# SYNTHESIS AND CYTOTOXICITY OF NEW 4-AZA-2,3-DIDEHYDROPODOPHYLLOTOXINS

**Nguyen Thi Thuy Hang[1, 2], Tran Thi Yen[1], Vu Dinh Hoang[3], Ngo Quoc Anh[1, 2, *]**

[1]*Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[2]*Graduate University of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[3]*Department of Pharmaceutical Chemistry, Hanoi University of Science and Technology, 1 Dai Co Viet, Bach Khoa, Hai Ba Trung, Ha Noi, Viet Nam*

[*]Email: *ngoqanh@ich.vast.vn*

**Abstract.** Podophyllotoxin is a lignan, first isolated from herbaceous plants of *Podophyllum* and possess a potent antiviral and antitumor agent. Podophyllotoxin and its analogs exhibit antineoplastic activities mainly by preventing the assembly of tubulin into microtubules or inhibiting the catalytic activity of DNA topoisomerase II. Recently, many series of novel conjugates of 4-aza-2,3-didehydropodophyllotoxin analogs were synthesized and demonstrated significant cytotoxicity. Some compounds inhibit tubulin polymerization comparable to podophyllotoxin. These compounds were also shown to arrest the cell cycle in the G2/M phase of cell cycle and to lead to caspase-3 dependent apoptotic cell death. In this paper, we report the synthesis of five new 4-aza-2,3-didehydropodophyllotoxins by a straightforward multi-component reaction in the hope of finding new structures with interesting anticancer activity. The novelty in our work is the preparation of quinoline structural compounds of podophyllotoxin derivatives, in two simple steps, having functional groups which are available for further modifications. The "one-pot" synthesis process increased performance as well as minimized the steps involved. The obtained compounds with podophyllotoxin frame have the corresponding yields of 60 - 83 %. Five new 4-aza-2,3-didehydropodophyllotoxins demonstrated comparable cytotoxicity against the Hep-G2, MCF7 cell lines with an $IC_{50}$ value of 52.2 - 261.2 μM, and with ellipsitine as the positive control.

*Keywords:* 4-aza-2,3-didehydropodophyllotoxins, podophyllotoxin, one-pot, cytotoxicity

*Classification numbers*: 1.2.4.

## 1. INTRODUCTION

Podophyllotoxin (**1**) is a lignan, first isolated by Podwyssotzki in 1880 from the dry roots and rhizomes of the perennial North American herbaceous plants of *Podophyllum* such as P. *hexandrum* and P. *peltatum*. Key chemical structures include podophyllotoxin,

deoxypodophyllotoxin, 4′-demethylpodophyllotoxin, 4′-demethylepipodophyllotoxin (DMEP), α-peltatin, β-peltatin, and their corresponding glycosides (Figure 1a) [1].

Podophyllotoxin is a potent plant-derived antiviral and antitumor agent. Critical modifications on the skeleton of this natural compound led to the development of anticancer drugs etoposide (**2**) and teniposide (**3**) (Figure 1b) which are approved for the treatment of testicular cancer, and curently used for the treatment of other types of cancer, including small cell lung cancer, testicular cancer, lymphoma, leukemia, and Kaposi's sarcoma [2 - 7]. Mechanisms of action of the antineoplastic activities of podophyllotoxin analogs include preventing the assembly of tubulin into microtubules or inhibiting the catalytic activity of DNA topoisomerase II, although other known and in some cases ambiguous mechanisms are also involved [4-10].
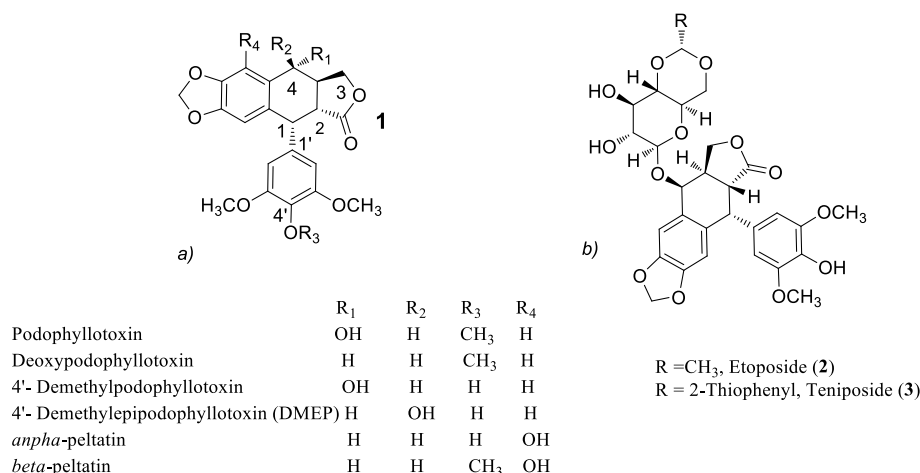
|  | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Podophyllotoxin | OH | H | CH$_3$ | H |
| Deoxypodophyllotoxin | H | H | CH$_3$ | H |
| 4'- Demethylpodophyllotoxin | OH | H | H | H |
| 4'- Demethylepipodophyllotoxin (DMEP) | H | OH | H | H |
| *anpha*-peltatin | H | H | H | OH |
| *beta*-peltatin | H | H | CH$_3$ | OH |

