shown in Figure 2 *b*. In Figure 2 *c* spectra of both the polystyrene film and the bubble are shown with expansion in the region 3150–2775 cm^{-1}. Applying the Lambert–Beer law of absorbance to both the thick film (38.1 μ) as well as the bubble and considering the peaks at 3025, 2920 and 1450 cm^{-1}, an average thickness of 315 nm is obtained for the bubble film.

The general features of the FTIR spectrum of the thin polystyrene bubble is same as that of a thick film. The total number of peaks occurring in the thick film and the thin bubble film are same. Also, the peaks for both the systems occur approximately at the same positions. This is obvious from Figure 2 *a*, *b*. This indicates that the general structural features of a thick polystyrene film is retained in a bubble with film thickness of about 300 nm. It may be noted here that the infrared absorption peaks due to a thin polystyrene bubble do not match exactly with those of the thick film. The positions and the intensities of peaks, relative to each other, are not necessarily the same. There may be small differences in the nature of bonding in a thin polystyrene bubble compared to that in a thick film giving rise to differences in the FTIR spectra. Also, there could be structural differences between a thick film and a thin bubble giving rise to different oscillator strengths and vibration frequencies.

The present study demonstrates that it is possible to make thin polystyrene films in the form of air bubbles. They are stabilized by keeping the air pressure inside the bubble higher than that outside. It would be important to dope these bubbles with different molecules and see if the properties of such dopands are different depending on the thickness of the films. Work in this direction is in progress in our laboratory.

Ordinary shampoo or detergent bubbles when encased in a container are fairly stable by themselves[4]. On the other hand, the polystyrene bubbles crunch spontaneously once the air flow is stopped. It may be possible that detergent bubbles are organized in a membrane-like structure and their stability originates from a combination of hydrophobic and hydrophilic interactions leading to a membrane-like organized structure. There is no such force acting in the polystyrene film and the network structure is not stable without any external force acting on it in the form of a support (in this case, the air flow). It is also worth mentioning here that the polystyrene bubble is stable even under high air flow whereas an ordinary soap bubble would not be stable under such conditions. Generation of bubbles with thinner films may reveal interesting features of thin polymer membranes in the air.

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Synthesis and anticancer activity of new derivatives of podophyllotoxin

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Six new 4-alklyamino compounds 6.1–6.4, 7.1–7.3, derived from podophyllotoxin have been synthesized and evaluated for their anticancer activity *in vitro*. All analogues of podophyllotoxin showed inhibitory activity against L1210 and K562 cells. Compounds 6.2, 6.3, 6.4, 7.1, 7.2 are as potent or more potent than VP-16 in their inhibition of L1210 cells. The inhibitory activities of all analogues against K562 cells are less potent than that of VP-16.

ETOPOSIDE (VP-16, 1), which is a semisynthetic glycoside derivative of podophyllotoxin (2), has been used in cancer chemotheraphy[1]. This compound causes extensive DNA strand breaks from inhibition of the nuclear enzyme DNA topoisomerase II (topo II), which functions in decatenating supercoiled DNA prior to transcription[2]. Changes in the 4β-O-glucosidic substituent of 1 are of interest for simple structures which may be as potent or more potent than 1 in inhibiting the human DNA topoisomerase II[3–5]. In our previous studies[6,7] we found that substitution of the glycoside moiety in 1 by a similar nitrogen-containing group at C-4β position led to some compounds which have anticancer activity comparable or superior to 1. Considering the useful biological activity in the human body and good water-solubility of amino acids, we introduced amino acid analogues into podophyllotoxin at the C-4 position and synthesized new derivatives of podophyllotoxin, compounds 6.1–6.4, 7.1–7.3. The synthesis route of target compounds is shown in scheme 2.

