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Syntheses and Biological Properties of Novel Aza-podophyllotoxin Analogs Possessing Pronounced Antitumor Activity

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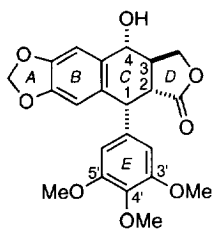
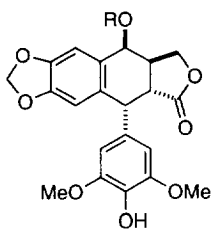
Abstract: 2,4-Diaza-4-desoxypodophyllotoxin analogs have been synthesized from substituted anilines. Some of them showed promising antitumor activities against vincristine-resistant P388 murine leukemia and B16 melanoma in vivo.

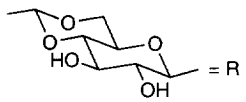
Podophyllotoxin (**1**) is an antitumor lignan isolated from the *Podophyllum* plants such as *P. peltatum* and *P. emodi* (Berberidaceae). The podophyllotoxin core structure possesses a dual mode of action, *i.e.*, the inhibition of DNA topoisomerase II and of microtubule assembly through binding to tubulin, both of which are considered to be responsible for its antitumor activity.[1] A glycoside analog, etoposide (**2**), possesses the former enzyme inhibitory activity and has been developed as an anticancer agent. Although the alternative property, a spindle poison, has been known for over 40 years,[2] no promising analog possessing this activity has yet appeared thus far partly due to the strict structure requirement for such activity. Recently, podophyllotoxin heterocyclic analogs have attracted much interest, and a number of synthetic and/or biological studies have been reported.[3] We have synthesized the diazapodophyllotoxin analogs **3a** and **4a** and found that they possess

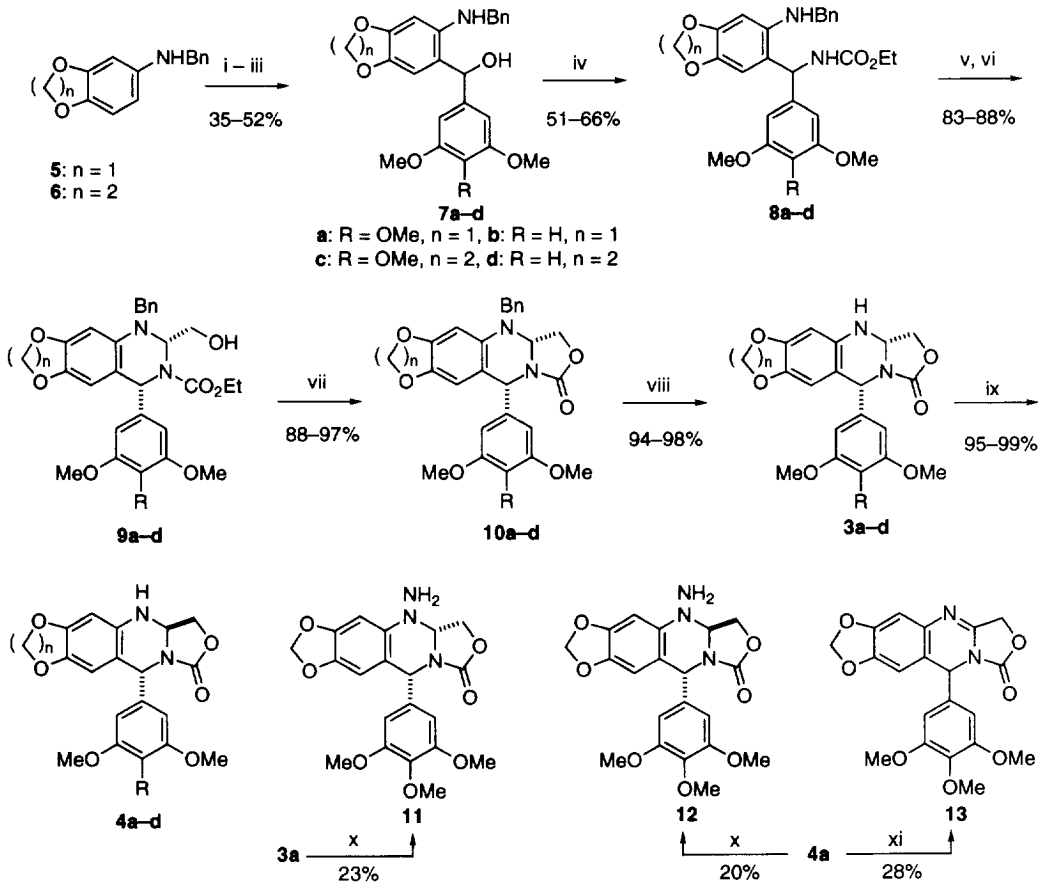


podophyllotoxin (**1**)



etoposide (**2**)





