Regioselective Synthesis and in Vitro Anticancer Activity of 4-Aza-podophyllotoxin Derivatives Catalyzed by L-Proline

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A series of 4-aza-podophyllotoxin derivatives have been synthesized regioselectively via the three-component reaction of aldehydes, aromatic amines, and tetronic acid catalyzed by L-proline. This method has the advantages of high yield, high regioselectivity, extensive adaptability, easy operation, and environmental friendliness. These compounds were also investigated in vitro, and some were found to have good anticancer activity.

Introduction

Podophyllotoxin (Figure 1) is an antitumor lignan that inhibits microtubule assembly.[1] Because of mostly unsuccessful attempts to use it for the treatment of human neoplasia, extensive structural modifications have been performed in order to obtain more potent and less toxic anticancer agents.[2] Among them, derivatives of 4-aza-podophyllotoxin (Figure 1), reported as powerful DNA topoisomerase II inhibitors, have recently attracted considerable interest. Takeya et al.[3] reported the synthesis of 4-aza-podophyllotoxin derivatives via three steps of condensation, cyclization, and reduction. Tratrat et al.[4] reported the one-pot synthesis from an aldehyde, aniline, and tetronic acid in refluxing ethanol. Tu et al.[5] also reported a three-component reaction for one-pot synthesis of 4-aza-podophyllotoxin derivatives in water at higher temperature under microwave irradiation conditions. However, these methods still suffer from drawbacks such as needing rigorous conditions and a special reaction instrument, and are limited to anilines. Therefore, there is a need for milder and more efficient methods for the one-pot synthesis of this type of compound.

Recently, organic reactions catalyzed by small organic molecules have drawn attention. In particular, cinchona alkaloids or L-proline and its derivatives have been used as catalysts in various reactions with excellent yields.[6–12] Recently, L-proline and its derivatives have been used in multicomponent dissymmetric Biginelli[13] and Hantzsch reactions.[14] We have previously reported the synthesis of heterocycles using L-proline as catalyst.[15] In continuing with our earlier work, we would like to report herein efficient access to the construction of 4-aza-podophyllotoxin derivatives using L-proline as catalyst.

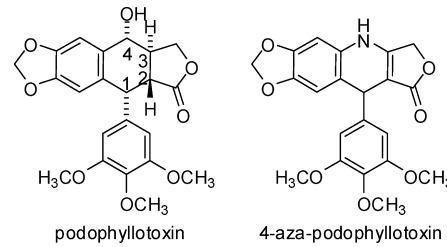
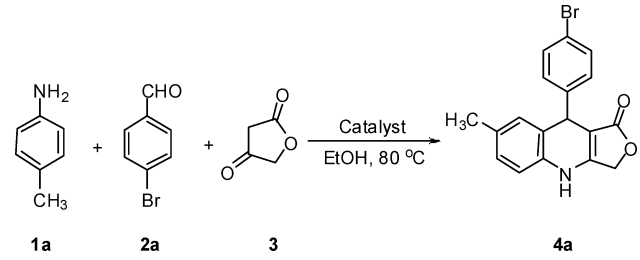


Figure 1. Structure of podophyllotoxin and 4-aza-podophyllotoxin.

Scheme 1. Model Reaction



Results and Discussion

In our initial study, the reaction of *p*-toluidine **1a**, 4-bromobenzaldehyde **2a**, and tetronic acid **3** (Scheme 1) was chosen as a model reaction to optimize the reaction conditions. The results are summarized in Table 1. The reaction was first carried out in ethanol in the absence and presence of several additives. It was found that only 35% of the target compound **4a** was obtained in the absence of additive (Table 1, entry 1). Some proton acid (Table 1, entries 2−4), Lewis acid (Table 1, entries 5−10), and bases (Table 1, entries 11−12) can catalyze this reaction with moderate yields. The best result was obtained when L-proline was used according to the yield and the reaction time (Table 1, entry 13). So L-proline was chosen as the catalyst for this reaction.

