Cytotoxicity Study on Some Synthetic Aromatic Esters of 4´-Demethyl-4-deoxypodophyllotoxin

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Received April 26, 2011: Revised June 13, 2011: Accepted June 28, 2011

Abstract: To search for potential anti-cancer analogs, 16 aromatic esters of 4´-demethyl-4-deoxypodophyllotoxin (**6a-p**) were synthesized and tested their cytotoxicity on six cancer cell lines. The results indicated that all derivatives showed potent cytotoxic activity against A-549 cell line with IC_{50} values of 0.00009-0.037 μg/mL.

Keywords: Aromatic ester, Cytotoxicity, Deoxypodophyllotoxin.

INTRODUCTION

Podophyllotoxin (**1**, Fig. **1**), one of the naturally occurring aryl tetrahydronaphthalene lignan lactones, is isolated as the main component from the roots and rhizomes of *Podophyllum* species such as *P. hexandrum* and *P. peltatum*, and has been used as a lead compound for drug design in the search for improved antitumor activity. Consequently, many semisynthetic derivatives of **1** have been developed and tested for anticancer activity over more than two decades, resulting in the commercial production of two antitumor drugs, such as etoposide (VP-16, **2**, Fig. **1**), and teniposide (VM-26, **3**, Fig. **1**) [1,2]. Deoxypodophyllotoxin (**4**, Fig. **1**), as an analog of **1**, has exhibited the antiproliferative and antitumor activities against diverse cell types [3-5]. Recently, You *et al.* reported that some alkyl and carboxylalkyl esters of 4´-demethyl-4-deoxypodophyllotoxin exhibited good antitumor activity [6]. The above encouraging results, therefore, prompted us in the present Letter to further study other aromatic esters of 4´-demethyl-4-deoxypodophyllotoxin as anticancer agents.

RESULTS AND DISCUSSION

Chemistry

As shown in Scheme **1**, 4-deoxypodophyllotoxin (**4**) was firstly obtained in a 66% yield by catalytic hydrogenolysis of **1** in the presence of 10% palladium/carbon [7]. Then regioselective 4´-demethylation of **4** with dry hydrobromide, followed by the mixed solvent system (water/acetone) and $BaCO_3$ could proceed readily to give the 4´-demethyl-4-deoxypodophyllotoxin (**5**) in a 60% yield [8]. Finally, 16 esters of 4´-demethyl-4-deoxypodophyllotoxin (**6a-p**) were obtained in 38-93% yields by the reaction of **5** with the corresponding acids containing aromatic rings in the presence of diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP). The structures of all the target compounds were well identified by ^{1}H NMR, MS, HRMS and m.p [9].

Biological Activity

Compounds **6a-p** were evaluated for their cytotoxic activity against six cancer cell lines, such as A-549, HT-29, SGC-7901, MDA-MB-231, U-251 and DU-145. Taxol was used as a positive control. The results of **6a-p** were presented in Table **1**. Interestingly, the derivatives showed the stronger cytotoxic activity on A-549 and DU-145 than other cell lines. Compared with other derivatives and taxol, especially compounds **6g** and **6p** exhibited the most potent cytotoxic activity against A-549 cell with the IC_{50} values of 0.00009 and 0.0001 μg/mL, respectively. It indicated that introduction of the *meta*-chloro atom on the phenyl ring of **6b** could lead to the more potent compound (IC_{50} 0.00009 for **6g** *vs.* IC_{50} 0.008 for **6b**), while introduction of the *orth or para*-chloro atom on the phenyl ring of **6b** led to the less potent compounds (IC_{50} 0.016 for **6f** and IC_{50} 0.037 for **6h** *vs.* IC_{50} 0.008 for **6b**). Meanwhile, introduction of the substituent R as the 4-pyridinyl group gave the tenfold more potent compound than that having 3-pyridinyl group. For example, IC_{50} values of **6n** and **6p** against A-549 cell were 0.001 and 0.0001 μg/mL, respectively.

EXPERIMENTAL SECTION

General procedure for the preparation of **4** and **5** was reported in our previous paper [9].

