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INDUCTION OF REVERSIBLE PROTEIN-LINKED DNA BREAKS IN HUMAN OSTEOGENIC SARCOMA CELLS BY NOVEL CYTOCIDAL COLCHICINE DERIVATIVES WHICH INHIBIT DNA TOPOISOMERASE II *IN VITRO*: ABSENCE OF CROSS-RESISTANCE IN A COLCHICINE-RESISTANT SUB-CLONE[1]

Kenneth F. Bastow,[a] Hiroshi Tatematsu,[b+] Ibrahim D. Bori,[a] Yasuhiro Fukushima,[b#] Li Sun,[b] Barry Goz,[c] and Kuo-Hsiung Lee[b*]

[a]*Division of Medicinal Chemistry and Natural Products,* [b]*Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA*

[c]*Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, NC 27599, USA*

[+]*Visiting Research Scholar, Present Address: Pfizer Pharmaceuticals, Inc. Nagoya Biochemical Laboratory, 5-Gochi, Taketoyo-cho, Chita-gun, Aichi 470-23, Japan*

[#]*Visiting Research Scholar, Present Address: Nitto Denko Corp. 1-1-25 Schimohozumi, Ibaraki, Osaka 567, Japan*

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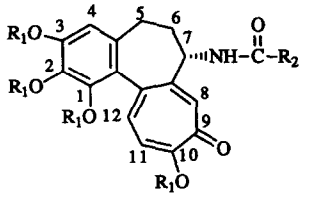
Abstract: Two colchicine derivatives gave dose-dependent cytocidal effects in human osteogenic sarcoma cells. Unlike colchicine, the analogues stimulated formation of intracellular protein-linked DNA breaks, they inhibited DNA topoisomerase II *in vitro*, and their cytotoxic action was not modulated by the P-glycoprotein drug-efflux pump.

Colchicine (**1**)(Fig. 1), a major alkaloid found in *Colchicum autumnale* has anti-inflammatory and anti-cancer properties,[2,3] and is used in the treatment of gout and Familial Mediterranean Fever.[3,4] Since binding to tubulin and inhibition of cellular mitosis probably account for the principal pharmacological actions of **1**, efforts at chemical modification have focused on the design of spindle toxins with improved therapeutic properties.[5-7] As a result of our continuing investigation of bioactive colchicinoids,[7,8] N-trifluoroacetyl-1,2,3,10-tetrademethyldeacetyl-colchicine (**2**)(Fig. 1) and N-(3',4',5'-trihydroxybenzoyl)-1,2,3,10-tetrademethyldeacetylcolchicine (**3**)(Fig. 1) were prepared and found to have interesting acitvities not shared by **1**. These properties



1: $R_1 = CH_3$, $R_2 = CH_3$

2: $R_1 = H$, $R_2 = CF_3$

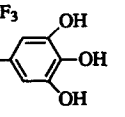
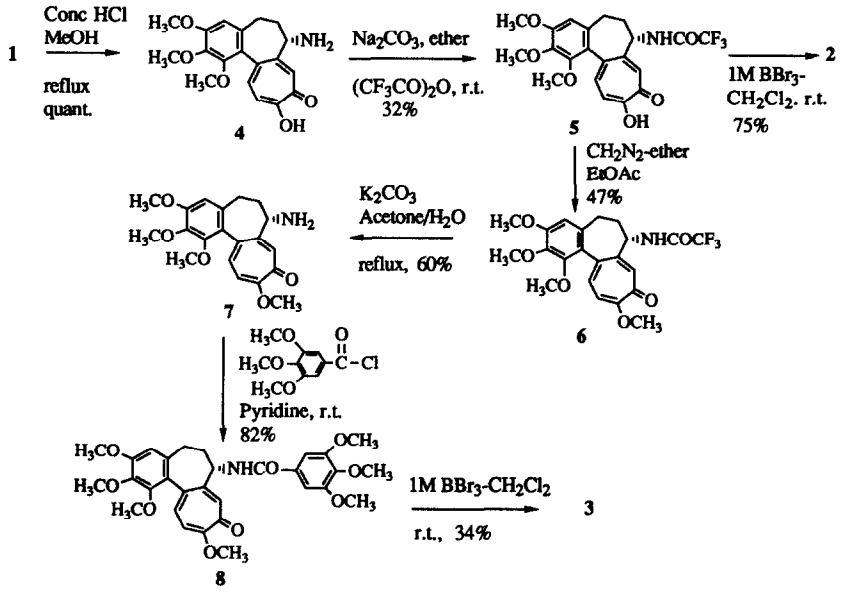
3: $R_1 = H$, $R_2 =$ 

