Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 12, pp. 1513-1518, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00179-0

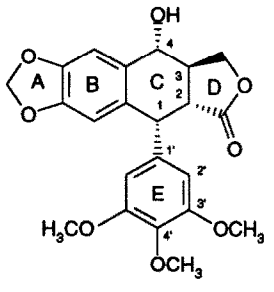
SYNTHESIS OF RING-A-OPENED ISOPICROPODOPHYLLINS AS POTENTIAL DNA TOPOISOMERASE II INHIBITORS

Ahmed Kamal*[1] and Mohsen Daneshtalab
Faculty of Pharmacy and Pharmaceutical Sciences
University of Alberta, Edmonton, Canada T6G 2N8

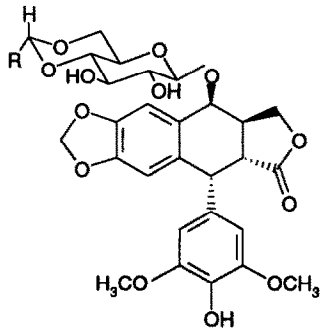
Kevin Atchison and Ronald G. Micetich
SynPhar Laboratories Inc., Edmonton, Alberta, Canada T6E 5V2

Abstract: Some representative ring-A-opened podophyllin lignans were synthesized employing the Michael Initiated Ring Closure methodology. These lignans exhibited considerable DNA topoisomerase II inhibition and were devoid of topoisomerase I inhibition.

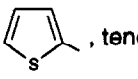
Podophyllotoxin (**1**) and its naturally occuring derivatives belonging to the class lignans are known to possess antitumor activity by inhibition of microtubule assembly and arresting cancer growth during mitosis.[2] The semisynthetic glycoside derivatives of epipodophyllotoxin, such as etoposide (VP-16, **2a**) and tenoposide (VM-26, **2b**) are extensively being used in clinic against small cell lung cancer, testicular cancer, lymphoma and leukemia.[3,4] However, it has been proposed that these semisynthetic derivatives do not interfere significantly with microtubules, but inhibit the catalytic activity of the nuclear enzyme DNA topoisomerase II (topo II), which functions in decatenating supercoiled DNA prior to transcription.[5,6] It has been found that the ring-A-opened compounds such as diphyllin (**3**) are much more effective against Sindbis virus than **1**.[7] The cis-picro-lactone (**4**) of podophyllotoxin is also shown to possess much less cytotoxicity than that to trans C/D ring juncture.[8]

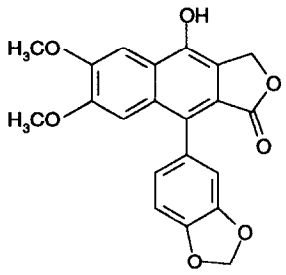


podophyllotoxin (1)

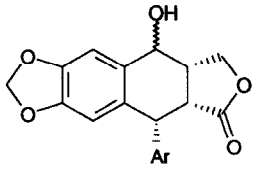


R = CH_3 , etoposide (2a)

R =  , tenoposide (2b)



diphyllin (3)



