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4-Aza-2,3-dehydro-4-deoxypodophyllotoxins: Simple Aza-podophyllotoxin Analogues Possessing Potent Cytotoxicity

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Abstract—4-Aza-2,3-dehydro-4-deoxypodophyllotoxin analogues **3a**–**n** were synthesized through quinolines **2a**–**n**. Comparison of their cytotoxicity against P-388 leukemia cells revealed that the steric effects of the ring B substituents on the activity are greater than the electronic effects, while the presence of a methoxy group on the ring E is not essential to exhibit potent cytotoxicity. Analogues **3a** and **3b** proved to be more than twice as cytotoxic as natural podophyllotoxin (**1**). © 2000 Elsevier Science Ltd. All rights reserved.

Podophyllotoxin (**1**) is an antitumor lignan mainly found in the plants *Podophyllum peltatum* and *P. emodi*. Its mode of action is the inhibition of microtubule assembly through binding to tubulin, which is an important target in the development of novel anticancer drugs.[1,2] Although various chemical modifications of podophyllotoxin (**1**) have been made,[1–6] the substituent effects of the two aromatic rings on the activity have not yet been systematically studied since its analogues, having different aromatic substituents, are difficult to prepare; the preparation requires selective chemical manipulation of the two aromatic rings. Alternatively, a total synthesis approach necessitates elaboration of the strained 1,2-*cis*-2,3-*trans* system of such compounds.[7] We report herein novel aza-podophyllotoxin analogues, which are readily prepared from commercially available materials in a few steps, and information regarding the structure–activity relationships of the substituents on the two aromatic rings towards cytotoxicity.

Previously, we reported that quinolines **2**, 4-aza-analogues of 1-arylnaphthalene lignans, are readily prepared from anilines, benzaldehydes and tetronic acid or 2,3-cyclopentanedione in good to excellent yields.[8] During our study of the chemical properties of analogue **2a**, we found that this compound was reduced by an excess amount of sodium cyanoborohydride in acetic acid at room temperature to provide 4-aza-2,3-dehydro-4-deoxypodophyllotoxin (**3a**) in excellent yield and that it possesses potent cytotoxicity. A comparison of the crystal structure of podophyllotoxin (**1**)[9] and the MM2*[10] energy minimized structure of analogue **3a** revealed that the two aromatic rings in analogue **3a** well mimic the topology of those in podophyllotoxin (**1**), which will account for the potent activity of **3a** (Fig. 1). Since quinolines **2** can be prepared in a very convergent manner, we could prepare novel aza-analogues **3b**–**n** having various substituents on the two aromatic rings through quinolines **2b**–**n** (Table 1).[8]

Cytotoxicity of analogues **2a**–**n** and **3a**–**n** was evaluated using P-388 leukemia cells, and that of podophyllotoxin (**1**) was also evaluated simultaneously for comparison. Although quinoline analogues **2a**–**n** generally showed very weak or no activity, 4-aza-justicidin B (**2e**) showed moderate activity of the IC_{50} value of 2.0 μg/mL, which is comparable to or even more active than the reported data for justicidin B (**4**),[11,12] a cytotoxic principle from several plants, of 3.3 μg/mL against the same cell line. On the contrary, dihydroquinoline analogues **3a**–**n** showed moderate to very potent activity. Comparison of the activity of **3a**–**c**,**f**–**h**, having the same ring E structure as podophyllotoxin (**1**), revealed the substituent effects of the ring B. Analogue **3a**, having the

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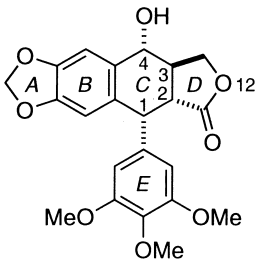

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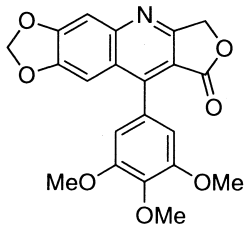
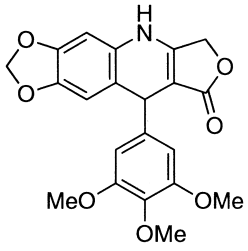


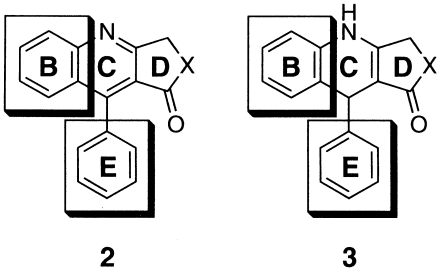
2a



3a

Table 1. Cytotoxicity of aza-ligan analogues **2a–n** and **3a–n** against P-388 leukemia cells

