Recent Advances in Semisynthesis, Biosynthesis, Biological Activities, Mode of Action, and Structure-Activity Relationship of Podophyllotoxins: An Update (2008-2010)

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Abstract: Podophyllotoxin, one of the well-known naturally occurring aryltetralin lignans, has been used as the lead-compound for the preparation of potent anticancer agents, such as etoposide, teniposide, and etopophos. In our previous review, we described the advances of podophyllotoxin derivatives from 2003 and 2007. In recent years, an increased number of interesting research work has been carried out on the podophyllotoxins. As a continuation, the present review summarizes and highlights the update advances of podophyllotoxin derivatives from 2008 and 2010 in regard to semisynthesis, biosynthesis, biological activities, mode of action and structure-biological activity relationship.

Keywords: Podophyllotoxin, anticancer activity, antiviral activity, insecticidal activity, semisynthesis, biosynthesis, mode of action.

INTRODUCTION

Podophyllotoxin (**1**, PPT), one of the well-known naturally occurring and antimitotic aryltetralin lignans, has been used as a lead-compound in the development of new potent anticancer drugs [1]. Its semisynthetic derivatives, etoposide (VP-16, **2**) and teniposide (VM-26, **3**), currently are most commonly used in frontline cancer chemotherapy as DNA topoisomerase II inhibitors (TOPI) against various cancers, including small cell lung cancer, testicular carcinoma, lymphoma, and Kaposi′s sarcoma [2]. To overcome the poor water-solubility and improve the pharmaceutical characteristics of **2**, etopophos (etoposide phosphate, **4**), the phosphate ester derivative of **2**, recently has been widely used in the treatment of a variety of neoplasms. In comparison to the parent compound, **1**, compound **4** was highly soluble in water and could be readily formulated for intravenous use, resulting in higher clinical application. However, **4** was easily converted *in vivo* into the active moiety, **2**, by dephosphorylation, and the mechanism of action of **4** was believed to be the same as that of **2**, therefore, **4** might share the same drug-resistance profile with **2** [3-5]. When they are used to treat with various cancers in the clinical test, in fact, their potential therapeutic applications are often hindered by the development of drug-resistance, myelosuppression and cytotoxicity towards normal cells. Consequently, extensive structural modifications on **1** to develop new drugs to overcome the aforementioned problems and improve antitumor activity are still highly desirable.

Recently, some excellent reviews on the distribution, sources, applications, synthesis, biological activities, mechanism of action and structure-activity relationship (SAR) of podophyllotoxins have been published [6,7]. In our previous review, we reported the advances of podophyllotoxins from 2003 and 2007 [8]. Since 2008, an increasing number of interesting research work has been carried out on **1** and its analogs. The present review summarizes and highlights the update advances of podophyllotoxin derivatives from 2008 and 2010 in regard to semisynthesis, biosynthesis, biological activities, mode of action and structure-biological activity relationship.

BIOLOGICAL ACTIVITIES

Besides PPT and its derivatives exhibiting anticancer and antiviral activities [6-8], they also showed other interesting activities, such as antioxidative activity [9], anti-trypanosomal activity [10], and anti-melanocortin-4 receptor (MC4R) activity [11]. On the other hand, some interesting insecticidal activities of podophyllotoxin derivatives against the larvae of *Mythimna separata* and *Brontispa longissima* have also been reported in recent years [12-18].

MODE OF ACTION

Mainly two types of mechanisms of action of PPT derivatives as anticancer agents have already been reported, *i.e.*, tubulin polymerisation inhibitors (TPI) for "podophyllotoxin-like" compounds and topoisomerase II inhibitors (TOPI) for "etoposide-like" compounds [19-22].