Scheme 1 *Reagents:* i, BCl_3, Et_3N, 1,2-dichloroethane, 0°C ~ room temp.; ii, 3,4,5-trimethoxybenzaldehyde or 3,5-dimethoxybenzaldehyde, Et_3N, room temp.; iii, NH_4OH; iv, ethyl carbamate, PPE, THF; v, $OHC–CO_2H$, THF; vi, $NaBH_4$, BF_3•OEt, THF; vii, MeONa, MeOH; viii, H_2, Pd/C, CH_2Cl_2–MeOH–AcOH; ix, CF_3CO_2H, $CHCl_3$; x, *O*-(2,4-dinitrophenyl)hydroxylamine, K_2CO_3, 1,4-dioxane; xi, benzoyl peroxide, $CHCl_3$.

unique antitumor activity.[4] To examine the potential of these lines of modifications, we prepared analogs **3a–d**, **4a–d**, and **11–13** and evaluated their biological properties.

The syntheses of the new analogs **3b–d** and **4b–d** have essentially followed the scheme used for **3a** and **4a** (Scheme 1).[4] The *N*-benzylanilines **5** and **6** were reacted with boron trichloride and triethylamine, and then with 3,4,5-trimethoxy- or 3,5-dimethoxybenzaldehyde. Successive hydrolysis of the boracyclic intermediates gave the benzhydryl alcohols **7a–d** which were reacted with ethyl carbamate in the presence of polyphosphate ester (PPE) to give the urethanes **8a–d**. Compounds **8a–d** were reacted with glyoxylic acid monohydrate, and successive reduction with borane produced the alcohols **9a–d**. Sodium methoxide treatment of **9a–d** afforded the oxazolones **10a–d**. Debenzylation gave the *cis* analogs **3a–d**, and successive treatment with trifluoroacetic acid afforded the *trans* analogs **4a–d**.[5] The reaction of **3a** and **4a** with *O*-(2,4-dinitrophenyl)hydroxylamine

Table 1. Antitumor Activity and Cytotoxicity of Podophyllotoxin (1) and The Diaza-podophyllotoxin Analogs

Compounds	mp (°C)	Antitumor activity against P388 leukemia[a] *T/C* (%)							Cytotoxicity L1210
		Dose[b]/ 2.5	5	10	25	50	100	250	IC_{50}[d]
1		111		109	111	toxic[c]			0.0036
3a	249–250		117	144	161	239			0.42
3b	211–212			123	137	159	214		0.22
3c	245–247		119	131	153	165	233	toxic[c]	0.052
3d	227–228 (dec.)		117	133	150	172	94	toxic[c]	0.080
4a	223–224	111	124	128	150	161	172	>326	0.050
4b	251–253			110	139	156	206	206	0.16
4c	245–246		139	144	156	177	toxic[c]		0.055
4d	196–197		114	131	147	199	toxic[c]		0.028
11	218–220			111	122	147	147	161	0.75
12	205–208		104	109	114	134	153	toxic[c]	0.090
13	204–206		104	109	109	111	109	144	0.54

[a] Single i.p. treatment on day 1. *T/C*, Median survival time of test animals/median survival time of control animals; 125% or above considered active. [b] mg/kg/injection. [c] toxic, *T/C* <85%. [d] μg/mL.

gave the *N*-aminoanalogs **11** and **12**, respectively. This amination provided a foothold which would facilitate the further derivatization of these analogs. Lead tetraacetate oxidation of **4a** afforded the dehydro analog **13**.

Podophyllotoxin (**1**) and all the analogs were assayed for *in vitro* cytotoxicity against L1210 murine leukemia cells and for antitumor activity against P388 leukemia in mice. The results are summarized in Table 1. Podophyllotoxin (**1**) was the most cytotoxic *in vitro* against L1210 leukemia cells among the compounds. For *in vivo* experiments, samples were administered i.p. on day 1 and the effects on the life span of mice bearing P388 leukemia (i.p.) were examined. Although podophyllotoxin (**1**), which was the most active *in vitro*, showed no activity with this schedule, both the *cis* analog **3a** and the *trans* analog **4a** showed potent antitumor activity. Analogs **3b–d** and **4b–d** also showed significant activity; this means that the ethylenedioxy group on ring B and the 3,5-dimethoxy group on ring E are compatible with the activity. The *N*-amino analogs **11** and **12** retained activity both *in vitro* and *in vivo*. The *trans* analogs were generally more cytotoxic than the corresponding *cis* analogs possibly due to the former being topologically more similar to podophyllotoxin (**1**) than the latter, which would also explain the dehydrated analog **13** showing reduced activity. Whether the improved antitumor activity of analogs *in vivo* may be due to pharmacokinetics or other factors remains to be clarified.