The 4β alkylamino acid benzyl ester demethylepipodophyllotoxin (6.1–6.4) were synthesized by direct nucleophilic substitution (SN_1) of appropriate L-α-amino

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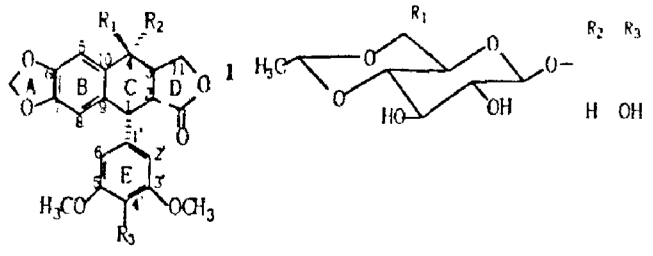
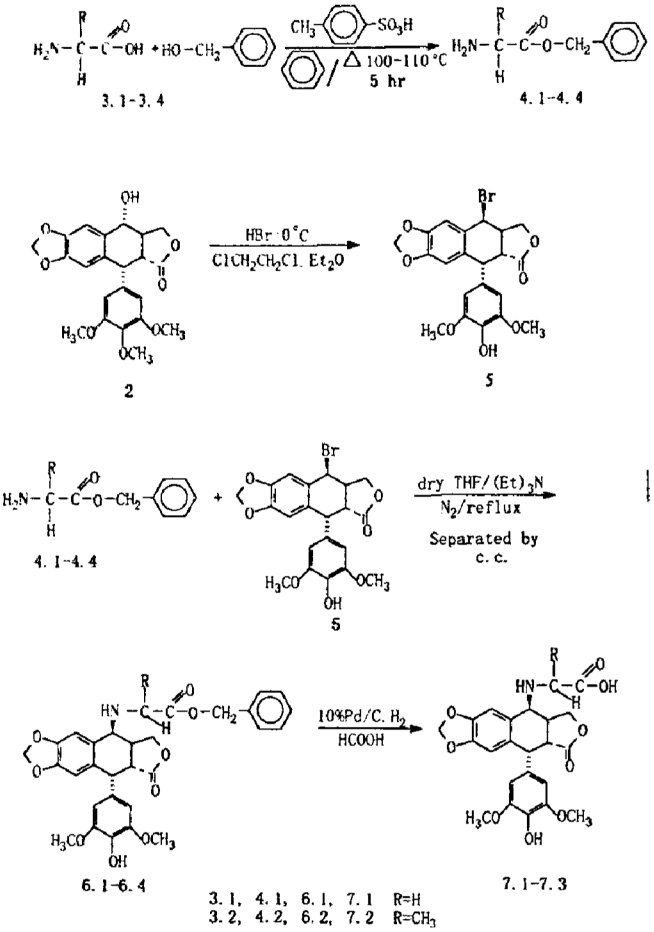


Scheme 1.



Scheme 2.

acid benzyl ester (4.1–4.1) with 4β-bromo-4-deoxy-4'-demethylepipodophyllotoxin (5) resulting from podophyllotoxin (2). The bulky C-1 α pendant aromatic ring E directed the substitution to be stereoselective in yielding the C-4β alkylamino isomers as the main products[4,5]. In some cases, C-4α isomers were also observed, but as minor products. The C-4β alkylamino isomers (6.1–6.4) were purified by column chromatography. The assignment of the configuration at C-4 position for compounds 6.1–6.4 and 7.1–7.3 was based on $J_{3,4}$ coupling constants. On the basis of Karplus dihedral angle rule in six-membered rings, the C-4β substituted compounds 6.1–6.4, 7.1–7.3 have $J_{3,4} \approx 4.0$ Hz as seen in 1 (refs 3, 4), due to a *cis* relationship between H-3 and H-4. The C-4α substituted compounds, however, like 2, have $J_{3,4} \geq 10.0$ Hz because H-3 is *trans* to H-4[3].

We have tested the inhibitory activities of compounds 6.1–6.4, 7.1–7.2 against L1210 and K562 cells *in vitro*. As illustrated in Table 1, compounds 6.2, 6.3, 6.4, 7.1, 7.2 are as potent or more potent than VP-16 in their inhibition of L1210 cell. The inhibitory activities of all analogues against K562 cells are less potent than that of VP-16. These results demonstrate the possibility of considerable simplification in the sugar structure of VP-16 and suggest further elaboration of the 4β-amino acid substituent to optimize the structure of this class of anticancer compounds. Further study for anticancer activity of synthesized compounds is in progress.

