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SHORT COMMUNICATION

4β-Isocyanopodophyllotoxins: valuable precursors for the synthesis of new podophyllotoxin analogues

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A new and efficient method for the synthesis of novel 4β-isocyanopodophyllotoxins as a valuable building block for the synthesis of versatile bioactive podophyllotoxin analogues under both classical and ultrasonic conditions was developed. In general, significant improvements in rates of reaction and yields of sonochemical reactions relative to the classical ones were observed.
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Isocyanides constitute a special group of starting materials useful for producing combinatorial libraries in isocyanide based multicomponent reactions (IMCRs) due to their unique reactivity involving the formation of α-adducts with nucleophiles and electrophiles (Dömling, 2006; Shaabani et al., 2008; El Kaim et al., 2008; Bremner & Organ, 2007; Bon et al., 2005; Nair et al., 2001). Nowadays, they are increasingly used as valuable building blocks for various biologically active molecule based multicomponent reactions in organic synthesis and medicinal chemistry (Touré & Hall, 2009; Dolle et al., 2009). Despite their promising applications in drug discovery, there is a very limited number of applicable diverse isocyanide building blocks. Thus, finding or designing efficient and practical bioactive isocyanide building blocks seems to be important.

Podophyllotoxin (*I*, (10*R*,11*R*,15*R*,16*R*)-16-hydroxy-10-(3,4,5-trimethoxyphenyl)-4,6,13-trioxatetracyclo[7.7.0.0^{3,7}.0^{11,15}]hexadeca-1,3(7),8-trien-12-one, Fig. 1), a naturally occurring bioactive lignan used as a molecular precursor for the development of potent antineoplastic drugs and antiviral agents (Bohlin & Rosen, 1996; Gordaliza et al., 2004; Liu et al., 2007),

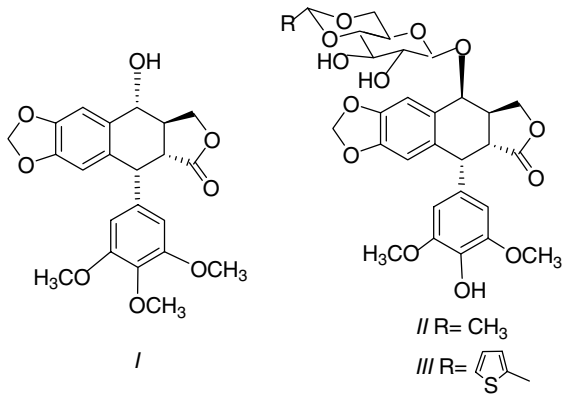

Fig. 1. Structures of podophyllotoxin (*I*), etoposide (*II*), and teniposide (*III*).

exhibits promising insecticidal activity (Liu et al., 2008; Xu et al., 2009). Extensive chemical transformations of podophyllotoxin led to the clinical introduction of two C-4β-glucoconjugate analogues of etoposide (VP-16, *II*) and teniposide (VM-26, *III*), their clinical success has greatly stimulated the interest in further study of the modification of C-4 substituent in

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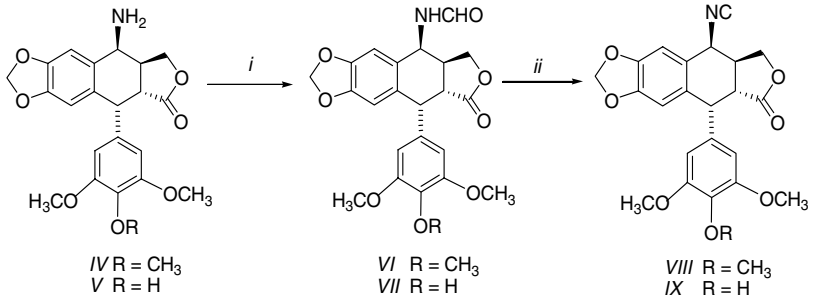

Fig. 2. Synthesis of 4β-isocyanopodophyllotoxins. Reaction conditions: *i*) ultrasonic reflux, 1 h, $HCO_2C_2H_5/CH_2Cl_2$; *ii*) *p*-TsCl/pyridine, ultrasonic, room temperature, 2 h.

I to achieve better antitumour activity. However, typical replacement of the C-4 position in *I* has been limited mainly to simple groups such as 4β-substituted ethers, esters and *N*-linked congeners. To enrich the limited set of podophyllotoxin derivatives typically employed in drug discovery, an efficient synthesis of 4β-isocyanopodophyllotoxins is reported herein; such key building blocks increase the range of available isocyanides and furthermore allow the synthesis of a versatile bioactive podophyllotoxin fragment in the MCR products, libraries created in the MCRs reactions and heterocycles built from isocyanides.

