	O
660	Me	p-NO_2	O
661	H	H	O
662	H	o-Me	O
663	H	m-Cl	O
664	H	p-Me	O
665	H	o-Cl	O
666	H	p-Cl	O
667	H	p-NO_2	O
668	Me	H	S
669	Me	p-Me	S
670	Me	p-Cl	S
671	H	H	S
672	H	p-Me	S
673	H	p-Cl	S

	IC_{50} (μM)						
Compound	**HT-29**	**HCT-15**	**502713**	**HOP-62**	**A549**	**MCF-7**	**SF-295**
ETO	5.31	7.07	3.88	4.80	7.63	2.51	5.69
654	9.65	1.91	0.96	NT[a]	1.0	0.90	0.93
655	0.34	0.53	0.32	0.50	1.42	1.60	3.79
656	0.53	1.46	0.72	4.18	1.67	1.84	8.86
657	8.25	5.11	20.8	NT	8.11	4.84	3.65
658	0.34	0.62	0.41	0.69	0.64	0.79	1.0
659	0.35	0.31	0.43	0.57	0.56	1.6	1.67
660	21.8	23.4	44.2	50.0	48.1	NT	50.6
661	1.46	3.22	4.07	4.32	4.12	3.68	1.21
662	4.72	4.33	NT	NT	5.06	4.22	9.15
663	3.37	3.31	7.56	6.5	4.26	4.74	16.6
664	2.86	4.83	22.0	7.87	6.22	4.79	1.22
665	2.88	3.53	4.69	10.7	5.3	4.74	4.64
666	8.87	4.79	41.6	NT	19.8	4.29	2.92
667	9.23	6.67	5.17	28.6	29.6	5.18	1.45
668	0.37	0.65	0.46	0.81	0.7	1.91	5.44
669	22.7	21.8	28.5	32.9	29.2	23.1	49.5
670	24.4	27.5	37.0	50.0	32.2	17.5	30.2
671	2.08	3.31	4.76	4.84	5.06	4.95	9.56
672	1.38	3.68	4.37	5.0	6.22	4.64	3.89
673	1.51	3.39	3.74	5.0	4.59	3.7	NT

[a] NT, not tested.

Table XXVII

Cytotoxicity Data for Triazole Analogs **674–682**

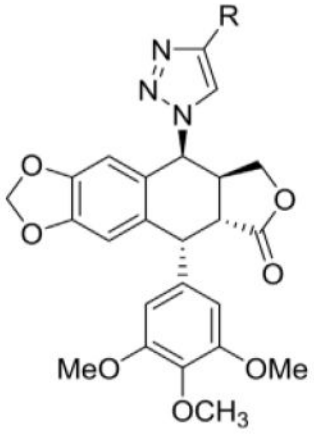


	IC_{50} (μM)					
Compound	**DU-145**	**PC-3**	**A549**	**HCT-15**	**HOP-62**	**SF-295**
ETO	2.97	100	7.63	1	4.8	5.69
674	25.8	127	125	15	NT[a]	26.8
675	42	221	556	NT	142	35.1
676	237	316	59.9	16	178	14.5
677	18.6	94.9	51.8	237	121	53.4
678	26.5	268	114	NT	NT	88
679	3.11	34.6	3	0.93	8.55	2.01
680	4.95	24.3	6.88	1	5.27	16.1
681	2.73	8.62	7.33	3.57	5.96	2.51
682	5.04	20.8	7.04	2.14	16.2	8.29

[a]NT, not tested.

