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New Podophyllotoxin Derivatives as Potential Anticancer Agents: Synthesis and Cytotoxicity of 4β-O-Propenylpodophyllotoxin Ethers

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NEW PODOPHYLLOTOXIN DERIVATIVES AS POTENTIAL ANTICANCER AGENTS: SYNTHESIS AND CYTOTOXICITY OF 4β-O-PROPENYLPODOPHYLLOTOXIN ETHERS

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Abstract: 4β-O-Propenylethers of podophyllotoxin **3a-e** were prepared from 4-chloro-4-deoxypodophyllotoxin **2** and allylic alcohols. Cytotoxicity of these compounds against KB cells is reported.

Podophyllotoxin **1** is the main constituent of podophyllin which is an alcoholic extract of dried roots of *Podophyllum peltatum* or *P. emodi*. Podophyllotoxin **1** exhibits its cytotoxic effect by inhibiting microtubule assembly through binding to tubulin. Several years of research in podophyllum lignans led to the discovery of two important anticancer agents etoposide and tenoposide which are partially protected glycosides of 4'O-demethylepipodophyllotoxin[1]. These anticancer agents do not inhibit microtubule assembly, but they exhibit their antitumor

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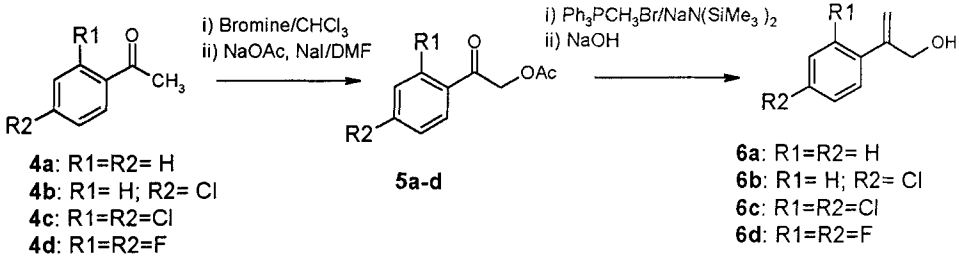
properties through interfering with catenation activity of topoisomerase-II by stabilizing the cleavable complex[2,3].

In an effort to find more selective anticancer agents with favorable water solubility and pharmacokinetic properties, we propose to synthesize new derivatives of podophyllotoxin with different moieties at C-4 position. In our proposed molecules the propenyl ethers **3** can serve as key intermediates. The double bond of **3** can be readily converted to an epoxide and can be opened by various nucleophiles. In this communication we would like to report the synthesis of 4β-O-propenyl ethers of podophyllotoxin.

Lithium salt of podophyllotoxin was generated at -60 °C from the reaction of podophyllotoxin and *n*-butyllithium in tetrahydrofuran. The reaction of lithium salt with either allyl bromide or epichlorohydrin did not yield any required ether. The product isolated from epichlorohydrin reaction was characterized as picropodophyllotoxin. It appears that under the reaction conditions podophyllotoxin **1** has isomerised to picropodophyllotoxin in which C/D ring junction has *cis* configuration. When podophyllotoxin **1** was reacted with allyl bromide in the presence of sodium hydride at 70 °C, 4α-O-allyl ether was obtained as a mixture (C/D *cis* and *trans*) of isomers. 4-Bromo-4-deoxypodophyllotoxin readily reacts with ethanol[5a] and benzyl alcohol[5b] to give respective ethers. Similar

reaction is not reported for allyl alcohols. It is proposed to prepare allyl ethers of podophyllotoxin **3** via chloropodophyllotoxin **2**. Dichloromethane solution of podophyllotoxin **1** on treatment with HCl gas at 0 °C gave 4-chloro-4-

