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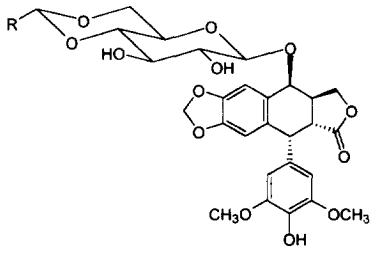
ANTITUMOR AGENTS. 177.[1] DESIGN, SYNTHESES, AND BIOLOGICAL EVALUATION OF NOVEL ETOPOSIDE ANALOGS BEARING PYRROLECARBOXAMIDINO GROUP AS DNA TOPOISOMERASE II INHIBITORS

Zheng Ji,[a] Hui-Kang Wang,[a] Kenneth F. Bastow,[a] Xiao-Kang Zhu,[a] Sung Jin Cho,[a] Yung-Chi Cheng,[b] and Kuo-Hsiung Lee[a,*]

[a]*Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599*
[b]*Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510*

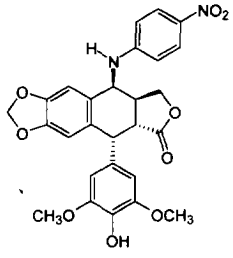
Abstract: Novel water-soluble 4β-amino-4'-*O*-demethylepipodophyllotoxin derivatives (**6-12**), designed to enhance minor groove binding ability, were synthesized and screened against NCI's in vitro disease-oriented human tumor cells. Among them, 4'-*O*-demethyl-4β-[*N*-(1'''-methyl-4'''-nitro-pyrrole-2'''-carbonyl)-4"-aminoanilino]-4-desoxypodophyllotoxin (**10**) and its HCl salt (**11**) were found to exhibit potent cytotoxic activities (average log GI_{50} = -6.91, -7.00, and -5.01 for **10**, **11**, and etoposide, respectively). Compounds **10** and **12** were further tested for their inhibitory activities against DNA topoisomerase II. Compound **10** again exhibited a superior activity profile compared to that of etoposide, displaying increased cytotoxicity against KB and KB-7d cells (ID_{50}/LD_{50} = 0.04/0.15 and 0.2/0.25 for KB and KB-7d cells, respectively), topoisomerase II inhibitory activity (12.5 μM), and cellular protein DNA complex formation (225%).

Introduction: Etoposide (VP-16, **1**) and teniposide (VM-26, **2**), semisynthetic glycosides of podophyllotoxin,[2] are both potent anticancer chemotherapeutic agents. GL-331 (**3**), discovered and developed in the author's laboratory, is another podophyllotoxin derivative that is currently in the phase II clinical trial for treatment of various cancers.[3] Unlike podophyllotoxin, which inhibits the assembly of microtubules, the primary mode of action of etoposide, teniposide, and GL331 is to stabilize the covalent topoisomerase II-DNA cleavage complex, break DNA strands, and cause cell death.[4,5]



