CHAPTER - I

INTRODUCTION

1. Background

Cancer disease is a growing public problem whose estimated worldwide new incidence is about 6 million per year. It is the second major cause of deaths after cardiovascular diseases. It is characterized by unregulated proliferation of cells. The search for natural products as potential anti-cancer agents dates back, at least, to the Ebers papyrus in 1550 BC, but the scientific period of this search is much more recent, beginning with the investigations by Hartwell and co-workers in late 1960 on the application of podophyllotoxin and derivatives as anticancer agents.

Research on the anticancer drug discovery has significant attention over the last few years. The term cancer chemoprevention coined by Sporn and coworkers in 1976 has been defined as a strategy for reducing cancer mortality by the prevention, delay or reversal of cancer by pharmaceutical agents capable of mediating the process of carcinogenesis. The number of attempts made to discover the anticancer drugs and evident that a similar mode of action to the structurally related compounds like combrestatin and other drugs. Tubulin and microtubules are the major targets of a number of clinically useful anticancer drugs that interrupts metaphase of the cell cycle where the lack of microtubules (primarily composed of tubulin) preventing the formation of the mitotic spindles.
tubulin is a protein molecule exists as a heterodimer of the two homologous α & β subunits. This dimer can couple together to make profilaments consists of the alternating α & β units, 12 or more profilaments can further link together to form pipe like structures called microtubules. These structures play an important role in a number of biochemical processes vital to cell survival and growth, one of these is the formation of the mitotic spindle without mitosis would not be able to takes place.

In the recent years, the number of the anticancer drugs 5-fluorouracil (5-FU) \(^5,6\), doxorubicin \(^7-10\), palcitaxol\(^11,12\), methotrexate\(^13,14\), campothecin\(^15-18\), cytarabine\(^19,20\), cis-platinum\(^21\), taxotere\(^22-26\) and combrestatin\(^27-31\) have been reported as clinical anticancer drugs. The two main groups of cytotoxic drugs used are the alkylating agents and the antimetabolites. Nitrogen mustards, ethylenediamine compounds and alkyl sulphonates are the main alkylating agents. Other compounds with an alkylating action are various nitro sources. Cisplatin and dicarbazine appear to act similarly. The antimetabolites may be subdivided in to folic acid, purine or pyrimidines antagonists. Several natural products or their derivatives are used for their actions as mitotic inhibitors; they include vinca alkaloids and the taxanes. Other drugs act as topoisomerase inhibitors, interfering with the coiling and uncoiling of DNA during replication. Drugs thought to act in this way include campothecin derivatives such as irinotecan and topotecan. And another one with same action includes podophyllotoxin\(^1\).
Among the various lignans isolated so far from the plant sources, podophyllotoxin 1 has emerged as a lead naturally occurring bioactive compound in the resin of the important medicinal plants *podophyllum emodi* (an Indian species) and *podophyllum peltatum* (a North American species) commonly known as American mandrake or mayapple (*Fig. 1*) and recently it has also been extracted from *podophyllum pleianthum* by David Jockson et al. They belong to the large family of the *Berberidaceae*.

*Fig. 1. Podophyllum peltatum*

Podophyllotoxin 1 was first isolated from podophyllin, a resin by Podwyssotzki (1880) 33. The related compounds obtained from the roots of podophyllum species include α-peltatin 2, β-peltatin 3 and their corresponding glycosides 4 to 7 currently extracts of the plants are used in topical medications for genital warts and some skin cancers. In China & Japan, the rhizomes of the podophyllum pleianthum are used to make a compound called Hakkakuren, which is used to treat snakebites and tumors of the genitals. Although the natural podophyllin resin was used in folk medicine, it was not until its antitumor activity was confirmed in the 1940s that synthetic studies 34 of the podophyllotoxin were
undertaken. Earlier efforts on synthetic, structural and mechanistic aspects of podophyllotoxin have provided much of the basis for the synthetic endeavors that followed.

\[
\begin{array}{cccc}
\text{Compound} & R & R' & R'' \\
2 & H & OH & OH \\
3 & H & OCH₃ & OH \\
4 & o-Glucosyl & OCH₃ & OH \\
5 & H & H & o-Glucosyl \\
6 & H & OCH₃ & o-Glucosyl \\
7 & o-Glucosyl & H & H \\
\end{array}
\]

In the early 1950s, Hartwell and Schrecker proposed its correct structure by a combination of chemical means. Interest in podophyllotoxin has been heightened by its potent antimitotic activity and its challenging stereochemistry. Gensler and coworkers did the pioneering work and published the first total synthesis in the 1960s. Several synthesis of racemic podophyllotoxin has been reported in the past two decades. Additionally, asymmetric total synthesis has been accomplished by Vander Walle et al., Meyers et al. and Jones et al.
Podophyllotoxin 1 and its derivatives has attracted our attention as both a challenging synthetic target and as they show broad spectrum of biological activities as anticancer, antioxidant and antitumor, cytotoxic, antiviral, antineoplastic and anti HIV drugs.