General Procedure for the Preparation of 6a-p

A mixture of the corresponding acid (0.3 mmol), diisopropylcarbodiimide (DIC, 0.3 mmol), 4-dimethylaminopyridine (DMAP, 0.1 mmol), and **5** (0.25 mmol) in dried dichloromethane (10 mL) were stirred at 0 °C. When the reaction was completed according to TLC analysis, the resulting suspension was filtered, and water (25 mL) was added to the solution of the above mixture, which was extracted with dichloromethane (30 mL × 4). Then the organic

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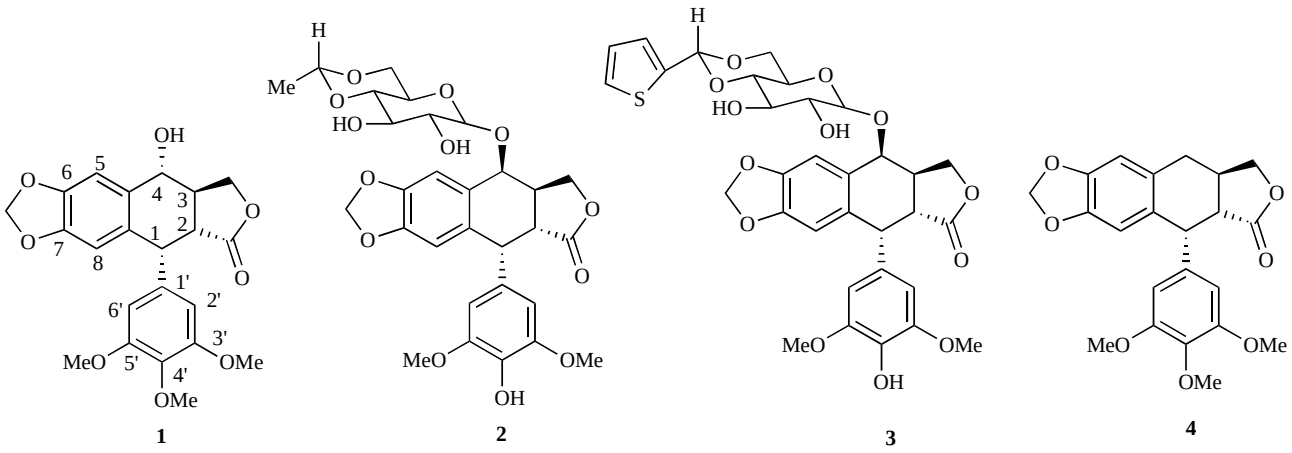
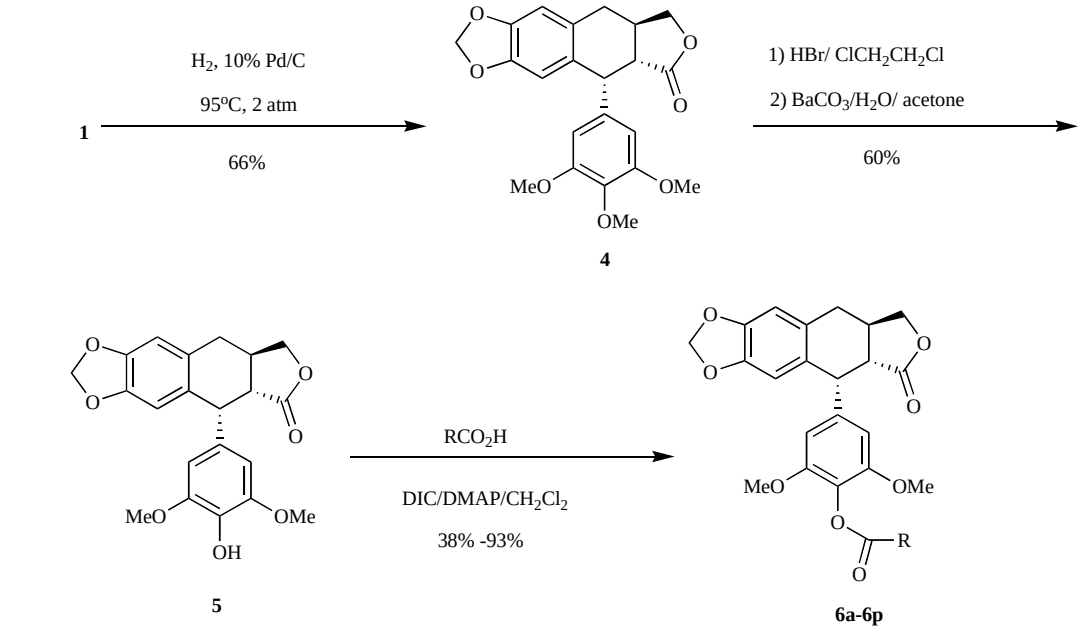
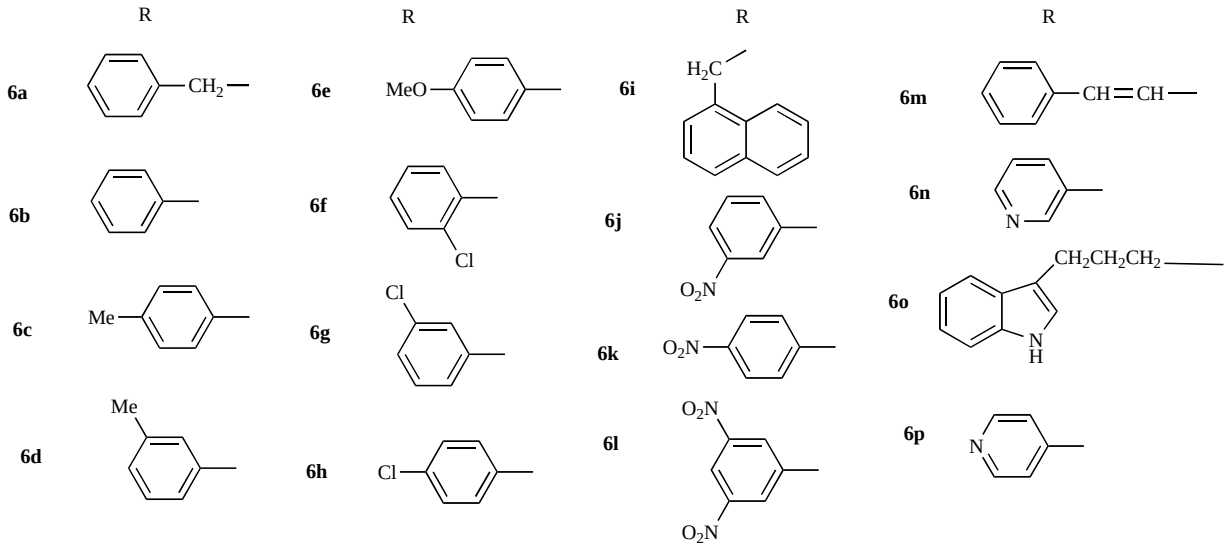


