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NOVEL D-RING ANALOGUES OF PODOPHYLLOTOXIN AS POTENT ANTI-CANCER AGENTS[#]

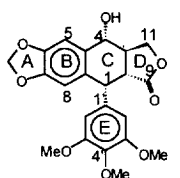
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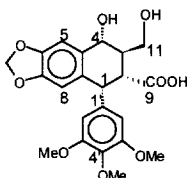
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Abstract : Several D-ring modified analogues of podophyllotoxin were prepared viz semi-synthesis starting from naturally occurring podophyllotoxin and determined their *in vitro* anti-cancer activity. Most of the analogues have shown good activity towards human cancer cell lines. © 1998 Elsevier Science Ltd. All rights reserved.

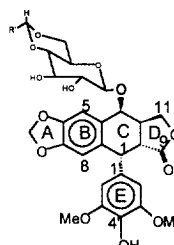
Among the various lignans isolated so far from the plant sources, podophyllotoxin¹, has emerged as a lead plant toxin that inhibits the assembly of microtubules¹. As the compound **1** was found to be highly cytotoxic for its clinical use against human cancers², extensive structural modifications of **1** have been undertaken which culminated into two semi-synthetic analogues of podophyllotoxin, namely, etoposide and teniposide. Although, these two compounds were widely used as anti-cancer drugs for small cell lung cancer, efforts for improving their clinical efficacy by overcoming the drug resistance, myelosuppression and poor bioavailability problems³ associated with them, were continued to be challenging. Consequently, the number of analogues of **1** increased considerably thereby the structural requirements for the better pharmacokinetic profile of podophyllotoxin **1** has become increasingly difficult⁴.



1 : Podophyllotoxin



2 : Podophyllilic acid



R' = Me, Etoposide

R' = , Teniposide

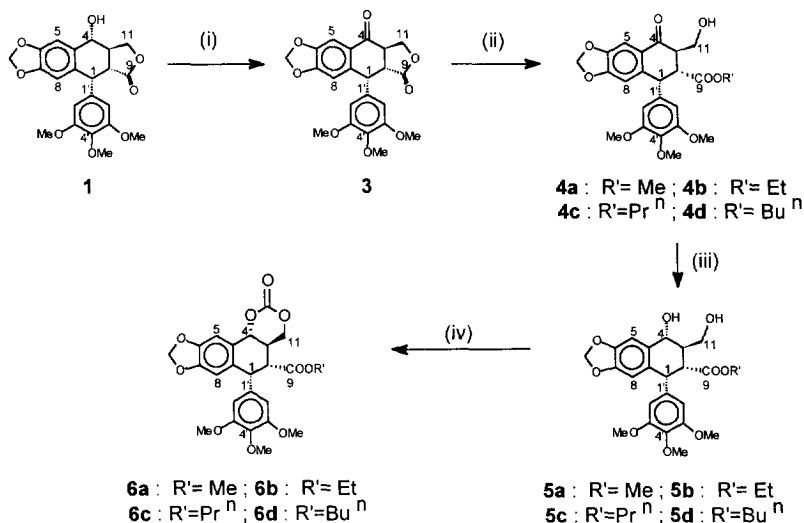
Most of these analogues prepared so far have the D-ring lactone intact. The number of analogues having D-ring lactone opened were limited presumably because the *trans* fused- γ -lactone⁵ was considered to be one of the essential features for these type of lignans to retain their anti-cancer activity. However, ethyl hydrazide derivative of podophyllilic acid **2** (SP-1)⁶ was found to possess potent anti-mytotic activity and its clinical efficacy was examined for some time and discontinued later due to severe side effects⁷. The importance of these compounds has once again gained momentum because of the recent discovery showing

[#]DRF's Publication No. : 31____Dedicated to my mentor Professor Goverdhan Mehta.

that the D-ring modified podophyllotoxins⁸ were found to possess immunosuppressive activities. Therefore, we were also interested in investigating certain D-ring analogues of podophyllotoxins prepared semi-synthetically starting from naturally occurring podophyllotoxin **1**. Some of these derivatives were found to be extremely potent against human colon and breast cancer cell lines. The synthesis and their in vitro anti-cancer activity of these analogues were reported herein.