Etoposide (VP-16, 1): R = CH_3

Teniposide (VM-26, **2**): R =

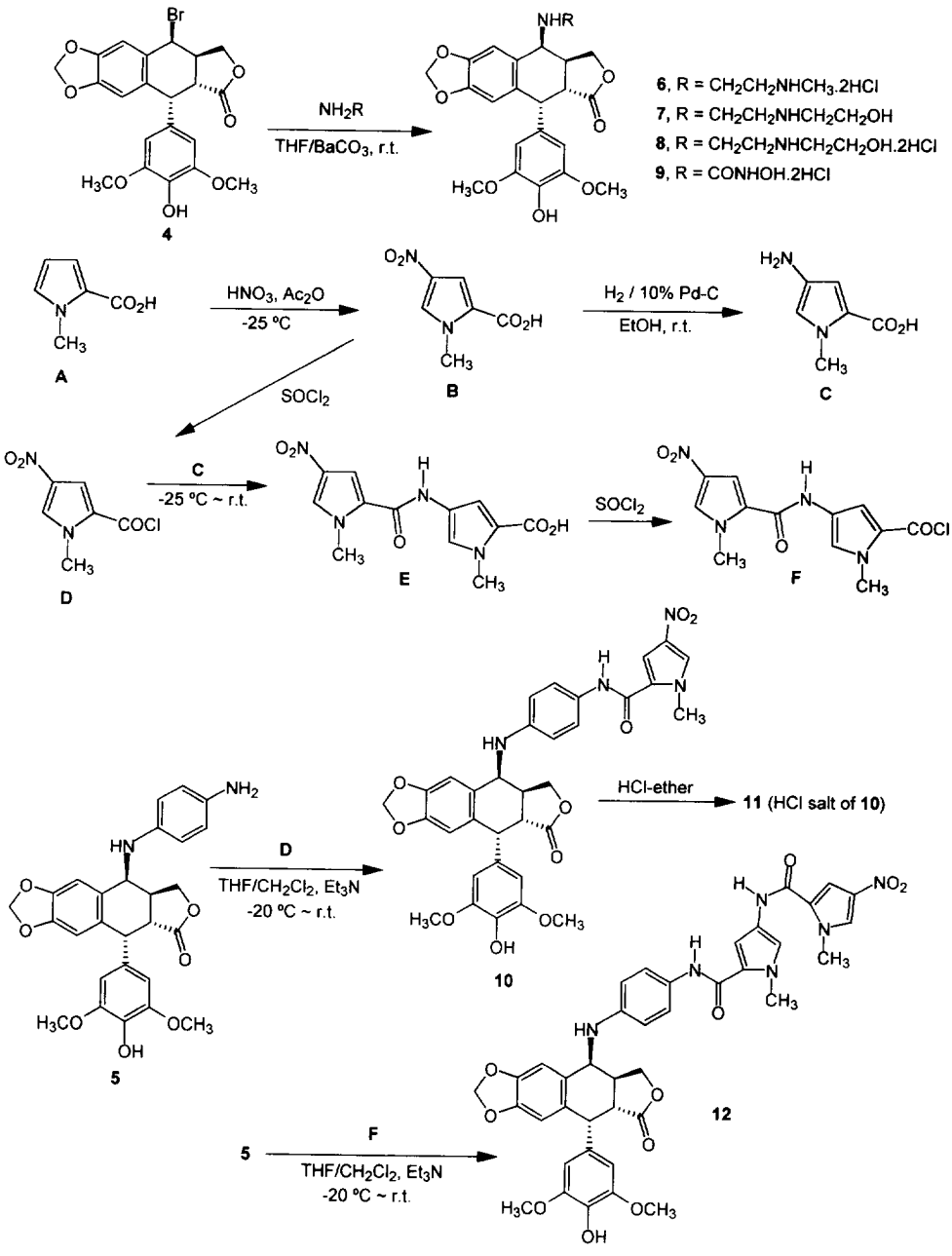


GL-331 (**3**)

Recently, we reported the development of three-dimensional quantitative structure-activity relationship of 101 analogs of 4'-*O*-demethylepipodophyllotoxin using the methodology of comparative molecular field analysis (CoMFA)[6] and q^2-GRS technique.[7,8] The steric and electrostatic contour plots of the final CoMFA/q^2-GRS model were compared with the DNA phosphate backbone environment of the DNA-4'-*O*-demethylepipodophyllotoxin analog complex. The comparison revealed that the CoMFA steric and electrostatic fields are compatible with stereochemical properties of the DNA backbone. On the basis of this study, we hypothesized that an increase in the minor groove binding ability of the epipodophyllotoxin derivatives should enhance the antitopoisomerase II activity.

As a part of our ongoing effort to understand the mechanism of enzyme inhibition and design inhibitors with clinical potential, we have investigated the proposed hypothesis by linking a known minor groove binding functional group to active compound **5**.[9] In the present study, the basic fragment, 1-methyl-4-nitro-2-pyrrolecarboxyl (see **D**) and 1-methyl-4-(1'-methyl-4'-nitro-2'-pyrrolecarboxamido)-4-amino-2-pyrrolecarboxyl (see **F**), which are structural components of the cytotoxic polypeptide netropsin,[10] were linked to the compound **5**. In addition, simple, straight alkylamino derivatives were also synthesized (**6-9**). These derivatives were then evaluated for their cytotoxic and inhibitory activities against various tumor cell lines and DNA topoisomerase II.