Substitution				Compound **2**	Compound **3**		
2/3	Ring B	Ring E	X	IC_{50} (μg/mL)	Yield (%)	Mp (°C)	IC_{50} (μg/mL)
a	**I**	**VII**	O	>100	94	271–274	0.0018
b	**II**	**VII**	O	80	98	288–292	0.0017
c	**III**	**VII**	O	>100	84	236–238	4.9
d	**III**	**VIII**	O	39	91	228–232	0.76
e	**III**	**XII**	O	2.0	90	287–290	0.77
f	**IV**	**VII**	O	29	97	135–139	2.6
g	**V**	**VII**	O	>100	80	260–262	0.0041
h	**VI**	**VII**	O	63	80	>300	0.92
i	**I**	**VIII**	O	40	93	291–293	0.048
J	**I**	**IX**	O	>100	70	289–293	0.0053
k	**I**	**X**	O	>100	85	294–298	0.13
l	**I**	**XI**	O	60	80	284–288	0.0053
m	**I**	**XII**	O	>100	98	286–289	0.030
n	**I**	**VII**	CH_2	71	52	265–268	0.028
					Podophyllotoxin (**1**)		0.0043



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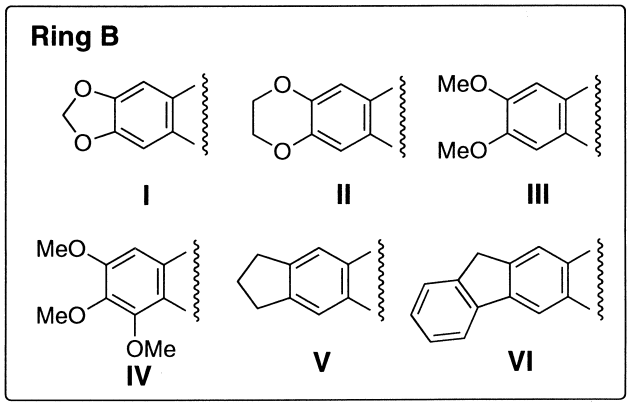
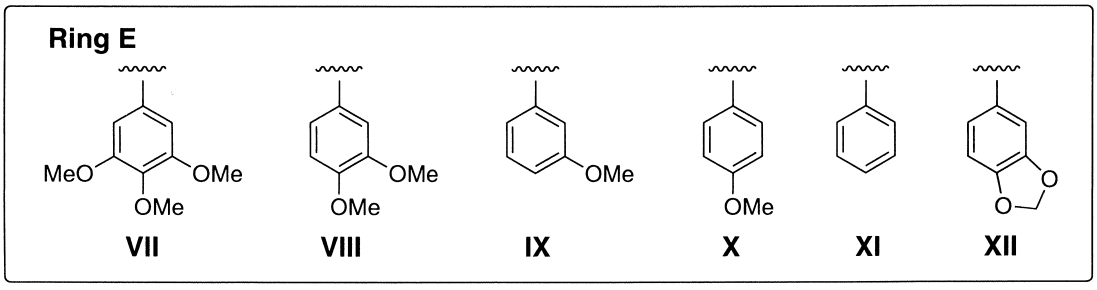




same 1,3-benzodioxole structure as **1**, and analogues **3b** and **3g** also having a cyclic ring A structure, 1,4-benzodioxane and indan, respectively, showed very potent activity, while analogues having a dimethoxyphenyl (**3c**), a trimethoxyphenyl (**3f**) or a fluorene (**3h**) unit showed moderate activity. These data suggested that the electronic effects are not important for the activity, while the steric effects are influential; although the electronic effects of the methylenedioxy, ethylenedioxy and *ortho*-dimethoxy groups are quite similar; analogue **3c**

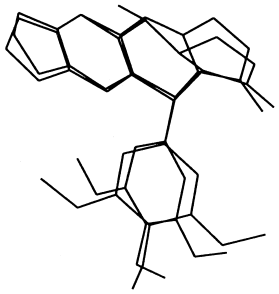
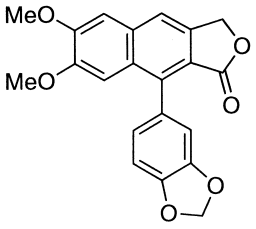


Figure 1. Superposition of the crystal structure of **1** and the MM2* minimized structure of **3a**.

having two free rotatable methoxy groups was more than 2700 times less toxic than analogues **3a** and **3b** having a cyclic ether structure, while sterically very similar but electronically dissimilar indan analogue **3g** was only 2.3 times less toxic than analogue **3a**. The reduced activity of fluorene analogue **3h** compared to that of indan analogue **3g** also supports this consideration. The same tendency was also seen in the analogues between **3d** and **3i**, and between **3e** and **3m** having a 3,4-dimethoxyphenyl or a 3,4-methylenedioxyphenyl group, respectively, as the ring E. Analogue **3f** having two trimethoxyphenyl groups as the rings B and E showed moderate activity.