Podophyllotoxin **1** was oxidised to podophyllotoxone **3** using pyridinium dichromate (PDC). Lactone ring opening of compound **3** was carried out in different alcohols under strong acid conditions to

Scheme 1

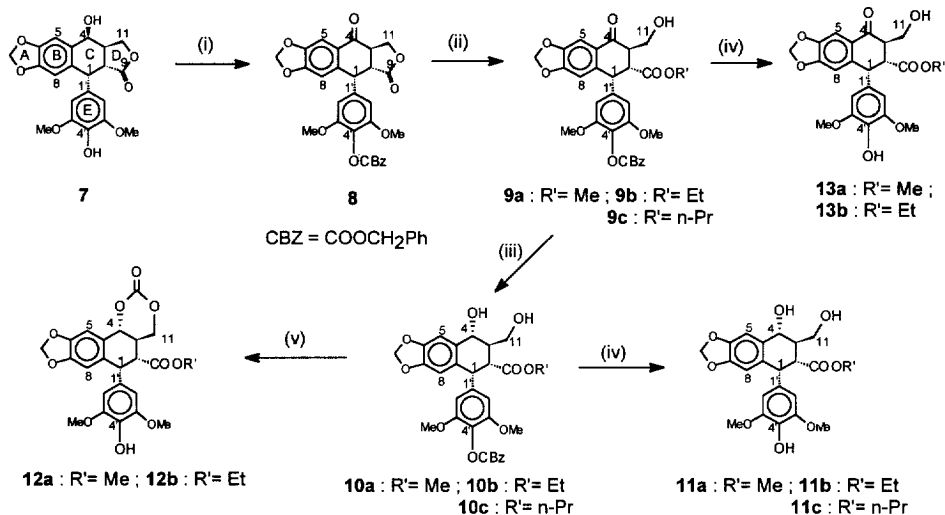


Reagents : (i) PDC, DCM, rt, 2h, 80% ; (ii) R'-OH where R' = Me, Et, n-Pr or n-Bu, H₂SO₄, 60°C, 45min., 60–65% ; (iii) NaBH₄, MeOH, rt, 0.5h, 85% ; (iv) 1,1-carbonyldiimidazole, C₆H₆, 80°C, 70% ;

furnish the corresponding esters **4a-4d** in 60–65% yield, Scheme 1. Stereoselective reduction of ketone in **4a-4d** was achieved with sodium borohydride to obtain exclusive formation of C-4 α hydroxy ester **5a-5d**⁹ in 85% yield. Reaction of the esters **5a-5d** with carbonyldiimidazole gave 4,11-carbonates **6a-6d**. Similarly, 4'-hydroxyderivative of the carbonates **12a,12b** were also prepared from 4'-demethyl epipodophyllotoxin **7** as shown in Scheme 2.

Protection of phenoxy group in **7** followed by the oxidation of C-4 hydroxyl group gave the ketone **8**. Opening of lactone moiety in **8** using alcohol and sulfuric acid gave the corresponding esters **9a-9c** in 65% yield. Debenzylation of these esters produced their 4'-hydroxy derivatives, Scheme 2. Stereoselective reduction of ketone in **9a-9c** using sodium borohydride provided C-4 α hydroxyesters **10a-10c** respectively. Hydrogenation of **10a-10c** with 10% Pd/C afforded the corresponding C-4' hydroxyl derivatives **11a-11c**.

Scheme 2



Reagents : (i) Py, DCM, ClCOOCH₂Ph, rt ; PDC, DCM, rt, 2h, 80% ; (ii) R'-OH where R' = Me, Et, n-Pr, H₂SO₄, 60°C, 45min. 65% ; (iii) NaBH₄, MeOH, rt, 0.5h, 85°C ; (iv) H₂ / 10%Pd-C, EtOAc, 70% ; (v) 1,1-carbonyldiimidazole, C₆H₆, 80°C, 75% ; H₂ / 10%Pd-C, EtOAc, 60%.

Table 1 : *In vitro* cytotoxicity (GI 50) data of podophyllotoxin analogues against colon cancer cell lines :

COMPOUND	COLON CANCER CELL LINES						
	COLO 205	HCC 2998	HCT 116	HCT 15	HT 29	KM 12	SW 620
4a	7.71	10.8	6.26	5.71	3.52	3.62	3.71
4b	2.22	5.63	4.35	3.99	3.36	4.21	2.07
5a	0.01	0.03	0.04	0.04	0.02	0.02	0.02
5b	14	14	24	13	17	8.4	26
5c	1.2	1.7	0.42	1.4	0.89	0.56	0.7
5d	1.9	1.8	3.7	2.0	2.5	1.86	2.2
11a	0.1	0.8	0.2	1.02	0.09	0.11	0.34
11b	1.16	1.3	1.7	2.07	0.88	0.78	1.17
Etoposide	18.1	3.46	6.16	---	15.13	5.62	---
1	---	0.02	0.01	0.02	0.01	0.01	---

All the above values were given in μ M concentrations. The term GI 50 stands for the concentration of the drug that produced 50% growth inhibition (GI50) of the cells in the cell line under study.