Podophyllotoxin 1  Picropodophyllin 8  β-apopicropodophyllin 9

The parent compound podophyllotoxin 1 was found to possess potent anticancer activity and its efficacy was examined for some time and discontinued latter due to severe side effects, unfavorable solubility and its ready epimerization to form inactive picropodophyllin 8. β-Apopicropodophyllin a dehydrated product of 1 containing cis fused lactone ring system showed pronounced antimitotic activity compare to parent compound 1.

Deoxy podophyllotoxin 10
Podophyllotoxin 1 and deoxy podophyllotoxin 10 are two well-known naturally occurring aryl tetralin lignans, are cytotoxic and their derivatives have potential clinical use as antitumor agents. These properties prompted the scientific community to perform numerous modification on the podophyllotoxin skeleton, in order to avoid the side effects, which led to the Pharmaceutical research of Sandoz (Switzerland); they developed two semi synthetic glycosides of 1, namely Etoposide 11, Tenoposide 12 and Etopophos 13. These are structurally different and have retained the anticipated anticancer activity and lost the undesired side effects. Structurally etoposide and tenoposide have an-OH group instead of –OCH₃ at C-4; and is glycosylated with opposite stereochemistry at C-4.
Etoposide and congeners induce a premitotic blockade in late S stage of the cell cycle because of the inhibition of DNA topoisomerase II (Topo II), an enzyme required for unwinding of DNA during replication. Etoposide binds to and stabilizes the DNA-protein complex preventing relegation of the double stranded breaks. However due to the typical adverse effects, such as anemia, hair loss and severe gastrointestinal disturbances, the application of them has been limited to a certain extent. Therefore being continued research on Podophyllotoxin is currently focused on structure optimization to generate derivatives with superior pharmacological profiles and broader therapeutic scope. D. Subrahmanyam et al. have reported the in vitro cancer studies on human colon and breast cancer cell line models of lactone ring modified analogues of podophyllotoxin, have pronounced activity. Ahmed kamal and V. Damaynathi have reported an important representative of arylnaphthalene lignans from aryl tetralin lignans by biotransformation.

Podophyllotoxin and its congeners have a 1-phenyl tetrahydroanthalene system. The basic skeleton of the lignan system is 2, 3-dibenzyl butane.
Among the naturally occurring interesting lignans, shows anti leukemic activity is steganacin 15 and its derivatives \(^{58, 59}\) are structurally similar to 1, has been reported \(^{60}\) to inhibit cleavage of sea urchin eggs. It also inhibits in vitro, calf and rabbit tubulin polymerization and causes a slow depolymerisation of existing microtubules. Futher, 15 blocks HeLa replication in mitosis, inhibits colchicines binding to purified tubulin \(^{59}\). In this latter work, a number of analogues of steganacin 15 were also examined for inhibiting and binding activity. The total synthesis of 15 and a series of derivatives have been described by Damon et al \(^{61}\), Hughes et al \(^{62}\), Kende et al \(^{63}\), Kende et al \(^{64}\), Krow et al \(^{65}\) and Zeigler et al \(^{66}\).

\[
\begin{align*}
&\text{H}_3\text{COCOH} \\
&\text{O} \\
&\text{OCH}_3 \\
&\text{H}_3\text{CO} \\
&\text{H}_3\text{C} \\
&\text{H}_3\text{CO} \\
&\text{O} \\
&\text{H}_3\text{CO} \\
&\text{O} \\
&\text{H}_3\text{C} \\
&\text{H}_3\text{CO} \\
&\text{O} \\
&\text{H}_3\text{C} \\
&\text{H}_3\text{CO} \\
&\text{O} \\
&\text{H}_3\text{C} \\
&\text{H}_3\text{CO} \\
&\text{O} \\
&\text{H}_3\text{C} \\
&\text{H}_3\text{CO} \\
&\text{O}
\end{align*}
\]

