ISSN 0973-3450 (Print)
JUC Vol. 19(1), 1-8 (2023). Periodicity 2-Monthly
ISSN 2319-8036 (Online)

JOURNAL OF ULTRA CHEMISTRY
An International Open Free Access Peer Reviewed Research Journal of Chemical Sciences and Chemical Engineering
website:- www.journalofchemistry.org
Estd. 2005

Synthetic analogues of podophillotoxin and their antimitotic activities

SADASHIVAMURTHY. B., DAKSHAYINI. C. and PRAGASAM ANTONY*

Maharani's Science College for Women, Mysuru, Karnataka (India)
*Corresponding author Email: brightbright56@gmail.com
http://dx.doi.org/10.22147/juc/190101

Acceptance Date 21st January, 2023, Online Publication Date 20th February, 2023

Abstract

Podophyllotoxin finds a lot of scopes in pharmaceutical field as it show wide biological activities. Several analogues of podophyllotoxin were reported variety of biological activities such as cathartic, cytotoxic, antimitotic, anticancer, antitropical skin disease, antimalarial, virucidal, fungicidal *etc*. Experiment was carried out to synthesize 6, 6a-dihydro-2, 3-dimethoxy-9-methyl-11bH benzo [c] fluoren - 5, 7 dione and Synthesis of 6, 6a-dihydro-3-methoxy-2, 9-dimethyl-11 bH benzo [c] fluoren - 5, 7 dione. The products were re-crystallized in ethanol and characterized by spectrometric methods, IR, NMR and Mass spectroscopy. A yield of 82.72 and 83.6 percent were recorded respectively. The anti-mitotic activities of the synthesized analogues were examined by the onion root tip method with reference to b- apopicropodophyllin and Podophyllotoxin. In the present work, the synthetic analogues D and E have shown increased anti-mitotic activities with ID_{50} of 2.005×10^{-6} M and 2.260×10^{-6}M respectively with reference to standard and control.

Key words : Podophyllotoxin, anti-mitotic, analogue, onion root, b- apopicropodophyllin.

1. Introduction

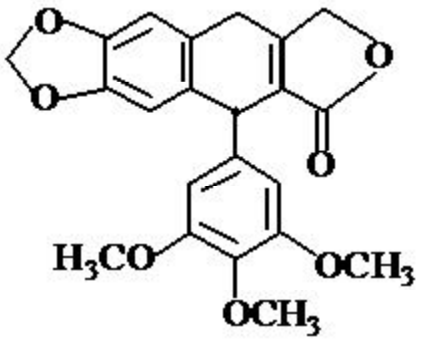
Podophyllotoxin (**a**)[1] is a strong anti-mitotic agent, which has been extracted from two important medicinal plants named Podophyllum emodi an Indian species and Podophyllum peltatum a North American species that belong to the family of berbideracea[2]. Recently it has also been extracted from other medicinal plant called podophyllum pleianthum by David Jackson *et al*[3]. The dried roots and rhizome of these plants are known as podophyllum, which on extraction from alcohol and after drying gives a resin termed podophyllin. In 1942 Kaplan cured the venereal

wart condyloma acuminatum with the topical application of podophyllin in oil. This led to the studies of the action of podophyllin on tumor tissues and to intensive chemical examination of the constituents of podophyllin[4].

a

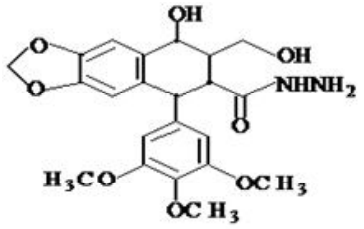
Podophyllotoxin and several of its analogues show wide variety of biological activities such as cathartic, cytotoxic, antimitotic, anticancer, antitropical skin disease, antimalarial, virucidal, fungicidal[5-8] *etc*. Podophyllotoxin derivatives and vinca alkaloids were the only drugs found markedly inhibit DNA ligases from normal cells[9]. Highly purified Podophyllotoxin (**a**) efficiently suppress in-vitro and in-vivo immune responses. Podophyllotoxin (**a**) in extremely low doses efficiently inhibits antibody responses to Sheep erythrocyte[10].

