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Antitumor agents. Part 232: Synthesis of cyclosulfite podophyllotoxin analogues as novel prototype antitumor agents☆

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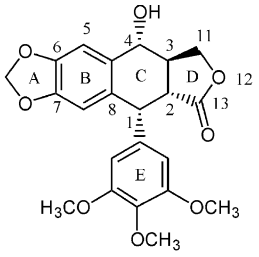
Abstract—An 11,13-*O*,*O*′-cyclosulfite GL-331 analogue (**7**) was synthesized in six steps from podophyllotoxin and evaluated as a potential antitumor agent. Compound **7** was significantly cytotoxic against human tumor cell lines, but showed no inhibition against human DNA topoisomerase II in vitro. This compound represents a novel prototype of antitumor podophyllotoxin analogues.

Podophyllotoxin (**1**)[2] is a bioactive lignan isolated from *Podophyllum peltatum* L., *P. emodi* W., or *P. pleianthum* H. Two semisynthetic derivatives of **1**, etoposide (**2**) and teniposide (**3**), are currently used in front-line cancer chemotherapy against various cancers, including small-cell lung cancer, testicular carcinoma, lymphoma, and Kaposi's sarcoma.[3,4] Although **1** is known as an antimicrotubule agent, **2** and **3** inhibit the catalytic activity of DNA topoisomerase II (topo II).[5,6]

The *trans*-fused γ-lactone D ring in **2** and its analogues are susceptible to metabolic inactivation through



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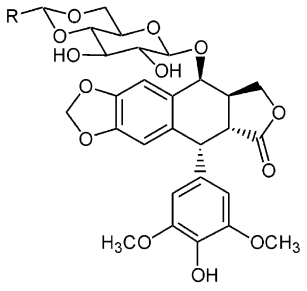
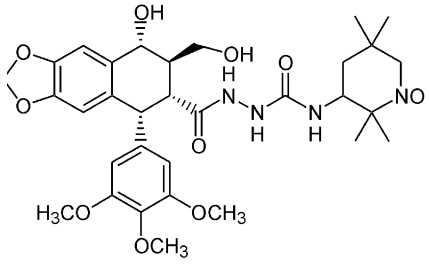


2. R = CH_3

3. R = 



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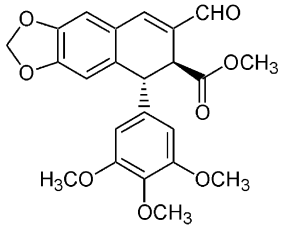

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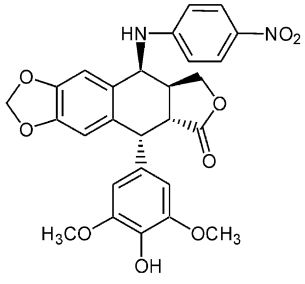