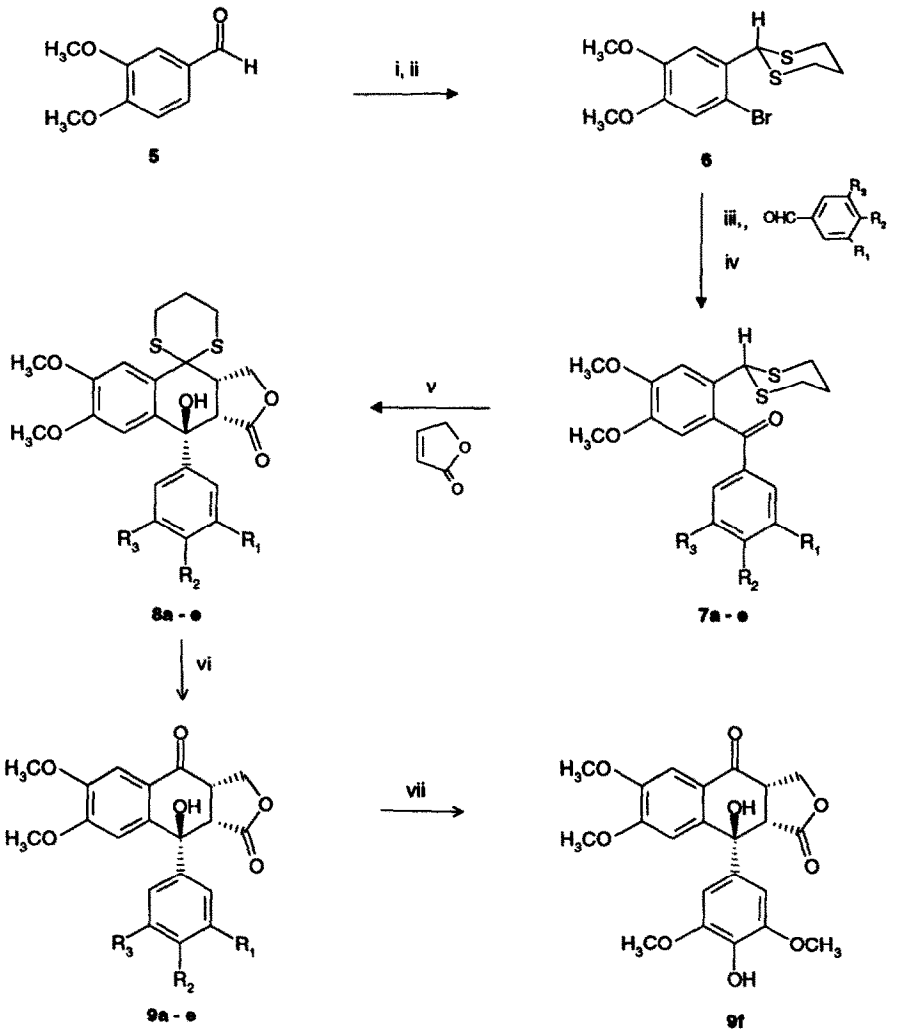
isopicropodophyllin (4)

Most reports in the literature are on the changes at the 4-position of the podophyllotoxin moiety.[9] However, in recent years there have been some attempts to modify the podophyllotoxin system itself, which has proven to have significant antitumor activity with reduced toxicity.[10,11] Based on this principle a series of ring-A-opened isopicropodophyllins have been synthesized to explore the possibility of a compound possessing topo II inhibitory activity with decreased toxicity.

These compounds were prepared by employing type II Michael Initiated Ring Closure (MIRC) strategy.[12,13] Thus, the first step was the bromination of 3,4-dimethoxybenzaldehyde (**5**) followed by the protection of the aldehydic group by 1,3-propanedithiol to give 2-bromo-4,5-dimethoxy dithiolane (**6**). This on transmelallation by n-BuLi and subsequent treatment with substituted aldehydes gave the corresponding alcohols, which upon benzylic oxidation with MnO_2 yielded the benzophenone intermediates (**7a-e**) as crystalline compounds. MIRC reaction between compounds **7** and (5H)-2-furanone led to the lignan precursors (**8**).

In a typical MIRC procedure; to a solution of dithiolane (**7a**, 3.8 gm, 0.01 mol) in dry THF (150 ml) was added LiHMDS (1.0 M, in THF, 10 ml) at -65°C to generate the anion (deep purple). To this anion was added furanone (0.84 gm, 0.01 mol) taken in THF (5 ml) gradually maintaining the temperature at -60°C. The reaction mixture was stirred for another 2h at this temperature and usual work up gave the single isomer of lignan precursor **8a** along with the starting material. These were separated by solubility differences in EtOAc-hexane (4:1) mixed solvent. In a similar manner **8b-e** were also prepared.