Fig. (1). Chemical Structures of Compounds **1-4**.





Scheme 1. The Synthetic Route of Compounds **6a-p**.

Table 1. Cytotoxicity of Deoxypodophyllotoxin Analogs 6a-p against Six Cancer Cell Lines

Compd	IC_{50} (μg/mL)					
	A-549	HT-29	SGC-7901	MDA-MB-231	U-251	DU-145
6a	0.006±0.0004	NA[a]	NA	NA	NA	NA
6b	0.008±0.0007	0.060±0.006	NA	NA	0.009±0.0007	0.073±0.001
6c	0.006±0.001	0.067±0.002	NA	NA	0.037±0.003	0.058±0.008
6d	0.006±0.0003	NA	NA	NA	0.008±0.0007	0.616±0.193
6e	0.010±0.002	NA	4.923±1.952	NA	NA	0.055±0.003
6f	0.016±0.006	0.081±0.003	NA	NA	NA	1.526±0.596
6g	0.00009±0.000006	NA	NA	NA	NA	0.075±0.013
6h	0.037±0.003	NA	NA	NA	NA	0.094±0.018
6i	0.033±0.006	0.021±0.005	0.072±0.003	NA	0.081±0.006	0.085±0.014
6j	0.005±0.0002	NA	0.008±0.0004	NA	NA	0.065±0.002
6k	0.001±0.0001	NA	NA	NA	0.006±0.0003	0.024±0.004
6l	0.007±0.0007	0.007±0.0003	NA	NA	NA	0.012±0.005
6m	0.007±0.0008	0.068±0.004	0.915±0.059	2.483±0.985	NA	0.078±0.016
6n	0.001±0.0002	NA	0.849±0.141	NA	NA	NA
6o	0.005±0.0006	NA	NA	NA	NA	0.010±0.001
6p	0.0001±0.00006	NA	NA	NA	NA	NA
Taxol	0.0008±0.0002	0.005±0.002	0.002±0.003	>10	0.008±0.005	0.05±0.02