Recently, Saitoh *et al*. demonstrated that PPT bound to a recombinant human papillomavirus (HPV) type1a E2 protein giving a K_D = 24.1 μM, and an E2/E7 interaction was inhibited by the addition of PPT [23]. Suh *et al*. found that the matrix metalloproteinases (MMP-9) expression and migration were strongly inhibited by deoxypodophyllotoxin (**5**, DPT, Fig. **2**) in tumor necrosis factor-α (TNF-α)-induced human aortic smooth muscle cells (HASMC). Meanwhile, the mRNA transcription of MMP-9 gene expression, and the TNF-α-induced phosphorylation of extracellular signal regulated kinase 1 and 2 (ERK1/2), p38 and c-Jun N-terminal

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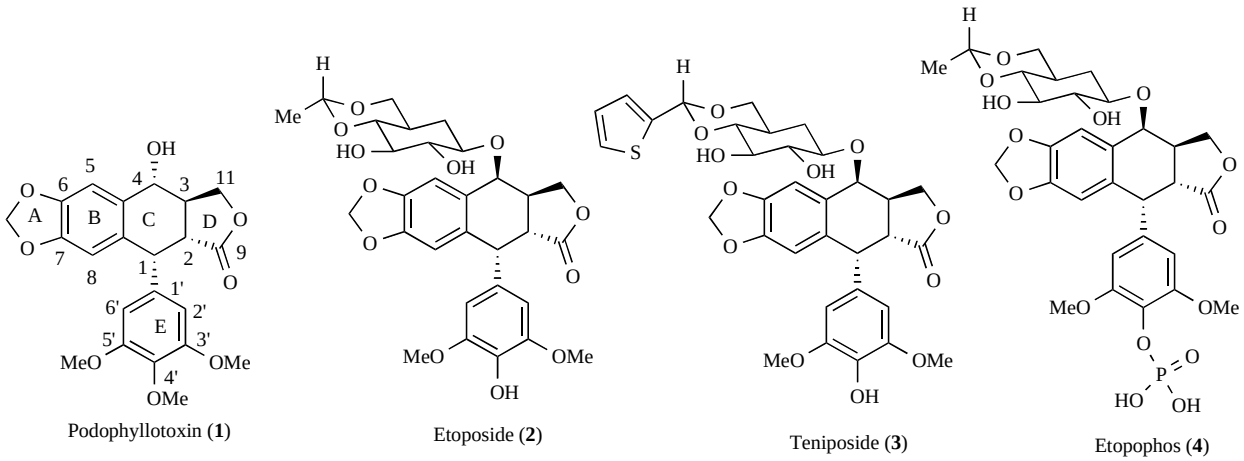


Fig. (1). Structures of podophyllotoxin (**1**), etoposide (**2**), teniposide (**3**) and etopophos (**4**).

kinase (JNK) were strongly inhibited by DPT [24]. Yong *et al.* evaluated DPT on the HeLa human cervix carcinoma cells and discovered that the mechanisms of action of DPT involved inhibition of tubulin polymerization and dysregulation of cyclin A and cyclin B1 expression, thus resulting in the mitotic cell cycle arrest, and activation of caspases-3 and -7 to promote apoptotic cell death [25]. Subsequently, Yong and co-workers further described that DPT caused cell cycle arrest of HeLa cells at G_2/M phase, followed by induction of apoptosis. The activation of ataxia-telangiectasia mutated (ATM), checkpoint kinase 2 (Chk2), and p53 might contribute to DPT-induced apoptosis, possibly through a mitochondria-mediated pathway [26]. Chen *et al.* reported that PPT did not affect intracellular calcium level and the phosphorylation state of p38, and PPT induced cyclic adenosine monophosphate (cAMP)-responsive element (CRE)-driven gene expression and CRE binding protein activation *via* protein kinas A (PKA) activation by a cAMP-independent mechanism [11].

BIOSYNTHESIS

PPT is used as a precursor for the chemical synthesis of the anticancer drugs like etoposide, teniposide, and etopophos. However, the availability of PPT is becoming increasingly limited because of intense collection from the wild, slow plant growth, poor reproduction, and delay of developing crop cultivation methods. Although total chemical synthesis of PPT and its analogs/derivatives have been accomplished [27-32], it is still not an option from a commercial point of view. Biotransformation and plant cell suspension cultures are good choices for the production of sufficient amounts of scientifically and commercially valuable compounds with the advantages of strict stereo- and region selectivity, mild reaction conditions and simple operation procedure.