R =CH$_3$, Etoposide (**2**)
R = 2-Thiophenyl, Teniposide (**3**)

*Figure 1.* a) Podophyllotoxin (**1**)**,** b) etoposide (**2**) teniposide (**3**).

Many years after its first clinical use, this aryltetralin lignan continues to be the subject of extensive research with the challenge to design novel derivatives with superior pharmacological profiles and broader therapeutic scope. Modifications of podophyllotoxin concern the removal of the methylenedioxy group to give hydroxy, methoxy, or other oxygenated substituents; replacement of the methylenedioxy ring with heteroaromatic ring systems in ring A; 5-oxygenation to give hydroxy or alkoxy groups in ring B; extensive C4 modifications, including C4 sugar- and nonsugar- (with O-, S-, N- and C- linkages) substituted derivatives, aromatization of C ring [8 - 19]. Recently, many series of novel conjugates of 4-aza-2,3-didehydropodophyllotoxins analogs were synthesized by a straightforward one-pot multi-component reaction of substituted anilines, substituted arylaldehydes, and tetronic acid (Scheme 1) and demonstrated cytotoxicity against five human cancer cell lines (breast, oral, colon, lung and ovarian) at nanomolar level. Some compounds exhibited tubulin polymerization inhibitory activity comparable to podophyllotoxin by inhibiting microtubule growth without accelerating microtubule decay. These compounds were shown to arrest the cell cycle in the G2/M phase of cell cycle and to lead to caspase-3 dependent apoptotic cell death [20 - 24].

In this paper, we report the elaboration of some new analogs of 4-aza-2,3-didehydropodophyllotoxins with various substituted anilines bearing methyl, methoxyl or chloro groups and evaluate their cytotoxicity on different cancer cells.

## 2. MATERIALS AND METHODS

### 2.1. Methods of determining structure

The NMR spectra were recorded using a Bruker DRX 500 spectrometer or Varian 400-MR spectrometer. All coupling constants (*J*) were expressed in Hz. The chemical shifts (*δ*) expressed in ppm relative to tetramethylsilane, using $CDCl_3$, DMSO as the solvent. The HRMS spectra were obtained using SCIEX-X500R QTOF LC/MS system. Column chromatography were performed using silica-gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) and thin layer chromatography (TLC) was performed using a precoated silica gel 60 F254 (0.25 mm, Merck).

### 2.2. Cytotoxic assay

Cytotoxic assays were performed according to a method developed by Monks et al, which is being used at the National Institute of Health (USA) as a standard method for the evaluation of the cytotoxic potential of compounds or extracts using a panel of human cancer cell lines. The cancer cell lines MCF7 (human breast cancer), Hep-G2 (Hepatocellular carcinoma) were provided by Prof. J. M. Pezzuto, Long Island University, USA and Prof. Jeanette Maier, University of Milan, Italy and used for the assays.