β-Apopicropodophyllin (**b**), a dehydrated isomerised product of podophyllotoxin acts as a much stronger antimitotic agent[11]. The total synthesis of β-apopicropodophyllin(**b**), a dehydrated isomerised product of podophyllotoxin acts as a much stronger antimitotic agent[11]. The total synthesis of β-apopicropodophyllin (**b**) was carried out by Gensler *et al*. in 1960[12]. Very recently the asymmetric total synthesis of β-apopicropodophyllin (**b**) was prepared for the first time from podophyllotoxin(**a**) by a three steps procedure by Schrecker and Hartwell-[13]. Recently, a convenient one step procedure for the preparation of β-apopicropodophyllin(**b**) from podophyllotoxin(**a**) in excellent yield have been reported by Murthy and coworkers[13].

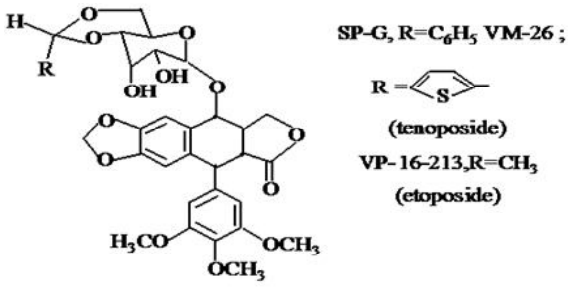


b

The sandoz company in Basel (Switzerland) have developed a number of semi synthetic derivatives mainly glycosides that have retained the desirable antimitotic activity and lost the undesired side effects. At least four of these VIZ: SP-1 and SP-G, VM-26 (tenoposide) and VP-16-213 (etoposide) are now in clinical use[14-18].

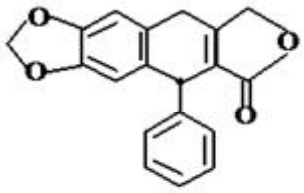


SP - 1



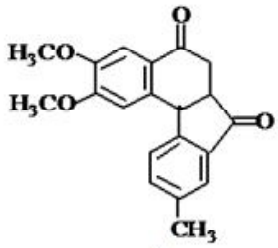
An interesting observation from Schreier and his coworkers is that tridemethoxy-β-apopicropodophyllin (**C**) a synthetic product, which

act as a strong antimitotic agent[11-35].

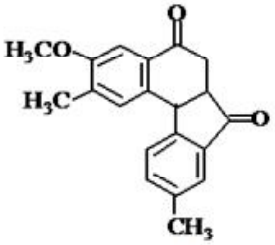


C

Hence it was decided to synthesize the analogues **D** and **E** by replacing tri-methoxy phenyl group with dimethoxy and methoxy methyl groups respectively by Gensler's method[12] and the anti-mitotic activity was studied in the present study.



D



E

Material and Methods

1. Synthesis of 6, 6a-dihydro-2, 3-dimethoxy-9-methyl-11bH benzo [c] fluoren - 5, 7 dione:

1.1 Conversion of 1a into 2a

A mixture of **3, 4 – dimethoxy - 4' – methyl – benzhydryl succinic acid 1a** (2g, 0.00558 mole) and thionyl chloride (40ml) was boiled for 5hours. The excess of thionyl chloride was distilled off to obtain **2a**.

1.2 Conversion of 2a into 3

A solution of **3, 4-dimethoxy-4'-methyl-benzhydryl succinoyl chloride 2a** (1.5g, 0.00379 mole) in dry dichloromethane (50ml) was added over a period of a magnetically stirred solution of anhydrous aluminum chloride (0.505g, 0.00379 mole) in dry dichlomethane at 0°C for 8h. The reaction mixture was treated with cold 5N HCl (50ml). The organic layer was washed with 10% NaOH solution (2x50ml) and finally with water. The solvent was removed by distillation gave a light yellow residue. The crude product was column chromotographed over silica gel (1cmx30) using chloroform as the eluant. The solvent was removed and evacuated at 50°C on a rotary evaporator gave white solid.

2. Synthesis of 6, 6a-dihydro-3-methoxy-2, 9-dimethyl-11bH benzo [c] fluoren - 5, 7 dione

2.1 Conversion of 1b into 2b :

2b was prepared from 4-methoxy-3-methyl-benzhydryl succinic acid 1b (2.0g, 0.005841 mole) and thionyl chloride (40ml) by refluxing for 5hours.

2.2 Conversion of 2b into 4

The 2b (1.5g, 0.003954 mole) was cyclised to 4 using anhyd. Aluminum chloride (0.5273g, 0.003954 mole) in dry dichloromethane (50ml) gave white crystalline solid.