Recently, Kour *et al.* reported the production of PPT by an endophytic fungus *Fusarium oxysporum* isolated from the medicinal plant *Juniperus recurva*. The results indicated that *F. oxysporum* can be a promising candidate for large scale production of PPT [33]. Anbazhagan *et al*. established the embryogenic cell and adventitious root culture systems in *Podophyllum peltatum* and analyzed PPT production [34]. Kusari *et al*. found an endophytic fungus from *Juniperus communis* L. Horstmann, as a novel producer of DPT. It would be interesting to further study the DPT production and regulation by the cultured endophyte in *J. communis* and in axenic cultures [35]. Tang *et al*. developed a novel biotransformation process of podophyllotoxin to produce picropodophyllotoxin (**6**, Fig. **2**) and podophyllic acid (**7**, Fig. **2**) when *Pseudomonas aeruginosa* CCTCC AB93066 was used as the biocatalyst [36].

SEMISYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP

The recent developments of semisynthesis and structure–activity relationship (SAR) of podophyllotoxin derivatives,

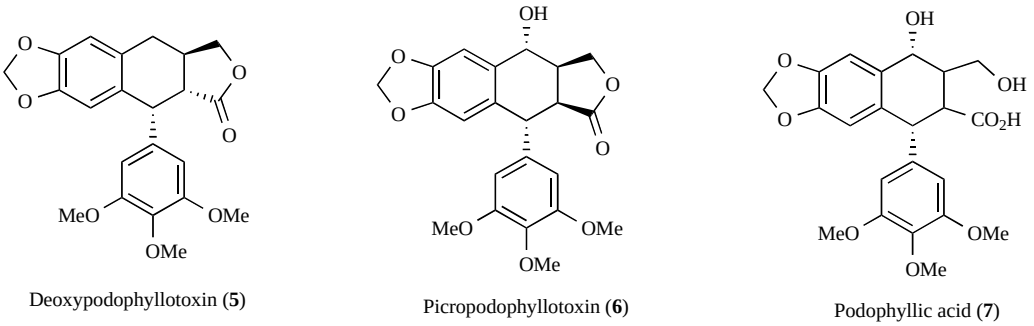
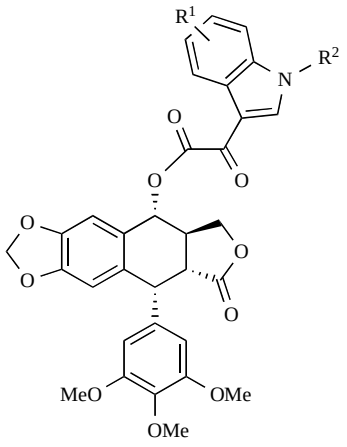
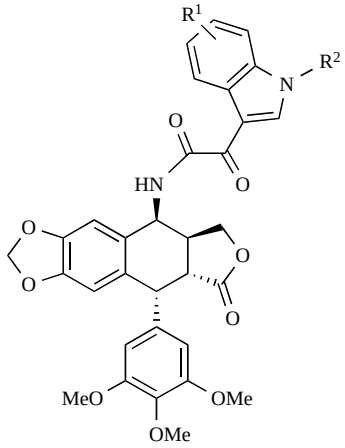


Fig. (2). Structures of deoxypodophyllotoxin (**5**), picropodophyllotoxin (**6**) and podophyllic acid (**7**).

Table 1. Biological Activity of 4*α*-*O*- and 4*β*-*N*-Indol-3-yl-glyoxyl-substituted Derivatives of Podophyllotoxin (8-17)



8-10



11-17

Compd	R^1	R^2	IC_{50}[a] (*μ*M)		GI_{50}[b] (*μ*M)	
			HeLa	SKOV3	K562	K562ADR
8	H	4-Chlorobenzyl	0.55	0.31	0.03	0.3
9	H	3,4-Dichlorobenzyl	0.65	3.34	0.1	0.22
10	H	Benzo[1,3]dioxol-5-ylmethyl	0.4	0.24	0.07	0.18
11	H	4-Chlorobenzyl	11.05	13.49	5.42	7.61
12	H	Benzo[1,3]dioxol-5-ylmethyl	5.72	9.5	0.94	33.85
13	5-Br	H	4.65	46.2	1.55	1.51
14	2-Me	H	3.62	7.96	0.89	>50
15	6-F	H	1.34	4.82	1.95	>50
16	5-MeO	H	0.94	3.04	0.21	0.42
17	6-MeO	H	2.45	7.85	3.04	3.09
PPT	/	/	0.1	0.17	0.03	0.17