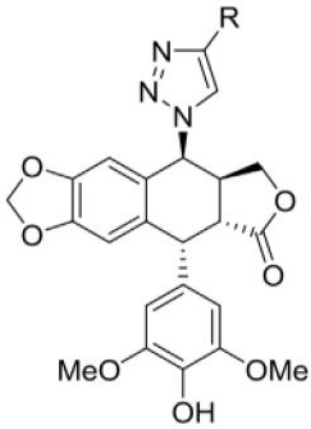
Table XXVIII

Cytotoxicity Data for Alkyltriazole Analogs **683–700**



683-691

	R
683	Ethyl
684	Propyl
685	Butyl
686	Pentyl
687	Hexyl
688	Heptyl
689	Octyl
690	Decyl
691	CH_2OH



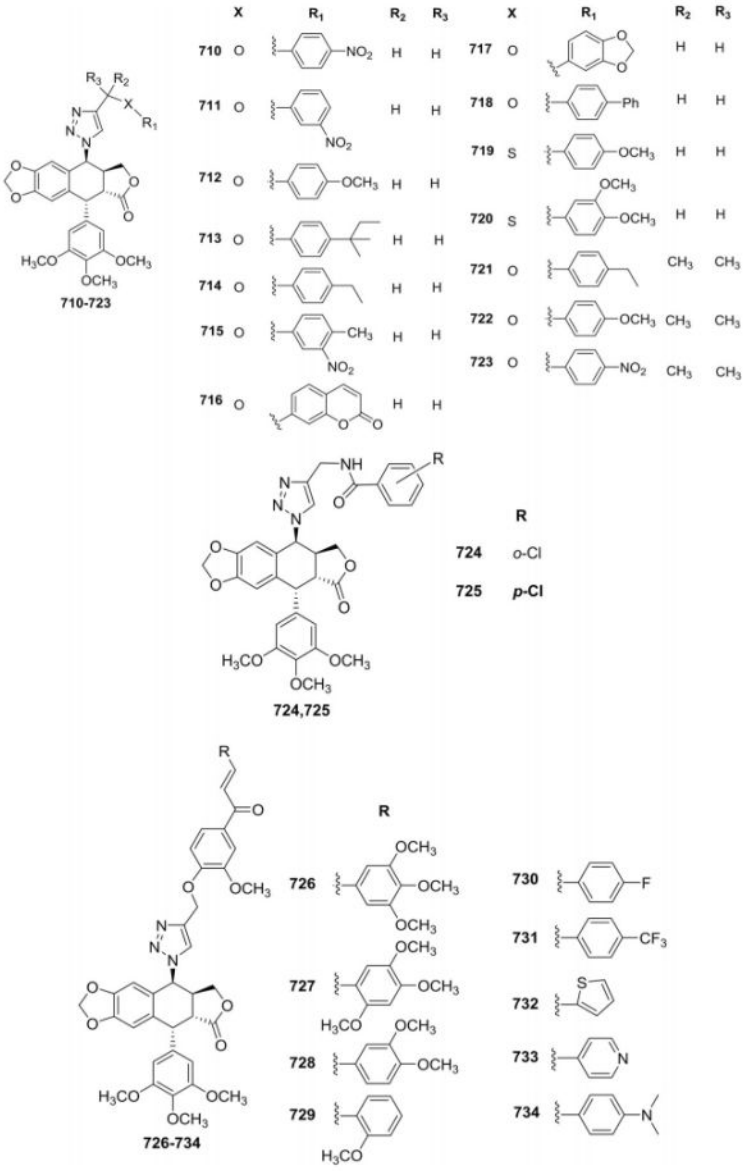
692-700

	R
692	Ethyl
693	Propyl
694	Butyl
695	Pentyl
696	Hexyl
697	Heptyl
698	Octyl
699	Decyl
700	CH_2OH

