Antitumor Agents. 185.† Synthesis and Biological Evaluation of Tridemethylthiocolchicine Analogues as Novel Topoisomerase II Inhibitors

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Several 1,2,3-tridemethyldeacetylthiocolchicine derivatives have been synthesized and evaluated for cytotoxic activity against various human tumor cell lines and for their inhibitory effects on DNA topoisomerases in vitro. Exhaustive demethylation of thiocolchicine analogues completely changes their biological profiles. Instead of displaying antitubulin activity, most target compounds inhibited topoisomerase II activity. Only compounds with a larger side chain, such as **15a**, **23a**, and **24a**, did not interfere with topoisomerase II enzymatic functions. The cytotoxicity of target compounds was reduced by 3 orders of magnitude compared to that of colchicine in most cell lines. The hydrophilicity of phenolic compounds might prevent drug passage through the cell plasma membrane and, thus, be responsible for the relatively weak cytotoxicity. To test this hypothesis, **27**−**30** were prepared from **16a** by protecting all hydroxy groups with esters with an aim to facilitate drug transportation. In vitro cytotoxicity assays indicated that **27** was more potent than its parent compound in all tested tumor cell lines and showed tissue selective cytotoxicity with a significant inhibitory effect against KB cells (IC_{50} = 2.7 μg/mL). Therefore, we propose that **27** acts as a prodrug, liberating **16a** to exert its antitopoisomerase activity and, finally, to cause cell death.

Introduction

Colchicine (**1**) (Figure 1), the major alkaloid isolated from *Colchicum autumnale* in 1820, is an ancient drug used to treat gout and familial Mediterranean fever.[2] Most biological effects of **1** are probably related to its powerful action on tubulin,[3] causing inhibition of microtubule polymerization and cell arrest at metaphase.[4] Although colchicine is a potent antitumor agent, it is not used clinically due to its severe toxicity.[5] Subsequently, much interest has focused on structural modifications in the hope of improving the therapeutic potential.[6−8]

Both the therapeutic and toxic effects of colchicinoids are likely caused by interaction with tubulin, resulting in a low therapeutic index.[9] We postulate that a conformational change in tubulin results when drugs bind to the colchicine domain, and this change may invariably lead to undesirable side effects. Consequently, further development of colchicine analogues may require targeting the analogues to either a different binding site on tubulin or a completely different intracellular target protein. The successful conversion of podophyllotoxin to etoposide offers an excellent example of the second possibility.

Podophyllotoxin (**2**) (Figure 2) is another antimitotic agent that binds at the colchicine domain.[10] Like colchicine, its medical uses are limited due to its high toxicity.[11] However, its semisynthetic derivatives, etoposide (**3**) and teniposide (**4**) (Figure 2), are less toxic and function as DNA topoisomerase II inhibitors with broad antitumor spectra (compare data for **2** and **3** in Table 1).[12−14] Because topoisomerase II appears to be a multidrug target in cancer chemotherapy,[15] it is of great interest to develop colchicine analogues that target topoisomerase II but have no or reduced antitubulin-related toxicity.

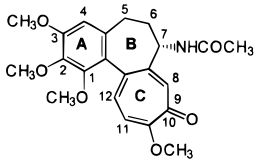

Figure 1. Structure of colchicine (**1**).

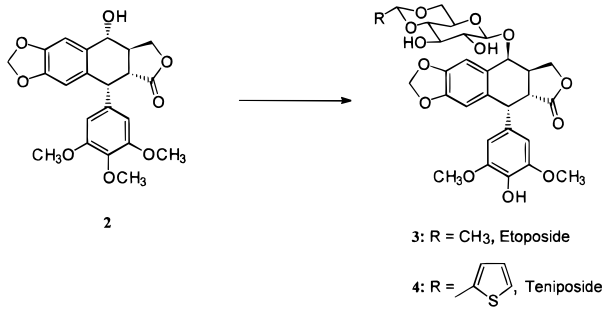

Figure 2. Structures of podophyllotoxin **2**, etoposide, and teniposide.