3. Anti-mitotic activity :

The antimitotic activities of the synthesized analogues of β- apopicropodophyllin were examined by the onion root tip method.

3.1 Reagents required :

3.2 Orcein Solution: 2g of orcein dissolved in 45% acetic acid at boiling temperature, cooled to room temperature and filtered. The filtrate was used for staining.

3.3 Hydrochloric acid: 0.1N :

3.4 Test Solution: Prepared by dissolving exactly known weight (0.001 to 0.003g) of synthetic analogue in 3ml of absolute ethanol and diluted with distilled water to 250 ml in a standard flask. All the tested synthesized products gave a clear solution in the above process.

3.5 Procedure: Onion base was immersed to an extent of about half a centimeter in a sample tube (7x3 cm) after removing the old roots from it and the immersion continued for two days for germination. After two days, germinated root tips were removed and placed on the sample tube containing fixing solvent (absolute ethanol-glacial acetic acid, 3:1v/v). After 24h fixing solvent was decanted carefully and the root tips were washed with preserving solvent (70% ethanol) and keep immersed in the same solvent. An onion was also allowed to germinate in a control solution (3 ml of absolute ethanol diluted with distilled water to 250 ml) without the synthetic analogue in exactly the same way as was done in preparing solution of synthetic analogues.

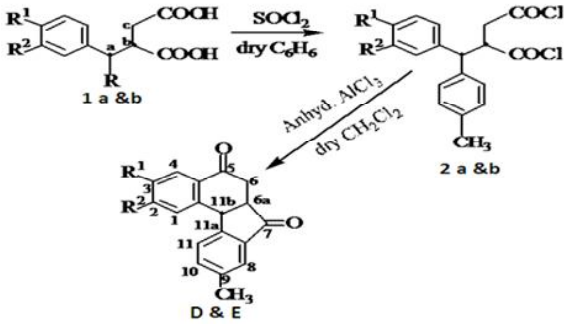
Root tips were placed on a clean watch glass containing stain solution (orcein solution-HCl solution 7:1 v/v) and heated on the flame until fumes come out. It was then cooled to room temperature. Root tips were then placed on the micro slide, a drop of stain solution was added and the root tips were squashed by a razor blade and slides were prepared.

Result and discussion

1. synthetic analysis of 6,6a-dihydro-2, 3-dimethoxy-9- methyl-11b h benzo [c] fluoren-5, 7-dione :

6,6a-dihydro-2, 3-dimethoxy-9- methyl-11b h benzo [c] fluoren-5, 7-dione **D** and 6,6a dihydro-3-methoxy -2, 9 dimethy-11bh benzo [c] fluoren 5, 7-dione **E** were prepared by friedel craft's intramolcular acylation reaction[36]**.** The experiment was carried out to synthesize 2a by refluxing a mixture of 3, 4 – dimethoxy - 4'– methyl – benzhydryl succinic acid **1a** and thionyl chloride for 5h. As the reaction is completed, the excess of thionyl chloride was distilled off. Finally, a pale yellow residue was obtained as gummy product **2a.** A yield of 82.72% (1.824g) was recorded. The product formation was confirmed by IR spectrum by not having peak for -OH group.

IR (Nujol): 1710 cm^{-1} for -C=O, and no -OH peak



a: $R^1 = R^2 = OCH_3$
b: $R^1 = OCH_3$, $R^2 = CH_3$
Scheme of synthesis

The compound **D** was obtained by pouring a solution of **3, 4-dimethoxy-4'-methyl-benzhydryl succinoyl chloride 2a** in dry dichloromethane into solution of anhydrous aluminum chloride over a period. The resulting mixture was magnetically stirred at 0°C for 8h. The reaction mixture was treated with cold 5N HCl (50ml) which results in the separation organic and aqueous layer. The organic layer was washed with 10% NaOH solution and finally with water. On distillation, the solvent was removed forming light yellow residue. The crude product was purified by column chromatography over silica gel using chloroform as the elutant. The elutant were collected and evacuated at 50°C using rotary evaporator gives white solid. It was re-crystallized from ethanol and a yield of 83.6% was recorded. Melting point of the re-crystallized product is 166-168°C. The final product was analysed by the following analytical techniques

IR (KBr): 1689 cm^{-1} (Indenone carbonyl), 1666 cm^{-1} (tetralone carbonyl), 1600 (aromatic C=C)
PMR ($CDCl_3$): δ2.4 (s, 3H, CH_3), δ2.8-3.0 (d,

J=6Hz, 2H, C_6-H), δ3.2-3.9 (m, 1H C_{6a}-H) δ4.0-4.1 (s, 6H, OCH3), δ4.7 (d, J=6Hz, 1H, C_{11b}-H,), δ7.1-7.8 (m, 5H, Ar-H);
Mass (m/z, % abundance): 322 (M^+, 98);
Anal. Calcd. For $C_{20}H_{18}O_4$; C, 74.5; H, 5.62%;
Found: C, 74.48; H, 5.59%.