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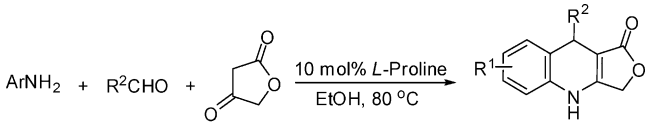
Table 1. Optimization of Catalysts, Solvents and Temperature in the Synthesis of **4a**

entry	additive (mol %)	solvent	T (°C)	time (h)	yield[a] (%)
1	none	ethanol	80	4	35
2	HCl (10%)	ethanol	80	1.5	51
3	H_2SO_4 (10%)	ethanol	80	1.5	49
4	*p*-TsOH (10%)	ethanol	80	2	47
5	$CaCl_2$ (10%)	ethanol	80	2	49
6	$FeCl_3 \cdot 6H_2O$ (10%)	ethanol	80	1.5	65
7	$CoCl_2 \cdot 6H_2O$ (10%)	ethanol	80	1.5	46
8	$AlCl_3$ (10%)	ethanol	80	2	60
9	$SnCl_4 \cdot 5H_2O$ (10%)	ethanol	80	2.5	76
10	$SnCl_2 \cdot 2H_2O$ (10%)	ethanol	80	2.5	70
11	Et_3N (10%)	ethanol	80	2	35
12	pyridine (10%)	ethanol	80	2	43
13	L-proline (10%)	ethanol	80	0.5	93
14	L-proline (5%)	ethanol	80	2	90
15	L-proline (15%)	ethanol	80	0.5	92
16	L-proline (20%)	ethanol	80	0.5	93
17	L-proline (10%)	ethanol	*b*	4	*c*
18	L-proline (10%)	ethanol	40	4	58
19	L-proline (10%)	ethanol	60	2	68
20	L-proline (10%)	methanol	65	1.5	92
21	L-proline (10%)	water	100	3	36
22	L-proline (10%)	DMF	150	2.5	83
23	L-proline (10%)	HOAc	120	2.5	90
24	L-proline (10%)	acetonitrile	70	3	79

[a] Isolted yield. [b] Room temperature. [c] Trace.

Table 2. Synthesis of 4-Aza-podophyllotoxin Derivatives **4** Catalyzed by L-Proline



1 **2** **3** **4**

entry	Ar (**1**)	R^2 (**2**)	product	time (h)	yield (%)
1	4-$CH_3C_6H_4$	4-BrC_6H_4	**4a**	0.5	93
2	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	**4b**	0.5	92
3	4-$CH_3C_6H_4$	4-ClC_6H_4	**4c**	0.5	89
4	4-$CH_3C_6H_4$	4-$NO_2C_6H_4$	**4d**	0.5	91
5	4-$CH_3C_6H_4$	3,4-$Cl_2C_6H_3$	**4e**	1	93
6	3-$CH_3C_6H_4$	4-$CH_3C_6H_4$	**4f**	0.5	91
7	3,4-$OCH_2OC_6H_3$	3,4,5-$(CH_3O)_3C_6H_2$	**4g**	0.15	95
8	3,4-$OCH_2OC_6H_3$	4-FC_6H_4	**4h**	0.2	92
9	3,4-$OCH_2OC_6H_3$	4-$CH_3OC_6H_4$	**4i**	0.15	94
10	3,4-$OCH_2OC_6H_3$	4-BrC_6H_4	**4j**	0.2	93
11	3,4-$OCH_2OC_6H_3$	4-ClC_6H_4	**4k**	0.2	92
12	naphthalene-1-yl	4-$CH_3OC_6H_4$	**4l**	0.5	93
13	naphthalene-1-yl	4-BrC_6H_4	**4m**	0.5	91
14	naphthalene-1-yl	4-ClC_6H_4	**4n**	1	92
15	naphthalene-1-yl	3,4-$OCH_2OC_6H_3$	**4o**	0.5	94
16	naphthalene-1-yl	4-$CH_3C_6H_4$	**4p**	0.5	92
17	naphthalene-1-yl	2,4-$Cl_2C_6H_3$	**4q**	1	91
18	naphthalene-1-yl	thiophen-2-yl	**4r**	0.5	93
19	quinolin-6-yl	4-$CH_3C_6H_4$	**4s**	0.5	91
20	quinolin-6-yl	4-ClC_6H_4	**4t**	1	93
21	3-Cl-4-$CH_3C_6H_3$	4-ClC_6H_4	**4u**	2	92
22	3-Cl-4-$CH_3C_6H_3$	4-BrC_6H_4	**4v**	2	94
23	3-Cl-4-$CH_3C_6H_3$	3,4-$Cl_2C_6H_3$	**4w**	3	92
24	3-Cl-4-$CH_3C_6H_3$	4-$NO_2C_6H_4$	**4x**	2	91