Since analogs **3a** and **4a** showed the most promising activity in terms of their *T/C* values, they were further evaluated using vincristine-resistant P388 leukemia (P388/VCR) and B16 melanoma. These results are summarized in Table 2. Although podophyllotoxin (**1**) showed only marginal (*T/C* = 125%) activity against P388/VCR, both **3a** and **4a** showed significant activity. Analogs **3a** and **4a** also showed potent antitumor

Table 2. Antitumor Activity of Podophyllotoxin (1) and Analogs 3a and 4a against P388/VCR Leukemia and B16 Melanoma

Compounds	P388/VCR[a] *T/C* (%)					B16 melanoma[b] *T/C* (%)		
Dose/	1	2.5	5	10	25	25	50	100
1	122	125	toxic[c]			110		
3a			137	toxic[c]		163	190	
4a		113	129	152	152		153	197

[a] Dose in mg/kg given i.p. on days 1–5. *T/C*, Median survival time of test animals/median survival time of control animals; 125% or above considered active. [b] Dose in mg/kg given i.p. on days 1, 5, and 9. [c] toxic, *T/C* <85%

activity against B16 melanoma, but podophyllotoxin (**1**) was inactive even at the upper dose limit (25 mg/kg, *cf.* Table 1).

To confirm the mode of action of analogs **3a** and **4a**, we examined the effects of the compounds on assembly of microtubules prepared from bovine brain.[6] The concentrations of podophyllotoxin (**1**), **3a** and **4a** necessary to inhibit microtubule assembly by 50% were 0.13, 1.7 and 0.42 μg/mL, respectively, which was correlated to the *in vitro* cytotoxicity against L1210 leukemia cells (see Table 1).

In conclusion, since some of the analogs expressed more pronounced antitumor activity over podophyllotoxin (**1**) in these experiments, the incorporation of hetero atoms within the podophyllotoxin core structure would constitute a possible approach for more promising analogs.

References and notes

1. (a) Jardine, I. *Anticancer Agents Based on Natural Product Models*; Cassady, J. M.; Douros, J. D., Ed.; Academic Press, New York, 1980, pp 319-351. (b) Ayres, D. C.; Loike, J. D. *Lignans. Chemical, Biological and Clinical Properties*; Cambridge University Press, Cambridge, 1990.
2. Cornman, I.; Cornman, M. E. *Ann. N.Y. Acad. Sci.* **1951**, *51*, 1443.
3. (a) Pearce, H. L.; Bach, N. J.; Cramer, T. L. *Tetrahedron Lett.* **1989**, *30*, 907. (b) Tomioka, K.; Kubota, Y.; Koga, K. *Tetrahedron Lett.* **1989**, *30*, 2953. (c) Tomioka, K.; Kubota, Y.; Koga, K. *J. Chem. Soc., Chem. Commun.* **1989**, 1622. (d) Van der Eycken, J.; Bosmans, J.-P.; Van Haver, D.; Vandewalle, M.; Hulkenberg, A.; Veerman, W.; Nieuwenhuizen, R. *Tetrahedron Lett.* **1989**, *30*, 3873. (e) Bosmans, J.-P.; Van der Eycken, J.; Vandewalle, M.; Hulkenberg, A.; Van Hes, R.; Veerman, W. *Tetrahedron Lett.* **1989**, *30*, 3877. (f) Lienard, P.; Royer, J.; Quirion, J.-C.; Husson, H.-P. *Tetrahedron Lett.* **1991**, *32*, 2489. (g) Itokawa, H.; Hitotsuyanagi, Y.; Takeya, K. *Heterocycles* **1992**, *33*, 537. (h) Reteurtre, F.; Madalengoitia, J.; Orr, A.; Cuzi, T. J.; Lehnert, E.; Macdonald, T.; Pommier, Y. *Cancer Res.* **1992**, *52*, 4478. (i) Tomioka, K.; Kubota, Y.; Koga, K. *Tetrahedron* **1993**, *49*, 1891. (j) Lienard, P.; Quirion, J.-C.; Husson, H.-P. *Tetrahedron* **1993**, *49*, 3995. (k) Madalengoitia, J. S.; Macdonald, T. L. *Tetrahedron Lett.* **1993**, *34*, 6237. (l) McCombie, S. W.; Tagat, J. R.; Metz, W. A.; Nazareno, D.; Puar, M. S. *Tetrahedron* **1993**, *49*, 8073. (m) Lehnert, E. K.; Miller, K. E.; Madalengoitia, J. S.; Guzi, T. J.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2411. (n) Hitotsuyanagi, Y.; Ichihara, Y.; Takeya, K.; Itokawa, H. *Tetrahedron Lett.* **1994**, *35*, 9401.
4. Hitotsuyanagi, Y.; Yamagami, K.; Fujii, A.; Naka, Y.; Tahara, T. *J. Chem. Soc., Chem. Commun.* **1995**, 49.
5. The stereochemical behavior of this reaction has been discussed in reference 4.
6. Loike, J. D.; Brewer, C. F.; Sternlicht, H.; Gensler, W. J.; Horwitz, S. B. *Cancer Res.* **1978**, *38*, 2688.

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