Published in final edited form as:

Bioorg Med Chem Lett. 2014 December 15; 24(24): 5627–5629. doi:10.1016/j.bmcl.2014.10.082.

Synthesis and evaluation of ether-linked demethylepipodophyllotoxin dimers

Norma Dunlap^a, Tracy L. J. Salyard^{a,1}, Anuradha Liyana Pathirana^a, Jeannie Stubblefield^a, Steven L. Pitts^b, Rachel E. Ashley^b, and Neil Osheroff^b

^aDepartment of Chemistry, Middle Tennessee State University, 1301 Main St., Murfreesboro, TN, USA

^bDepartment of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, USA

Abstract

A series of novel ether-linked dimers of demethylepipodophyllotoxin are topoisomerase II poisons that exhibit higher levels of double-stranded vs. single-stranded DNA cleavage than their corresponding monomers. The dimers also have higher levels of tumor cell cytotoxicity than the monomers, lending support to the two-drug model for interaction of demethylepipodophyllotoxins with human topoisomerase II α .

Keywords

demethylepipodophyllotoxin; topoisomerase; cytotoxicity

Podophyllotoxin is a toxic microtubule inhibitor, isolated from *Podophyllum peltatum linnaeus*, commonly known as the Mayapple plant.^{1–3} Epimerization at C4 of podophyllotoxin, and substitution at that position with various nucleophiles has led to the demethylepipodophyllotoxins. Demethylation at C4' of the epipodophyllotoxins affords compounds with useful antitumor activity, which act as topoisomerase II poisons rather than microtubule inhibitors. Numerous analogs bearing amine and ether substituents at C4 have been reported, and several semi-synthetic drugs derived from podophyllotoxin, including

¹current address: Department of Chemistry, Vanderbilt University, Stevenson Center, Nashville, TN, USA

Supplementary data

Experimental details for compound synthesis, for cytotoxicity assays and DNA cleavage assays are available in Supporting Information. This Supporting information is available free of charge via the Internet at <http://sciencedirect.com>.

etoposide and teniposide, are used clinically for the treatment of cancer (Figure 1).⁴⁻⁶ Others, such as the amine derivative TOP-53 have shown superior antitumor activity to etoposide and advanced to clinical trials, although TOP-53 itself was shown to have unacceptable levels of toxicity.⁷

DNA topoisomerase II is an essential enzyme that removes knots and tangles from DNA by generating transient double-stranded breaks in the nucleic acid backbone.⁸ The mechanism of enzyme action involves the formation of a covalent DNA-topoisomerase II cleavage complex. Drugs that act as topoisomerase II poisons form a ternary drug-DNA-topoisomerase II complex and stabilize the covalent DNA cleavage complex.⁸ The mechanism of action of the demethylepipodophyllotoxin analogs involves an increase in topoisomerase II-mediated DNA breaks, and etoposide inhibits the ability of topoisomerase II to ligate the cleaved DNA.⁸ It has been suggested that the activity of etoposide depends on one molecule of etoposide binding at the scissile bond on each strand of DNA to effect double-stranded cleavage, requiring two drugs per event.⁹ Evidence suggests that the two drugs act independently of each other with human topoisomerase II α .⁹ One implication for drug design, is that covalently linked dimers of demethylepipodophyllotoxin analogs might show increased levels of double-stranded cleavage, and may display increased cytotoxicity as compared to the monomers.

Although many C4 substituted demethylepipodophyllotoxins have been reported, there is only one report of demethylepipodophyllotoxin dimers, which were linked by rigid aryl diamines.¹⁰ Linking groups included phenyl, naphthyl, biphenyl, and fluorenyl diamines, with some of the dimers having useful tumor cytotoxicity. Syntheses of several novel, flexible, ether-linked dimers are reported here, including assays for the DNA cleavage activity and cytotoxicity of these compounds. These linked demethylepipodophyllotoxin analogs provide further insight into the two-drug model and may potentially provide novel antitumor agents.

The dimers of 4'-*O*-demethylepipodophyllotoxin were prepared *via* a one-pot synthesis. Treatment of podophyllotoxin with iodotrimethylsilane, followed by addition of the appropriate diol, afforded the ether-linked dimers **1-3** as shown in Scheme 1.¹¹ In a similar fashion, the corresponding monomers (**4-6**) were prepared in order to provide comparison to the dimers.

The dimers and monomers, as well as etoposide, were assayed for their DNA cleavage activity and cytotoxicity. DNA cleavage was observed in the presence of human topoisomerase II α and all dimers induced high levels of double-stranded DNA cleavage with very low levels of single-stranded cleavage (Figure 2). While the benzyl ether monomer **6** displayed a similar pattern to dimers, the alkyl ether monomers **4** and **5** induced very little DNA cleavage. A summary of the relative amounts of double-stranded vs. single-stranded DNA cleavage by the analogs at 100 μ M also is shown. Comparing alkyl dimers (**1-3**) to monomers (**4-6**) there is a pattern of a higher ratio of double-strand to single-strand cleavage for the dimers over the monomers, although the difference is not as pronounced for the more rigid benzyl ethers.

