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SHORT COMMUNICATION

A novel, stereoselective and practical protocol for the synthesis of 4β-aminopodophyllotoxins

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Ritter reaction of podophyllotoxins with chloroacetonitrile and subsequent cleavage of the chloroacetyl group in the resulting chloroacetamide with thiourea under both classical heating and ultrasonic conditions is an efficient procedure for the synthesis of 4β-aminopodophyllotoxins. In general, significant improvements in the rates of reaction and yields of the sonochemical reactions relative to the classical heating reactions were observed.

Keywords: 4β-aminopodophyllotoxins, anticancer drugs, Ritter reaction, ultrasound

Semisynthetic analogues of naturally occurring podophyllotoxin (*I*) have drawn renewed interest in recent years as a result of the development of etoposide (VP-16, *II*) and teniposide (VM-26, *III*) as anticancer drugs (Canetta et al., 1982; Bohlin & Rosen, 1996; Gordaliza et al., 2004). These semisynthetic glucoconjugates of epipodophyllotoxin block the catalytic activity of DNA topoisomerase II and concurrent enzyme-mediated production of lethal DNA strand breaks leading to DNA damage and cytotoxicity (Wilstermann et al., 2007; Berger et al., 1996; Hande, 1998; Burden & Osheroff, 1998). Their clinical success as well as intriguing mechanism of action has greatly stimulated the interest in further studies on the modification of the C-4 substituent of *I* for better antitumor activity. Some nonsugar substituted analogues, particularly nitrogen containing congeners were found to exhibit pharmacological properties superior to VP-16 and some of them were brought into clinical evaluations (Cho et al., 1996a, 1996b; Kamal et al., 2003, 2005). In view of the important biological activities as well as of the significant clinical role of *N*-substituted derivatives, 4β-aminopodophyllotoxins are of considerable interest as attractive synthetic targets since they serve as important precursors to construct a variety of biologically useful *N*-substituted derivatives of 4β-aminopodophyllotoxins with powerful antineoplastic and insecticidal properties (Roulland et al., 2002; Hansen et al., 1993; Li et al., 2006). Convenient methods available for the conversion of podophyllotoxins to 4β-aminopodophyllotoxins and the key step in the sequence involve the azidation of podophyllotoxins through a HN_3 (or NaN_3)/Lewis acids system and further reduction of 4β-azidopodophyllotoxins by catalytic hydrogenation, H_2/HCO_2NH_4, TMSCl/NaI, $FeSO_4/NH_3$, SmI_2, Zn/HCO_2NH_4, and the biocatalytic method (Liu et al., 2007; Yu et al., 1999; Kamal et al., 1997, 1998; Chen et al., 2000; Xu et al., 2008). Although some good results were obtained using these protocols, most of these methods often suffer from one or more limitations with respect to general applicability, stereoselectivity, ready availability, operational convenience, the main disadvantage of these methods being the use of hazardous and explosive azides (HN_3 or NaN_3) as reactive reagents, which makes them unsuitable not only for large-scale synthesis of this class

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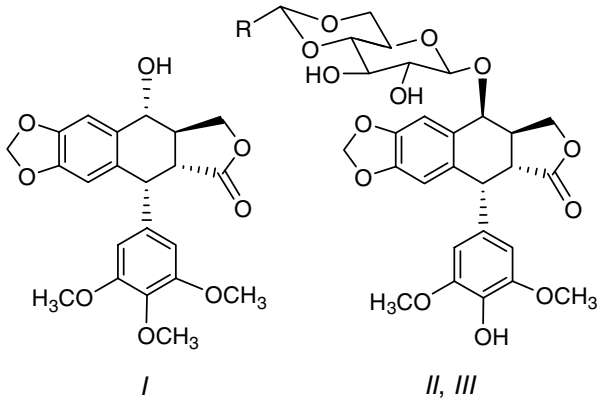


I *II, III*

Fig. 1. Structure of podophyllotoxin (*I*), etoposide (*II*, R = methyl), and teniposide (*III*, R = 2-thienyl).