The cells were cultured as a monolayer in Dulbeco's Modified Eagle Medium (DMEM) or RPMI-1640 (depending on the cell lines) with contents including 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate and supplemented with fetal bovine serum (FBS) 10 %. The MCF7 medium was further added with 0.01 mg/mL bovine insulin. The cells were subcultured after 3 - 5 days at ratio of 1:3 and incubated at 37 $^{\circ}$C, 5 % $CO_2$ and 100 % humidity. The inhibitory rate of cell growth (IR) was calculated using the following equation

$$IR = 100 \text{ \%} - [(ODt - OD0) / (ODc - OD0)] \times 100$$

where: ODt : average OD value on day 3; OD0: average OD value at time-zero; ODc: average OD value of the blank DMSO control sample. The cytotoxicities were calculated and expressed as inhibition concentration at 50 % ($IC_{50}$ values). Ellipticine was served as a positive control. In our experiments, the isolated compounds were dissolved in DMSO 100 % at 4 mg/mL as stock solution. The final concentration of testing compound for screening assay is 20 μg/mL. The $IC_{50}$ values of promising agents will be determined by testing a series of sample concentrations at 100, 20, 4 and 0.8 μg/mL. Experiments were carried out in triplicate to ensure data accuracy. The TableCurve 2Dv4 software was used for data analysis and for $IC_{50}$ calculation. The $IC_{50}$ values should have small deviations throughout the experiments.

### 2.3. Synthetic methods

The amines (**1**) (1 equiv.) was added to a solution of 1 (1.0 mmol) and tetronic acid (**2**) (1 equiv.) in dioxane (5 mL). The resulting mixture was stirred under reflux conditions for 6 h. Then, the mixture was concentrated in vacuo to remove dioxane giving a residual. The solution of 2 TFA (5 mL), aromatic aldehydes (**4**) (1.0 equiv) and *p*-chloranil (**5**) (1.0 mmol) was stirred at room temperature for 30 min, then the product of reaction 1 was added, and the mixture was refluxed for another 6 h. Reaction progress was monitored by TLC until no starting material remained. After the reaction was completed, 25 mL of water was added to quench the reaction and the aqueous layer was extracted with $CH_2Cl_2$ (20 mL × 3). Finally, the combined organic

phase was washed with brine (25 mL × 2), dried on anhydrous sodium sulfate, and concentrated to provide a crude product, which was then purified by column chromatography (10 - 30 % EtOAc in hexane) to give the corresponding (**6**).

### 7-methoxy-9-(3,4,5-trimethoxyphenyl)furo[3,4-b]quinolin-1(3H)-one (6a)

R = *p*-OMe ($C_{21}H_{19}NO_6$), colorless solid (65 %) with solvent (ethyl acetate) column chromatography: *n*–hexan = 15/1-10/1. ^{1}H NMR (500 MHz, DMSO) δ 3.79 (s, 9H, 3-OMe); 3.81 (s, 3H, OMe); 5.48 (s, 2H, CH$_2$); 6.85 (s, 2H, Ph); 7.24 (d, J＝3.0, 1H, Ph); 7.68 (dd, J＝3.0, J＝9.3, 1H, Ph); 8.13 (d, J＝9.3, 1H, Ph); ^{13}C NMR (125 MHz, CDCl$_3$): δ 55.4; 56.2 (2C); 60.3; 69.1; 105.2; 107.6 (2C); 113.6; 124.4; 127.0; 127.2; 130.4; 138.0 146.7; 148.1; 152.5; 157.6; 161.9; 166.6. HR-MS, *m/z* = 382.1266 ([M+H]$^+$, $C_{21}H_{20}NO_6^+$; calc. 382.1285).

### 5-methoxy-9-(3,4,5-trimethoxyphenyl)furo[3,4-b]quinolin-1(3H)-one (6b)

R = *o*-OMe ($C_{21}H_{19}NO_6$), pale yellow solid (73 %) with ethyl acetate column chromatography: *n*–hexan = 15/1-10/1. ^{1}H NMR (500 MHz, DMSO) δ 3.84 (s, 9H, 3-OMe); 3.86 (s, 3H, OMe); 5.53 (s, 2H, CH$_2$); 6.90 (s, 2H, Ph); 7.28 (d, *J*＝2.8, 1H, Ph); 7.72 (dd, *J*＝2.8, *J*＝9.0, 1H, Ph); 8.17 (d, *J*＝9.0, 1H, Ph); ^{13}C NMR (125 MHz, CDCl$_3$): δ 55.9; 56.6 (2C); 60.6; 69.5; 105.7; 108.1 (2C); 114.2; 125.4; 127.5; 127.8; 131.0;138.5;147.3; 148.6; 153.1; 158.2; 162.4; 168.2. HR-MS, *m/z* = 382.1266 ([M+H]$^+$, $C_{21}H_{20}NO_6^+$; calc. 382.1285).

