Bioorganic & Medicinal Chemistry Letters, Vol.2, No.10, pp. 1213-1218, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

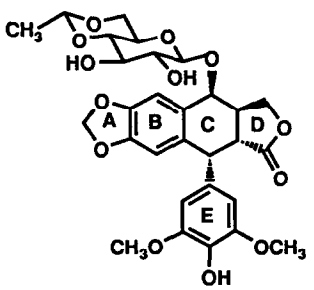
SYNTHESIS AND BIOLOGICAL EVALUATION OF 4'-DESHYDROXY-4'-METHYL ETOPOSIDE AND TENIPOSIDE ANALOGS

Mark G. Saulnier*, Karen L. LeBoulluec, Byron H. Long, Dolatrai M. Vyas,
Alfred R. Crosswell, and Terrence W. Doyle
Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, CT 06492.

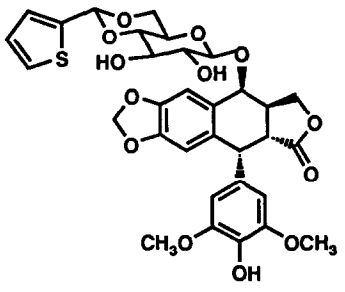
(*Received* 24 *February* 1992)

ABSTRACT: *The E-ring 4'-deshydroxy-4'-methyl analogs of the clinical antitumor agents etoposide and teniposide were synthesized from the corresponding 4'-triflates. These compounds display significant antitumor activity against murine P388 leukemia in vivo but are only 1/5 as cytotoxic against a human colon tumor cell line (HCT-116) grown in culture. These analogs also show inferior activity vis-a-vis etoposide and teniposide when evaluated for their ability to inhibit purified human topoisomerase II.*

The semisynthetic podophyllotoxin analog etoposide (VP-16, **1**) is a clinically prescribed antineoplastic drug, particularly effective for the treatment of testicular and small cell lung cancers.[1] As one of the least toxic of all chemotherapeutic agents, the combination of orally administered VP-16 with cisplatin shows further utility towards inoperable non-small cell lung cancer.[2] The somewhat more potent congener, teniposide (VM-26, **2**), has additional activity for treating childhood leukemia and brain gliomas.[3] The mechanism of action for **1** and **2** involves topoisomerase II (topo II) inhibition resulting in DNA strand cleavage and cell death.[4] Additionally, *in vivo* or *in vitro* oxidative activation of VP-16 and VM-26 leads to their corresponding ortho-quinones, which are implicated as well in the overall SAR profile for these drugs.[5]

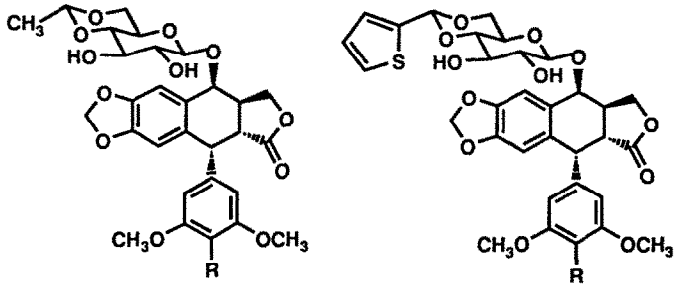


1 (VP-16)



2 (VM-26)

We have previously reported the synthesis and biological evaluation of 4'-deshydroxyetoposide **3**, which displayed good in vitro cytotoxic potency against a human colon tumor cell line (HCT-116) and significant in vivo antitumor activity in a P388 murine leukemia screen, yet was inactive against purified topo II using a P4 phage DNA assay, while still capable of producing DNA strand breaks in a whole cell assay.[6] Our data implicated a third mechanism of action, which could relate to direct DNA binding, since metabolism of **3** to an ortho-quinone would be highly unlikely to occur *in vivo* or *in vitro*. The introduction of a CH_3 moiety in place of the H atom at the 4'-position of **3** would certainly rule out bioactivation to ortho-quinone species, as well as to begin to develop the SAR for carbon substitution at this site. We now report the synthesis and biological evaluation of the etoposide and teniposide 4'-deshydroxy-4'-methyl analogs **4** and **5**, along with their 4'-vinyl (**6** and **7**) and 4'-allyl (**8** and **9**) homologues.