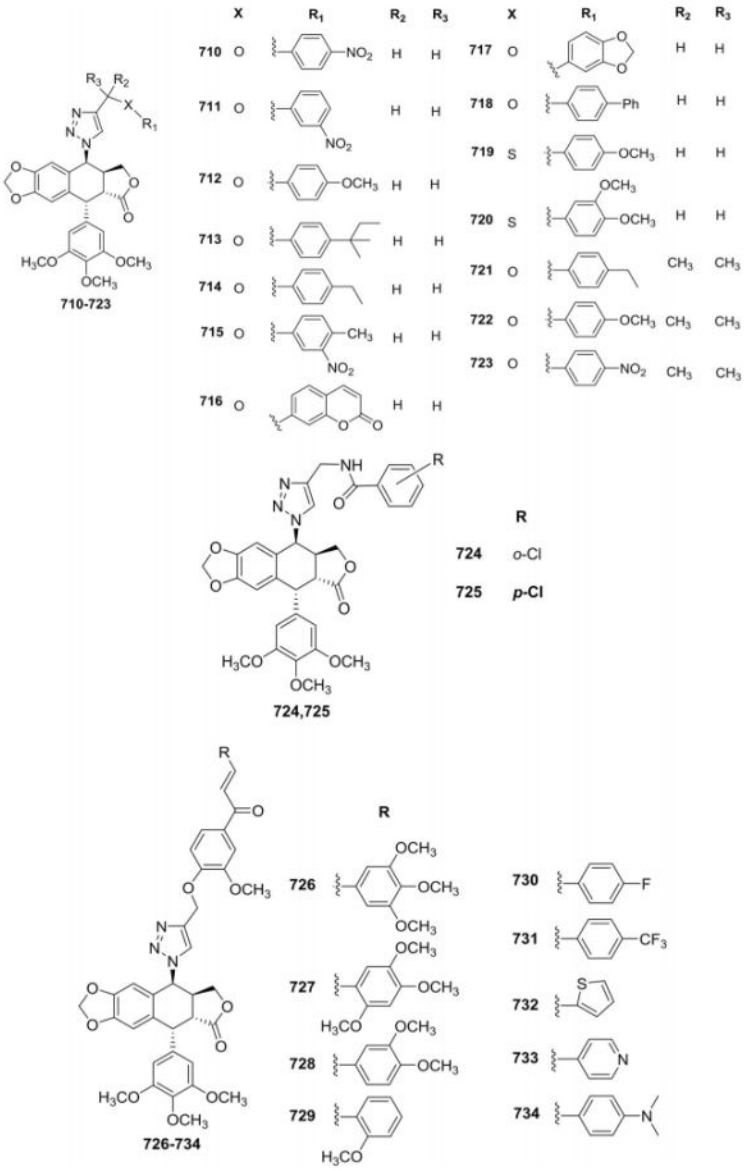
	IC_{50} (μM)					
Compound	**SF-295**	**A549**	**PC-3**	**Hep-2**	**HCT-15**	**MCF-7**
ETO	13.5	5.62	17.5	2.15	7.15	19
683	1.8	35	0.03	0.06	0.4	0.4
684	3.6	39	5.1	1.6	0.03	1.4
685	8.1	82	6.6	9.6	11	9.8
686	24	39	8.4	7.7	11	9.5
687	10	35	6.4	10	27	8.6
688	25	36	23	9.8	45	6.4
689	14	>100	17	18	>100	9.3
690	6.2	>100	5.5	5.8	29	5.7
691	2.1	>100	0.06	0.06	15	0.01
692	4.8	17	0.06	0.05	1.9	0.04
693	2.3	27	0.2	2.9	0.8	5.7
694	21	35	12	17	34	20
695	4.3	26	4.7	21	2.1	14
696	18	26	8.7	>100	11	19
697	10	45	5.8	6	15	13
698	5.7	>100	5.1	3.8	13	11
699	13	30	19	19	15	11
700	15	18	8.2	6.7	18	0.6