Finally, reaction of the esters **10a,10b** with 1,1-carbonyldiimidazole followed by hydrogenation provided the 4'-hydroxycarbonates **12a,12b**¹⁰.

Table 2 : *In vitro* cytotoxicity (GI 50) data of podophyllotoxin analogues against breast cancer cell lines :

COMPOUND	BREAST CANCER CELL LINES						
	MCF-7	MCF7-ADR	MDA-MB 231	HS 578T	MDA-MB 435	MDA-N	BT-549
4a	4.11	17.5	16.5	1.89	1.64	2.24	29
4b	3.86	5.14	5.99	1.56	1.33	1.70	5.45
5a	0.03	0.04	0.03	0.01	0.01	0.01	0.06
5b	23	14	18	27	4.4	3.5	>30
5c	0.8	3.2	>30	2.13	0.4	1.45	>30
5d	2.0	5.6	>30	----	1.6	11.5	>30
11a	0.3	0.1	0.1	3.12	0.1	0.1	0.5
11b	2.17	----	6.78	1.2	0.84	1.06	4.91

All the above values were given in μM concentrations. The term GI 50 stands for the concentration of the drug that produced 50% growth inhibition (GI50) of the cells in the cell line under study.

***In Vitro* Cytotoxicity :** Most of the compounds were tested at National Cancer Institute(NCI), Bethesda, USA for *in vitro* anti-cancer activity against 60 human tumor cell line assay. Some of the compounds were tested at our in-house facility against 6 human cancer cell lines taking one cell line from each cancer subtype following NCI's *in vitro* assay protocol¹¹. Based on the data obtained from NCI, the ketones **4a**, **4b** were found to be active whereas their 4'-hydroxyderivatives **13a** and **13b** were completely inactive even at 100 μM conc.. In the case of 4,11-diols, all the compounds **5a** -**5d**, **11a** and **11b** showed impressive activity against most of the colon and breast cancer cell lines as shown in tables 1 and 2. Among these esters, compound **5a** showed superior activity than the corresponding 4'-hydroxyderivative **11a**. However, in the case of ethyl ester, compound **5b** is much less potent than the corresponding 4'-hydroxyderivative **11b**. Surprisingly, the D-ring lactone opened diol derivative **5a** is equipotent to podophyllotoxin **1** in most of the cell lines tested, tables 1 and 3. However the other diol derivatives **5c**, **5d**, **11a**, and **11b** are less potent than **1** but more potent than etoposide, Table 1. These results shows that the D-ring lactone of podophyllotoxin **1** is not essential for its activity. Moreover, these compounds showed good sensitivity towards colon and breast cancer cell lines as shown in Tables 1 and 2.

Table 3 presents the *in vitro* cytotoxicity data of D-ring lactone opened diol-esters and the corresponding cyclic carbonates along with podophyllotoxin **1**, podophyllilic acid **2** and etoposide. Etoposide and podophyllilic acid **2** did not show impressive *in vitro* activity except against ovarian and melanoma cell lines. Both the diol-esters and the 4,11-cyclic carbonates have showed good activity in most of the cell lines in comparison to podophyllilic acid **2**. When we compare the activity of open diols vs the corresponding carbonates, **6b** and **12b** showed improved activity than the diols **5b** and **11b** respectively, table 3. Overall the compound **5a** showed exceptionally better activity in all the cell lines. Since these compounds have C-4

substitution in α configuration just as in podophyllotoxin **1**, they might show tubulin binding properties. Evaluation of these characteristics and other pharmacokinetic studies of these derivatives are in progress.