3, R = H
4, R = CH_3
6, R = $CH{=}CH_2$
8, R = $CH_2CH{=}CH_2$

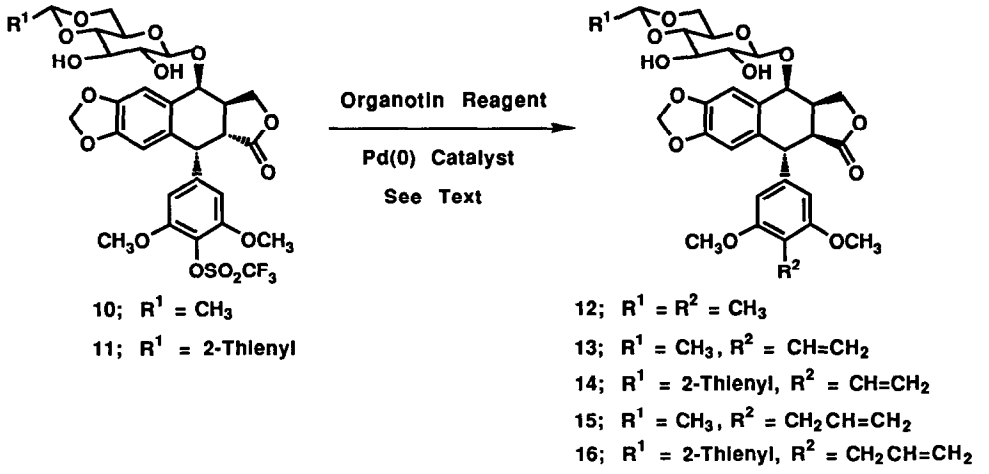
5, R = CH_3
7, R = $CH{=}CH_2$
9, R = $CH_2CH{=}CH_2$

Etoposide **1** and teniposide **2** are converted to their corresponding 4'-triflates **10** (76%) and **11** (86%) [$(CF_3SO_2)_2NPh$, $EtN(i\text{-}Pr)_2$, CH_3CN, 40 °C, 7 days]. A recent report by Saa described a catalytic system (0.15 equiv $PdCl_2(Ph_3P)_2$, 0.4 equiv Ph_3P, 8 equiv LiCl, refluxing DMF) for the successful palladium(0)-catalyzed cross coupling of 2,6-dimethoxy phenol triflates with organostannane reagents.[7] Using Me_4Sn under these conditions, the etoposide 4'-triflate **10** is cleanly converted to the 4'-methyl analog (83%), but not without suffering concomitant epimerization of the trans lactone to the thermodynamically more stable, biologically inactive cis (picro) lactone isomer **12**.[8] Generation of the enolate of **12** (LDA, THF, -78 °C), followed by kinetic protonation (HOAc, -78 °C), provides a ca 3:1 mixture of recovered **12** and the desired trans lactone isomer **4** after purification by preparative TLC.

In similar fashion (Scheme I), the etoposide and teniposide 4'-triflates **10** and **11** are transformed, using vinyltributyltin, into the corresponding picro 4'-vinyl analogs **13** (48%) and **14** (49%). Likewise, allyltributyltin affords the picro 4'-allyl

derivatives **15** (63%) and **16** (70%). Epimerization (*vide supra*) of **13-16** gives the desired trans lactone isomers **6-9**.

SCHEME I



10; $R^1 = CH_3$

11; R^1 = 2-Thienyl

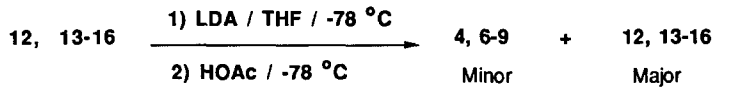
12; $R^1 = R^2 = CH_3$

13; $R^1 = CH_3$, $R^2 = CH{=}CH_2$

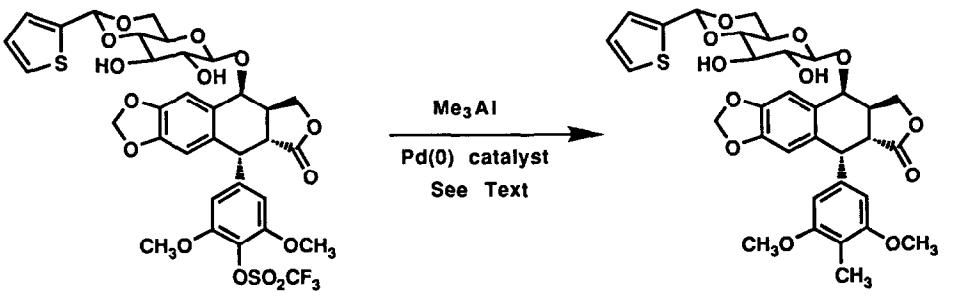
14; R^1 = 2-Thienyl, $R^2 = CH{=}CH_2$

15; $R^1 = CH_3$, $R^2 = CH_2CH{=}CH_2$

16; R^1 = 2-Thienyl, $R^2 = CH_2CH{=}CH_2$



Alternatively, the use of 2 equiv of Me_3Al (0.15 equiv $PdCl_2(Ph_3P)_2$, 0.5 equiv Ph_3P, 3 equiv LiCl, refluxing DMF, 2 h), in lieu of Me_4Sn, delivers a much improved ratio (8:1) of trans:picro lactone isomers **4** (40%) and **12** (5%) using the etoposide triflate **10**: The corresponding teniposide 4'-triflate **11** converts exclusively to the desired trans 4'-methyl analog **5** in 48% yield following flash chromatography over silica gel.