Figure 1. Structures of 1, 2, and 3

include the ability to induce intracellular protein-linked DNA breaks,[9] to inhibit DNA topoisomerase II *in vitro*,[10] and to be cytotoxic,[11,12] but not affected by overexpression of P-glycoprotein.[13]

The syntheses of compounds **2** and **3** are outlined in Scheme I. Hydrolysis of **1** with concentrated hydrochloric acid in methanol afforded 10-demethyldeacetylcolchicine (**4**),[14] which, after acylation with trifluoroacetic acid anhydride,[15] gave 10-demethyl-N-trifluoroacetyldeacetylcolchicine (**5**). N-Deacetylcolchicine (**7**) was obtained by hydrolyzing N-trifluoroacetyldeacetylcolchicine (**6**) which was prepared by methylation of **5** with diazomethane.



Scheme I. Syntheses of Compounds 2 and 3

Condensation of **7** with 3,4,5-trimethoxybenzoylchloride in pyridine at room temperature afforded N-(3',4',5'-trimethoxybenzoyl)-deacetylcolchicine (**8**) in good yield. Exhaustive demethylation of **5** and **8** with borontribromide in dichloromethane at room temperature gave **2** and **3**, respectively.[16] The structures of all intermediates and final products are secured by spectroscopic and analytical data.[17]

Compounds **1**-**3** inhibited the growth of human osteogenic sarcoma (Hos) cells,[18] with **2** and **3** being more than three orders of magnitude less toxic than **1**.[19] The plating efficiency of cells[12] was markedly affected after brief exposure to cytotoxic concentrations, and LD_{50} values of 14 ± 1 and 18 ± 3 μM were determined following a one-hour treatment with compounds **2** and **3**, respectively.

Under similar conditions, compounds **2** and **3** induced protein-linked DNA breaks[9] in a dose-dependent fashion, and this effect was rapidly reversed after drug-media were replaced with fresh media (Fig. 2). In contrast, compound **1** at 50 μM gave no effect.

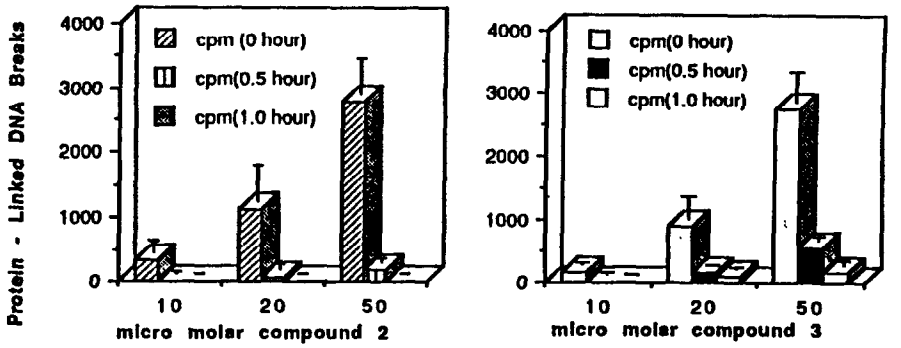


Figure 2. Induction and Reversibility of Protein-Linked DNA Breaks in Hos Cells. Cells were treated with compounds for one-hour, then drug media were replaced with fresh media. Protein-Linked DNA breaks were detected at 0, 0.5, and 1.0 hour after media replacement.[9] Data shows cpm recovered for duplicate treatments each at three drug concentrations.