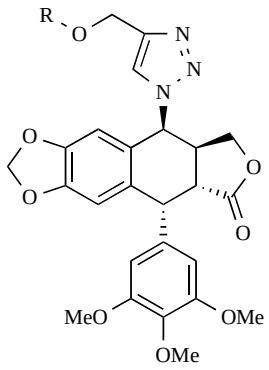
[a]IC_{50}: concentration causing 50% reduction in total cell number; [b]GI_{50}: concentration producing 50% cell growth inhibition.

are presented in chronological order to demonstrate the sequential progress in this area.

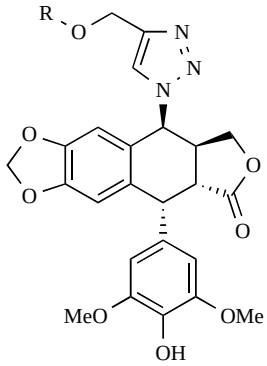
To overcome multidrug resistance (MDR) and lower the toxicity of PPT derivatives, Yu *et al*. synthesized 4*α*-*O*- and 4*β*-*N*-indol-3-yl-glyoxyl-substituted derivatives of podophyllotoxin (**8**-**17**), and tested their biological activity against a panel of four human cancer cell lines including HeLa (cervix), SKOV3 (ovary), K562 (leukemia) and K562ADR (adriamycin resistant leukemia) *in vitro*. As shown in Table **1**, the activity of compounds **9**, **10**, **13**, **16** and **17** against K562ADR was comparable to those against the common K562 cell line. The *O*-linked derivatives (esters) of PPT showed more potent activity than the corresponding *N*-linked congeners (amides) (*e.g.*, **8** vs **11**; **10** vs **12**). Generally, introduction of the electron-donating group at the C-5 position on the indole ring would lead to the more potent compound than the one bearing electron-withdrawing group (*e.g.*, **16** vs **13**), whereas the position of the methoxyl group of **16** was transferred from C-5 to C-6 position to afford **17**, the cytotoxicity of which was decreased sharply [37].

Reddy *et al*. prepared nine bulky 4*β*-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxin derivatives (**18**-**26**), and their cytotoxicities were evaluated against six human cancer cell lines, such as DU-145 (prostate), PC-3 (prostate), A-549 (lung), HOP-62 (lung), HCT-15 (colon), and SF-295 (CNS) (Table **2**). Among all the synthesized derivatives, compound **23**, which contained a dimethoxy moiety on the ring E and a glucose moiety on the triazolyl ring, exhibited the most promising activity. Compound **19** in which the sugar moiety was per-acylated showed less potent activity than the one having free sugar (**19** vs **18**). The presence of a hydroxy moiety on the ring E was essential for the activity, for example, compounds containing the dimethoxy moiety (**23**-**26**) were more active than those having the trimethoxy moiety (**18**-**22**). Generally, compounds **23**-**26** exhibited the promising activity against two cell lines, DU-145 and HCT-15 [38].

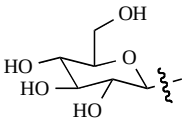
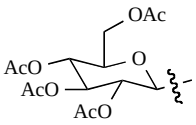
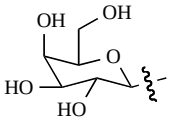
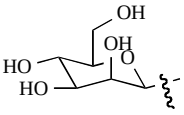
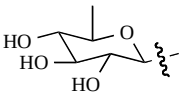
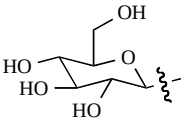
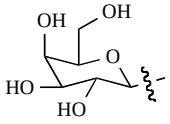
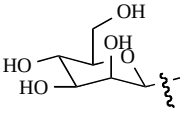
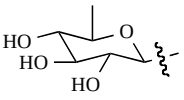
Table 2. Cytotoxicities of 4*β*-[(4-Substituted)-1,2,3-triazol-1-yl]podophyllotoxin Derivatives (18-26) (IC_{50}, *μ*M)