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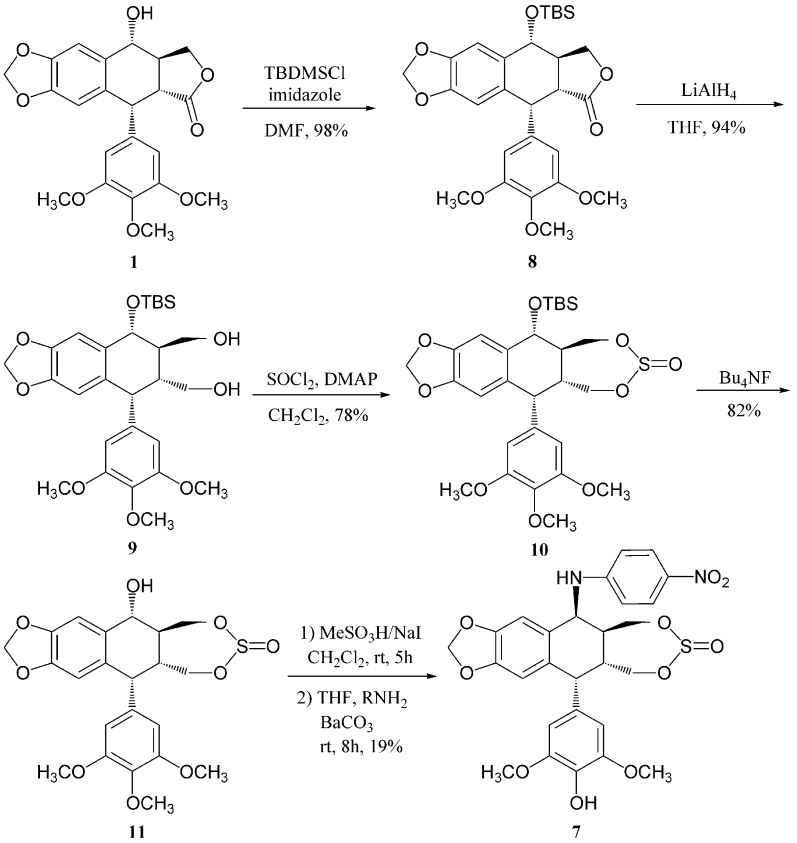
C_2-epimerization or hydrolysis to give the inactive *cis*-fused lactone or open-ring hydroxy acid metabolites, respectively.[7] However, SAR studies indicated that the *trans*-lactone ring is essential to maintain topo II inhibition, presumably because it forces the molecules into a twisted conformation that ensures optimal interaction with the enzyme. Therefore, previous efforts to circumvent the metabolic pathways have primarily focused on bioisosteric approaches to minimize conformational alteration.[7]

Recently, a spin-labeled analogue of **1**, GP-11 (**4**) was reported as a low immunosuppressive antitumor agent, which increases the mitotic index and results in G2/M, and to a lesser extent, S arrest.[8] In addition, methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**5**) was found to be a highly selective antitumor agent against HT-29 colon carcinoma.[9] Several aldehydes related to this compound but with different configurations have been synthesized and evaluated for their cytotoxic activities in neoplastic cell lines. All of them lacked the lactone ring but maintained their cytotoxicity at, or under, the μM level.[10]

These results reaffirm the importance of D ring structure in **1**-related pharmacological profiles and the feasibility of producing clinically useful agents with dramatically modified D rings. Accordingly, we have designed and synthesized a 11,13-*O,O′*-cyclosulfite analogue (**7**) of GL-331 (**6**), which is a 4β-arylamino analogue of **1** currently under Phase II clinical evaluation against several forms of cancer, especially etoposide-resistant malignancies.[11] In this modification, the lactone D ring is replaced with a seven-membered sulfite ring and the *trans*-configuration is retained.

As shown in Scheme 1, compound **11** was synthesized in four steps from podophyllotoxin (**1**). Compound **1** was treated initially with *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole to afford **8**, in which the 4-hydroxy group is protected. Reduction of **8** with lithium aluminum hydride ($LiAlH_4$) in tetrahydrofuran yielded diol **9**.[12] Subsequent ring closure with thionyl chloride and 4-dimethylaminopyridine (DMAP) in methylene chloride provided the seven-membered cyclic sulfite ester **10**. Compound **10** was a mixture of diastereomers with respect to the orientation of the lone pair electrons of the sulfur atom.[13] Deprotection of **10** with tetrabutylammonium fluoride (Bu_4NF) in tetrahydrofuran gave **11**, which can be separated by silica gel column chromatography to afford optical active diastereomers **11a** and **b**.[14] The 4β-(*p*-nitroanilino) derivative **7** was prepared from **11** (a mixture of two diastereomers) according to the previously described procedure.[15]

Compound **7** was evaluated against multiple human tumor cell lines (HTCL) and showed significant inhibition against HTCL replication in vitro (Table 1).[16] Although compound **7** was similar to GL-331 in overall potency, its spectrum of activity and dose–response profile against certain HTCL differed from those of

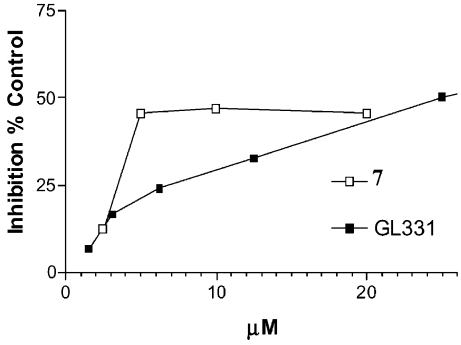


Scheme 1. Synthesis of **7** from podophyllotoxin.