Finally, deprotection [14] of dithiolane lignans (**8**) afforded the desired 1-aryl substituted podophyllin lignans (**9a-e**), while **9f** was obtained by the hydrogenation of **9e**. Their relative stereochemistry was established by n.O.e. difference experiments.[15]



i. Br_2, AcOH, 3h, 79%; ii. 1,3-propanedithiol, p-TsOH, C_6H_6, 90°C, 1.5 h, 97%, iii. n-BuLi, THF, -78°C, ArCHO, 88-94%; iv. MnO_2, CH_2Cl_2, 94-96%; v. LiHMDS, THF, -65°C, 2-furanone, 36-49%; vi. $CaCO_3$, $HgCl_2$, CH_3CN (aq.), 1h, 55-65%; vii. H_2, 5% Pd/C, MeOH, 95%.

Table 1. Inhibition of DNA topoisomerase II activity (calf thymus)

9	R_1	R_2	R_3	Conc., μM	Inhibition of DNA topo II[a]; %
a					
	OCH_3	OCH_3	H	100	++ [b]
				250	+++
b					
	-O-CH_2-O-		H	100	++
				250	+++
c					
	OCH_3	OCH_3	OCH_3	100	-
				250	+
d	-CH=CH-CH=CH-		H	100	++
				250	++++
e	OCH_3	$OCH_2C_6H_5$	OCH_3	100	+
				250	++
f	OCH_3	OH	OCH_3	100	+++
				250	+++
	VP-16			50	++
				100	+++
				250	++++

[a]Measured by visualizing pBR322 DNA's topological state by agarose gel electrophoresis (Fig.1), these results were corroborated by an ethidium fluorescence assay[18]; [b]+, ++, +++, ++++, and - denotes <15%, 25-35%, 40-50%, 90-100%, and 0% inhibition.

2 4 7 10 13 16 19 22 25 28

Fig.1 Agarose gel electrophoresis for the inhibition of topo II by podophyllin lignans (**9**).

DNA Topoisomerase II was prepared from calf thymus as previously described.[16,17] Enzyme activity was assayed using pBR322 DNA substrate under catalytic relaxation reaction conditions as previously described.[18] The dithiolone lignans (**8 a-f**) showed either no or mild topo II inhibition. Whereas, compound 9 caused substantial topo II inhibition as indicated by the results given in Table 1. The agarose gel electrophoresis results indicate this more clearly (Fig. 1). Lanes 1 and 2 contain super coiled pBR322 DNA in the absence of topo II, wherein the upper band is nicked open circular (oc) DNA and the lower brighter band is super coiled covalently closed circular (ccc) DNA. Lanes 3 and 4 show ccc DNA relaxed by topo II. There are various topoisomers and the upper "bright" band co-migrates with nicked DNA, which is also relaxed. Lanes 5 to 10 show the inhibitory effects of VP-16 and VM-26 (increasing concentrations, i.e. 50, 100 and 250 μM, respectively). The bright middle band is linear DNA. Lanes 11 to 28 exhibit the effects of the podophyllin lignans (**9 a-f**) at concentrations 50, 100 and 250 μM, respectively. A linear DNA band is not obvious in any of these lanes where substantial inhibition is observed (note lane 22, 250 μM **9d**).

However, the activity is not very remarkable in comparison to VP-16 or VM-26 but this study has given some insight and lead in the design of podophyllins with possible reduced toxicity and still retaining the topo II inhibitory effect. All the compounds, like VP-16 and VM-26 were devoid of topo I inhibition. Further structural modifications and aromatization of ring C is in progress to arrive at an understanding of the structure-activity correlation for podophyllin lignan analogs.

References and Notes:

1. Visiting Scientist on sabbatical from the Indian Institute of Chemical Technology, Hyderabad 500 007, India. (Address for correspondence.)

2. Jardine, I., Podophyllotoxins, In *Anticancer Agents Based on Natural Product Models*; Cassidy, J.M.; Douros, J.D., Eds.; Academic Press:New York, 1980; p. 323.

3. O'Dwyer, P.; Leyland-Jones, B.; Alonso, M.T.; Marsoni, S.; Wittes, R.E., *N. Engl. J. Med.* **1985**, *312*, 692.

4. Bellel, R.E.; Catalano, R.B.; Mastrangelo, M.J.; Bred, D.; Koons, L.S.; *Cancer Treatment Reports* **1987**, *62*, 445.