18-22

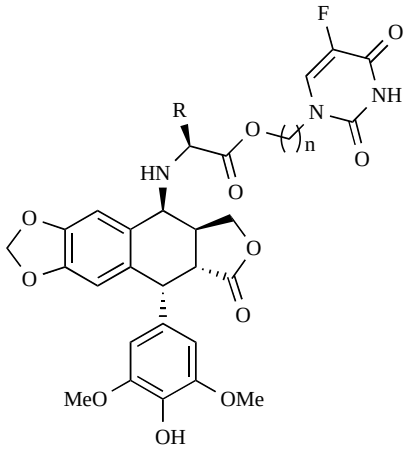


23-26

Compd	R	DU-145	PC-3	A-549	HCT-15	HOP-62	SF-295
18		25.8	127	125	15	ND[a]	26.8
19		42	221	556	ND[a]	142	35.1
20		237	316	59.9	16	178	14.5
21		18.6	94.9	51.8	237	121	53.4
22		26.5	268	114	ND[a]	ND[a]	88
23		3.11	34.6	3	0.93	8.55	2.01
24		4.95	24.3	6.88	1	5.27	16.1
25		2.73	8.62	7.33	3.57	5.96	2.51
26		5.04	20.8	7.04	2.14	16.2	8.29
VP-16	/	2.97	100	7.63	1	4.8	5.69

[a]ND: not determined.

Table 3. Cytotoxicities of Novel Conjugates of Podophyllotoxin and 5-FU (27-38) (IC_{50}, μM)



27-38

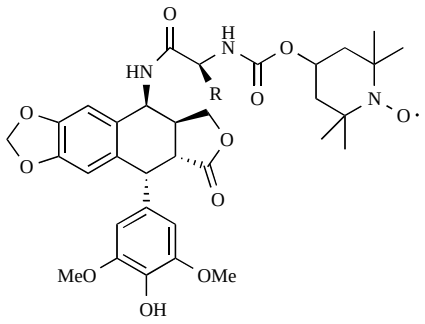
Compd	R	n	K562	AGS	HL-60	A-549	log*P*
27	Me	3	19.5	40.4	22.3	2.59	-0.07
28	$CHMe_2$	3	13.5	62.5	5.8	1.92	0.34
29	CHOHMe	3	30.8	140.8	23.2	6.95	-0.12
30	CH_2CHMe_2	3	9.5	59.2	0.13	0.01	0.33
31	$CH(Me)CH_2Me$	3	5.1	56.2	0.24	0.18	0.59
32	CH_2CH_2SMe	3	8.8	24.8	0.99	0.29	0.23
33	CH_2Ph	3	5.8	23.6	0.31	0.48	0.65
34	CH_2Ph(*p*-OH)	3	28.6	114.7	26.6	21.7	0.07
35	CH_2CHMe_2	4	5.3	34.5	0.73	<0.01	0.35
36	CH_2Ph	4	9.2	23.7	0.42	0.3	0.43
37	CH_2CHMe_2	5	6.0	23.5	0.31	0.53	0.51
38	CH_2Ph	5	13.2	38.8	0.04	<0.01	0.29
5-FU	/	5	>100	>100	65.3	50.5	/
VP-16	/	/	>100	50.8	2.75	7.38	0.69

As shown in Table **3**, Chen *et al.* synthesized twelve novel conjugates (**27-38**) of podophyllotoxin and 5-fluouracil (5-FU), and evaluated their cytotoxicities against a panel of four tumor cell lines, such as human premyelocytic leukemia (HL-60), human erythromyeloblastoid leukemia (K562), human gastric cancer (AGS) and human lung carcinoma (A-549). And their octanol-water partition coefficients (log*P*) were also tested. For the log*P* values of **27-38** were lower than that of VP-16, they showed superior water solubility than VP-16. Among compounds **27-34**, the activity profiles were markedly affected by the substituent of the amino acids. For example, the cytotoxicities of compounds **29** and **34**, containing hydroxyl groups in the amino acid side-chains, were decreased sharply as compared to **28** and **33**, respectively. The different lengths of alkyl linkages of target compounds did not obviously affect their cytotoxicities (**30** vs **35** vs **37**; **33** vs **36** vs **38**) [39].