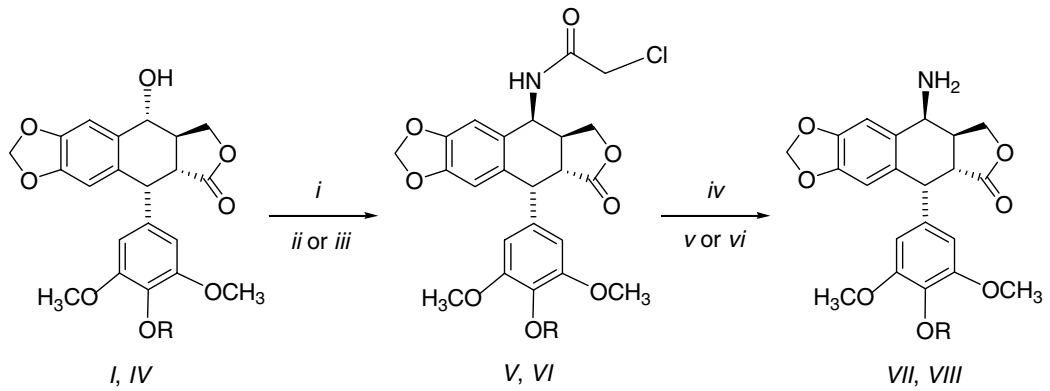
of biologically active molecules but for the synthesis of screening libraries for drug discovery or for industrial production.

Due to the above reasons and also as a part of our ongoing research program for the synthesis of podophyllotoxin congeners of biological significance, a facile and efficient synthetic route for the preparation of stereoselective 4β-aminopodophyllotoxins by a modified Ritter reaction and smooth cleavage of the chloroacetyl group with thiourea under standard conditions using a simple ultrasonic technique is described in this paper. It is worth to mention that ultrasound as a non-thermal energy transfer source is well known to enhance reaction rates, yields, and selectivity in organic synthesis and has found widespread application in synthetic organic chemistry. Significant enhancement in the reaction rates and yields of sonochemical reactions relative to the classical heating reactions was observed. Thus, the Ritter reaction with $ClCH_2CN$ was carried out with podophyllotoxin (*I*) or its derivative *IV* in the presence of 60 mass % of $MsOH/Al_2O_3$ to give 4β-chloroamidopodophyllotoxins (*V*, *VI*); subsequent cleavage of the chloroacetyl group with thiourea gave the corresponding 4β-aminopodophyllotoxins (*VII*, *VIII*) under both classical heating and ultrasonic conditions in moderate to good yields (Fig. 2). The desired compounds (*VII*, *VIII*) were characterized by m.p., IR, ^{1}H and ^{13}C NMR, MS, and HRMS analyses.

Melting points were taken on a Kofler melting point apparatus and are uncorrected. IR spectra were obtained on an NIC-5DX spectrophotometer and mass spectral analysis was performed on ZAB-HS and Bruker Daltonics APEXII49e instruments. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AM-400 spectrometer using TMS as reference. Sonication was performed in a Shanghai Branson-CQX ultrasonic cleaner with the frequency of 25 kHz and the nominal power of 500 W. The reaction flask was located in the maximum energy area in the cleaner, where the surface of reactants (reaction vessel) is slightly lower than the level of water; addition or removal of water was used to control the temperature of the water bath. All chemicals were commercially available (Sigma–Aldrich Co., USA) and were used without further purification. Podophyllotoxin (*I*) and 4′-demethylpodophyllotoxin (*IV*) were isolated from the Chinese medicinal herb *Podophyllum emodi* Wall var. Chinesis Sprague.