Chemistry: The compounds **6-9** were synthesized (Scheme 1) from 4β-bromo-4'-demethyl-epipodophyllotoxin (**4**) with corresponding amines according to a previously published method.[11] Compounds **10**[12] and **12** were synthesized (Scheme 1) by condensation of compound **5**, and the corresponding fragments, 1-methyl-4-nitro-2-pyrrolecarbonyl chloride (**D**) and 1-methyl-4-(1'-



Scheme 1. Synthesis of Etoposide Analogues 6-12

Table 1. Inhibition of in vitro tumor cell growth[a,b] by selected synthetic 4-substituted epi-podophyllotoxin analogs

Compound No.	Cytotoxicity log GI_{50} (M)[c]									Average		
	MOLT-4	NCI-H23	HCC-2998	SF-295	M14	OVCAR-8	ACHN	DU-145	MCF7	log GI_{50}[d]	log TGI[d]	log LC_{50}[d]
6	< -8.00	-6.79	-6.57	-7.53	-6.40	-6.51	-7.18	-6.44	-7.84	-6.62	-5.52	-4.41
7	-7.57	-6.18	-5.47	-5.99	-5.39	-5.62	-6.47	-5.64	-7.02	-5.73	-4.67	-4.08
8	< -8.00	-5.88	-5.90	-6.75	-5.40	-5.58	-5.63	-5.56	-7.41	-5.87	-4.92	-4.18
9	-5.31	-5.03	-5.39	-5.30	-4.43	-4.46	-5.22	-5.14	-5.11	-5.01	-4.03	-4.00
10	< -8.00	-7.31	-6.65	-7.29	-7.34	-7.01	-7.93	-7.77	< -8.00	-6.91	-5.71	-4.28
11	< -8.00	-7.41	-6.61	-6.86	-7.20	-6.90	-7.85	-7.84	< -8.00	-7.00	-6.28	-5.80
12	< -8.00	-6.14	-5.22	-5.31	-5.26	-6.12	-6.64	-6.43	-7.32	-5.93	-4.54	-4.09
1 (Etoposide)[e]	-5.99	-5.08	-4.74	-5.00	-6.09	-4.78	-6.06	-6.07	-5.36	-5.01	-4.11	-3.28

[a]Data obtained from NCI's in vitro disease-oriented human tumor cells screen (see ref 15 for detail). [b]MOLT-4, leukemia cell line; NCI-H23, non-small cell lung cancer cell line; HCC-2998, colon cancer cell lines; SF-295, CNS tumor cell lines; M14, melanoma; OVCAR-8, ovarian cancer cell lines; ACHN, renal cancer cell line; DU-145, prostate cancer cell line; and MCF7, breast cancer cell line. [c]Log concentrations that reduced cell growth to 50% of level at start of experiment. [d]Average log GI_{50}, log TGI (total growth inhibition), and log LC_{50} values calculated from all cell lines tested. [e]Data taken from NCI Web site (http://epnws1.ncifcrf.gov:2345/dis3d/drugs/main/141540.html)

Table 2. Biological evaluation of compounds 10 and 12 as DNA topoisomerase II inhibitors

Compound No.	Cytotoxicity: ID_{50} / LD_{50} (μM)[a]		Inhibition of DNA Topoisomerase II activity[b]	Cellular protein DNA complex formation, %[c]	
	KB	KB-7d	IC_{100} (μM)	(2.5 μM)	(25 μM)
1 (Etoposide)	0.20 / 3	25 / ND[d]	100	100	100
10	0.04 / 0.15	0.2 / 0.25	12.5	225	100
12	0.08 / >10	0.2 / >10	100	30	26