2. synthetic analysis of 6, 6a-dihydro-3-methoxy-2, 9-dimethyl-11bH benzo [c] fluoren - 5, 7 dione :

The compound **2b** was prepared from **4-methoxy-3-methyl-benzhydryl succinic acid 1b** and thionyl chloride. A yield of 82.2% was obtained.

The **2b** was cyclised by pouring the solution of 2b in dry dichloromethane over a period into anhydrous Aluminum chloride in dry dichloromethane. White crystalline solid was obtained having an yield of 92% yield. The crude compound was re-crystallized in ethanol and the melting point is recorded as 102-104^{0}C. The final product was analysed by the following analytical techniques
IR (KBr): 1702 cm^{-1} (Indenone C=O), 1674 cm^{-1} (tetralone C=O), 1590 cm^{-1} (aromatic C=C) PMR ($CDCl_3$): δ2.3-2.5 (bs, 6H, CH_3), δ2.75-2.95 (d, J=6Hz, 2H, C_6-H), δ3.1-3.8 (m, 1H, C_{6a}-H) δ4.0 (s, 3H, OCH_3), δ4.6 (d, J=6Hz, 1H, C_{11b}-H), δ7.0-7.7 (m, 5H, Ar-H);
Mass (m/z, % abundance): 306 (M^+, 96);
Anal. Calcd. For $C_{20}H_{18}O_3$; C, 78. 41; H, 5.92%;
Found: C, 78.34; H, 5.85%.

3. Anti-mitotic activity :
Assay:

The prepared slide was mounted for observation under a compound microscope. The total numbers of cells and the number of dividing cells were counted. The percent of the number of dividing cells compared to the control and the percent inhibition of mitosis by the test antimitotic agent at a given concentration against a control were calculated.

The inhibition study for each synthetic product was done for three different concentrations. The statistical data are presented in the table.

A graph of concentration, versus percent inhibition for each test compound was drawn. The concentration needed for 50% inhibition (ID_{50}) was shown from the bar graph and ID_{50} values for the synthetic derivatives for antimitotic activity are tabulated.

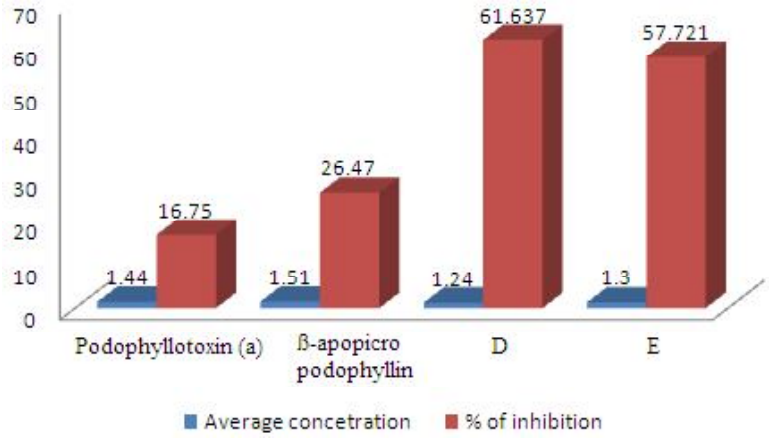


Figure 1: Percent inhibition versus concentration

Table 1. Biological assay and anti-mitotic activity of D and E

Compound	Conc. In mol/ l x 10^{-6}	Total No. of dividing cells	Total no. of cells	%. of dividing cells	Average	%. of dividing cells compare control	%. of inhibition compare to control	ID50M (($X10^{-6}$ mol/ l)
Control	-	19 18 20	104 93 88	18.269 19.354 22.727	20.116	100	0.0	-
Podophyllo-toxin (a)	1.44	18 46 52	202 230 245	8.991 20.000 21.224	16.712	83.25	16.75	4.29
ß-apopicro-podophyllin	1.51	23 34	209 183	11.005 18.579	14.792	73.53	26.47	2.87
D	1.24 2.48 3.72	18 25 48	281 343 514	6.405 7.288 9.338	7.677	38.163	61.637	2.005
E	1.3 2.61 3.92	35 31 47	512 341 490	6.835 9.090 9.591	8.505	42.279	57.721	2.260