Scheme-I

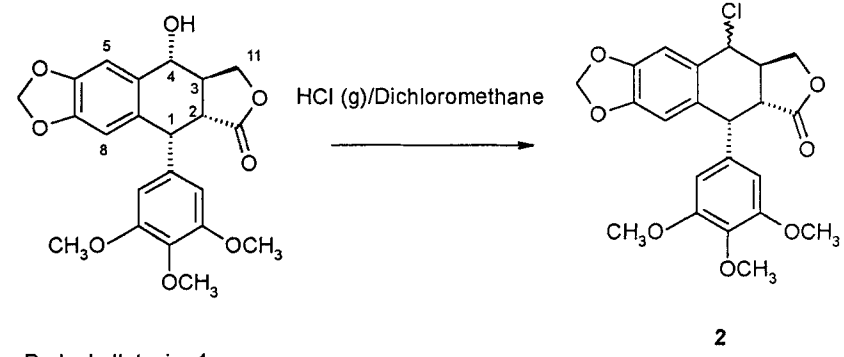


4a: R1=R2= H
4b: R1= H; R2= Cl
4c: R1=R2=Cl
4d: R1=R2=F

5a-d

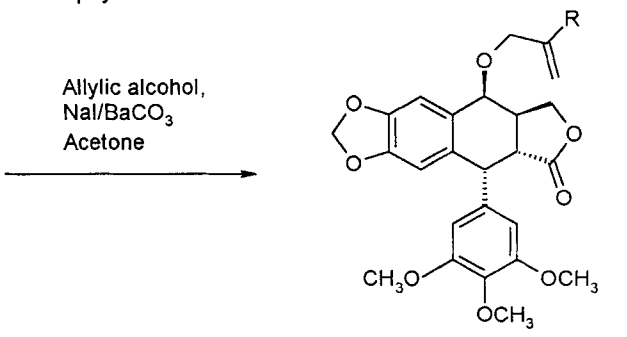
6a: R1=R2= H
6b: R1= H; R2= Cl
6c: R1=R2=Cl
6d: R1=R2=F

Scheme-II



Podophyllotoxin **1**

2



3a ; R = H
3b ; R = Ph
3c ; R = 4-Chlorophenyl
3d ; R = 2,4-Dichlorophenyl
3e ; R = 2,4-Diflurophenyl

deoxypodophyllotoxin **2** in quantitative yield as a mixture of 4α and 4β isomers.The reaction of chloropodophyllotoxin with allyl alcohol in the presence of barium carbonate and sodium iodide gave allyl ether **3a** in 70% yield **(Scheme-II).** The required allylic alcohols **6a-d** were prepared from respective acetophenones **4a-d** following the sequence of reactions described in **scheme I** [4]. Aceotophenone was converted to bromoacetophenone with bromine in dichloromethane. The resulted bromide was substituted with acetoxy group by reacting with sodium acetate in the presence of sodium iodide. Wittig reaction of **5** with methyltriphenylphosphonium bromide followed by basic hydrolysis yielded allyl alcohol **6**. The reaction of chloropodophyllotoxin **2** and allylic alcohols **6a-d** yielded allyl ethers **3b-e** in 45-55% yield. The products isolated has C-4β configuration. The substitution of chloride by alkoxy group takes place via a carbonium ion intermediate. Presence of bulky C-1α phenyl group dictates the substitution to be stereoselective to give C-4β isomer[5].

KB Cytotoxicity of 4β-O-propenyl ethers of podophyllotoxin:

Compound	*Cytotoxicity ID_{50} (μg/ml)	Compound	*Cytotoxicity ID_{50} (μg/ml)
3a	0.41	**3e**	5.7
3b	0.66	**Etoposide**	0.83
3c	4.0	**Adriamycin**	0.031
3d	0.56		

* ID_{50} was drug concentration to afford 50% reduction in cell number after a 3 day incubation period.

In conclusion allyl ethers of podophyllotoxin **3a-e** are prepared in moderate to good yields and characterized from their spectral data. These compounds can serve as key intermediates for the proposed modifications in podophyllotoxin analogs. It is important to note that the stereochemistry of C/D ring junction in **3a-e** is intact. Cytotoxicity[6] of these ethers against KB cells has been determined and is comparable to etoposide. To find out the mechanism responsible for the toxicity needs further investigation.

EXPERIMENTAL

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. The ^{1}H NMR are recorded on Brucker AC-200E spectrometer and chemical shifts are reported as δ (ppm) down field from tetramethylsilane. Mass spectra were determined on Associate Electrical Industries (AEI) MS-9 spectrometer at the Department of Chemistry, University of Alberta. Kieselgel 60 (230-400 mesh) of E Merk was used for column chromatography.

4-Chloro-4-deoxypodophyllotoxin(2):

A solution of podophyllotoxin (1.0 g, 2.41 mmol) in dichloromethane (50 ml) was saturated with dry HCl gas at 0 °C and stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the resulting product was co-evaporated with hexane to yield **2** (950 mg, 91%) as a colorless solid.

4β-O-(2-Propenyl)podophyllotoxin (3a):

To a mixture of 4-chloro-4-deoxypodophyllotoxin **2** (216 mg, 0.5 mmol) and allyl alcohol (58 mg, 1.0 mmol) in acetone (10 ml), barium carbonate (197 mg, 1.0 mmol) and sodium iodide (150 mg, 1.0 mmol) were added. The resulting reaction mixture was heated under reflux for 3-4 h. The solvent was removed under vacuum, the residue was extracted with ethyl acetate (3X30 ml), combined organic extract was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under vacuum, the resulting product was purified on a column of silica gel (Ethyl acetate: Hexane) to yield the title compound as colorless solid (160 mg, 70%).
mp 88-90 °C,

^{1}H NMR ($CDCl_3$)δ 2.85 (m, 1H, CH), 3.74 (s, 6H,2XOCH_3), 3.8 (s, 3H, OCH_3), 4.1 (m, 2H, $COOCH_2$), 4.36 (m, 2H, OCH_2), 4.52 (d, 1H, CH), 4.62 (d, 1H, CH), 5.2-5.4 (m, 2H, vinylic), 5.85 (m, 1H, vinylic), 5.97 (m, 2H, OCH_2O), 6.26 (s, 2H, Ar-H), 6.55 (s, 1H, Ar-H), 6.80 (s, 1H, Ar-H); MS (LR POSFAB) 455.1 $(M+H)^+$; Anal. Calcd. for $C_{25}H_{26}O_8$: C, 66.07; H, 5.77. Found: C, 65.89 ; H, 5.71.