As depicted in Table **4**, Zhang *et al*. reported the cytotoxicities of seven novel spin-labeled amino acid-linked derivatives of podophyllotoxin (**39-45**) against three tumor cell lines, *e.g.*, A-549, HL-60, and RPMI-8226 (human multiple myeloma). Introduction of different alkyl or aryl substituents at the α-carbon of $_L$-amino acid side-chains of **39** afforded compounds **40-45** (except **44**), which showed superior or comparable activities against A-549, HL-60, and RPMI-8226 compared to VP-16. Remarkably, compounds **41-43** and **45** exhibited significant cytotoxic activities against RPMI-8226 with the IC_{50} values in the range of 0.06–0.09 μM. In the meantime, the log*P* values of **39-45** were lower than that of VP-16, and it demonstrated that their water solubility was better than that of VP-16 [9].

As outlined in Table **5**, Zhang *et al*. further synthesized eight new spin-labeled derivatives of deoxypodophyllotoxin

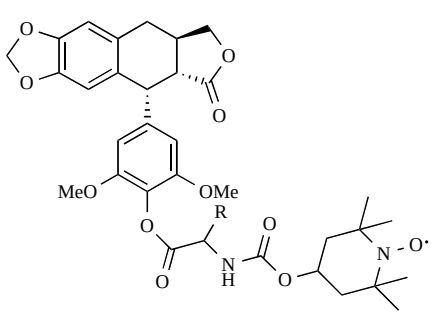
Table 4. Cytotoxicities of Novel Spin-Labeled Podophyllotoxin Derivatives (39-45) (IC_{50}, μM)



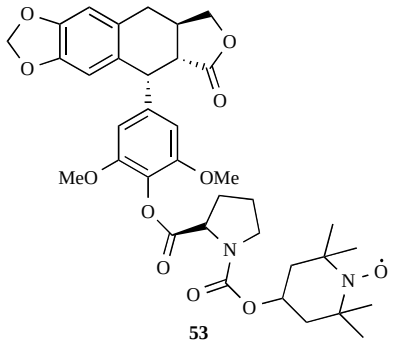
39-45

Compd	R	A-549	HL-60	RPMI-8226	log*P*
39	H	0.21	0.32	0.33	0.11
40	Me	0.12	0.22	0.26	0.12
41	$CHMe_2$	0.15	0.24	0.061	0.15
42	CH_2CHMe_2	0.19	0.16	0.089	0.21
43	CHOHMe	0.21	0.21	0.078	0.10
44	CH_2CH_2SMe	0.42	0.67	0.48	0.161
45	CH_2Ph	0.21	0.21	0.09	0.183
VP-16	/	0.29	0.42	0.14	0.68

Table 5. Cytotoxicities of Novel Spin-Labeled Deoxypodophyllotoxin Derivatives (46-53) (IC_{50}, μM)



46-52



53

Compd	R	A-549	RPMI-8226	HL-60
46	H	0.62	0.073	0.0087
47	Me	0.67	0.044	0.012
48	$CHMe_2$	0.55	0.16	0.024
49	CH_2CHMe_2	0.77	0.11	0.085
50	$CHMeCH_2Me_2$	0.83	0.058	0.036
51	CH_2Ph	0.50	0.031	0.018
52	CH_2Ph(*p*-OH)	0.27	0.043	0.032
53	/	0.60	0.13	0.14
DPT	/	0.80	0.23	0.11
VP-16	/	0.83	0.70	0.18

Table 6. The Ratio of Polymerized/Unpolymerized Tubulin of Novel Thiocolchicine-Podophyllotoxin Conjugates (54-62)[a]

Compd	P/U (mean ± SEM[c])	Compd	P/U (mean ± SEM)	Compd	P/U (mean ± SEM)
TCC[b]	0.87 ± 0.14	**56**	1.13 ± 0.23	**60**	1.29 ± 0.29
PPT	0.80 ± 0.11	**57**	1.83 ± 0.45	**61**	1.20 ± 0.10
54	0.74 ± 0.17	**58**	0.75 ± 0.16	**62**	1.36 ± 0.29
55	0.98 ± 0.04	**59**	1.51 ± 0.21	control	1.82 ± 0.06

[a]Tubulin (3 mg/mL) was polymerized in the absence or presence of 10 μM solution of selected compounds and ratios of polymerized/unpolymerized tubulin reported; [b]TCC: thiocolchicine; [c]SEM: standard error of the mean.