Table XXIX

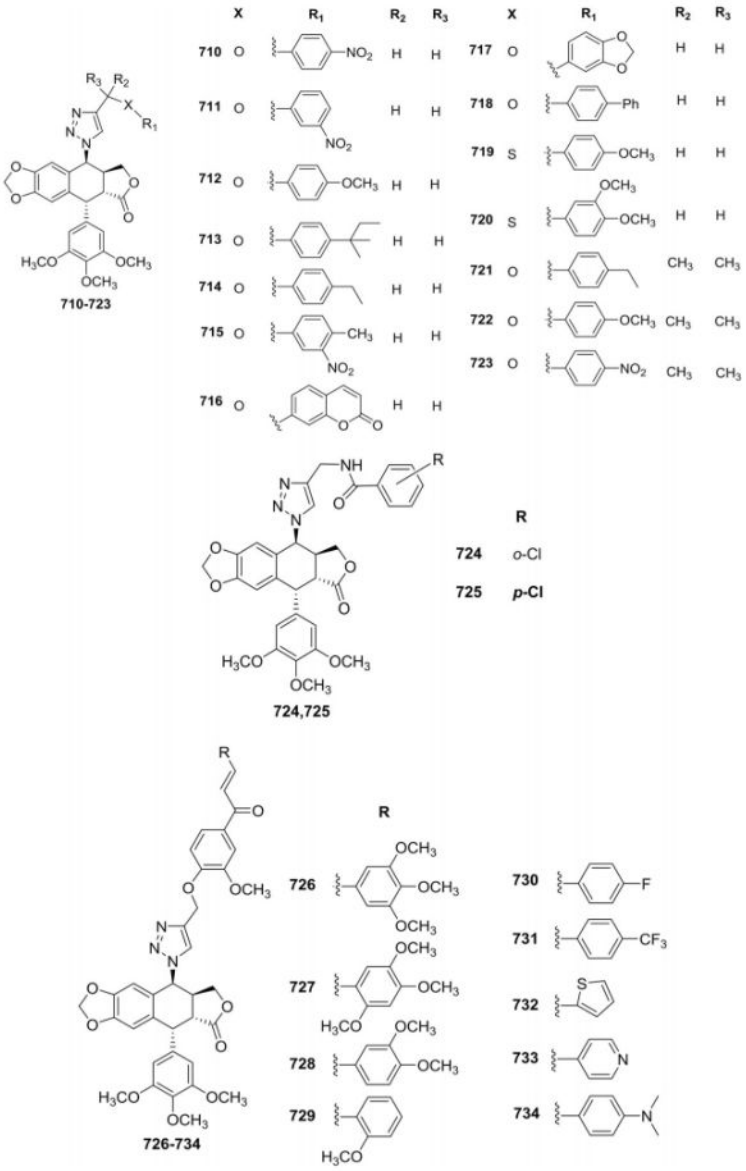
Cytotoxicity Data for Triazole Analogs **710–734**