11 **5**

We have previously described the details of the experimental protocol for the *in vitro* cytotoxicity assay in the context of 4'-deshydroxy etoposide **3**.[6] Compound **3**

(4'-H) had shown the ability to overcome resistance in our cytotoxicity screen using human colon tumor sensitive (HCT-116) and resistant (HCT/VP35) cells grown in culture (resistance ratio = 0.5).[6,9] In comparison, **4** (4'-CH_3) does not exhibit this quality, giving a ratio of IC_{50} values[10] of 5.7 for the HCT/VP35 vs HCT-116 cell lines. Overall, the 4'-CH_3 analogs of etoposide **4** and teniposide **5** are $1/5^{th}$ as cytotoxic as **1** (VP-16) and **2** (VM-26), and the vinyl and allyl analogs **6-9** are only $1/50^{th}$ as cytotoxic towards the etoposide sensitive HCT-116 cells.

In regards to the *in vivo* antitumor activity[11], both of the 4'-CH_3 analogs **4** and **5** are significantly active in the P388 murine leukemia screen, whereas the 4'-vinyl and 4'-allyl derivatives of etoposide (**6** and **8**) are totally inactive at the highest doses tested (100 mg/kg/dose).[12]

Compounds **1-9** were evaluated for their abilities to stabilize the transient intermediate formed between DNA and topoisomerase II (topo II) during DNA strand passage. For this purpose, we used a quantitative assay based on the formation of linear DNA from covalently closed, circular PM2 phage DNA in the presence of purified human topo II. The data is summarized in Table I. The 4'-methyl analogs **4** and **5** are $1/12^{th}$ and $1/7^{th}$ as potent, and the alkenyl analogs, **6** and **8**, and **7** and **9**, are $1/18^{th}$ and $1/9^{th}$ as potent as their respective parents **1** and **2** for action on topo II. The 4'-methyl analogs **4** and **5** also display preferentially enhanced DNA cleavage at fewer sites than VP-16 or VM-26. This observation could explain why **4** and **5** show significant in vivo antitumor activity. However, a proposed third mechanism of action, which could relate to direct DNA binding[6] (*vide supra*), may be operative in the overall biological profile of etoposide, teniposide, and related congeners.

TABLE I. DNA-Topo II Intermediate Stabilization by VP-16 and VM-26 4'-Modified Analogs.

4'-GROUP	COMPD	DSB[a]	COMPD	DSB[a]
OH	1	305	2	346
H	3	27		
CH_3	4	26	5	49
VINYL	6	17	7	36
ALLYL	8	17	9	42

[a] Double strand DNA breakage reflecting stabilization of enzyme-DNA intermediates. Values are expressed in ng of linear DNA per unit of enzyme per mM of drug.

In conclusion, we have described the first synthesis and biological evaluation of etoposide and teniposide analogs for which the 4'-hydroxyl group has been replaced with a carbon bearing substituent. As for the related 4'-hydrogen-substituted analog **3**, which we have previously described[6], the 4'-methyl analogs of etoposide and teniposide retain modest antitumor activity and comparable potency relative to the

parent drug. However, these 4'-methyl-substituted analogs **4** and **5** do not overcome etoposide acquired-resistance in a human colon tumor cell line *in vitro*, a desirable property that was previously noted for the related 4'-hydrogen-substituted derivative **3**. The ability of the 4'-CH_3 and 4'-H analogs to stabilize the intermediate formed between DNA and topo II is comparable, yet significantly diminished vis-a-vis etoposide and teniposide. Substitution at the E-ring 4'-position by the sterically larger vinyl and allyl moieties, results in complete loss of *in vivo* antitumor activity and greatly diminished *in vitro* cytotoxic potency.

While topo II inhibition remains a relevant target, such carbon substituted analogs as **4-9** described herein cannot derive their biological effects from the ortho-quinone mechanism (*vide supra*). In addition, since topo II inhibition is greatly diminished (relative to etoposide or teniposide), the interesting pharmacological properties of compounds **3-5** are consistent with a contributory *third mechanism of action* for these agents, and probably etoposide and teniposide as well.[13]

Acknowledgement: We thank David R. Langley and Henry Wong for discussions.

References and Notes

1. Issel, B. F.; Muggia, F. M.; Carter, S. K. *Etoposide (VP-16) Current Status and New Developments*; Academic Press: New York, **1984**.

2. a) Splinter, T.; Kok, T.; Kho, S.; Lameris, H.; Kate, F.; Dalesio, O.; Dolman, B.; Palmen, F.; Bouvy, J.; Burghouts, J.; Simonis, F.; Harper, P.; Rankin, E.; Reijswoud, I.; Hoogenhuijze, J. *Semin. Oncol.* **1986**, *13*, 97. b) Klastersky, J. *Semin Oncol.* **1986**, *13*, 104.