\textbf{Steganacin 15.}

The antimitotic activity, pharmacology and toxicity of podophyllotoxin 1 analogues have been reviewed \(^{67}\).
The characteristic features of the Podophyllotoxin molecule is the transfused lactone ring imparting considerable rigidity to the geometry of the hydroaromatic ring. As a result, trimethoxy phenyl group takes an axial conformation, which is very close to the neighbouring groups. The molecular model of podophyllotoxin also reveals that transfused lactone ring B is not only rigid but also strained. Where as in picropodophyllin 8 cis fused lactone ring allows considerable flexibility and trimethoxy phenyl ring can move in to less crowded space. These factors contribute more in promoting the ready base catalysed epimerization of Podophyllotoxin\textsuperscript{76} at its C\textsubscript{2} position to a diastereomer picropodophyllin 8.

Similarly, in the case of β-apopicropodophyllin 9 ring B will assume a planar configuration.
The strong antimitotic activity of podophyllotoxin 1 and several of its analogues has led to the investigation of structure activity relationship of...
podophyllotoxin by modification of lactone ring, by modifying the substituents of ring C, ring A.

Based on P-815 mastocytoma cell culture of the mouse, Schreir et al. has summarized the structural requirements for biological activity among the Podophyllotoxin analogues.

Schreir’s generalization

\[ \text{1-phenyl-1, 2, 3, 4-tetrahydronaphthalene} \]

**Fig-6**

1) Ring A must posses either a methylene dioxy ring or free phenolic hydroxyls.

2) Ring C contains three methoxy groups or no methoxy groups, with the exception of the 4'-sustituent, which may be a phenolic hydroxyl instead of a methoxy group.

3) The presence of substituents at positions 2, 3 and 4 of the ring B is not vital to the activity of a compound. If substituents are present, the stereochemistry and nature of substitution determines the magnitude of activity.

a) If a lactone exists, it must be closed with hydroxy methyl group at position 3.
b) Substitution imparting rigidity to ring B in addition to holding ring C in an axial position favors strong activity.

4) For maximum activity, the configuration must be ‘L’ according to the convention of Klyne. The absolute configuration at position 1, 2 and 3 would be ‘R’.

In the light of the above studies, one can postulate the mechanism of action at the molecular level. The assumption is that the strained lactone system of I acts to acylate a critical cell constituent as an N-H, S-H or OH function and thereby blocks the function of such a molecule the strain in the lactone ring is removed by acylation process. Biochemically the acylation could destroy the activity of an essential cell constituent. Thus the function of N-mustards (alkylation) and the function of penicillins. Acylation involve biologically critical bond formations with release of ring strain in the reagent as shown (Fig. 7).

\[ \text{N-CH}_2\text{CH}_2\text{Cl} \rightarrow \text{N-Ch}_{2}\text{Cl} \]

\[ \text{N-CH}_2\text{Cl} \text{ Strained} \]

\[ \text{RCOHN} \rightarrow \text{RCONHCH} \]

\[ \text{trans peptidase} \]

\[ \text{COOH} \]

\[ \text{trans peptidase} \]

\[ \text{Fig. 7} \]
With a view to avoid the carbonyl group of the lactone ring which causes epimerization of the unstable transfused lactone ring in podophyllotoxin 1 to the cis-lactone as in picropodophyllin 8, when the product becomes biologically inactive, the carbonyl group was thought to be modified to the methylene group. Gensler et al., \textsuperscript{72b} and recently C. A. Murthy et al., \textsuperscript{73-75} have synthesized several non-lactone analogs of podophyllotoxin 1, screened for the antimitotic activity and found that some compounds are more active than the parent compound podophyllotoxin 1 and some are less active. The fact that some of the analogs retained their activity, despite the absence of the lactone ring is contradictory to the hypothesis that biological activity involves acylation.