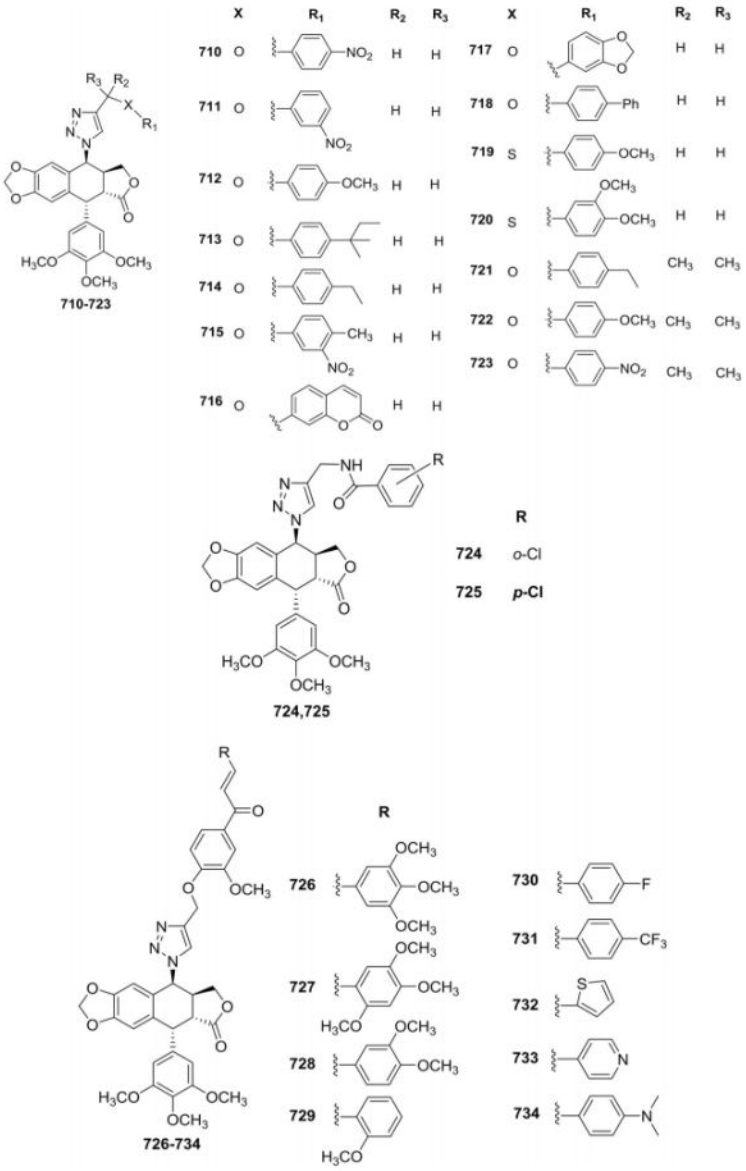
	IC_{50} at 48 hr (μM)[a]			
Compound	**HepG2**	**MKN-45**	**NCI-H1993**	**B16**
ETO	>10.00	6.34 ± 0.89	7.93 ± 0.77	>10.00
710	3.87 ± 0.23	6.10 ± 0.98	59.60 ± 2.34	30.00 ± 1.87
711	1.05 ± 0.04	0.99 ± 0.01	54.50 ± 4.78	10.00 ± 1.89
712	0.98 ± 0.04	1.05 ± 0.06	10.70 ± 1.26	2.85 ± 0.67
713	19.00 ± 1.89	7.62 ± 0.87	64.00 ± 4.76	66.60 ± 4.56



Compound	IC_{50} at 48 hr (μM)[a]			
	HepG2	MKN-45	NCI-H1993	B16
714	4.18 ± 0.56	4.26 ± 0.65	8.25 ± 0.89	>80.00
715	3.80 ± 0.19	3.11 ± 0.22	4.13 ± 0.34	4.53 ± 0.55
716	7.78 ± 0.77	1.45 ± 0.13	6.92 ± 0.55	4.35 ± 0.65
717	0.25 ± 0.01	0.93 ± 0.04	0.85 ± 0.05	2.93 ± 0.32
718	0.15 ± 0.01	0.22 ± 0.01	0.24 ± 0.03	0.54 ± 0.09
719	0.26 ± 0.02	0.13 ± 0.02	0.49 ± 0.05	2.52 ± 0.33
720	0.31 ± 0.08	0.44 ± 0.04	1.45 ± 0.12	0.90 ± 0.35
721	0.18 ± 0.01	0.31 ± 0.02	0.29 ± 0.05	0.57 ± 0.01



Compound	IC_{50} at 48 hr (μM)[a]			
	HepG2	MKN-45	NCI-H1993	B16
722	1.72 ± 0.09	1.72 ± 0.09	3.00 ± 0.14	2.50 ± 0.34
723	3.14 ± 0.19	3.14 ± 0.78	2.04 ± 0.43	3.35 ± 0.12
724	8.55 ± 0.98	4.20 ± 0.98	7.25 ± 0.98	8.25 ± 0.65
725	4.85 ± 0.56	3.10 ± 0.81	3.90 ± 0.56	4.10 ± 0.45
726	2.80 ± 0.09	1.05 ± 0.05	1.63 ± 0.07	2.48 ± 0.32
727	>80.00	>10.00	>10.00	>80.00
728	1.61 ± 0.23	1.01 ± 0.05	2.14 ± 0.07	1.56 ± 0.09
729	9.65 ± 0.98	6.80 ± 0.66	>10.00	>20.00