In our continuing investigation of bioactive colchicinoids, *N*-(trifluoroacetyl)-1,2,3,10-tetrademethyldeacetyl-

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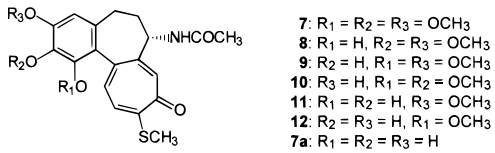
Table 1. Evaluation of Phenolic Thiocolchicines as Inhibitors of Tubulin and Topoisomerase II in Vitro



7: $R_1 = R_2 = R_3 = OCH_3$
8: $R_1 = H, R_2 = R_3 = OCH_3$
9: $R_2 = H, R_1 = R_3 = OCH_3$
10: $R_3 = H, R_1 = R_2 = OCH_3$
11: $R_1 = R_2 = H, R_3 = OCH_3$
12: $R_2 = R_3 = H, R_1 = OCH_3$
7a: $R_1 = R_2 = R_3 = H$

	IC_{50} (μM)	
compd	DNA topo II inhibition[a]	tubulin polymerization[b]
1	IN[c]	1.5[d]
2	IN	1.0[e]
3	38	IN
7	IN	0.65
8	IN	4
9	IN	2
10	IN	2
11	>150	4
12	38	4
7a	9	>40

[a] Measured as ATP-dependent unknotting of P4 DNA compared to enzyme and DNA control reactions. Etoposide (100 μM) completely inhibited the unknotting activity. [b] Concentration which inhibits tubulin polymerization by 50% compared to the control. [c] IN = inactive. [d] Data from ref 8. [e] Data from ref 10.

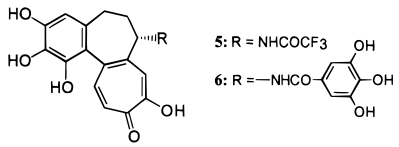


Figure 3. Structures of demethylisocolchicine analogues.

isocolchicine (**5**) and *N*-(3′,4′,5′-trihydroxybenzoyl)-1,2,3,-10-tetrademethyldeacetylisocolchicine (**6**) (Figure 3) demonstrated interesting activities not shared by **1**.[16] These properties include the ability to induce intracellular protein-linked DNA breaks, inhibit DNA topoisomerase II in vitro, and exert cytotoxicity that is not affected by overexpression of p-glycoprotein.[17] This encouraging discovery brought a new vision for additional investigation of colchicinoids.

The major goal of the present work is to determine the structural requirements and B ring side chain modifications for specific inhibition of DNA topoisomerase II activity. Therefore, regioselectively and exhaustively demethylated thiocolchicine analogues were synthesized and evaluated in vitro for topoisomerase I and II inhibitory, antitubulin, and cytotoxic activities. Limited mechanism of action studies of selected compounds are also reported in this paper.

Chemistry

Monophenols **8** and **10** were prepared according to published methods.[18,19] An improved procedure of regioselective demethylation of thiocolchicine to phenol **9** and catechols **11** and **12** has been reported recently by our laboratory.[20]

As shown in Scheme 1, all demethylated compounds were synthesized from thiocolchicine (**7**) and deacetylthiocolchicine (**13**), which were prepared from **1** by literature procedures.[21] Reaction of **13** with 4-aminobenzoic acid/DCC afforded **14**, which was then acylated with (*S*)-(−)-camphanic chloride to give **15**.[22,23] Similarly, *N*-acyldeacetylthiocolchicinoids **16**−**26** were obtained by acylation of **13** with various commercially available acyl chlorides. Finally, exhaustive demethylation of **7** and **13**−**26** with excess boron tribromide gave rise to the corresponding demethylated **7a** and **13a**−**26a**.[24] Reaction of compound **14a** with HCl formed its water-soluble hydrochloride salt **14b**. Esterification of compound **16a** with the appropriate acid chlorides and with methyl chloroformate in the presence of pyridine and DMAP[25] afforded the esters **27**−**29** and carbonate **30**, respectively (Scheme 2).