The above study of the antimitotic activity of podophyllotoxin 1 and β-apopicropodophyllin 9 \textsuperscript{76} shows that the molecule possibly acts on a cell constituent not by a covalent bond formation but by a non-covalent combination. There are evidences of neutral compounds like steroids binding to the proteins by hydrophobic forces.\textsuperscript{77,78}

\textbf{Colchicine 16}
Vinblastin 17

There has been growing evidence for the speculation that spindle poisons such as colchicines 16, vinblastine 17 and podophyllotoxin 1 are potent antimitotic agents and they act by destroying the assembly and the function of microtubules that constitute the spindle in the cell. It has also been demonstrated that these spindle poisons bind non-covalently to the tubulin. The protein building block of microtubules and that, this binding inhibits mitosis in the cell. This binding does not disrupt intact microtubules but rather prevents the polymerization reaction. This colchicine binding site on tubulin is also a high-affinity binding site for 1, which is thus similarly a metaphase poison and produces similar morphological effects which are practically indistinguishable from those of colchicine 16 however the mechanisms of binding are somewhat different. Inhibition of colchicin 15 binding to tubulin by 1 is competitive and is prevented in a concentration-dependent manner. The tubulin binding affinities in mouse brain was found in study for analogues of 1 with substitutions in the ring B, C and E.
Podophyllotoxin 1 competitively inhibit nucleoside transport in mammalian cells (Eg. HeLa cells) by inhibiting the facilitated diffusional component of nucleoside transport. This effect of 1 is greater than that of 16 but occurs at higher concentrations than that required to arrest cell division in mitosis. The marked similarity observed between the mode of action of the aryl tetralin lignans as anticancer and antiviral agents is noteworthy. Owing to their ability to bind tubulin, these lignans disrupt the cellular cytoskeleton and thus interfere with viral replication. In addition to tubulin binding, synthetic podophyllotoxin analogues show inhibition of reverse transcriptase, which may be, exploited to selectively combat RNA viruses such as human immuno deficiency virus (HIV). But still speculation has been made about β-apopicropodophyllin 9 derivatives with substituents such as a nucleophilic amino group or an electrophilic epoxide ring which might act through covalent bonding on same critical cell constituent.
2. Research objectives

The traditional source of podophyllotoxin, the endangered, *podophyllum emodi*, *podophyllum peltatum* and other species, becomes scarcer, the demand for the compound continues to increase. In addition, numerous new podophyllotoxin derivatives and analogues are currently under development. This growing demand for podophyllotoxin exerts severe pressure regarding the development of new analogues of podophyllotoxin and its derivatives as anticancer agents.

More than fifty years after the first medicinal application of the antimitotic activity of podophyllotoxin was proposed, this aryl tetralin lignan continues to be the subject of extensive research. The introduction of etoposide 11, tenoposide 12 and etopophos 13, other various analogues and derivatives of 1 in to the armory of antitumor drugs is an excellent example of the manner in which useful pharmaceuticals may be developed from folk remedies. Continued efforts to rationally synthesis a better anticancer drug based on the aryl tetralin framework is still underway.

Gensler and coworkers 72 have screened the activity of 1 and 18-23 with TA3 mammary carcinoma cells in tissue culture have shown good activity in spite of the absence of lactone ring (*Table-1*). C.A.Murthy and coworkers 73-75 have synthesized several analogues of β-apopicropodophyllin 9 such as epoxide 28,
keto epoxide 30 with modified lactone ring and allylic amine 29 showed strong antimitotic activity which was determined by onion root tip method (*Table-2*).

\[
\begin{align*}
\text{Hydroxy ether 18} & \quad \text{Deoxy ether 19} \\
\text{Hydroxy thio ether 20} & \quad \text{Deoxy thio ether 21} \\
\text{Deoxy ketone 22} & \quad \text{Deoxy cyclopentane 23}
\end{align*}
\]

\[R = 1', 2', 3'-\text{trimethoxy phenyl}\]
Table: 1. Studies conducted with TA3 mammary carcinoma cells in tissue culture.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID$_{50}$ μmoles/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td>18</td>
<td>6.0</td>
</tr>
<tr>
<td>19</td>
<td>6.0</td>
</tr>
<tr>
<td>20</td>
<td>6.0</td>
</tr>
<tr>
<td>21</td>
<td>6.0</td>
</tr>
<tr>
<td>22</td>
<td>1.5</td>
</tr>
<tr>
<td>23</td>
<td>5.0</td>
</tr>
</tbody>
</table>
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Beta-Apopicropodophyllin homolactone 24