### 5-amino-9-(3,4,5-trimethoxyphenyl)-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one (6c)

R = *o*-NH$_2$ ($C_{20}H_{20}N_2O_5$), bright yellow solid (83 %) with column chromatography of dichlomethane solvent: Methanol = 15/1-10/1. ^{1}H NMR (500 MHz, DMSO) δ 3.56 (s, 3H, OMe); 3.59 (s, 6H, 2-OMe); 4.88 (d, *J*＝3.8 Hz, 2H, CH$_2$); 4.98 (d, *J*＝4.3 Hz, 1H, CH); 5.98 (d, *J*＝4.5 Hz, 1H, Ph); 6.42 (s, 2H, NH$_2$); 6.61 (dd, *J*＝7.5 Hz, *J*＝2.0 Hz, 1H, Ph); 6.74 (td, *J*＝8.9 Hz, *J*＝2.0 Hz, 2H, Ph); 6.90 (dd, *J*＝2.0, *J*＝7.5 Hz, 1H, Ph); 9.80 (s, 1H, NH); ^{13}C NMR (125 MHz, CDCl$_3$): δ 56.1 (2C); 57.8; 60.3; 66.4; 97.2; 105.2 (2C); 119.9; 121.2; 123.4 (2C); 131.9; 136.6; 138.0;140.0; 152.8 (2C); 159.1; 173.3. HRMS, m/z = 367.1287 ([M - H]$^-$, $C_{20}H_{17}NO_5^-$; calc. 367.1299).

### 7-methyl-9-(3,4,5-trimethoxyphenyl)furo[3,4-b]quinolin-1(3H)-one (6d)

R = *p*-Me ($C_{21}H_{19}NO_5$), dark yellow solid (68 %) with *n*-hexane solvent column chromatography: Ethylacetate= 15/1-10/1. ^{1}H NMR (500 MHz, DMSO) δ 2.48 (s, 3H, Me); 3.77 (s, 6H, 2-OMe); 3.81 (s, 3H, OMe); 5.49 (s, 2H, CH$_2$); 6.76 (s, 2H, Ph); 7.66 (s, 1H, Ph); 7.82 (dd, *J*＝8.5 Hz, *J*＝2.0 Hz, 1H, Ph); 8.08 (d, *J*＝8.5 Hz, 1H, Ph); ^{13}C NMR (125 MHz, CDCl$_3$): δ 21.7; 55.6 (2C); 60.6; 69.6; 108.1 (2C); 113.9; 126.6; 126.7; 127.6; 129.2; 135.2; 137.6; 138.4;149.6 (2C); 153.0 (2C); 163.7; 168.1. HRMS, m/z = 366.1310 ([M+H]$^+$, $C_{21}H_{20}NO_5^+$; calc. 366.1336).

### 6-chloro-9-(3,4,5-trimethoxyphenyl)furo[3,4-b]quinolin-1(3H)-one (6e)

R = *m*-Cl ($C_{20}H_{16}ClNO_5$), bright yellow solid (60 %) with n-hexane solvent column chromatography: Ethylacetate = 10/1 - 5/1. ^{1}H NMR (500 MHz, DMSO) δ 3.72 (s, 6H, 2-OMe); 3.78 (s, 1H, OMe); 552 (s, 2H, CH$_2$); 6.67 (s, 2H, Ph); 7.79 (dd, *J*＝7.8 Hz, *J*＝1.5 Hz, 1H, Ph); 7.94 (m, 1H, Ph); 8.20 (dd, J＝8.5 Hz, J＝1.5 Hz, 1H, Ph); ^{13}C NMR (125 MHz, CDCl$_3$): δ 56.5 (2C); 60.8; 69.1; 107.6 (2C); 116.4; 124.0; 129.8; 131.3 (2C); 132.6; 132.9 (2C); 138.3; 150.2; 152.5; 152.7 (2C); 164.5; 167.2. HR-MS, *m/z* = 386.0775 ([M+H]$^+$, $C_{20}H_{17}ClNO_5^+$; calc. 386.0790).