Available methods for the preparation of isocyanides mainly involve the dehydration of *N*-substituted formamides using phosgene, diphosgene, phosphorus oxychloride in combination with bases, and the reaction of primary amines with chloroform in the presence of strong bases (Katritzky et al., 1993; Doemling & Ugi, 2000). Although some good results were obtained, most of these methods often suffer from one or more limitations with respect to general applicability, chemoselectivity, ready availability, operational convenience, and the use of toxic phosgene and diphosgene as reactive reagents. These limitations make their application difficult not only in large-scale synthesis of isocyanides and screening libraries for drug discovery but disadvantage them also from a general industrial standpoint. Due to the above reasons and also as a part of our ongoing project aiming at the development of novel multicomponent reactions based on podophyllotoxin congeners of biological significance, a facile and reliable access to isocyanopodophyllotoxins is required. After the screening of various dehydrating agents, including $POCl_3/NEt_3$, $CHCl_3/KOH$, $SOCl_2/DMF$, phenyl chlorothionoformate, Tf_2O/Hünig's base, the easily available and cheap *p*-toluene sulfonyl chloride (TsCl) in combination with pyridine was selected as a mild and high-yielding alternative to the synthesis of isocyanopodophyllotoxins under classical conditions in the simple ultrasonic technique The results of our study are reported in this paper. It is worth to mention that ultrasound as a non-thermal energy transfer source is well known to enhance the reaction rates/yields/selectivity in organic synthesis and that it has found wide application in synthetic organic chemistry. Significant enhancement in the reaction rates and yields was observed when using the sonochemical reactions relative to the classical ones.

In a typical procedure, the starting material of 4β-aminopodophyllotoxins (*IV*, *V*) is prepared from podophyllotoxin by employing the above procedures (Yu et al.,1999; Liu et al., 2010), then, formylation of 4β-aminopodophyllotoxins with ethyl formate proceeds followed by the dehydration of 4β-formamido-4-deoxypodophyllotoxins (*VI*, *VII*) with *p*-toluene sulfonyl chloride in combination with pyridine under both classical and ultrasonic conditions to give the corresponding 4β-isocyanopodophyllotoxins (*VIII*, *XI*) in moderate to good yields. In general, significant improvement in the reaction times and yields was observed when ultrasonic conditions were applied. The desired compounds (*VIII*, *XI*) were characterized by m.p., IR, ^{1}H NMR, ^{13}C NMR, MS, and HRMS analyses. A noteworthy aspect of this reaction is that the γ-lactone ring is intact under ultrasonic conditions though this reagent is known to form the corresponding *cis*-fused lactone (Roulland et al., 2002). Moreover, the assignment of configuration at the C-4 position in 4β-isocyanopodophyllotoxins (*VIII*, *XI*) was based on the $J_{3,4}$ coupling constants. According to the Karplus dihedral angle rule in six-membered rings, in C-4-α-substituted compounds, $J_{3,4} \geq 8.5$ Hz as H-3 is in trans position to H-4, whereas in C-4β-substituted compounds, $J_{3,4} < 4.5$ Hz due to the *cis* relationship between H-3 and H-4 (Wang et al., 1990).The overall sequence is shown in Fig. 2.

In conclusion, a novel, mild and efficient synthesis of 4β-isocyanopodophyllotoxins by dehydration of the corresponding formamide under both ultrasonic and classical conditions with practical applicability is described for the first time. In general, significant enhancement in the reaction rates and yields for the sonochemical reactions relative to the classical ones were observed. This procedure provides valuable building blocks for the synthesis of versatile biologically significant podophyllotoxin fragments in the MCR products, libraries created in the MCRs reac-

Table 1. Spectral data of newly prepared compounds

Compound	Spectral data
VIII	IR, $\tilde{\nu}$/cm^{-1}: 2130 (NC), 1779 (lactone), 1588, 1506, and 1485 (aromatic C═C), 930 (OCH_2O) ^{1}H NMR ($CDCl_3$) δ: 6.91 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.26 (s, 2H, H-2′,6′), 6.03 (dd, $J = 7.9$ Hz, $J = 1.1$ Hz, 2H, OCH_2O), 4.99 (d, $J = 4.4$ Hz, 1H, H-4), 4.68 (d, $J = 5.1$ Hz, 1H, H-1), 4.39 (m, 2H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′,5′ —OCH_3), 3.20 (q, 1H, H-2), 2.96 (m, 1H, H-3) ^{13}C NMR: ($CDC1_3$) δ: 173.01, 160.01, 152.78, 149.27, 147.95, 137.64, 134.32, 131.59, 125.13, 110.53, 108.71, 108.33, 101.95, 67.69, 60.73, 56.35, 53.53, 43.53, 41.37, 35.24 MS(EI) (m/z): 424 (M+1) HRMS (m/z) for $C_{23}H_{21}NO_7$ $[M+NH_4]^+$: calc. 441.1656, found 441.1652
XI	IR, $\tilde{\nu}$/cm^{-1}: 3447 (OH), 2130 (NC), 1745 (lactone), 1600, 1500, and 1480 (aromatic C═C), 933 (OCH_2O) ^{1}H NMR ($CDCl_3$) δ: 6.88 (s, 1H, H-5), 6.50 (s, 1H, H-8), 6.32 (s, 2H, H-2′,6′), 5.98 (dd, $J = 7.9$ Hz, $J = 1.1$ Hz, 2H, OCH_2O), 4.92 (d, $J = 4.4$ Hz, 1H, H-4), 4.68 (d, $J = 5.1$ Hz, 1H, H-1), 4.37 (m, 2H, H-11), 3.78 (s, 6H, 3′,5′-OCH_3), 3.15 (q, 1H, H-2), 2.80 (m, 1H, H-3) ^{13}C NMR: ($CDC1_3$) δ: 175.40, 160.01, 152.78, 149.27, 146.30, 137.64, 134.12, 133.95, 125.13, 110.53, 108.71, 108.33, 101.95, 67.69, 60.73, 56.35, 53.53, 43.70, 41.37, 38.02 MS(EI) (m/z): 410 (M+1) HRMS (m/z) for $C_{22}H_{19}NO_7$ $[M+NH_4]^+$: calc. 427.1843, found 427.1825