Cyclic ether 25

Cyclic ketone 26

Cyclic amide 27

Epoxide 28

Allylic amine 29

Keto epoxide 30

Lactone 31

R = 3', 4' & 5' - trimethoxy phenyl
Diketone 32

33

34

35

36

37

38
Table-2 Anti-mitotic activity by onion root tip method.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID50 μM/L</th>
</tr>
</thead>
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<td>3.7</td>
</tr>
<tr>
<td>8</td>
<td>1.6</td>
</tr>
<tr>
<td>9</td>
<td>2.5</td>
</tr>
<tr>
<td>24</td>
<td>6.5</td>
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<td>25</td>
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<tr>
<td>26</td>
<td>4.0</td>
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<tr>
<td>27</td>
<td>1.4</td>
</tr>
<tr>
<td>28</td>
<td>1.0</td>
</tr>
<tr>
<td>29</td>
<td>1.7</td>
</tr>
<tr>
<td>30</td>
<td>1.62</td>
</tr>
<tr>
<td>31</td>
<td>2.62</td>
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<tr>
<td>32</td>
<td>1.9</td>
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<tr>
<td>33</td>
<td>2.6</td>
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<tr>
<td>34</td>
<td>4.4</td>
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<tr>
<td>35</td>
<td>3.7</td>
</tr>
<tr>
<td>36</td>
<td>2.1</td>
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<tr>
<td>37</td>
<td>1.6</td>
</tr>
<tr>
<td>38</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Angeles Castro et al.,90-93 have synthesized several podophyllotoxin derivatives 39-41 lacking the methylene dioxy group or with different functionalization of A-ring of podophyllotoxin skeleton and evaluated their cytotoxic activities on selective four human neoplastic cell culture such as P-388 (Marine leukemia), A-549 (human lung carcinoma), HT-29 (human colon carcinoma) and MEL-28 (Malignant human carcinoma) (Table-3).
$R = 1', 2', 3'-\text{trimethoxy phenyl}$

Table-3: Cytotoxicity activity of podophyllotoxin modified in A-ring (IC$_{50}\mu M$)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human cancer cell line</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-388</td>
<td>A-549</td>
<td>HT-29</td>
<td>MEL-28</td>
</tr>
<tr>
<td>1</td>
<td>0.012</td>
<td>0.02</td>
<td>0.012</td>
<td>--</td>
</tr>
<tr>
<td>39</td>
<td>0.003</td>
<td>0.005</td>
<td>0.005</td>
<td>--</td>
</tr>
<tr>
<td>40</td>
<td>0.62</td>
<td>0.62</td>
<td>1.2</td>
<td>0.62</td>
</tr>
<tr>
<td>41</td>
<td>2.0</td>
<td>2.0</td>
<td>5.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

All the above given values were in $\mu M$ concentration. The term $GI_{50}/IC_{50}$ stands for the concentration of the drug that produced 50% growth inhibition of the cell in the cell line under study.
Ahmed kamal et al have reported the synthesis and invitro cytotoxicity assay of novel analogues of 1 in selected human cancer cell lines (Table- 4).

**Table-4: Invitro cytotoxicity in selected human cancer cell lines.**

<table>
<thead>
<tr>
<th>Cancer cell line / Panel</th>
<th>42a</th>
<th>42b</th>
<th>42c</th>
<th>42d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>0.36</td>
<td>0.04</td>
<td>--</td>
<td>0.41</td>
</tr>
<tr>
<td>CCRF-CEMSR</td>
<td>0.34</td>
<td>0.03</td>
<td>0.15</td>
<td>0.27</td>
</tr>
<tr>
<td>Normal cell lung NCI-H522</td>
<td>0.25</td>
<td>0.09</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>Colon KM12</td>
<td>0.25</td>
<td>0.03</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>CNS</td>
<td>0.34</td>
<td>0.05</td>
<td>0.27</td>
<td>0.36</td>
</tr>
<tr>
<td>SF-295</td>
<td>0.25</td>
<td>0.02</td>
<td>0.18</td>
<td>0.26</td>
</tr>
<tr>
<td>SF-539</td>
<td>0.35</td>
<td>0.02</td>
<td>0.21</td>
<td>0.48</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACHN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>0.34</td>
<td>0.02</td>
<td>0.51</td>
<td>--</td>
</tr>
</tbody>
</table>
Reaction Schemes

Scheme-1
53a-c (Cis/ trans) → 54a-c

5% Na-Hg → 55a-c

5% aq. NaOH →

Anhyd. AlCl₃

R¹ = R² = OCH₃

a : R = p-NO₂-C₆H₄
b : R = p-Cl-C₆H₄
c : R = p-F-C₆H₄

EtOH/H⁺ → 56a-c

Scheme-2
**INTRODUCTION**

**Scheme-3**

\[
\begin{align*}
R^1 = R^2 &= \text{OCH}_3 \\
a : R &= p-\text{NO}_2-\text{C}_6\text{H}_4 \\
b : R &= p-\text{Cl}-\text{C}_6\text{H}_4 \\
c : R &= p-\text{F}-\text{C}_6\text{H}_4
\end{align*}
\]
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Scheme-4
Hydroxy methylation