In addition to DNA cleavage, the dimers and monomers were tested for cytotoxicity in an adenocarcinoma cell line, and for cytotoxicity in normal cells. In all cases, the dimers (**1-3**) were more cytotoxic to cancer cells than both the monomers (**4-6**) and etoposide. Furthermore, they exhibited a higher selectivity ratio (Table 1).

In conclusion, several flexible ether-linked dimers of demethylepipodophyllotoxin were synthesized and compared to their corresponding monomeric ethers. As predicted, all dimers induced higher levels of double-stranded DNA cleavage than the monomers in the presence of human topoisomerase II α . This observation lends credence to the two-drug model for demethylepipodophyllotoxin-induced DNA cleavage by topoisomerase II.⁹ In addition, all dimers were more cytotoxic against tumor cells than the corresponding monomers and displayed better selectivity for tumor cells vs. normal cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Support from the Chemistry Department and the Molecular Bioscience program at MTSU is acknowledged for T.S., A.L.P and J. S. Support from National Institutes of Health Grant GM033944 is acknowledged for N.O. and from National Science Foundation Graduate Research Fellowship DGE-0909667 is acknowledged for R.E.A.

References

1. MacRae WD, Hudson JB, Towers BHN. *Planta Med.* 1993; 55:531. [PubMed: 2559420]
2. Kuhn M, von Wartburg A. *Helv Chim Acta.* 1969; 52:948–955. [PubMed: 5798941]
3. Desbne S, Giorgi-Renault S. *Curr Med Chem – Anti-Cancer Agents.* 2002; 2:71–90.
4. Hande KR. *Eur J Cancer.* 1998; 34:1514–1521. [PubMed: 9893622]
5. Meresse P, Dechaux E, Monneret C, Bertounesque E. *Curr Med Chem.* 2004; 11:2443–2466. [PubMed: 15379707]
6. Lee KS, Beer AS, Wang ZQ, Mori M, Kuo YH, Liu SY, Chang JY, Han FS, Cheng YC. *J Med Chem.* 1990; 33:1364–1368. [PubMed: 2158562]
7. Utsugi T, Shibata J, Sugimoto Y, Aoyage K, Wierzbicki K, Kobunai T, Terada T, Oh-hara T, Tsuruo T, Yamada Y. *Cancer Res.* 1996; 56:2809–2814. [PubMed: 8665518]
8. Deweese JE, Osheroff N. *Nucleic Acids Res.* 2009; 37:738–748. [PubMed: 19042970]
9. Bromberg KD, Burgin AB, Osheroff N. *J Biol Chem.* 2003; 278:7406–7412. [PubMed: 12473657]
10. Kamal A, Laxman E, Ramesh Kanna GB, Reddy PSMM, Rehana T, Arifuddin M, Neelima K, Kondapi AK, Dastidar SG. *Bioorg Med Chem.* 2004; 12:4197–4209. [PubMed: 15246096]
11. Duca M, Guianvarc’h D, Meresse P, Bertounesque E, Dauzon D, Kraus-Berthier L, Thiriot S, Leonce S, Pierre A, Pfeiffer B, Renard P, Arimondo PB, Monneret C. *J Med Chem.* 2005; 48:593–603. [PubMed: 15658872]

Figure 1.
Structures of podophyllotoxin, etoposide and TOP-53.

Figure 2. Results of DNA cleavage assay, and comparison of double-strand vs. single-strand DNA cleavage at 100 μM .

Scheme 1.

Synthesis of dimers and monomers. Reagents and conditions: (a) TMSI, CH_2Cl_2 , 0° , 2h (b) Ba_2CO_3 , $\text{HO}-(\text{CH}_2)_n-\text{OH}$, 25° , CH_2Cl_2 , 18h (c) Ba_2CO_3 , ROH, 25° , CH_2Cl_2 , 18h

Table 1

Cytotoxicity of compounds **1-6** in cancer cells (A549) and normal cells (L6).

Compound	IC ₅₀ values ^a (μM)		
	A549	L6	selectivity ^b
1	0.07 ± 0.02	0.30 ± 0.18	4.3
2	0.12 ± 0.03	0.30 ± 0.07	2.5
3	0.36 ± 0.14	0.60 ± 0.51	1.7
4	2.87 ± 1.05	4.62 ± 0.29	1.6
5	5.97 ± 2.60	4.55 ± 1.31	0.76
6	3.86 ± 0.86	4.55 ± 0.01	1.2
etoposide	2.87 ± 0.90	18.99 ± 4.37	6.7

^aValues reported are from average of three trials. A549 is an adenocarcinoma cell line; L6 is a normal rat skeletal muscle cell line.

^bRatio of IC₅₀ for L6/A549.