(DPT) (**46**-**53**) by the reaction of 4′-demethyl-4-deoxypodophyllotoxin (DDPT) with *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl) amino acids. Their biological activities were tested against three tumor cell lines (*e.g*., HL-60, RPMI-8226, and A-549) *in vitro*. The vast majority of compounds exhibited more potent cytotoxicities than DPT and VP-16, that is, introduction of $_L$-amino acids with a stable nitroxyl radical into DPT could lead to the compounds with improved antitumor activity. Especially, compounds **51** and **52**, containing the benzyl substituents at the α-carbon of $_L$-amino acid side-chains, showed the potent cytotoxicities against all three tumor cell lines. Interestingly, the cytotoxic activities of compounds **46**-**53** against three tumor cell lines decreased in the order HL-60>RPMI-8226>A-549 [40]. Notably, as compared with the above-mentioned spin-labeled derivatives of PPT (**39**-**45**), **46**-**53** exhibited more potent cytotoxic activities against HL-60 and RPMI-8226 cell lines. It suggested that introduction of $_L$-amino acids containing a nitroxyl radical on the C-4′ position of DPT could lead to the more active anticancer compounds.

Passarella *et al*. synthesized nine homo- and heterodimers (**54**-**62**) by the condensation of thiocolchicine and/or podophyllotoxin with six different dicarboxylic acids as described in Fig. (**3**). As shown in Table **6**, in general, thiocolchicine homodimers exhibited a much greater ability to disrupt tubulin polymerization *in vitro* than dimers containing podophyllotoxin (**54**, **55** and **58** vs **59**-**62**). Interestingly, the homodimers connected by different spacers showed different effects on tubulin polymerization. The nature of the linker unit had an important influence on the biological activity of the dimmer. The presence of two amino groups on the spacer improved the water solubility, but it reduced the ability to inhibit tubulin polymerization (**56** vs **55**). Obviously, the spacer unit displayed a significant effect on the biological activity. Therefore, the design of conjugate compounds was to create new biologically active molecules in which the spacer could be useful to improve the solubility and to modulate the efficacy of well-known drugs [41].