# 3. RESULTS AND DISCUSSION

Some new 4-aza-2,3-didehydropodophyllotoxins were synthesized in two steps in Scheme 2. The intermediates of the reaction between aniline derivatives and tetroic acid were condensed with trimethoxyl benzadehyd in an "one pot" reaction. The novelty in our work is the preparation of quinoline structural compounds of podophyllotoxin derivatives, in two simple steps, having functional groups which are available for further modifications. The novel furan compounds were prepared by the reaction between commercially available substituted anilines and tetronic acid in dioxane (step 1). Step 2 is a reaction between novel furan, aldehyde and catalyst p-chloranilin TFA (Scheme 2). The desired quinoline derivatives of podophyllotoxin were obtained with good yield (60 – 83 %). All structures were elucidated with the help of $^1$H, $^{13}$C-NMR and HR-MS.
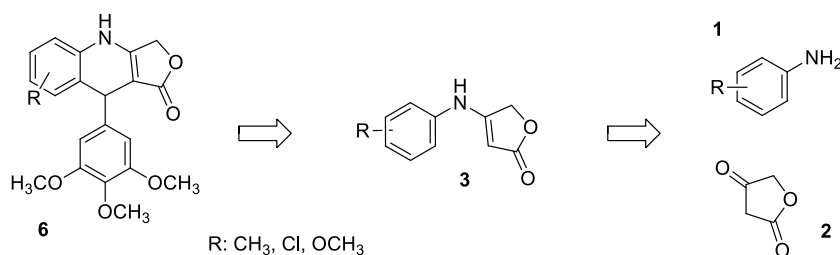


*Scheme 1.* Retrosynthesis of 4-aza-2,3-didehydropodophyllotoxins.
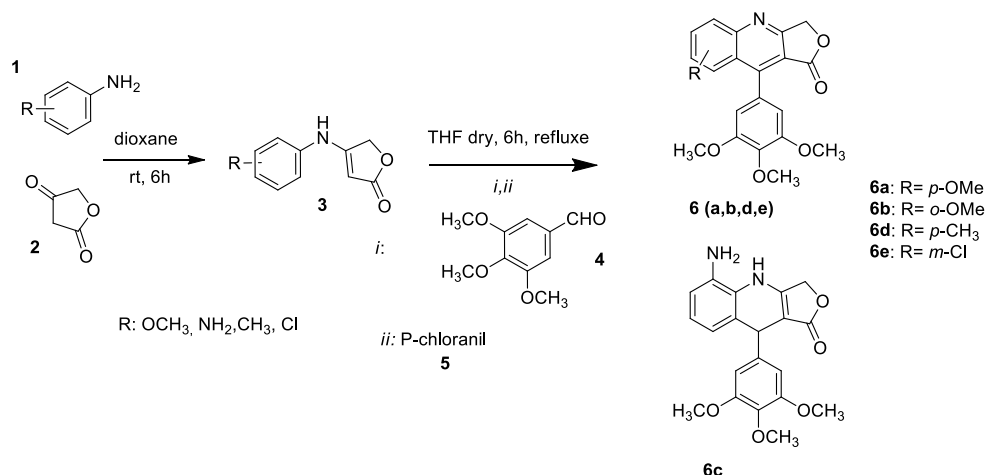


*Scheme 2.* Synthesis of 4-aza-2,3-didehydropodophyllotoxins.

This method is continuous, fast and effective, producing clean products with high yields. The structure of the obtained products was determined by $^1$H-NMR, $^{13}$C-NMR and HR-MS spectroscopic methods. Spectral data confirmed that five new substances were synthesized for the first time.