Table 3 : *In vitro* cytotoxicity(GI 50) data of D-ring analogues of **1** :

Compound	CANCER CELL LINES					
	SK-OV3	MCF-7 ADR	DU-145	A 498	H 522	M-14
5a	0.03	0.04	0.04	0.03	0.01	0.04
11a	0.34	0.1	0.47	0.40	0.10	0.86
5b	29.9	14.3	>30	>30	---	14.9
11b	3.35	---	1.63	1.10	0.1	2.1
6a	2.0	20	>30	10	>30	70
6b	1.5	8.0	0.09	0.2	2.0	6.0
6c	0.9	2.0	0.5	0.3	>30	2.0
12a	0.2	1.0	0.5	0.4	>30	0.4
12b	30	1.5	0.6	0.4	0.2	0.2
1	0.9	0.6	0.04	0.08	0.7	0.07
2	< 0.01	55	40	>100	50	< 0.01
Etoposide	< 0.01	25	2.0	8.0	0.9	< 0.01

All the above values were given in μ M concentrations. The term GI 50 stands for the concentration of the drug that produced 50% growth inhibition (GI50) of the cells in the cell line under study. Representative cancer cell lines are Ovarian (SK-OV-3), ADR resistant Breast cancer(MCF7-ADR), Prostate(DU-145), Renal(A 498), Lung (H 522) and Melnoma (M-14).

In summary, a number of D-ring analogues of podophyllotoxin **1** are prepared from naturally occurring podophyllotoxin **1** and their *in vitro* anti-cancer activity was determined. The fact that most of these compounds have shown good activity towards human cancer cell lines emphasise that the open D-ring lactone derivatives of podophyllotoxin too have potential for pursuing further studies towards the development of better drug candidate. *In vivo* efficacy study of some of these compounds is in progress.

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- 9) A patent application no. Fr. 1,395,088, April 9, **1965**, by Sandoz Ltd., describing the preparation of podophyllinic acid esters was appeared in Chemical Abstracts: *CA*, **1965**, *63*, 5653.; According to this reference, podophyllinic acid methyl ester was prepared from **1** by methanolysis using ZnCl_2 .
- 10) All the new compounds have satisfactory analytical data. Spectral data of selected compounds are given here: **Compound 4b**: mp: 86-88°C; IR: 3515, 1738, 1669, 1590, 1479, 1248, 1126 cm^{-1} ; ^1H NMR (CDCl_3 , 200MHz): δ 7.52(s, 1H), 6.58(s, 1H), 6.13(s, 2H), 6.04(s, 1H), 6.02(s, 1H), 4.57(d, J=5Hz, 1H), 4.20(dd, J=11Hz, 2Hz, 1H), 4.07(q, J=8Hz, 2H), 3.78(s, 3H), 3.72(s, 6H), 3.57(dd, J=6Hz, 2H), 3.15-3.00(m, 1H), 1.20(t, J=7Hz, 3H); **Compound 6c**: mp: 210°C; IR: 1764, 1720, 1484, 1220, 1130, 1037 cm^{-1} ; ^1H NMR (CDCl_3 , 200MHz): δ 7.10(s, 1H), 6.41(s, 1H), 6.14(s, 2H), 5.96(s, 2H), 5.19(d, J=10Hz, 1H), 4.80(dd, J=10Hz, 6Hz, 1H), 4.49(d, J=6Hz, 1H), 4.17(t, J=12Hz, 1H), 3.86(t, J=7Hz, 2H), 3.80(s, 3H), 3.74(s, 6H), 3.05(dd, J=12Hz, 6Hz, 1H), 3.0-2.80(m, 1H), 1.51(q, J=7Hz, 2H), 0.84(t, J=7Hz, 3H); **Compound 11b**: mp: 220°C; IR: 3524, 1704, 1612, 1486, 1227, 1115, 1039, cm^{-1} ; ^1H NMR (CDCl_3 , 200MHz): δ 7.07(s, 1H), 6.38(s, 1H), 6.25(s, 2H), 5.91(s, 2H), 5.40(br s, D_2O exchangeable, 1H), 4.77(d, J=8Hz, 1H), 4.29(d, J=6Hz, 1H), 4.18-3.92(m, 3H), 3.79(s, 6H), 3.80-3.62(m, 2H), 2.97(dd, J=12Hz, 6Hz, 1H), 2.58-2.40(m, 1H), 1.16(t, J=7Hz, 3H); **Compound 12a**: mp: 180°C; IR: 3512, 1750(br), 1485, 1244, 1218, 1121, 1036, 779 cm^{-1} ; ^1H NMR (CDCl_3 , 200MHz): δ 7.10(s, 1H), 6.41(s, 1H), 6.15(s, 2H), 5.95(s, 2H), 5.50(s, D_2O exchangeable, 1H), 5.20(d, J=10Hz, 1H), 4.80(dd, J=10Hz, 6Hz, 1H), 4.48(d, J=6Hz, 1H), 4.15(t, J=12Hz, 1H), 3.80(s, 6H), 3.57(s, 3H), 3.07(dd, J=12Hz, 6Hz, 1H), 2.95-2.70(m, 1H).
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