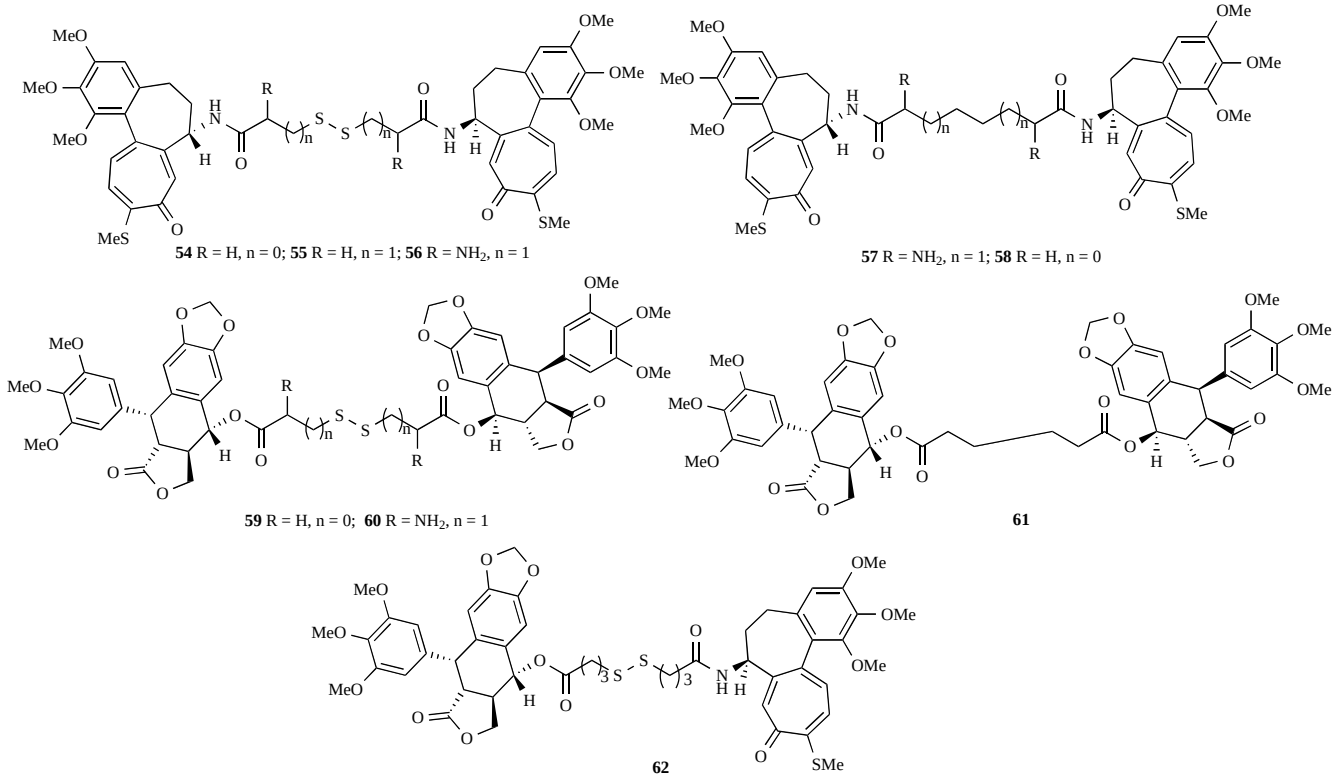


Fig. (3). Structures of novel thiocolchicine-podophyllotoxin conjugates (**54**-**62**).

CONCLUSION

PPT has been used as a lead-compound for drug design to obtain more efficient anticancer agents, and its three potent and promising derivatives, such as **2**, **3** and **4**, have been widely used in frontline cancer chemotherapy. However, their therapeutic uses are often hindered by the development of drug-resistance, myelosuppression and cytotoxicity towards normal cells. Consequently, to overcome the poor water-solubility and improve the pharmaceutical characteristics, extensive structure-activity relationship studies have been recently carried out on the podophyllotoxins through numerous chemical modifications on their cyclolignan skeleton.

In this mini-review, the advances of podophyllotoxin and its derivatives from 2008 and 2010 in regard to semisynthesis, biosynthesis, biological activities, mode of action and structure-biological activity relationship were summarized.

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ABBREVIATIONS

ATM = Ataxia-telangiectasia mutated

cAMP = Cyclic adenosine monophosphate

Chk2 = Checkpoint kinase 2

CRE = cAMP-responsive element

DDPT = 4′-Demethyldeoxypodophyllotoxin

DPT = 4-Deoxypodophyllotoxin

ERK1/2 = Extracellular signal regulated kinase 1 and 2

5-FU = 5-Fluorourcial

GI_{50} = Concentration producing 50% cell growth inhibition

HASMC = Human aortic smooth muscle cells

HPV = Human papillomavirus

IC_{50} = 50% Cytotoxic concentration, concentration of drug that causes 50% reduction in total cell number

JNK = c-Jun N-terminal kinase

MC4R = Melanocortin-4 receptor

MDR = Multidrug resistance

Me = Methyl

MeO = Methoxyl

MMP = Matrix metalloproteinases

PKA = Protein kinas A

PPT = Podophyllotoxin

SAR = Structure-activity relationship

SEM = Standard error of the mean

TCC = Thiocolchicine

TNF-α = Tumor necrosis factor-α

TOPI = Topoisomerase II inhibitor

Topo II = DNA topoisomerase II

TPI = Tubulin polymerization inhibitor

VM-26 = Teniposide

VP-16 = Etoposide

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