The cytotoxicity of five compounds and ellipticine as a positive control were evaluated for their inhibition rate of cell growth against two human tumor cell lines (Hep-G2 and MCF7) by a method developed by Monks *et al*. and the results are summarized in Table 1, showing that new compounds containing amino, chlorine groups indicated an average and more prominent activity with MCF7 than the others.

187

*Table 1.* The cytotoxicity of new compounds against two human cancer cell lines Hep-G2, MCF7.

| Entry | Compound | R | $IC_{50}$ (µM) | |
|:---:|:---:|:---:|:---:|:---:|
| | | | **Hep-G2** | **MCF7** |
| 1 | **6a** | *p*-OCH$_3$ | 261.2 ± 2.6 | 217.4 ± 5.6 |
| 2 | **6b** | *o*-OCH$_3$ | 205.1 ± 5.0 | 148 ± 15.9 |
| 3 | **6c** | *o*-NH$_2$ | 104.8 ±10.3 | 75.3 ±6.2 |
| 4 | **6d** | *p*-Me | 98.9 ± 10.8 | 77.7 ± 5.5 |
| 5 | **6e** | *m*-Cl | 88.2 ± 9.1 | 52.2 ± 6.3 |
| | Ellipticine | | 2.1 ± 0.1 | 1.5 ± 0.12 |

## 4. CONCLUSIONS

We have successfully synthesized five new toxins 4-aza-2,3-didehydropodophyllotoxins by a straightforward multi-component reaction process with the yield of 60 – 83 %. The obtained products demonstrated comparable cytotoxicity against the Hep-G2, MCF7 cell lines with an $IC_{50}$ value of 52.2 - 261.2 µM. The research will be continued in the near future with the expectation of finding highly selective anti-cancer substances.

*CRediT authorship contribution statement.* These authors contributed equally to this work.

*Declaration of competing interest.* The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES

1. Wratten S. J., Wolfe M. S., Andersen R. J., and Faulkner D. J. - Antibiotic Metabolites from a Marine Pseudomonad, Antimicrob. Agents Chemother. **11** (3) (1977) 411-414.

2. Berger J. M. and Wang J. C. - Recent developments in DNA topoisomerase II structure and mechanism, Curr. Opin. Struct. Biol. **6** (1) (1996) 84-90.

3. Sakurai H., Miki T., Imakura Y., Shibuya M. - Metal- and photo-induced cleavage of DNA by podophyllotoxin, etoposide, and their related compounds, Mol. Pharmacol. **40** (6) (1991) 965-973.

4. Huang T. S., Shu C. H., Shih Y. L., Huang H. C. - Protein tyrosine phosphatase activities are involved in apoptotic cancer cell death induced by GL-331, a new homolog of etoposide, Cancer Lett. **110** (1996) 77-85.

5. Huang T. S., Shu C. H., Yang W. K., Whang-Peng J. - Activation of CDC 25 phosphatase and CDC2 kinase involved in GL-331- induced apoptosis, Cancer Res. **57** (14) (1997) 2974-2978.

6. Cho S. J., Kashiwada Y., Bastow K. F. - Antitumor agents 164. Podophenazine, 2″ ,3″ - dichloropodophenazine, ben-zopodophenazine, and their 4β-p-nitroaniline derivatives as novel DNA topoisomerase II inhibitors, J. Med. Chem. **39** (7) (1996) 1396-1402.

7. Wang Z. Q., Kuo Y. H., Schnur D., Bowen J. P. - Antitumor agents 113. New 4β-arylamino derivatives of 4′-O-demethylepipodo-phyllotoxin and related compounds as potent inhibitors of human DNA topoisomerase II, J. Med. Chem. **33** (9) (1990) 2660-2666.

8. Zhou X. M., Wang Z. Q., Chang J. Y., Chen H. X. - 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. **34** (12) (1991) 3346-3350.

9. Lee K. H., Beers S. A., Mori M., Wang Z. Q. - 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. **33** (5) (1990) 1364-1368.

10. Chang J. Y., Han F. S., Liu S. Y., Wang Z. Q., Lee K. H. - Effect of 4β-arylamino derivatives of 4′ -O-demethylpodophyllotoxin on human DNA topoisomerase II, tubulin polymerization, KB cells, and their resistant variants, Cancer Res. **51** (1991) 1755-1759.