We also evaluated the amount of L-proline required for this reaction. The results from Table 1 (entries 13−16) show that 10 mol % L-proline at reflux in ethanol is sufficient to initiate the reaction. Higher loading of the catalyst had no significant influence on the reaction yield. To find the optimum reaction temperature, the reaction was carried out with 10 mol % L-proline at room temperature, 40 °C, 60 °C, and reflux

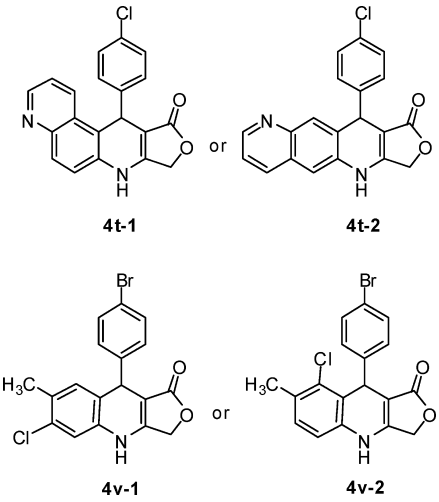


Figure 2. Possible structures of products **4t** and **4v**.

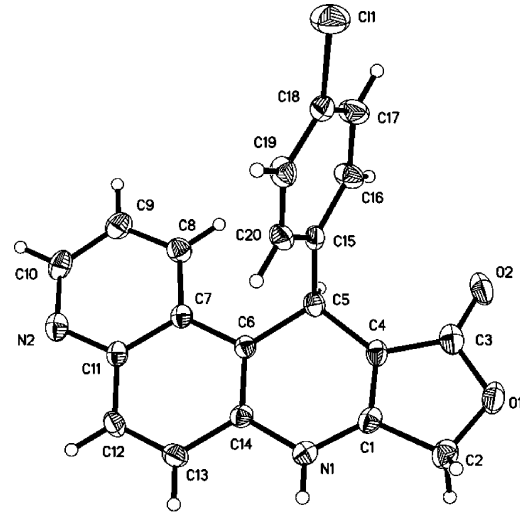


Figure 3. The molecular structure of product **4t**.

temperature, resulting in the isolation of **4a** in a trace amount, 58%, 68%, and 93% yields (Table 1, entries 17−19 and 13), respectively. Thus, 10 mol % L-proline and a reaction temperature at reflux were optimal conditions. In addition, methanol, water, DMF, HOAc, and CH_3CN (Table 1, entries 20−24) were also tested as solvents. In these cases, product **4a** was formed in slightly lower yields (Table 1, entries 20−24).

Under these appropriate reaction conditions (2 mL of ethanol, 80 °C, 10 mol % L-proline), a series of 4-aza-podophyllotoxin derivatives (**4**) were synthesized. The results are summarized in Table 2.

As shown in Table 2, this protocol can be applied not only to the aromatic aldehydes with either electron-withdrawing groups (such as nitro and halide groups) or electron-donating groups (such as hydroxyl and alkoxyl groups) but also to heterocyclic aldehydes under the same conditions. Importantly, a wide range of aromatic amines including 3,4-(methylenedioxy)aniline, *p*-toluidine, *m*-toluidine, 1-naphthylamine, quinolin-6-amine, and 3-chloro-4-methylaniline were employed successfully in this reaction with excellent results.

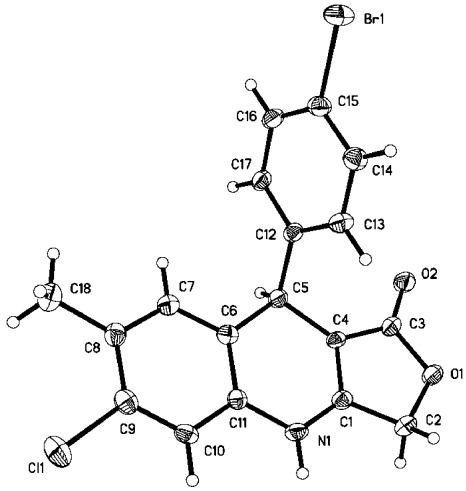


Figure 4. The molecular structure of product **4v**.