General procedure for the preparation of 4β-aminopodophyllotoxins (*VII*, *VIII*)

Method A (classical heating): Stirred mixture of podophyllotoxin (*I*, 4 mmol) and homogeneous mixture of $MsOH/Al_2O_3$ (60 mass %, 1 g) in $ClCH_2CN$ (10 mL) were heated at 60 °C for 5 h. After completion of the reaction as indicated by TLC, the solvent was evaporated under reduced pressure and the residue was poured onto ice water (20 mL) and extracted with ethyl acetate (3 × 50 mL). Combined organic extracts



I, IV *V, VI* *VII, VIII*

Fig. 2. Synthesis of 4β-aminopodophyllotoxins. Reaction conditions: *i*) $ClCH_2CN$, $MsOH/Al_2O_3$; *ii*) classical heating, 60 °C, 5 h; *iii*) sonication, 60 °C, 2 h; *iv*) thiourea, AcOH; *v*) classical heating, 80 °C, 12 h; *vi*) sonication, 80 °C, 4 h. R = CH_3 for *I*, *V*, and *VII*; R = H for *IV*, *VI*, and *VIII*.

were dried over anhydrous Na_2SO_4, the mixture was filtered and the filtrate was evaporated under reduced pressure to give the crude product which was purified by flash column chromatography using $CHCl_3$/MeOH ($\varphi_r = 9.5 : 0.5$) as an eluent affording the pure compound *V* in a 50 % yield. Subsequently, a solution of amide *V* (5 mmol) and thiourea (0.46 g, 6 mmol) in AcOH (50 mL) was heated at 80 ℃ for 12 h, neutralized with 20 mass % of aqueous $NaHCO_3$ and extracted with $CHCl_3$ (3 × 30 mL). Combined extracts were washed with brine (30 mL), dried ($MgSO_4$), filtered, and concentrated under diminished pressure. The residue was purified by column chromatography to give compound *VII* as a white solid in a 50 % yield. Analogously, amide *VI* (52 % yield) and subsequently amine *VIII* (55 % yield) were prepared starting from the podophyllotoxin derivative *IV*.

Method B (ultrasound irradiation): To a stirred mixture of podophyllotoxins (4 mmol) and $ClCH_2CN$ (10 mL) in a round-bottomed flask equipped with a condenser, homogeneous mixture of $MsOH/Al_2O_3$ (60 mass %, 1 g) was added and the mixture was irradiated by an ultrasonic generator in a water bath at 60 ℃ for 2 h. The progress of reaction was monitored by TLC followed by the same work-up as described in method A to give pure *V* in an 80 % yield. Subsequently, a solution of amide *V* (5 mmol) and thiourea (0.46 g, 6 mmol) in AcOH (50 mL) was sonicated at 80 ℃ for 4 h followed by the same work-up as described in method A to give compound *VII* as a white solid in an 82 % yield. Analogously, amide *VI* and subsequently amine *VIII* were prepared starting from the podophyllotoxin derivative *IV* in 82 % and 85 % yields, respectively.

For compound *VII*: m.p. 110–112 ℃; IR (KBr), ν_{max}/cm^{-1}: 3430 (NH_2), 1775 (lactone), 1590, 1550, and 1484 (C═$C_{aromatic}$), 935 (OCH_2O); ^{1}H NMR ($CDC1_3$, 400 MHz), δ: 6.84 (s, 1H, H-5), 6.47 (s, 1H, H-8), 6.36 (s, 2H, H-2′, H-6′), 5.97 (s, 2H, OCH_2O), 4.58 (d, 1H, $J = 5.1$ Hz, H-1), 4.32 (m, 2H, H-11), 4.25 (d, 1H, $J = 4.0$ Hz, H-4), 3.80 (s, 3H, 4′-OCH_3), 3.72 (s, 6H, 3′,5′-OCH_3), 3.32 (q, 1H, H-2), 2.92–2.63 (m, 1H, H-3), 1.84 (d, 2H, 4-NH_2); ^{13}C NMR ($CDC1_3$, 100 MHz), δ: 175.4, 147.6, 147.2, 146.3, 134.1, 133.9, 131.2, 131.1, 110.2, 108.6, 107.9, 101.3, 68.1, 56.4, 48.9, 43.7, 40.2, 37.9; EIMS (100 eV), *m*/*z*: 414 (M + 1); HRMS, *m*/*z* calculated for $C_{22}H_{23}NO_7$: 414.1366 $[M + H]^+$, found: 414.1365 $[M + H]^+$.