11. Terada T., Fujimoto K., Nomura M. - Antitumor agents 3. Synthesis and biological activity of 4β-alkyl derivatives con-taining hydroxyl, amino, and amido groups of 4′-O-demethyl-4-deoxypodophyllotoxin as antitumor agents, J. Med. Chem. **36** (12) (1993) 1689-1699.

12. Zhou X. M., Lee K. J. H., Cheng J., Wu S. S. - Antitumor agents 144. New γ -lactone ring-modified arylamino etoposide analogs as inhibitors of human DNA topoisomerase II, J. Med. Chem. **37** (2) (1994) 287-292.

13. Tomioka K., Kubota Y., and Koga K. - Synthesis and antitumor activity of podophyllotoxin aza-analogues, Tetra. Lett. **30** (22) (1989) 2953-2954.

14. Roulland E., Bertounesque E., Huel C., Monneret C. - Synthesis of picropodophyllotoxin homolactone, Tetra. Lett. **41** (35) (2000) 6769-6773.

15. Van Vliet D. S., Tachibana Y., Bastow K. F. - Antitumor agents: 207. Design, synthesis, and biological testing of 4β−anilino-2-fluoro-4′-demethylpodophyllotoxin analogues as cytotoxic and antiviral agents, J. Med. Chem. **44** (9) (2001) 1422-1428.

16. Saulnier M. G., Langley D. R., Kadow J. F., Senter P. D. - Synthesis of etoposide phosphate, BMY-40481: a water-soluble clinically active prodrug of etoposide, Bioorg. Med. Chem. Lett. **4** (21) (1994) 2567-2572.

17. Hu H., Liu S. Y., Cheng Y. C., Lee K. H. - Antitumor agents 123. Synthesis and human DNA topoisomerase II inhibitory activity of 2′-chloro derivatives of etoposide and 4β-(arylamino)-4′-O-demethylpodophyllotoxins, J. Med. Chem. **35** (5) (1992) 866-871.

18. Cho S. J., Tropsha A., Suffness M. - Antitumor agents 163. Three-dimensional quantitative structure-activity relationship study of 4′-O-demethylepipodophyllotoxin analogs using the modified CoMFA/q2-GRS approach, J. Med. Chem. **39** (7) (1996) 1383-1395.

19. Kamal A., Suresh P., Mallareddy A., Kumar B. A., Reddy P. V., Raju P., Tamboli J. R., Shaik T. B., Jain N., Kalivendi S. V. - Synthesis of a new 4-aza-2, 3-didehydropodophyllotoxin analogues as potent cytotoxic and antimitotic agents, Bioorganic and Medicinal Chemistry **19** (2011) 2349-2358.

20. Botes M. G., Pelly S. C., Blackie M. A. L. -. Synthesis of 4-Azapodophyllotoxins with Anticancer Activity by Multicomponent Reactions (Review), Chem Heterocycl Comp **50** (2014) 119-138.

21. Shi F., Zeng X. N., Tu S. J. - Facile synthesis of new 4-aza-podophyllotoxin analogs via microwave-assisted multi-component reactions and evaluation of their cytotoxic activity, Bioorg. Med. Chem. Lett. **21** (2011) 7119-7123.

22. Semenova M. N., Kiselyov A. S., Semenov V. V. - Polyalkoxybenzenes from Plants. 5. Parsley Seed Extract in Synthesis of Azapodophyllotoxins Featuring Strong Tubulin Destabilizing Activity in the Sea Urchin Embryo and Cell Culture Assays, J. Med. Chem. **54** (2011) 7138-7149.

23. Kumar A., Kumar V., Alegria A. E., Malhotra S. V. - N-Hydroxyethyl-4-aza-didehydropodophyllotoxin derivatives as potential antitumor agents, Eur. J. Pharm. Sci. **44** (2011) 21-26.

24. Kamal A., Reddy T. S., Polepalli S., Paidakula S., Srinivasulu V., Reddy V. G., Jain N., Shankaraiah N. - Synthesis and biological evaluation of 4-aza-2, 3-dihydropyridophenanthrolines as tubulin polymerization inhibitors, Bioorg. Med. Chem. Lett. **24** (2014) 3356-3360.