All melting points were taken on a Kofler melting point apparatus and uncorrected IR spectra were obtained on NIC-5DX spectrophotometer[1]. ^{1}HNMR spectra were obtained by using either an AM-400 or AC-80 NMR spectrometer, all chemical shifts were reported in ppm from TMS. The elemental analyses were determined on a German Elementar Vario EL instrument. Mass spectral analyses were taken on a VG ZAB-HS instrument. Electron-spin resonance were taken on a Bruker ER-200D-SRC instrument. Optical rotations were determined on a J-20C spectropolarimeter.

L-α-amino acid benzyl ester (4.1–4.4) was prepared according to literature procedures[8]. In a 100 ml 3-neck round-bottom flask 0.05 mol L-α-amino acid 4.1–4.4 and 20 ml benzyl alcohol, were dissolved in 50 ml of benzene, and 11.4 g TsOH·H_2O (0.06 mol) was added. A water-cooled reflux condenser and a Dean–Stark trap was attached to remove water and the solution was refluxed in an oil bath (100–110°C) for about 5 h until all the water was removed from reaction mixture. The reaction solution then was cooled ether and petroleum ether were added and the white solid was precipitated. This was collected by suction and pressed firmly on the filter. The crude products were recrystallized from anhydrous ethanol–ether and a white solid was obtained.

Compound 4.1: yield 83%, m. p. 128–130°C;
Compound 4.2: yield 80%, m. p. 110–112°C;
Compound 4.3: yield 93%, m. p. 152–153°C;
Compound 4.4: yield 80%, m. p. 170–172°C;

The physical constants and spectroscopic data of compounds 4.1–4.4 were in accordance with literature[8].

4β-bromo-4-deoxy-4'-demethy-lepipodophyllotoxin (5) was prepared according to literature procedures[9], 15 g was suspended in 141 ml of 1,2-dichloroethane and 15 ml ether and cooled to 0°C. A current of dry hydrobromide was passed until an increased weight of 38.5 g was obtained. After standing at 0–2°C for 20 h, the solvent was removed under vacuum. The residue was recrystallized from acetone, weight 8.3 g (5), yield 54.2% m. p. 184–195°C. The physical constants and

Table 1. Biological evaluation of podophyllotoxin analogues

Compounds	IC_{50}* (ug/ml)	
	L1210	K562
VP-16	0.083	0.00022
6.1	0.30	0.0043
6.2	0.019	0.0013
6.3	0.0086	0.0040
6.4	0.017	0.0024
7.1	0.023	0.096
7.2	0.091	0.0047

*IC_{50} was the concentration of drug that affords 50% reduction in cell number.

spectroscopic data of compound 5 were in accordance with literature[9].

To a solution of 4β-bromo-4-deoxy-4'-demethylepipodophyllotoxion (5 g, 10.8 mmol) in dry THF (100 ml) was added triethylamine (3.6 ml) and a solution of L-α-amino acid benzyl ester (13 mmol) in dry THF (12 ml) and trethylamine (3.6 ml). The mixture was refluxed under N_2. After TLC analysis showed that most of the starting material had been converted, the reaction was stopped. Then the mixture was filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–acetone 50:1 as eluent and recrystallized in methanol to produce a white solid.

To a solution of compounds 6.1–6.4 (0.2 mmol) in ethanol (10 ml) and chloroform (10 ml) was added 10% palladium on activated carbon (50 mg) and formic acid. Hydrogen gas was passed through the mixture stirred at r.t until hydrogen absorption ceased. Then the mixture was filtered, and the filtrate was evaporated under reduced pressure. Crystallization from methanol gave a white solid.