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In the case of the substituent effects of the ring E, comparison among compounds **3a,i–m** was informative. Interestingly, analogue **3l** having an unsubstituted phenyl group as the ring E has fairly potent activity; only 2.9 times less toxic than analogue **3a**. Comparison of the activity of analogues **3a,i–l** suggested that a *meta*-methoxy group may enhance or not influence the activity, while a *para*-methoxy group reduces the activity since the potency of the activity of the analogues is in the order of **3a** > **3j** = **3l** > **3i** > **3k**.

Cyclic ketone analogue **3n** was about 15 times less toxic than the lactone analogue **3a**. Thus, an oxygen atom appears to be more effective to promote potent activity than a methylene group at position 12.[13]

Podophyllotoxin (**1**) possesses four chiral centers on the ring C carbon atoms, and its 1,2-*cis*-2,3-*trans* system maintains the two aromatic rings with appropriate topology, and the absolute configuration at the C-1 center is critical to express the activity.[14] Analogues **3a** and **3b**, possessing only one chiral center, are more than twice as cytotoxic as natural (–)-podophyllotoxin (**1**), although they are racemic compounds. The results obtained from this study would provide useful information for designing novel podophyllotoxin analogues having potent activity.

References and Notes

1. Jardine, I. In *Anticancer Agents Based on Natural Product Models*; Cassady, J. M.; Dourous, J. D., Eds.; Academic Press: New York, 1980; pp 319–351.
2. Ayres, D. C.; Loike, J. D. In *Lignans: Chemical, Biological and Clinical Properties*; Cambridge University Press: Cambridge, 1990; pp 85–95.
3. For a review, see: Ramos, A. C.; Peláez-Lamamié de Clairac, R.; Medarde, M. *Heterocycles* **1999**, *51*, 1443.
4. Terada, T.; Fujimoto, K.; Nomura, M.; Yamashita, J.; Wierzba, K.; Kobunai, T.; Takeda, S.; Minami, Y.; Yoshida, K.; Yamaguchi, H.; Yamada, Y. *Chem. Pharm. Bull.* **1993**, *41*, 907.
5. Van Vliet, D. S.; Lee, K. H. *Tetrahedron Lett.* **1999**, *40*, 2259.
6. Zhu, X. K.; Guan, J.; Tachibana, Y.; Bastow, K. F.; Cho, S. J.; Cheng, H. H.; Cheng, Y. C.; Gurwith, M.; Lee, K. H. *J. Med. Chem.* **1999**, *42*, 2441.
7. Ward, R. S. *Synthesis*, **1992**, 719.
8. Hitotsuyanagi, Y.; Kobayashi, M.; Fukuyo, M.; Takeya, K.; Itokawa, H. *Tetrahedron Lett.* **1997**, *38*, 8295. New compounds **2b**, **2g** and **2h** were prepared according to the method described therein. **2b**: Y. 68%, mp 284–286 °C; **2g**: Y. 96%, mp 280–282 °C; **2h**: Y. 95%, mp >300 °C.
9. Andersen, K. V.; Buchardt, O.; Hansen, H. F.; Jensen, R. B.; Larsen, S. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1871.
10. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
11. Pettit, G. R.; Cragg, G. M.; Suffness, M. I.; Gust, D.; Boettner, F. E.; Williams, M.; Saenz-Renauld, J. A.; Brown, P.; Schmidt, J. M.; Ellis, P. D. *J. Org. Chem.* **1984**, *49*, 4258.
12. Joseph, H.; Gleye, J.; Moulis, C.; Mensah, L. J.; Roussakis, C.; Gratas, C. *J. Nat. Prod.* **1988**, *51*, 599.
13. A similar tendency in activity was also observed for related compounds. Deoxypodophyllotoxin cyclic ether, where the lactone carbonyl group of 4-deoxypodophyllotoxin was substituted by a methylene unit, was 8–11 times more cytotoxic than the 4-deoxypodophyllotoxin analogue having a cyclopentane ring D structure. Gensler, W. J.; Murthy, C. D.; Trammel, M. H. *J. Med. Chem.* **1977**, *20*, 635; Loike, J. D.; Brewer, C. F.; Sternlicht, H.; Gensler, W. J.; Horwitz, S. B. *Cancer Res.* **1978**, *38*, 2688.
14. Tomioka, K.; Kubota, Y.; Koga, K. *Tetrahedron* **1993**, *49*, 1891.