Table 3. Studies on the Reuse of L-Proline in the Preparation of **4a**

round	yield(%)
1	92
2	93
3	92
4	91
5	93
6	92
7	90
8	90

When unsymmetrical aromatic amines are used in the reaction, there may be two different structures of products. For example, possible structures of product **4t** and **4v** are shown in Figure 2. But only one product was obtained in this reaction. The structures of **4t** and **4v** were established by X-ray crystallographic analysis. The molecular structures of **4t** and **4v** are shown in Figure 3 and Figure 4, respectively.

According to the results of X-ray crystallographic analysis, the structures of **4t** and **4v** are **4t-1** and **4v-1**, respectively. The regioselectivity in the formation of **4t** and **4v** are explained from the kinetic espect: for quinolin-6-amine, the reactivity of its 5-position is higher than that of its 7-position, and for 3-chloro-4-methylaniline, the effect of steric hindrance plays a critical role. It also can be explained from the thermodynamic effect; the structures of **4t-1** and **4v-1** are more stable than **4t-2** and **4v-2**, respectively.

All the products **4** were characterized by mp, IR, and ^{1}H NMR spectra, as well as HRMS.

Apart from the mild conditions of the process and its excellent results, the simplicity of product isolation and the possibility to recover and recycle the L-proline as catalyst offer a significant advantage. Because L-proline is soluble in the reaction medium (ethanol) and the desired products are less soluble in ethanol, the products can be directly separated by cooling to room temperature and filtering after the reaction is completed. The filtrate containing L-proline can directly be recovered and recycled. Studies using **1a**, **2a**, and **3** as model substrates showed that the recovered reaction solution could be successively recycled in subsequent reactions without any decrease of yields (Table 3). It is shown that after the reaction solution has been recycled seven times, catalytic efficiency of L-proline did not change.

Based on the literature,[4,17] the proposed mechanism for the synthesis of 4-aza-podophllotoxin derivatives **4** is described in Figure 5. We suggest that L-proline catalyzes the formation of iminium ion **8** in a reversible reaction with tetronic acid **3**. The higher reactivity of the iminium ion compared with the carbonyl species could facilitate the addition of aniline **1**, via intermediate **9**, and after the elimination of L-proline, **10** might be produced as intermediate. The product **4** can be produced by tautomerization of intermediate **10**.

It is noted that the compounds obtained are recemic ones. L-Proline plays a key role in this reaction. According to above proposed mechanism, L-proline not takes part in the generation of the chiral center. So stereoselection is not achieved.

Finally, selected compounds were screened for anticancer activity by the sulforhodamine B (SRB) method. The cells used in this study were human liver cancer cell line $HepG_2$.[16] The results of the prescreening are listed in Table 4. It could be seen that some products (e.g., **4b**, **4e**, **4f**, **4i**, **4j**, **4k**, **4p**, **4v**, and **4w**) had the stronger inhibitory effects than 4-aza-podophyllotoxin **4g**, and to our happiness, the inhibition rates

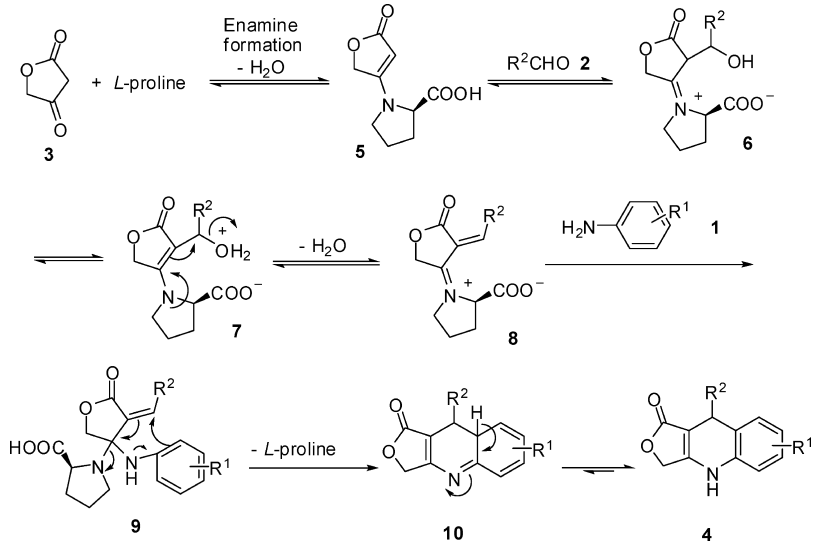


Figure 5. Possible mechanism in the synthesis of product **4**.