Compound 6.1: yield 28%; m. p. 155–157°C; $[\alpha]^{15}_D = -64.1°$ (C = 0.52, $CHCl_3$); MS (EI): 547 (M^+, 6%), 456 (6%), 382 (100%); ^1H NMR ($CDCl_3$, TMS, 400 Hz) δ ppm: 7.37–7.40 (m, 5H, C_6H_5), 7.25 (S, 1H, H_5), 6.46 (S, 1H, H_8), 6.26 (S, 2H, H_2, H_6), 5.97 (S, 2H, OCH_2O), 5.22 (S, 2H, –COO$\underline{CH_2}$-Ph),4.53 (d, 1H, H_1), 4.33 (t, 1H, H_{11}), 4.20 (q, 1H, H_{11}), 3.91 (d, J = 3.82 Hz, 1H, H_4), 3.76 (S, 6H, 3', 5'-OCH_3), 3.32 (m, 3H, H_2, –$\underline{CH_2}$COO–), 2.77 (m, 1H, H_3), 1.63 (1H, –NH–): IR (KBr) cm^{-1}: 3353 (NH, OH), 1763 (γ-lactone), 1744 (ester), 1609, 1519, 1479 (aromatic C = C); Anal. calculated for $C_{30}H_{29}NO_9 \cdot 0.5\ H_2O$ (%), C 64.75 H 5.40 N 2.52 found C 64.87 H 5.69 N 2.14.

Compound 6.2: yield 20%; m. p. 170.5–175°C: $[\alpha]^{15}_D = -52°$ (C = 0.50, $CHCl_3$); MS (FAB): 562 (M^+ + 1, 13%), 382 (51%), 154 (100%); ^1H NMR ($CDCl_3$, TMS, 80 Hz) δ ppm: 7.37 (S, 5H, C_6H_5), 6.71 (S, 1H, H_5), 6.48 (S, 1H, H_8), 6.26 (S, 2H, $H_{2'}$, $H_{6'}$), 5.96 (S, 2H OCH_2O), 5.40 (S, 1H, –OH), 5.18 (S, 2H, –COO$\underline{CH_2}$–Ph), 4.53 (d, H, H_1), 4.25-4.35 (m, 2H, H_{11}), 3.96 (d, J = 3.6 Hz, 1H, H_4), 3.77 (S, 6H, 3', 5'-OCH_3), 3.15-3.50 (m, 4H, –$\underline{CH_2}$COO–, H_2, H_3), 2.16 (d, 1H, –NH–); 1.41 (d, 3H, $-CH_3$) IR (KBr) cm^{-1}: 3357 (NH, OH), 1757 (γ-lactone), 1716 (ester), 1610, 1519, 1482 (aromatic C = C): Anal. calculated for $C_{31}H_{31}NO_9$ (%), C 66.31 H5.53 N 2.50 found C 66.70 H 5.82 N 2.27.

Compound 6.3: yield 25%; m. p. 139–141°C; $[\alpha]^{15}_D = -67.3°$ (C = 0.505, $CHCl_3$); MS (FAB): 603 (M^+, 14%), 382 (100%), 222 (98%), 185 (100%); ^1H NMR ($CDCl_3$, TMS, 80 Hz) δ ppm: 7.37 (S, 5H, C_6H_5), 6.67 (S, 1H, H_5), 6.48 (S, 1H, H_8), 6.24 (S, 2H, $H_{2'}$, $H_{6'}$), 5.95 (S, 2H, OCH_2O), 5.38 (S, 1H, –OH), 5.18 (S, 2H, –COO$\underline{CH_2}$-Ph), 4.53 (d, H, H_1), 4.21-4.32 (m, 2H, H_{11}), 3.92 (d, J = 3.5 Hz, 1H, H_4), 3.76 (S, 6H, 3', 5' –OCH_3), 3.26-3.43 (m, 4H, –CH_2COO–, H_2, H_3), 2.17 (d, 1H, NH–); 1.61 (m, 1H, >CH.), 0.81-0.91 (T, 8H, $-CH_2$–, 2 × $-CH_3$); IR (KBr) cm^{-1}: 3356 (NH, OH), 1758 (γ-lactone), 1735 (ester), 1609, 1520, 1481 (aromatic C = C); Anal. calculated for $C_{34}H_{37}NO_9$ (%), C 67.66 H 6.14 N 2.32 found C 67.88 H 6.37 N 2.03.