[a]ID_{50} is the concentration of drug which affords 50% reduction in cell number after two days of continuous treatment; LD_{50} is the drug concentration that reduces by half colony forming ability of cells treated for three hours then replated. Mean values were from two-independent experiments. ID_{50} data for KB-7d taken from Ferguson et al. (15). [b]Each compounds was examined at 100, 50, 25, 12.5, and 6.25 μM. The IC_{100} value is the lowest test concentration that completely inhibited enzyme activity. [c]Each compound was tested in KB cells at various concentrations (range 40 to 0.125 μM). Normalized values relative to etoposide standard are from a representative experiment; protein-DNA complexes were induced 2- and 35-fold at 2.5 and 25 μM etoposide respectively. [d]ND = Not tested.

methyl-4'-nitro-2'-pyrrolecarboxamido)-4-amino-2-pyrrolecarbonyl chloride (**F**), which were prepared based on the literature method.[10,13]

Biological Assay and Results: The compounds **6-12** were submitted to National Cancer Institute (NCI) for in vitro disease-oriented human tumor cells screening.[14] The cytotoxic activities of target compound against nine cell lines out of 60 cell lines tested are summarized in Table 1. The results shown that all compounds, except **9,** which displays almost equal potency to etoposide, were about 10- to 100-fold more potent than etoposide. Among them, compound **10** and its HCl salt (**11**) were the most potent cytotoxic agents with average log GI_{50} values about -7, especially against leukemia (MOLT-4) and breast cancer (MCF7) cells with log GI_{50} values of < -8.00. A comparison of the average log LC_{50} of these compounds with etoposide revealed that the newly synthesized analogues were approximately 6- to 330-fold less lethal to the tested cells.

The compounds **10** and **12** were further tested for their inhibitory activities against DNA topoisomerase II by our in-house assay.[15] The relative activities of compounds **10** and etoposide against DNA topoisomerase II and cell growth inhibition are consistent with a mode of action for **10** as a "cleavable-complex" type inhibitor (Table 2). Growth of an etoposide resistant cell line, KB-7d, is less cross-resistant to **10** than to etoposide (5- and 125-fold respectively). Moreover, KB-7d cells are no more twofold less susceptible to compound **10**'s cytotoxic action than KB cells. Compound **12** appears to be a weak cleavable-complex type topoisomerase II inhibitor by comparison to etoposide and **10**. Although **12** is a potent inhibitor of KB cell growth, the analogue is substantially less cytotoxic than **10**, and it is unclear from the present work whether topoisomerase II is an intracellular drug target for compound **12** (Table 2).

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12. 4'-*O*-Demethyl-4β-[*N*-(1'''-methyl-4'''-nitro-pyrrole-2'''-carbonyl)-4''-aminoanilino]-4-desoxypodophyllotoxin (**10**): yield 53%; yellow crystals from CH_2Cl-MeOH; mp 211-213 °C; $[\alpha]D_{26}$ -93.0 (*c* 0.03, MeOH); IR (KBr) 3360 (OH), 3130, 2900, 1750 (C=O), 1600, 1475 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.87 (s, 1H, CON*H*), 8.29 (s, 1H, N*H*), 8.18 (br, 1H, 5'''-H), 7.61 (br, 1H, 3'''-H), 7.43 (d, *J* = 8.4 Hz, 2H, 3'', 5''-H), 6.78 (s, 1H, 5-H), 6.70 (d, *J* = 8.4 Hz, 2H, 2'', 6''-H), 6.55 (s, 1H, 8-*H*), 6.27 (s, 2H, 2', 6'-H), 6.01 (s, 1H, 12-α*H*), 5.98 (s, 1H, 12-β*H*), 4.85 (dd, *J* = 4.0, 6.7 Hz, 1H, 4-H), 4.50 (d, *J* = 4.0 Hz, 1H, 1-H), 4.36 (t, *J* = 7.3 Hz, 11-α*H*), 3.95 (s, 3H, N-CH_3), 3.73 (dd, *J* = 8.4, 10.5 Hz, 11-β*H*), 3.65 (s, 6H, 3',5'-OCH_3), 3.30 (dd, *J* = 4, 13.5 Hz, 1H, 2-*H*), 3.01 (m, 1H, 3-*H*). Anal. calcd. for $C_{33}H_{30}N_4O_{10}\cdot H_2O$: C 60.00, H 4.88, N 8.48; Found C 59.93, H 4.85, N 8.38.
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