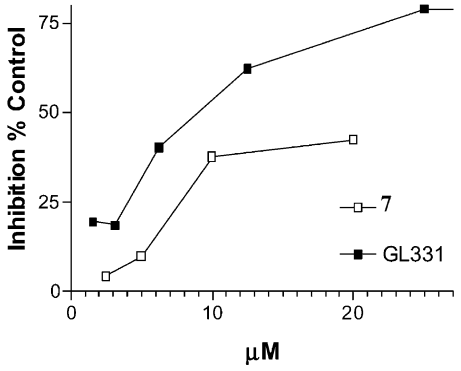
Table 1. Inhibition of HTCL replication by GL-331 and **7**

Compd	ED_{50} (μM)[a]										
	KB	KB-7d	KB-VIN	MCF-7	HCT-8	1A9	SK-MEL-2	PC-3	HOS	U87-MG	A549
6	3.5	1.5	4.1	24.5	1.6	<1.6	3.0	8.9	<1.6	5.5	<0.1
7	5.1	2.9	7.1	2.5[b]	10.0	4.2	7.9	>20[b]	6.0	8.0	8.0

[a] ED_{50} is the concentration of drug that afforded 50% reduction in cell number after a 3-day incubation. Cell lines: KB, nasopharyngeal; KB-7d, etoposide resistant; KB-VIN, vincristine resistant; MCF-7, breast; HCT-8, ileocecal; 1A9, ovarian; SK-MEL-2, melanoma; PC-3, prostate; HOS, bone; U-87-MG, glioblastoma; A549, lung.
[b] Plateau dose–response was observed (refer to Fig. 1).



a. **7** and GL-331 *vs* MCF-7 cell replication
7: ED_{50} ~ 2.5 μM
(46% plateau up to 20 μM)
GL-331: ED_{50} = 24.5 μM



b. **7** and GL-331 *vs* PC-3 cell replication
7: ED_{50} >20 μM
(43% plateau)
GL-331: ED_{50} = 8.9 μM

Figure 1. Different dose–response effects of **7** and GL-331.

GL-331. Plateau dose–response was observed in the replication of drug-treated MCF-7 and PC-3 cells (Table 1 and Fig. 1).

In spite of the significant activity against HTCL replication, compound **7** was inactive in topoisomerase II inhibition in vitro assays (Fig. 2).[17] The unique dose–response profile, activity spectrum, and inability to induce topoisomerase II-mediated DNA breaks suggested that compound **7** might be a novel prototype of antitumor podophyllotoxin analogues with a mechanism different from GL-331. Further synthetic and biological investigation of this series of compounds is underway.

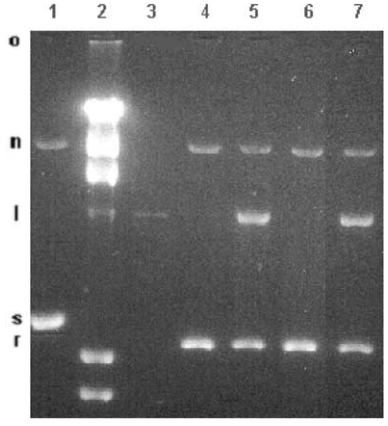


Figure 2. In vitro topoisomerase II inhibition by compounds GL-331, **7**, and etoposide. Lane 1: pBR322 DNA (predominantly supercoiled), Lane 2: M. Wt. markers, Lane 3: linear BR322 DNA, Lane 4: Enzyme control, Lane 5: GL-331, Lane 6: compound **7** and Lane 7: etoposide. o: Origin, n: Nicked, l: Linear, s: Supercoiled, and r: Relaxed.