[a]NA: No activity at 10 μg /mL.

phase was combined, dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified by preparative thin-layer chromatography (PTLC) to give the pure target products in 38-93% yields. The example data of **6a-f** and **6h-p** were shown in our previous paper [9]. The spectra data of **6g** were as follows:

6g: Yield: 64%, white solid, m.p. 247-249 ^oC; ^1H-NMR (400 MHz, $CDCl_3$) δ: 8.18 (s, 1H, H-2″), 8.07 (d, J = 8.0 Hz, 1H, H-6″), 7.51 (d, J = 8.0 Hz, 1H, H-4″),7.43 (t, J = 8.0 Hz, 1H, H-5″), 6.68 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.43 (s, 2H, H-2′, 6′), 5.94 (d, J = 8.4 Hz, 2H, OCH_2O), 4.67 (s, 1H, H-1), 4.48 (m, 1H, H-11), 3.92 (m, 1H, H-11), 3.69 (s, 6H, 3′, 5′-OCH_3), 3.07 (m, 1H, H-4), 2.76 (m, 3H, H-2, 3, 4). MS (ESI-TRAP), *m/z* (%): 545 ($[M+Na]^+$, 29). HRMS: Calcd for $C_{28}H_{27}NO_8Cl$ ($[M+NH_4]^+$), 540.1420; found, 540.1426.

Cell-Growth Inhibition Assay

Cell growth and viability were measured with the sulphorhodamine B (SRB) assay [10]. Cells were cultured in RPMI 1640 medium (Sigma) containing 10% FBS. Aliquots of 90 μL were seeded in 96-well flat-bottomed microtiter plates (Greiner) with 3.3–7.7×10^4 cells/mL. Twenty-four hours later, 10 μL compound, dissolved in DMSO and diluted with the medium, were added to the well in triplicate with a final concentration of 10 μg/mL. After incubation at 37 ^oC and 5% CO_2 for another 48 h, cells were fixed by the addition of 50% ice-cold trichloroacetic acid and left at 4 ^oC for 1 h. After washing, air-drying, and staining for 15 min with 100 μL 0.4% SRB in 1% glacial acetic acid, excessive dye was removed by washing with 1% glacial acetic acid. After plates were air-dried, SRB was resuspended in 100 μL 10 mM Tris buffer, and the absorbance was measured at 560 nm with a Plate Reader (Molecular Devices, PECTRAMAX340). At the highest tested concentration of 10 μg/mL, if the cell viability is less than 50%, then the dose–response curves were performed with at least four concentrations (dilution ration 10:1 or 5:1), and the IC_{50} values (50% inhibitory concentration) was calculated according to the Reed-Muench method. Results are expressed as mean IC_{50} values ± standard deviation (n = 3). Taxol, clinically used as an anti-cancer natural drug, was used as a positive control.

CONCLUSION

In conclusion, we have reported 16 aromatic esters of 4´-demethyl-4-deoxypodophyllotoxin as anticancer agents. In general, the derivatives showed the potent cytotoxic activity against A-549 and DU-145 cell lines.

ACKNOWLEDGEMENTS

This work was financially supported by the Program for New Century Excellent University Talents, State Education Ministry of China (NCET-06-0868), and Special Funds of Central Colleges Basic Scientific Research Operating Expenses (QN2009045). We also would like to acknowledge the financial support from the National Natural Science Foundation of China (30725048) and the Foundation of Chinese Academy of Sciences (West Light Program, KSCX2-YW-R-177).

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