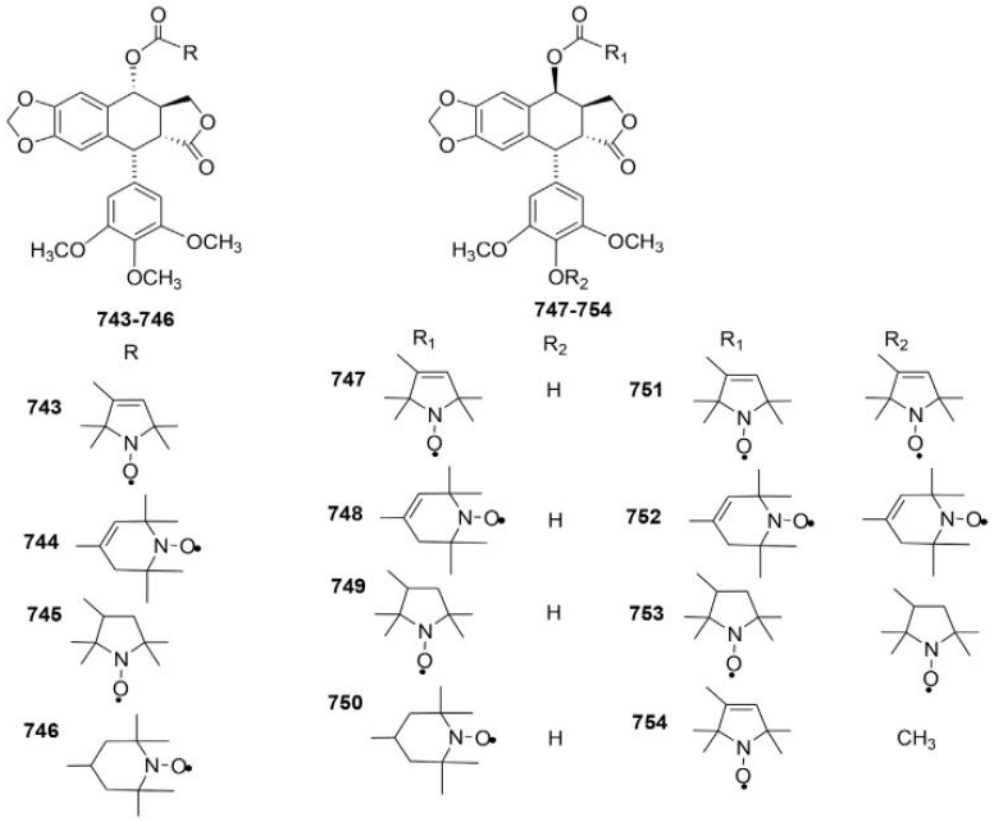


Compound	IC_{50} at 48 hr (μM)[a]			
	HepG2	MKN-45	NCI-H1993	B16
730	0.81 ± 0.04	0.69 ± 0.04	0.85 ± 0.11	0.53 ± 0.01
731	2.39 ± 0.11	2.34 ± 0.22	8.20 ± 0.99	3.45 ± 0.45
732	2.20 ± 0.15	2.33 ± 0.19	4.37 ± 0.76	2.18 ± 0.88
733	11.95 ± 1.01	>10.00	>10.00	>10.00
734	2.26 ± 0.44	0.91 ± 0.04	2.04 ± 0.17	2.93 ± 0.22

[a]Cytotoxicity results are expressed as IC_{50} values, the compound concentration producing a 50% cell growth inhibition and represent the mean ± SD of three independent experiments.