The antimitotic activity data was recorded as shown in the Table 1. From the table it is clear that the anti-mitotic activities of the newly synthetic analogues D and E showed an increased trend in compare to Control, Podophyllotoxin (a) and ß-apopicropodophyllin. The maximum activity for D and E of ID_{50} were 2.005 x 10^{-6}M and 2.260 x 10^{-6} M relatively to that of Podophyllotoxin (a) and ß-apopicropodophyllin having ID_{50} with 4.290 x 10^{-6}M and 2.870 x 10^{-6}M. It is conspicuously believed that the nucleophilic functional entities in the cell constituents might easily attack the electrophilic lactone ring moiety. The anti-mitotic activities of the newly synthetic compounds mainly based on the size of the substituents attached to the aromatic rings.

Conclusion

Podophyllotoxin, its derivatives and analogues finds a lot of scopes in pharmaceutical field as it show wide biological activities. Several researchres worked on the analogues of podophyllotoxin and its derivatives for variety of biological activities such as cathartic, cytotoxic, antimitotic, anticancer, antitropical skin disease, antimalarial, virucidal, fungicidal[5-18]. In the present work the analogues of podophyllotoxin 6, 6a-dihydro-2, 3-dimethoxy-9-methyl-11bH benzo [c] fluoren - 5, 7 dione and 6, 6a-dihydro-3-methoxy-2, 9-dimethyl-11bH benzo [c] fluoren - 5, 7 dione. The products were re-crystallized in ethanol and characterized by spectrometric methods, IR, NMR and Mass spectroscopy. A yield of 82.72 and 83.6 percent were recorded respectively. The anti-mitotic activities of the synthesized analogues were examined by the onion root tip method with reference to β- apopicropodophyllin and Podophyllotoxin. The synthetic analogues D and E have

shown increased anti-mitotic activities with ID_{50} of 2.005 x 10^{-6} M and 2.260 x 10^{-6}M respectively with reference to standards of ID_{50} with 4.290 x 10^{-6}M and 2.870 x 10^{-6}M. It is strongly believed that the nucleophilic functional groups in the cell constituents might easily attack the electrophilic lactone ring moiety. The anti-mitotic activities of the newly synthetic compounds mainly based on the size of the substituents attached to the aromatic rings.

Future Scope of the work :

The derivatives and analogues of Podophyllotoxin show wide biological activities. Hence the exploration of new analogues gives conspicuous importance in medicinal field and lead innovative researches.

The authors are grateful to CDRI Lucknow and IIT Chennai for providing NMR, Mass and CH analysis data and sincerely acknowledge the support of the principal Maharani's Science College for Women, Mysuru.

Reference :