Table 4. Inhibition Rates of Compounds **4** in Certain Concentration on $HepG_2$ Cells

product	inhibition rate (%)
4a	74.63
4b	80.22
4c	72.39
4d	77.61
4e	79.97
4f	82.64
4g	78.73
4h	76.49
4i	80.97
4j	80.60
4k	80.22
4l	67.06
4m	73.74
4n	78.63
4o	76.41
4p	82.19
4q	76.41
4r	71.51
4t	65.30
4u	74.63
4v	85.76
4w	87.98

of new compounds **4v** and **4w** were the highest among them. Further studies about the activities of these products will be carried out.

Conclusion

In conclusion, we have developed an efficient and convenient method for the preparation of 4-aza-podophyllotoxin derivatives by the three-component reaction of aldehydes, aromatic amines, and tetronic acid catalyzed by L-proline. A variety of substrates can participate in the process with good yields. The procedure used commercially available starting materials and is suitable for library synthesis and drug discovery efforts. The short reaction time and easily available materials render this method particularly attractive for the efficient preparation of biologically and medicinally interesting molecules. Importantly, almost all of the compounds exhibited good anticancer activity.

Experimental Section

General Information. Melting points are uncorrected. Infrared spectra were recorded on a Tensor 27 spectrometer in KBr with absorption in cm^{-1}. ^1H NMR spectra were recorded on Varian 300-MHz and Bruker DPX 400-MHz spectrometer as DMSO-d_6 solution. J values are in hertz. Chemical shifts are expressed in δ downfield from internal tetramethylsilane. HRMS were obtained using TOF-MS instrument. X-ray crystallographic analysis was performed with a Smart-1000 CCD diffractometer.

General Procedure for the Synthesis of 4-Aza-podophyllotoxin Derivatives 4. A mixture of aromatic amine **1** (1 mmol), aldehyde **2** (1 mmol), tetronic acid **3** (1 mmol), L-proline (10 mol %) and ethanol (2 mL) in a 10 mL round-bottom flask was stirred at 80 °C for given times. After completion of the reaction, the reaction mixture was cooled to room temperature. The precipitate was collected by filtration and purified by recrystallization from EtOH and DMF to give products **4**.

9-(4-Bromophenyl)-7-methyl-4,9-dihydrofuro[3,4-*b*]quinolin-1(3*H*)-one (4a). Mp > 300 °C (lit.[5] mp > 300 °C). IR (KBr) ν: 3232, 1711, 1637, 1545, 1500, 1349, 1205, 1036, 810 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) (δ, ppm): 9.92 (s, 1H, NH), 7.43 (d, J = 8.4 Hz, 2H, ArH), 7.13 (d, J = 8.7 Hz, 2H, ArH), 6.94 (d, J = 7.8 Hz, 1H, ArH), 6.82 (d, J = 8.1 Hz, 2H, ArH), 4.97 (s, 1H, CH), 4.92 (d, J = 15.6 Hz, 1H, CH_2), 4.83 (d, J = 15.6 Hz, 1H, CH_2), 2.11 (s, 3H, CH_3). HRMS: found, m/z 355.0209 (M^+), calcd for $C_{18}H_{14}{}^{79}BrNO_2$, M, 355.0208.

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Supporting Information Available. Experimental details, spectroscopic characterization for compound **4**, and crystal data of **4t** and **4v**. This material is available free of charge via the Internet at http://pubs.acs.org.

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D. Q.; Kim, S. H.; Huang, Z. B.; Ji, M. *Aust. J. Chem.* **2008**, *61*, 547–551. (c) Li, Y. L.; Chen, H.; Shi, C. L.; Shi, D. Q.; Ji, S. J. *J. Comb. Chem.* **2010**, *12*, 231–237.

(16) The compounds were dissolved in DMSO as 100, 10, or 20 mg/mL stock solutions before use and stored at −20 °C. $HepG_2$ cells were incubated in MEM culture medium containing 10% blood serum. The cells were plated in flat-bottomed 96-well plates (5000 cells/well) and cultured for 24 h in controlled atmosphere. Then certain concentrations of products (100, 10, or 20 mg/mL in DMSO) were diluted 1000 times and added to the culture using DMSO as control solution. After 24 h, the absorbencies were read at 592 and 570 nm with SRB assay.

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