4β-O-[2-(Phenyl)-2-propenyl]podophyllotoxin (3b): Colorless solid, 52% yield, mp 125-127 °C; ^{1}H NMR ($CDCl_3$)δ: 2.8 (m, 1H, CH), 3.44 (dd, 1H, CH), 3.72 (s, 6H, 2XOCH_3), 3.8 (s, 3H, OCH_3), 4.0 (m, 2H, $COOCH_2$), 4.4-4.7 (2d and ABq merged, 4H, 2XCH and OCH_2), 5.44 (s, 1H, vinylic), 5.56 (s, 1H, vinylic), 6.0 (m, 2H, OCH_2O), 6.2 (s, 2H, Ar-H), 6.54 (s, 1H, Ar-H), 6.8 (s, 1H, Ar-H), 7.4 (m, 5H, Ar-H). MS (LR POSFAB) 531.1 $(M+H)^+$; Anal. Calcd. for $C_{31}H_{30}O_8$: C, 70.18; H, 5.7. Found: C, 69.85; H, 5.59.

4β-O-[2-(4-Chlorophenyl)-2-propenyl]podophyllotoxin (3c): Colorless solid, 45% yield, mp. 101-103 °C; ^{1}H NMR ($CDCl_3$)δ: 2.8 9 (m, 1H, CH), 3.34 (dd, 1H, CH), 3.7 (s, 6H, 2XOCH_3), 3.78 (s, 3H, OCH_3), 4.35-4.7 (2d and ABq merged, 4H, 2XCH, OCH_2), 5.38 (s, 1H, vinylic), 5.56 (s, 1H, vinylic), 6.0 (m, 2H, OCH_2O), 6.23 (s, 2H, Ar-H), 6.55 (s, 1H, Ar-H), 6.77 9s, 1H, Ar-H), 7.32 (m, 4H, Ar-H); MS (LR POSFAB) 565.2: Anal. Calcd. for $C_{31}H_{29}ClO_8$: C, 65.90; H, 5.17. Found: C, 65.75; H, 4.95.

4β-O-[2-(2,4-Dichlorophenyl)-2-propenyl]podophyllotoxin (3d): Off-white solid, 55% yield, mp. 102-104 °C; ^{1}H NMR ($CDCl_3$)δ: 2.85 (m, 1H, CH), 3.38 (dd, 1H, CH), 3.73 (s, 6H, 2XOCH_3), 3.79 (s, 3H, OCH_3), 4.0-4.25 (m, 2H, $COOCH_2$), 4.4 (ABq, 2H, OCH_2), 4.48 (d, 1H, CH), 4.58 (d, 1H, CH), 5.24 (s, 1H, vinylic), 5.53 (s, 1H, vinylic), 5.98 (m, 2H, OCH_2O), 6.2 (s, 2H, Ar-H), 6.55 (s, 1H, Ar-H), 6.74 (s, 1H, Ar-H), 7.15 (d, 1H, Ar-H), 7.22 (dd, 1H, Ar-H), 7.4 (d, 1H, Ar-H); MS (LR POSFAB) 600.2 $(M+H)^+$; Anal. Calcd. for $C_{31}H_{28}Cl_2O_8$: C, 62.11; H, 4.71. Found: C, 62.11; H, 4.65.

4β-O-[2-(2,4-Difluorophenyl)-2-propenyl]podophyllotoxin (3e): Amorphous solid, 48% yield, mp. 149-151 °C; ^{1}H NMR ($CDCl_3$)δ:2.85 (m, 1H, CH), 3.4 (dd, 1H, CH), 3.7 (s, 6H, 2XOCH_3), 3.8 (s, 3H, OCH_3), 3.9-4.2 (m, 2H, $COOCH_2$), 4.3-4.55 (d, ABq merged, 3H, CH and OCH_2), 4.6 (d, 1H, CH), 5.4 (s, 1H, vinylic), 5.5 (s, 1H, vinylic), 5.95 (m, 2H, OCH_2O), 6.2 (s, 2H, Ar-H), 6.55 (s, 1H, Ar-H), 6.75 (s, 1H, Ar-H), 6.90 (m, 2H, Ar-H), 7.3 (m, 1H, Ar-H). MS (LR POSFAB) 567.1 $(M+H)^+$; Anal. Calcd. for $C_{31}H_{28}F_2O_8$: C, 65.72; H, 4.98. Found C, 65.36; H, 4.64

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