tions and heterocycles built from isocyanides.

Melting points were taken on a Kofler melting point apparatus (Triumph Company, Germany) and are uncorrected; IR spectra were obtained on a NIC-5DX spectrophotometer (Nicote Company, USA); mass spectral analysis was performed on a ZAB-HS and Bruker Daltonics APEXII49e instrument (Bruker Company, USA). Optical rotations were determined on a Perkin–Elmer Model 341 spectropolarimeter (Taike Company, China). NMR spectra were recorded on a Bruker AM-400 spectrometer (Bruker Company, USA) at 400 MHz using TMS as the reference. Sonication was performed in a Shanghai Branson-CQX (Shanghai Branson Company, China) ultrasonic cleaner at the frequency of 25 kHz and nominal power of 500 W. The reaction flask was located in the maximum energy area of 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. Chemicals are commercially available and were used without further purification. The starting materials of 4β-aminopodophyllotoxins (*IV*, *V*) were prepared by the above procedures in our laboratory (Yu et al., 1999; Liu et al., 2010).

General procedure for 4β-isocyanopodophyllotoxins (*VIII*, *XI*) preparation: in method A (classical method), to a solution of 0.24 mmol of 4β-aminopodophyllotoxins (*IV*, *V*) in 10 mL of CH_2Cl_2, 5 mL of ethyl formate were added and the reaction mixture was refluxed for 4 h. The solvents were then evaporated under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, $\varphi_r = 98 : 2$) to give 4β-formamido-4-deoxypodophyllotoxins (*VI*, *VII*) in ≈ 45–47 % yields. Subsequently, to a cooled and stirred solution of 0.31 mmol of formamide (*VI*, *VII*) in pyridine (5 mL), 0.77 mmol of *p*-toluenesulfonyl chloride was added. After 5 min, the ice-bath was removed and the mixture was stirred at room temperature for 8 h. The yellow solution slowly turned brown. The solution was quenched by an addition of water (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was washed with a saturated aqueous solution of $NaHSO_4$ (2 × 15 mL); the extracts were dried ($MgSO_4$), filtered, the reaction solvent was evaporated and chromatographed on silica-gel (hexane/ethyl acetate, $\varphi_r = 9 : 1$, then 8 : 2, and 7 : 3) to give compounds *VIII* and *XI* as white solids in ≈ 35–37 % yields.

In method B (ultrasound irradiation), to a solution of 0.24 mmol of 4β-aminopodophyllotoxins (*IV*, *V*) in 10 mL of CH_2Cl_2, 5 mL of ethyl formate were added and the above mixture was irradiated by an ultrasonic generator in a water bath at 30 ℃ for 1 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the solvents were evaporated under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, $\varphi_r = 98 : 2$) to give 4β-formamido-4-deoxypodophyllotoxins (*VI*, *VII*) in ≈ 78–80 % yields. Subsequently, to a cooled and stirred solution of 0.31 mmol of formamide (*VI*, *VII*) in 5 mL of pyridine, 0.77 mmol of *p*-toluenesulfonyl chloride was added. After 5 min, the ice-bath was removed and the mixture was sonicated at room temperature for 2 h. The yellow solution slowly turned brown. The solution was quenched by an addition of water (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was washed with a saturated aqueous solution of $NaHSO_4$ (2 × 15 mL), the extracts were dried ($MgSO_4$), filtered, concentrated, and chromatographed on silica-gel (hexane/ethyl acetate, $\varphi_r = 9 : 1$, then 8 : 2, and 7 : 3) to give compounds *VIII* and *XI* as white solids in ≈ 65–67 % yields. Characterization data of newly prepared compounds: *VIII*, yield: 67 %, m.p. = 115–117 ℃; *XI*, yield: 65 %, m.p. = 187–189 ℃. Spectral data are listed in Table 1.

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