Compound 6.4: yield 23%; m. p. 137.5–139°C; $[\alpha]^{15}_D = -36.6°$ (C = 0.54, $CHCl_3$); MS (FAB): 637 (M^+, 8%), 383 (32%), 154 (88%), 91 (100%); ^1H NMR ($CDCl_3$, TMS, 80 Hz) δ ppm: 7.00-7.47 (m, 1H, C_6H_5), 6.42-6.45 (d, 2H, H_5, H_8), 6.20 (S, 2H, $H_{2'}$, $H_{6'}$), 5.92 (S, 2H, OCH_2O), 5.35 (S, 1H, –OH), 5.11 (S, 2H, –COO$\underline{CH_2}$-Ph), 4.40 (d, H, H_1), 4.14-4.22 (m, 2H, H_{11}), 3.95 (d, J = 3.3 Hz, 1H, H_4), 3.74 (S, 6H, 3', 5'-OCH_3), 2.57-3.16 (m, 5H, H_2, H_3, –$\underline{CH_2}$COO–$\underline{CH}$–), 2.16 (S, 1H, –NH–); IR (KBr) cm^{-1}: 3351, 3324 (NH, OH), 1763 (γ-lactone), 1738 (ester), 1609, 1510, 1482 (aromatic C = C); Anal. calculated for $C_{37}H_{35}NO_9$ (%), C 69.70 H 5.49 N 1.57 found C 69.87 H 5.77 N 1.36.

Compound 7.1: yield 83.8%; m. p. 170–174°C; $[\alpha]^{15}_D = -67.3°$ (C = 0.505, CH_3COCH_3/CH_3OH); MS (EI): 457 (M^+, 4%), 413 (68%), 382 (100%); ^1H NMR (DMDO-d_6 TMS, 80 Hz) δ ppm: 7.24 (S, 1H, H_5), 6.52 (S, 1H, H_8), 6.25 (S, 2H, $H_{2'}$, H_6), 6.00 (S, 2H, OCH_2O), 4.43 (d, 1H, H_1), 3.87-4.12 (m, 3H, H_{11}, H_4), 3.62 (S, 6H, 3', 5', $-OCH_3$), 3.02-3.21 (m, 2H, H_2, H_3), IR (KBr) cm^{-1}: 2600-3500 (acid-OH), 1776 (γ-lactone), 1612, 1510, 1485 (aromatic C = C): Anal. calculated for $C_{23}H_{23}NO_9 \cdot HCOOH$ (%), C 57.49 H 4.97 N 2.79 found C 57.55 H 4.52 N 2.40.

Compound 7.2: yield 80%; m. p. 202–204°C; $[\alpha]^{15}_D = -11.5°$ (C = 0.5, CH_3COCH_3/CH_3OH); MS (EI): 471 (M^+, 1%), 148 (30%); ^1H NMR ($CDCl_3$, TMS, 80 Hz) δ ppm: 6.72 (S, 1H, H_5), 6.50 (S, 1H, H_8), 6.27 (S, 2H, $H_{2'}$, $H_{6'}$), 5.97 (S, 2H, OCH_2O), 5.41 (S, 1H, –OH), 4.21-4.51 (m, 4H, H_1, H_{11}, H_4), 3.78 (S, 6H, 3', 5', $-OCH_3$), 3.22-3.68 (m, 2H, H_2, H_3), 2.19(S, 1H, –NH–), 1.27-1.83 (m, 4H, $\underline{H_3C}$–$\underline{CH}$–CO_2H); IR (KBr) cm^{-1}: 2600-3500 (acid-OH), 1776 (γ-lactone), 1612, 1510, 1485 (aromatic C = C): Anal. calculated for $C_{24}H_{25}NO_9 \cdot H_2O$ (%), C 58.89 H 5.52 N 2.86 found C 59.27 H 5.16 N 2.52.