Since a class of clinically useful DNA topoisomerase poisons is known to cause the accumulation of DNA-enzyme covalent intermediates in treated cells,[20] the effects of compounds **2** and **3** on DNA topoisomerase II *in vitro* catalytic activity was investigated. As shown in Fig. 3, compounds **2** and **3** inhibited enzyme activity, being, respectively, about 2-fold and 17-fold more potent than the epipodophyllotoxin derivative VP-16. In contrast, compound **1** gave no effect at 200 μM.

Overproduction of a plasma-membrane-associated protein known as P-glycoprotein can confer resistance to a selecting drug as well as crossresistance to a variety of structurally and functionally unrelated drugs.[13] Compounds **2** and **3** were tested against a sub-clone, Hos col^R selected using **1**.[22] The resistant cells were 38-fold resistant to **1** and as shown in Fig. 4A, Hos col^R overproduced P-glycoprotein based on a Western blot immunostained using a specific monoclonal antibody.[23] The EC_{50} values for growth inhibition by compounds **2** and **3** were 26 μM and 28 μM, respectively, which were similar to the values determined using the parent Hos cells.[19] In addition, **2** and **3** induced similar levels of protein-linked DNA breaks in both cell lines, unlike VP-16 which was about 5-fold less effective in Hos col^R (Fig. 4B).

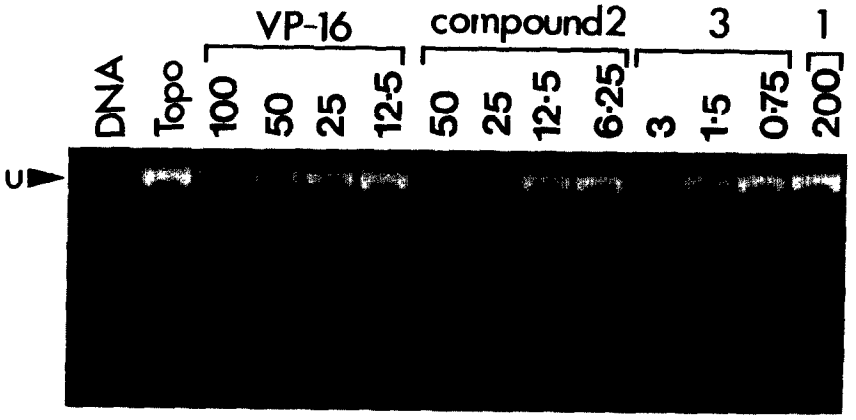


Figure 3. Inhibition of Hos DNA Topoisomerase II *in vitro* Catalytic Activity. Enzyme[21] was measured, using the P4 DNA unknotting assay,[10] in the presence and absence of 2-fold serial dilutions of compounds. The inhibitor VP-16 was included as the positive drug control. "U" shows the position of the unknotted DNA product formed in the reactions.