For compound *VIII*: m.p. 227–229 ℃; IR (KBr), ν_{max}/cm^{-1}: 3360 (OH), 3290 (NH_2), 1745 (lactone), 1600, 1500, and 1480 (C═$C_{aromatic}$), 933 (OCH_2O); ^{1}H NMR ($CDC1_3$, 400 MHz), δ: 6.81 (s, 1H, H-5), 6.50 (s, lH, H-8), 6.30 (s, 2H, H-2′, H-6′), 5.98 and 5.95 (2s, 2H, OCH_2O), 4.56 (d, 1H, $J = 5.2$ Hz, H-l), 4.30 (d, 2H, $J = 10.0$ Hz, H-11), 4.18 (d, 1H, $J = 4.0$ Hz, H-4), 3.78 (s, 6H, 3′,5′-OMe), 3.15 (dd, 1H, $J = 5.2$ Hz, $J = 14.0$ Hz, H-2), 2.80 (m, 1H, H-3); ^{13}C NMR ($CDC1_3$, 100 MHz), δ: 175.4, 147.6, 147.3, 146.3, 134.1, 133.9, 131.2, 131.1, 110.2, 108.6, 107.9, 101.3, 68.1, 56.4, 48.9, 43.7, 40.2, 38.02; EIMS (100 eV), *m*/*z*: 400 (M + 1); HRMS, *m*/*z* calculated for $C_{21}H_{21}NO_7$: 400.1822 $[M + H]^+$, found: 400.1825 $[M + H]^+$.

It is necessary to note that this modified Ritter reaction is a highly stereoselective process as it affords nearly pure 4β-aminopodophyllotoxins in quantitative yield in contrast to the already reported chemical protocol wherein α and β isomers were obtained in the ratio of 2 : 5, respectively. The β-selectivity in the present method is probably due to the epimer formed at C-4 which seems to be directed by steric hindrance of the bulky pseudoaxial trimethoxyphenyl E-ring acting on the α-side of the 4-carbocation of podophyllotoxins. It is shown in literature (Keller-Juslen et al., 1971; Tian et al., 1997; Wang et al., 1990) that biological activity is generaly retained or predominant in the case of 4β-substituted podophyllotoxin congeners when compared to their α-isomers, thus illustrating the importance of the β-isomers synthesis. Assignment of the configuration at the C-4 position to 4β-aminopodophyllotoxins (*VII*, *VIII*) was based on the $J_{3,4}$ coupling constants. According to the Karplus dihedral angle rule in six-membered rings, the C-4α-substituted compounds have $J_{3,4} \geq 8.5$ Hz as the H-3 atom is *trans* to the H-4 one, whereas C-4β-substituted compounds have $J_{3,4} < 4.5$ Hz due to the *cis* relationship between the H-3 and H-4 atoms (Wang et al., 1990).

In conclusion, a novel, mild, and efficient synthesis of 4β-aminopodophyllotoxins by a modified Ritter reaction under both sonication and classical heating conditions with practical applicability has been described. In general, significant enhancement in the reaction rates and yields of the sonochemical reactions relative to the classical heating reactions was observed. This procedure provides a significant improvement over the existing methods for obtaining 4β-aminopodophyllotoxins. As such, the modified version of the Ritter reaction opens up an easy way of preparing various new biologically significant *N*-substituted derivatives of 4β-aminopodophyllotoxins.

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