57a-c

$\text{NaBH}_4$

$\text{MeOH}$

58a-c

59a-c

$\text{NaBH}_4$

$\text{MeOH}$
R¹=R²=OCH₃

a : R= p-NO₂-C₆H₄

b : R= p-Cl-C₆H₄

c : R= p-F-C₆H₄

Scheme-5
Scheme 6
Targeted synthetic analogues 43-48 of β-apopicropodophyllin.
3. Results

The analogues 43, 44, 45, 46, 47 and 48 of podophyllotoxin were synthesized and their biological activities were determined to find out the correlation between structure activity relationship in the hope of arriving at some better anticancer agents. These could eventually be tested in the clinical field.

A. Antimitotic assay.

The relative order of antimitotic activity of podophyllotoxin 1 found to be same in both p-815-mastocytoma cell culture test and onion root tip method of finding the antimitotic activity should be quite valid and reliable for the determination of the preliminary antimitotic activity of the presently synthesized analogues 43, 44, 45, 46, 47 and 48.

It is interesting to note from the data in the Table-5, that some of the synthetic analogues have shown comparable antimitotic activity notably compounds such as 44, 45, 47 and 48 with ID50 values 1.95, 1.85, 2.3 and 2.0μM respectively. In fact, 43-48 have shown better antimitotic activity.
Table-5. Antimitotic (cytotoxicity) assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID$_{50}$ (µMole/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure 43" /></td>
<td>2.15</td>
</tr>
<tr>
<td><img src="image" alt="Structure 44" /></td>
<td>1.95</td>
</tr>
<tr>
<td><img src="image" alt="Structure 45" /></td>
<td>1.85</td>
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</table>
### Table-5.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID&lt;sub&gt;50&lt;/sub&gt; (µMole/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Compound 46" /></td>
<td>2.45</td>
</tr>
<tr>
<td><img src="image" alt="Compound 47" /></td>
<td>2.3</td>
</tr>
<tr>
<td><img src="image" alt="Compound 48" /></td>
<td>2.0</td>
</tr>
</tbody>
</table>
On comparing the antimitotic activity of very closer analogues 43-48 appear that the size of the substituents attached to the ring-A and C will affect the activity.

B. Anticancer assay.

The anticancer activity of synthesized analogues 43, 44, 45, 46, 47 and 48 of podophyllotoxin 1 was using the short-term in vitro cytotoxicity towards B16F10 (mouse melanoma) cells as a preliminary screening technique by (cell viability test) MTT assay for their cytotoxicity.

All the compounds were found to have considerable antiproliferative activity in the cell viability test.

The analogues 44 and 45 have shown maximum anticancer activity by inhibiting the cell proliferation up to 64% and 73% respectively, whereas rest of the analogues moderate inhibition of cell proliferation. The IC50 values of 43, 44, 45, 46, 47 and 48 were given in the Table-6.
**Table 6.** Anticancer (Antiproliferative) assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% of inhibition of cell proliferation for 2.5 µM</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; values(µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.21</td>
<td>3.6</td>
</tr>
<tr>
<td>9</td>
<td>52.01</td>
<td>2.78</td>
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<td>65.18</td>
<td>1.77</td>
</tr>
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<td>45</td>
<td>72.12</td>
<td>1.57</td>
</tr>
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<td>46</td>
<td>53.17</td>
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<tr>
<td>47</td>
<td>56.00</td>
<td>2.02</td>
</tr>
<tr>
<td>48</td>
<td>59.21</td>
<td>1.82</td>
</tr>
</tbody>
</table>
4. Prospects

In view of the strong antimitotic activity of many non-lactonic derivatives and analogs of podophyllotoxin, it would be worthwhile to modify the lactone ring to synthesize the following types of compounds to study their biological activities.

Where

\[ X = \text{O or NH or CO or SO} \]

Further, a synthetic study on modifying the C-4 substituents of podophyllotoxin in ring-C by synthesizing the following compounds with a view to study the biological activities would be worthwhile.

Where

\[ X = \text{O or NH or CO or SO} \]

\[ R = -\text{CH}_3, \text{C}_2\text{H}_5, \text{C}_3\text{H}_7 \]