5. Wang, Z.Q.; Shen, Y.C.; Chen, H.X.; Chang, J.Y.; Guo, X.; Cheng, Y.C.; Lee, K.H., *Pharm. Res.* **1993**, *10*, 343.

6. Terada, T.; Fujimoto, K.; Nomura, M.; Yamashita, J.; Kobunai, T.; Takeda, S.; Wierzba, K., *Chem. Pharm. Bull.* **1992**, *40*, 2720.

7. MacRae, W.D.; Hundson, J.B.; Towers, G.H.N.; *Planta Med.* **1989**, *55*, 531.

8. Ayers, D.C.; Lim, C.K.; *Cancer Chemother. Pharmacol.* **1982**, *7*, 99.

9. Wang, Z.Q.; Hu, H.; Chen, H.X.; Cheng, Y.C.; Lee, K.H.; *J. Med. Chem.* **1992,** *35*, 871.

10. Chen, Y.Z.; Wang, Y.G.; Li, J.X.; Tian, X.; Jia, Z.P.; Zhang, P.Y., *Life Sciences* **1989**, *45*, 2569.

11. Wang, J.Z.; Tian, X.; Tsumura, H.; Shimura, K.; Ito, H., *Anti-Cancer Drug Design* **1993**, *8*, 193.

12. A review on cyclizations by Michael addition reactions see Thebtaranonth, C.; Thebtaranonth, Y. *Tetrahedron*, **1990**, *46*, 1385.

13. A recent application of MIRC methodology for the regioselective total synthesis of taiwanin E.; Harrownen, D.C., *Tetrahedron Lett.*, **1991**, *32*, 3735.

14. *General procedure for deprotection:* To a solution of **8a** (1 gm) in 5% aqueous acetonitrile was added $CaCO_3$ (1.5 gm) and $HgCl_2$ (1.5 gm) and stirred for 1h at room temperature. After the completion of the reaction, it was filtered through a celite bed and the clear filtrate was evaporated without raising the temperature above 30°C (rise in temperature often led to traces of C-ring aromatized lignan). The residue was later dissolved in ethylacetate, washed twice with water followed by brine. Then drying over anhydrous Na_2SO_4 and evaporating under vacuum gave the crude deprotected product. This on subjecting to column chromatography (EtOAc-hexane, 2:3) afforded **9a** in 64% yield.

15. All new compound gave satisfactory spectral and analytical data e.g. **9c** ^1H NMR (200 MHz, $CDCl_3$ 7.51 (1H, s, AR), 7.28 (1H, s, Ar), 6.40 (2H, s, Ar), 5.82 (1H, s, OH), 4.7 (1H, d, J=9.2 Hz, C<u>H</u>HOCO), 4.32 (1H, dd, J=9.2, 5.8 Hz, CH<u>H</u>OCO), 3.98 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.73 (6H, s, 2-OCH_3), 3.46 (1H, d, J=7.4 Hz, $CHCO_2$) and 3.14 (1H, dd, J=7.4, 5.8 Hz, C<u>H</u>CH_2) ppm; Irradiation of the signal at 5.82 caused an n.O.e. enhancement at 3.46 ppm (12%); m/e found: M^+, 444.1424; $C_{23}H_{24}O_9$ requires 444.1420.

16. Drake, F.H.; Zimmerman, J.P.; McCabe, F.L.; Bartus, H.F.; Per, S.R.; Sullivan, D.M., Ross, W.E.; Mattern, M.R.; Johnson, R.K.; Crooke, S.T.; Mirabellie, C.K.; *J. Biol. Chem.* **1987**, *262*, 16,739.

17. Schomburg, U.; and Grosse, F.; *Eur. J. Biochem.* **1986**, *160*, 451.

18. Andrea, J.E.; Adachi, K.; Morgan, A.R., *Molecular Pharmacol.* **1991**, *40*, 163.

(*Received in USA* 28 *October* 1993; *accepted* 6 *May* 1994)