Acknowledgements

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12. The protocol used to synthesize **9** was similar to that used for its 4′-demethylated congener as previously described in: Zhou, X. M.; Lee, K. J. H.; Cheng, J.; Wu, S. S.; Chen, H. X.; Guo, X.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem*. **1994**, *37*, 287.
13. Compound **10**: To a solution of **9** (300 mg, 0.564 mmol) in 10 mL of dry CH_2Cl_2 was added DMAP (60 mg, 0.48 mmol) and $SOCl_2$ (0.15 mL, 1.99 mmol), and the mixture was stirred at room temperature for 1.5 h. The mixture was then diluted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography using EtOAc–hexane (1:3) as an eluent to give 255 mg (78%) of **10**: white solid, mp 119–120 °C, $[\alpha]_D^{27.4}$ −193.05 (*c* 0.47, $CHCl_3$); IR (film) 2960, 1590, 1504, 1483, 1329 cm^{-1}; MS *m*/*e*: 578 $[M]^+$; ^1H NMR ($CDCl_3$) δ 6.91 (s, 1/2 H, 5-H), 6.90 (s, 1/2 H, 5-H), 6.344 (s, 1/2 H, 8-H), 6.341 (s, 1/2 H, 8-H), 6.21 (s, 2H, 2′, 6′-H), 5.91 (d, 2H, *J* = 1.8 Hz, OCH_2O), 4.78–4.70 (m, 1/2 H, 13-H), 4.50–4.35 (m, 2H, 4-H, 13-H), 4.21–3.86 (m, 3H, 1-H, 11-H_2), 3.84 (s, 3/2H, 4′-OCH_3), 3.83 (s, 3/2H, 4′-OCH_3), 3.78 (s, 3H, 3′, 5′-OCH_3), 3.60–3.51 (m, 1/2 H, 13-H), 2.50 (m, 1/2 H, 2-H), 2.38 (m, 1/2 H, 2-H), 2.33 (m, 1/2 H, 3-H), 2.20 (m, 1/2 H, 3-H), 0.97 (s, 9/2H, *t*-BuSi), 0.96 (s, 9/2H, *t*-BuSi), 0.30 (s, 3H, CH_3-Si), 0.19 (s, 3H, CH_3-Si). Anal. calcd $C_{28}H_{38}O_9SiS$. 1/2 H_2O.
14. Compound **11**: 200 mg (0.35 mmol) of **10** in 5 mL of THF was added 365 mg (1.38 mmol) of Bu_4NF. The reaction mixture was stirred at room temperature for 2 h. After the reaction was completed as monitored by TLC, the reaction mixture was evaporated to dryness and CH_2Cl_2 was added. The solution was then washed with H_2O and dried over anhydrous Na_2SO_4. After the solvent was removed in vacuo, the crude products was chromatographed on silica gel (eluent: EtOAc:hexane = 1:1) to separate the two diastereoisomers **11a** and **11b**. Compound **11a**: white solid, mp 129–130 °C (dec.), $[\alpha]_D^{29.2}$ +71.9 (*c* 0.16, CH_2Cl_2); IR (film) 3500 (OH), 2938, 1591, 1507, 1203, 1125 cm^{-1}; MS *m*/*e*: 487 $[M + Na]^+$; ^1H NMR ($CDCl_3$) δ 7.03 (s, 1H, 5-H), 6.36 (s, 1H, 8-H), 6.17 (s, 2H, 2′, 6′-H), 5.93 (s, 2H, OCH_2O), 4.80 (dd, 1H, *J* = 10.5, 12.0 Hz, 13-H), 4.50–4.29 (m, 3H, 4-H, 11-H_2), 4.09–3.98 (m, 1H, 1-H), 3.83 (s, 3H, 4′-OCH_3), 3.78 (s, 6H, 3′,5′-OCH_3), 3.55 (dd, 1.5H, *J* = 10.3, 12.5 Hz, 13-H), 2.50 (m, 1H, 2-H), 2.40–2.24 (m, 1H, 3-H), 2.17 (d, 1H, *J* = 8.1 Hz, OH). Anal. calcd $C_{22}H_{24}O_9S$ 1/2 H_2O. Compound **11b**: white solid, mp 122–123 °C (dec.), $[\alpha]_D^{28}$ −215.7 (*c* 0.055, CH_2Cl_2); IR (film) 3500 (OH), 2938, 