Compound 7.3: yield 80%; m. p. 201–203°C; MS (EI): 513 (M^+, 5%), 381 (18%); 153 (100%); ^1H NMR

(DMSO-d_6 TMS, 80 Hz) δ ppm: 6.79 (S, 1H, H_5), 6.47 (S, 1H, H_8), 6.17 (S, 2H, $H_{2'}$, $H_{6'}$), 5.98 (S, 2H, OCH_2O), 4.15-4.45 (m, 3H, H_1, H_{11}), 3.90 (d, 1H, $J = 3.3$ Hz, H_4), 3.60 (S, 6H, 3', 5', $-OCH_3$), 1.58 (m, $-CH-$), 0.89-0.91 (T, 8H, $2 \times CH_3$, CH_2-). IR (KBr) cm^{-1}: 2500–3500 (acid-OH), 3152 (NH), 1779 (γ-lactone), 1619, 1513, 1487 (aromatic C = C). Anal. calculated for $C_{27}H_{31}NO_9 \cdot H_2O$ (%), C 61.01 H 6.2 N 2.64 found C 61.35 H 5.86 N 2.31.

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Knowledge, attitude, beliefs and practices (KABP) study related to malaria and intervention strategies in ethnic tribals of Mandla (Madhya Pradesh)

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In India, malaria control programmes among tribal belts failed to make any dent as the perceptions of the tribals regarding control and treatment of malaria and Government strategies are at variance. Therefore, a knowledge, attitude, belief and practices (KABP) study was undertaken among 'gond' ethnic tribals of Mandla district to assess their knowledge related to malaria transmission and its control.

Surveys revealed that the tribals call this 'fever with shivering and rigour' as *Attrala* and they did not appreciate the presentation of malaria control programmes in the first place. About 98% tribals believed malaria was transmitted by drinking or bathing in contaminated water. First line of treatment is through 'guniyas' the village traditional healers, failing which injections were given by unlicensed practitioners (quacks) in the market place. Primary Health Care system is their last resort. Tribals did have knowledge about mosquito breeding in stagnant water (43%) yet all efforts were made to store rain water around their houses and in agricultural fields. Further, they did not understand the relevance of DDT spray for control of mosquito/malaria. Therefore, there is an urgent need to build up information, education and communication (IEC) programmes for greater acceptance of the malaria control programme.

VAST tracts of forests with tribal settlement have persistent malaria problem. According to an estimate in 1987, 54 million tribals of various ethnic origins residing in forested areas and accounting for 7% of the total population contributed 30% of total malaria cases, 60% of total *Plasmodium falciparum* cases and 50% of malaria deaths in the country[1]. Traditionally, National Malaria Eradication Programmes (NMEP) have focussed their efforts on treatment of the malaria parasite and residual insecticide spraying for control of vectors. These measures failed to achieve desired results, as they did not take into account economy-based human activities and their socio-cultural practices[2,3]. Hence a knowledge, attitude, beliefs and practices (KABP) study was undertaken in ethnic tribals of Mandla in Madhya Pradesh from May to November 1994 with regard to the perception of tribals about malaria control and its treatment to learn how they might influence the success of control measures. An understanding of their beliefs and behaviour may be crucial for developing more effective alternate workable strategies. To the best of our knowledge this is the first preliminary study of its kind in the Indian subcontinent.

The malaria programme in India has an established network in the district, state and national levels. Two rounds of indoor residual spraying and active and passive case detections have been the main malaria control activities in practice in this area. At the district level, the responsibility for implementation of the programme lies with the Chief Medical Officer assisted by a District Malaria Officer. The Primary Health Centre (PHC) is the most peripheral rural facility. The medical officer, incharge of PHC has overall responsibility for surveillance and laboratory services and supervision of spray. The PHC is divided into sections, according to the population, with each section having a population of 3000 for tribal areas. Each section also has one multipurpose worker (MPW), the grass root level government functionary. Sections are further subdivided into 12 working day schedules. The MPW visits villages as per schedule and collects blood smears from all persons with fever and cases with history of fever. He also collects blood smears from fever treatment depots (FTDs) and drug distribution centres (DDCs) in his area during

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