1. V. Podwyssotzki, Pharmakologische studien uber *Podophvllum peltatum. Arch. Exp. Pathol. Pharmakol*., *13,* 29 (1880).
2. J.L. Hartwell and A. W. Schrecker; *Fortschr. Che. Org. Naturist*, *13,* 83 (1958).
3. E. David Jackson and Paul Dewick; Tumour-inhibitory aryltetralin lignans from Podophyllum pleianthum. *phytochemistry*, *24,* 2407 (1985).
4. I. Jardine, Anticancer agents based on natural products models; J. M Cassary and J. D. Dourous (ed). Academic press, New York, N.Y. 319 (1980).
5. E. E. Smissman *et al*., *J. Med. Chem*., *19,* 148 (1976).
6. W.F. Jungi and H.J. Senn, *cancer chemother. Rep., 59,* 737 (1975).
7. G. Rivera *et al*., *Cancer Chemother Rep*., *59,* 743 (1975).
8. Wei Zhaoand *et al*. Challenges and potential for improving the druggability of podophyllotoxin-derived drugs in cancer chemotherapy. *Nat. Prod. Rep.*, *38,* 470-488 (2021).
9. C. David Jean *et al.*, *Cancer Rev*., *45,* 2177 (1985).
10. C. Brigati and B. Sander, *J. immunopharmacol*, *7,* 285 (1985).
11. E. A. Schreir, *152nd National Meeting of the American Chemical Society*, New York, N. Y., Sep. 1996, Abstract sect. P. No. 34, Dr. Schreir very kindly made the full text of his lecturer available to us.
12. W. J. Gensler *et al*., *J Am. Chem. Soc*., *82,* 1714 (1960).
13. A. W. Schrecker and J. L. Hartwell, *J. Am. Chem. Soc*., *74,* 5676 (1952).
14. B. F. Issell, *Cancer Chemother, Pharmacol*, *7,* 73 (1982).
15. F. Cavalli *Ibid*, *7,* 81 (1982).
16. F.R. Macbeth *ibid, 7,* 87 (1982).
17. C. C. Huang et al, *Cancer Res*., *33,* 3121 (1973).
18. Gupta and B. Singh, *J. Natl. Cancer Ins*, *73(1),* 241 (1984).
19. M. Rozeneweig, D.D. VonHoff and J.E. Hennoy, *Cancer*, *40,* 334 (1977).
20. M.G. Kelly and J.L. Hartwell, *J. Natl. Cancer. Inst*., *14,* 459 (1961).
21. Zinnia Shah and *et al*. Recent advances of podophyllotoxin/epipodophyllotoxin hybrids in anticancer activity, mode of action, and structure-activity relationship, *Biomolecules, 11(4),* 603 (2021).
22. Decembrino, D., Raffaele, A., Knöfel, R. *et al.* Synthesis of (−)–deoxypodophyllotoxin and (−)–epipodophyllotoxin via a multi-enzyme cascade in *E. coli*. *Microb Cell Fact, 20,* 183 (2021).
23. Zhang W, Liu C, Li J, Liu R, Zhuang J, Feng F, Yao Y and Sun C.Target Analysis and

Mechanism of Podophyllotoxin in the Treatment of Triple-Negative Breast Cancer. *Front. Pharmacol.* 2020, 11, 1211. doi: 10.3389/fphar.2020.01211

24. W.J. Gensler, C. A. Murthy and M.H. Trammer, *J. Med. Chem.*, *20,* 635 (1977).
25. C.A. Murthy and K.M. Lokanath Rai, *Indian J. Chem.* 1985, 248, 502., 1987, 26B, 131., *Idem. Current Science*, 1986, 55, 702.
26. C. A. Murthy and S. Shashikanth, *Indian J. Chem*., 1985, 24B, 670., 1989, 25B, 718.
27. C.A. Murthy and P.M. Radhakrishna, *Indian J. of Hetero cyclic Chem*., 1992, I.203; *Idem, current science*, 1988, 57, 797.
28. J. Steinhardt and J.A. Reynolds, *Mutiple equalibria in proteins*, Academic press, 84 (1969).
29. R.E, Jordan, N. Hewitt, W.Lewis, H. Kagen and C.F. Ranzblan, *Biochem*., *13,* 3497 (1974).
30. L. Wilson and I. Mirza, *J. Cell Biol*., *58,* 709 (1973).
31. L. Wilson, J. R. Bamburg, C.O. Mizes, L.M. Grishan and K. M. Creevell, Fed, Proc., *33,* 158 (1973).
32. J. B. Olmsted and G. G. Borisy, *Anal. Rev. Biochem, 42,* 507 (1973).
33. Panel Jiaqi Xiao, Meixiang Gao, Zhou Sun, Qiang Diao, Peng Wang, Feng Gao. Recent advances of podophyllotoxin/epipodophyllotoxin hybrids in anticancer activity, mode of action, and structure-activity relationship. *European Journal of Medicinal Chemistry. 208,* 112830 (2020).
34. B. Rosen and K. Leander, Appl., 86, 04062 (Patent) C. A.1987, 106, 061211.
35. Geng Huan Wang, He Ping Shen, Xuan Huang, Xiao Hong Jiang, Cheng Sheng Jin, Zheng Min Chu. Effect of Podophyllotoxin Conjugated Stearic Acid Grafted Chitosan Oligosaccharide Micelle on Human Glioma Cells. *Journal of Korean Neurosurgical Society, 63(6),* 698-706 (2020).
36. B. Sadashivamurthy, Y.B. Basavaraju, A.D. Satisha, K.H. Hemakumar. New tetralone acid intermiediate for the synthesis of ß-apopicropodophyllin analogues. *Bulgarian Chemical communication*, *39(4),* 264-268 (2007).