1591, 1507, 1203, 1125 cm^{-1}; MS *m*/*e*: 487 $[M + Na]^+$; ^1H NMR ($CDCl_3$) δ 7.01 (s, 1H, 5-H), 6.34 (s, 1H, 8-H), 6.16 (s, 2H, 2′, 6′-H), 5.91 (s, 2H, OCH_2O), 4.54 (dd, 1H, *J* = 3.5, 12.5 Hz, 4-H), 4.44–4.30 (m, 2H, 13-H_2), 4.07–3.97 (m, 2H, 11-H_2), 3.88 (dd, 1H, *J* = 3.0, 12.0 Hz, 1-H), 3.82 (s, 3H, 4′-OCH_3), 3.76 (s, 6H, 3′,5′-OCH_3), 2.39 (m, 1H, 2-H), 2.22 (m, 1H, 3-H), 1.80 (m, 1H, OH). Anal. calcd $C_{22}H_{24}O_9S$. 3/4 H_2O.
15. The protocol used to prepare **7** was similar to that previously described in: Kamal, A.; Laxman, N.; Ramesh, G. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2059. Compound **7**: 49 mg (0.1 mmol) of a mixture of **11a** and **11b** was dissolved in 2 mL of dry CH_2Cl_2. Sodium iodide (45 mg, 0.3 mmol) was added and the mixture was stirred for 5 min. $MeSO_3H$ (29 mg, 0.3 mmol) was added in dropwise at 0 °C and stirred for 5 h at room temperature. Nitrogen was bubbled through the solution to drive off the excess HI; the solution was then evaporated in vacuo and used for the next step without further purification. To the above crude product, anhydrous $BaCO_3$ (40 mg, 0.2 mmol) and 4-nitroaniline (17 mg, 0.12 mmol) in 2 mL of dry THF under nitrogen were added and stirred for 8 h at room temperature. The reaction mixture was diluted with EtOAc, filtered, washed with water and brine, dried over anhydrous Na_2SO_4, and purified by column chromatography (eluent: EtOAc:hexane = 1:1) to afford 11 mg (19%) of **7**: Yellow power. Mp 144–146 °C, $[\alpha]_D^{30}$ −192.5 (*c* 0.2, acatone); IR (film) 2936, 1585, 1366, 1216, 1115 cm^{-1}; MS *m*/*e*: 570 $[M-H]^+$; ^1H NMR ($CDCl_3$) δ 8.15 (d, 1H, *J* = 9.0 Hz, 3″,5″-H), 8.13 (d, 1H, *J* = 9.0 Hz, 3″,5″-H), 6.72 (d, 1H, *J* = 9.0 Hz, 2″, 6″-H), 6.70 (d, 1H, *J* = 9.0 Hz, 2″, 6″-H), 6.60 (s, 1/2 H, 5-H), 6.58 (s, 1/2 H, 5-H), 6.38 (s, 1/2 H, 8-H), 6.37 (s, 1/2 H, 8-H), 6.10 (s, 1H, 2′, 6′-H), 6.09 (s, 1H, 2′, 6′-H), 5.92, 5.90 (d, 2H, *J* = 1.4 Hz, OCH_2O), 4.98 (m, 1H, NH), 4.41–4.25 (m, 2H, 4-H, 13-H), 4.24–4.11 (m, 2H, 1-H, 13-H), 4.02–3.96 (m, 2H, 11-H_2), 3.80 (s, 3H, 3′,5′-OCH_3), 3.79 (s, 3H, 3′,5′-OCH_3), 2.80 (m, 1/2 H, 2-H), 2.62 (m, 1H, 3-H), 2.55 (m, 1/2 H, 2-H).
16. Cell growth inhibition was assayed using the sulforhodamine B (SRB) protocol developed by Rubinstein et al. (Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, M. R. *J. Natl. Cancer Inst*. **1990**, *82,* 1113). Drug exposure was for 3 days, and the ED_{50} value was interpolated from dose–response data.
17. The plasmid DNA relaxation assay was carried out according to the procedure described previously (Krishnan, P.; Bastow, K. F. *Anti-Cancer Drug Des*. **2000**, *15*, 255). Assays were performed with drug concentrations of 50 μM.