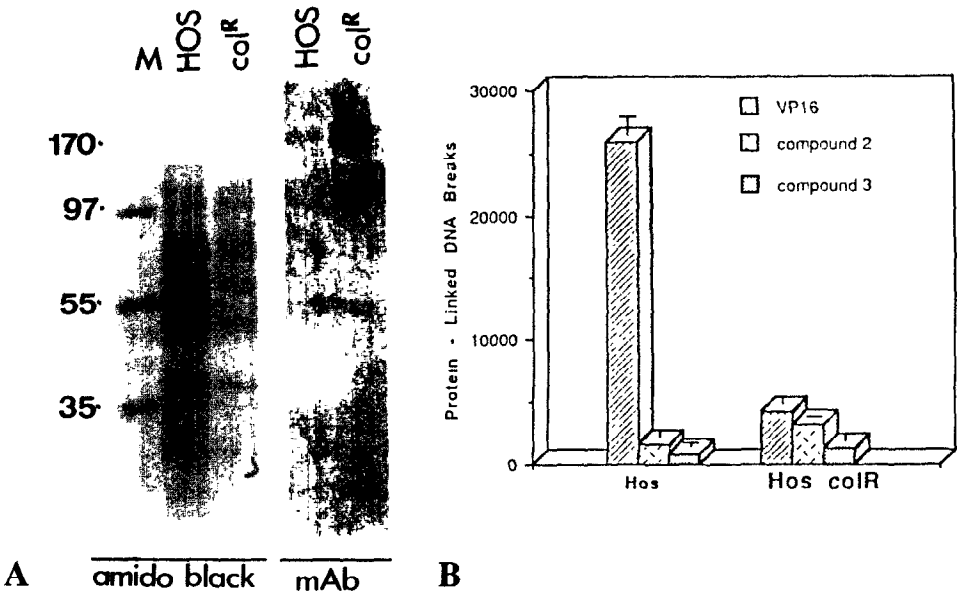


Figure 4. Immunoblot Analysis of P-glycoprotein levels and Induction of Protein-linked DNA Breaks in Hos and Hos col^R cells. A. Plasma membrane preparations were fractionated and transferred to a nitocellulose filter. Total proteins were stained with amido black as a control for sample load and P-glycoprotein was immunolocalized on a duplicate filter using a specific mAb. Markers for molecular mass (kDa) are given in the right margin. B. Cells were treated with 20 μM compounds for one hour and protein-linked DNA breaks were then detected.[9] The data shows cpm recovered for duplicate treatments.

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17. Compound **2**: $[\alpha]_D^{20}$ -304° (c 0.11, EtOH); ^{1}HNMR (Acetone-d_6) δ 2.22-2.50 (m, 4H, H-5,6), 4.77 (dd, J = 11.3, 5.92 Hz, 1H, H-7), 6.42 (s, 1H, H-4), 7.36 (d, J = 11.9 Hz 1H, H-11), 7.56 (s, 1H, H-8), and 7.78 (d, J = 11.9 Hz, 1H, H-12); Anal. ($C_{18}H_{14}O_6NF_3 \cdot 2H_2O$) calc. C 49.89, H 4.19, N 3.23; found C 49.95, H 3.98, N 3.11.

    Compound **3**: mp. 234-236 °C(dec); $[\alpha]_D^{20}$ -153° (c 0.10, EtOH); IR (KBr) 3260 (broad peak, OHs) and 1600 (CO) cm^{-1}; UV(EtOH) 246 (ε 32000) and 350 (ε 17000) nm; ^{1}HNMR (Acetone-d_6) δ 2.22-2.48 (m, 4H, H-5,6), 4.85 (m, 1H, H-7), 6.41 (s, 1H, H-4), 7.04 (s, 2H, H-2',6'), 7.25 (d, J = 11.8 Hz, 1H, H-11), 7.68 (d, J = 11.8 Hz, 1H, H-12), and 8.15 (d, J = 6.83 Hz, HN-7); Anal. ($C_{23}H_{19}O_9N \cdot 3/2H_2O$) calc. C 57.50, H 4.62, N 2.92; found C 57.07, H 4.43, N 2.61.
18. Chemically transformed human osteogenic sarcoma (TE85, clone F-5), American Type Culture Collection No.: CRL1547.

19. The growth inhibition assay was done according to a published procedure[11] and EC_{50} values of 8.25 ± 0.75 nM, 28 μM, and 21 μM were determined for compounds **1**-**3**, respectively.
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22. The Hos col^R cells were selected in two successive steps using 25 nM then 75 nM of **1**, and the EC_{50} value of **1** for the resistant su-clone was 315 ± 15 nM.
23. Plasma membranes were prepared using the buffers of Yang, L-Y.; Trujillo, J. M. *Cancer Res.* **1990**, *50*, 3218. Fifty four microgram amounts were separated by electrophoresis on a 7.5% SDS-PAGE gel and blotted using standard procedures. P-glycoCHEK™ C219 mAb was obtained from Centocor Diagnostics, Centocor, Inc., Malvern, PA 19355 and was used for staining according to the manufacturers recommendations.