Table XXX

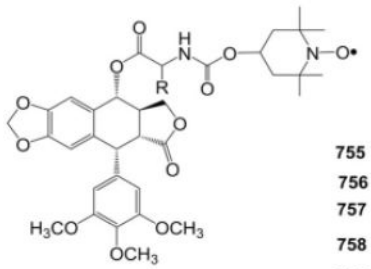
Biological Data for Spin-Labeled Compounds**743–754**



	Cytotoxic activity (IC_{50}, μM)		Antioxidative activity		
Compound	**P388**	**A549**	**Liver**	**Kidney**	**Heart**
ETO	1.18	7.14	46.38	64.63	82.73
743	0.82	0.85	7.92	8.80	8.76
744	0.18	8.42	13.55	10.35	10.47
745	0.01	6.43	15.41	15.42	13.39
746	<0.01	6.86	10.59	10.96	10.88
747	0.02	0.30	8.98	7.82	3.8
748	0.09	0.46	4.19	5.10	4.84
749	0.07	0.90	5.50	5.54	9.07
750	<0.01	0.13	5.26	4.96	5.54
751	0.34	>10	4.26	3.04	5.50
752	0.03	0.28	2.93	5.47	2.93
753	0.24	8.19	5.54	5.41	6.20
754	0.04	0.42	7.32	7.64	7.56

Table XXXI

Cytotoxicity Data for Spin-Labeled Compounds **755–759**



755-759

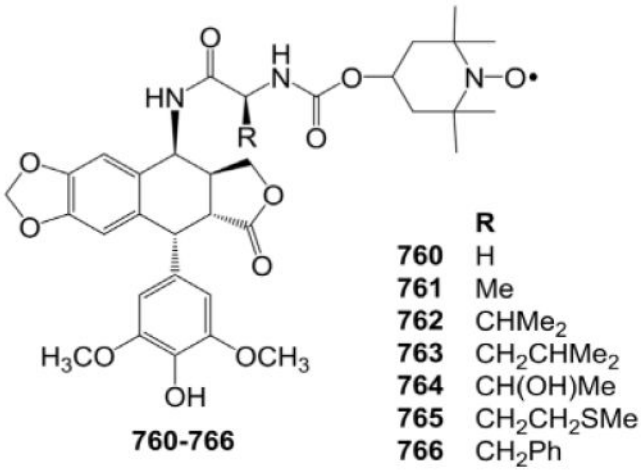
	R
755	H
756	CH_3
757	$CH_2CH_2SCH_3$
758	$CH(CH_3)CH_2CH_3$
759	CH_2Ph

	IC_{50} (μM)[a]				
Compound	**K562**	**HL-60**	**SPCA-1**	**Lewis**	**L-1210**
ETO	3.29×10^{-2}	4.44×10^{-2}	3.2×10^{-2}	7.2×10^{-2}	3.49×10^{-2}
755	1.08×10^{-3}	1.96×10^{-3}	0.248	0.190	1.57×10^{-2}
756	1.52×10^{-2}	1.52×10^{-2}	5.7×10^{-2}	0.155	8.64×10^{-3}
757	9.64×10^{-3}	1.08×10^{-4}	9.7×10^{-2}	6.6×10^{-2}	5.37×10^{-3}
758	1.67×10^{-2}	5.52×10^{-4}	0.127	9.3×10^{-2}	1.50×10^{-2}
759	1.48×10^{-2}	1.71×10^{-4}	7.6×10^{-2}	7.3×10^{-2}	1.63×10^{-4}

[a] IC_{50}, concentration of drug that affords 50% reduction in cell number using the MTT method with drug exposure for 48 hr.

Table XXXII

Biological Data for Spin-Labeled Compounds **760–766**



	Cytotoxic activity (IC_{50}, μM)			Antioxidative activity		
Compound	**A-549**	**HL-60**	**RPMI-8226**	**Liver**	**Kidney**	**Heart**
ETO	0.29	0.42	0.14	43.89	23.77	40.67
760	0.21	0.32	0.33	7.47	8.9	7.19
761	0.12	0.22	0.26	7.64	7.68	6.39
762	0.15	0.24	0.061	8.69	8.88	8.04
763	0.19	0.16	0.089	8.85	8.47	–
764	0.21	0.21	0.078	7.12	7.45	8.79
765	0.42	0.67	0.48	6.85	7.55	5.89
766	0.21	0.21	0.090	6.55	9.63	7.39