3. a) Stahelin, H.; Wartburg, A. von. *Progress in Drug Research*; Jucker, E., Ed.; **1989**, *33*, 238-241. b) Haaxma-Reiche, H.; Berendsen, H. H.; Postmus, P. E. *J. Neuro-Oncol* **1988**, *6*, 231.

4. a) Wozniak, A.J.; Ross, W.E. *Cancer Res.* **1983**, *43*, 120. b) Long, B.H.; Minocha, A. *Proc. Am. Assoc. Cancer Res.* ***1983***, *24*, 321. c) Minocha, A.; Long, B. H. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 165. d) Long, B. H.; Brattain, M. G.; see Chapter 5 in ref 1. e) Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K. M.; Liu, L. F. *J. Biol. Chem.* **1984**, *259*, 13560. f) Ross, W.; Rowe, T.; Glisson, B.; Yalovich, J.; Liu, L. F. *Cancer Res.* **1984**, *44*, 5857.

5. a) Sinha, B. K.; Trush, M. A.; *Biochem. Pharmacol.* **1983**, *32*, 3495. b) Sinha, B. K.; Myers, C. E. *Ibid.* **1984**, *33*, 3725. c) Sinha, B. K.; Trush, M. A.; Kalyanaraman, B.*Ibid.*.**1985**, *34*, 2036. d) Haim, N.; Nemec, J.; Roman, J.; Sinha, B. K. *Ibid.* **1987**,

36, 527. e) Haim, N.; Nemec, J.; Roman, J.; Sinha, B. K. *Cancer Res.* **1987**, *47*, 5835.

6. Saulnier, M. G.; Vyas, D. M.; Langley, D.R.; Doyle, T. W.; Rose, W.C.; Crosswell, A. R.; Long, B. H. *J. Med. Chem.* **1989**, *32*, 1418.

7. Martorell, G.; Garcia-Raso, A.; Saa, J. M. *Tetrahedron Lett.* **1990**, *31*, 2357.

8. Cis (picro) vs trans lactone isomers are readily distinguished by their characteristic ^{1}H NMR spectra. See, Brewer, C. F.; Loike, J. D.; Horwitz, S. B.; Sternlicht, H.; Gensler, W. J. *J. Med. Chem.* **1979**, *22*, 215.

9. The HCT/VP35 cell line was determined to have normal levels of the 170K P-glycoprotein but diminished levels of topo II, thus suggesting that a multidrug resistance mechanism may not be operative. See, Long, B. H. ; Wang, L. ; Lorico, A. ; Wang, R. C. C. ; Brattain, M. G. ; Casazza, A. M. ; *Cancer Research* **1991**, *51*, 5275.

10. The IC_{50}, in ug/mL, is the dose that reduces by 50%, after 72 h, cell growth *in vitro* as compared to controls.

11. Descriptions of the protocol for these experiments have been previously described in detail. See, a) Rose, W. C. *Anticancer Res.* **1986**, *6*, 557. b) Rose, W. C.; Schurig, J. E.; Huftalen, J. B.; Bradner, W.T. *Cancer Res.* **1983**, *43*, 1504.

12. The %T/C refers to the median survival time of drug-treated mice compared to saline-treated controls. A T/C=125% denotes the minimum criteria for activity. The 4'-methyl VP-16 analog **4** gave a T/C = 271% (120 mg/kg/dose) vs 343% for VP-16 (120 mg/kg/dose). The 4'-methyl VM-26 analog **5** displayed T/C=165% (200 mg/kg/dose) vs 240% for VP-16 (80 mg/kg/dose). Tumor cells were inoculated ip on day 1 and drug was administered ip on days 5 and 8.

13. All new compounds in this communication gave satisfactory analytical and spectroscopic data in full accord with their structures. 300 MHz ^{1}H NMR ($CDCl_3$) for 4'-methyl etoposide **4**: 6.82 (s, 1H), 6.53 (s, 1H), 6.17 (s, 2H), 5.95 (d, 2H), 4.91 (d, 1H, J=3.3 Hz,), 4.72 (dd, 1H, J=9.9 Hz), 4.61-4.56 (m, 2H), 4.39 (t, 1H, J=9.7 Hz), 4.21-4.13 (m 2H), 3.65 (s, 6H), 3.58-3.49 (m, 2H,), 3.41-3.27 (m,6H), 2.96-2.84 (m, 1H), 1.98 (s, 3H),1.36 (d, 3H, J=4.9 Hz). High resolution mass spectrum, calcd for $C_{30}H_{34}O_{12}$; 587.2129 $(M+H)^+$. Found; 587.2135.