Biological Results and Discussions

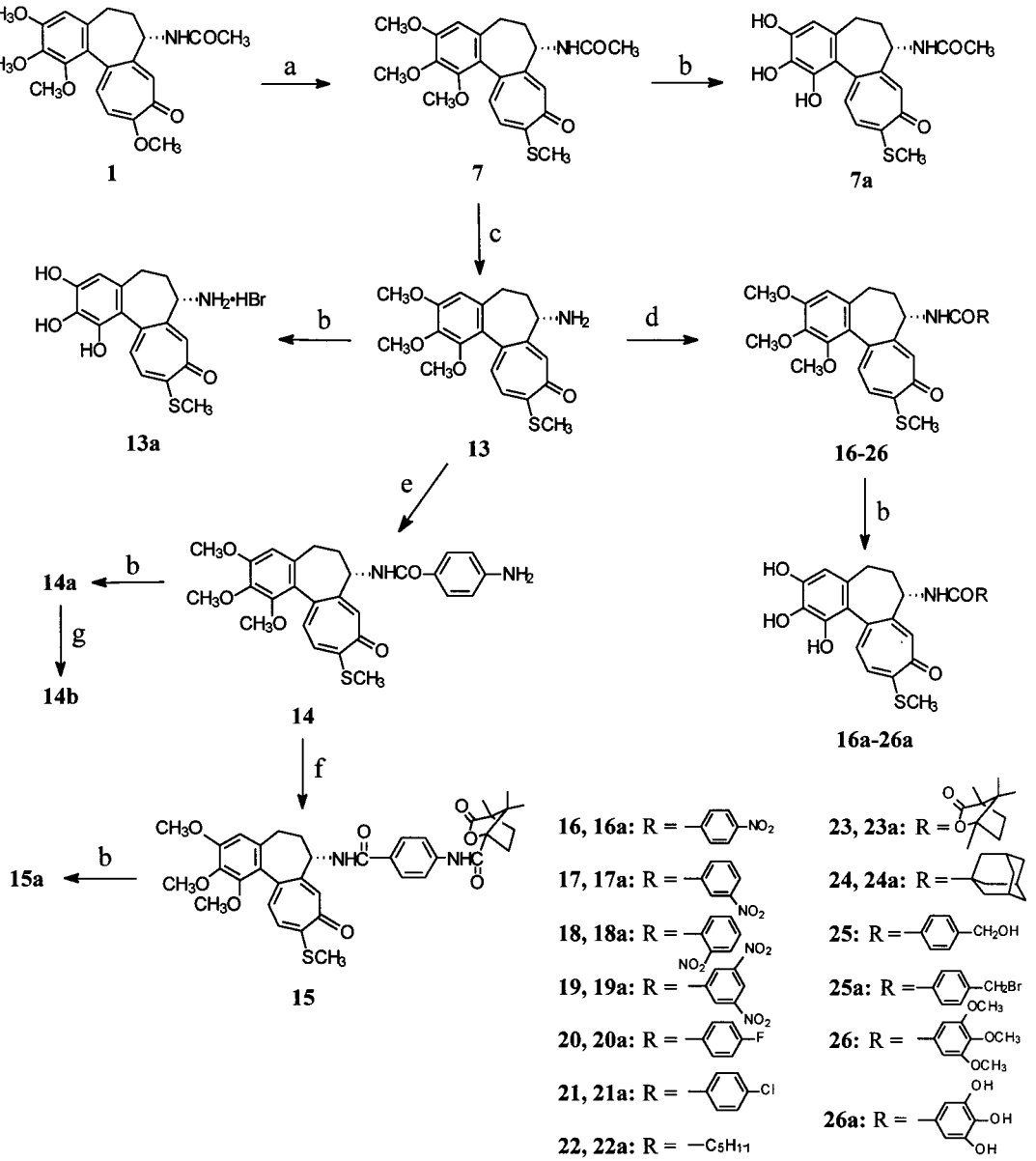
Table 1 shows the in vitro activities of phenolic congeners (**8**−**13**) of thiocolchicine against mammalian DNA topoisomerase II and tubulin polymerization. All phenolic A ring derivatives had reduced inhibitory effects on tubulin polymerization, with removal of all three methyl groups (**7a**) being the most deleterious (IC_{50} > 40 μM). In contrast, in the topoisomerase II inhibition assay, the completely demethylated **7a** displayed the highest activity (IC_{50} = 9 μM, 4-fold more active than etoposide). Catechol **12** was as inhibitory as etoposide; however, monophenols **8**−**10** and catechol **11** did not inhibit topoisomerase II. Interestingly, as more methyl ether groups on A ring are cleaved, tubulin inhibition decreases and topoisomerase II inhibition increases. Therefore, complete demethylation of the A ring is optimal for topoisomerase II inhibitory activity in vitro.

To extend these SAR studies, we prepared several exhaustively demethylated thiocolchicine derivatives with variable side chains at C-7. Most modifications of the C-7 substituent resulted in compounds active toward topoisomerase II. Complete inhibition of enzyme activity was observed at drug concentrations of 100 μM in preliminary in vitro topo II screening (Table 2). Thus, side chain modifications in the B ring are tolerated in the topoisomerase II enzyme inhibition assay. A few exceptions are compounds **15a**, **23a**, and **24a**, which possess a large side chain. Their inactivity suggests that increasing the steric bulk of the nitrogen substituent decreases the inhibitory activity of these molecules toward topoisomerase II. The target compounds were also screened against topoisomerase I; however, only compounds **16a** and **26a** interacted with both topoisomerases. In addition, all target compounds did not interfere with tubulin polymerization.

Table 3 shows the in vitro cytotoxic activities of selected target compounds against five panels of human tumor cell lines. For comparison, colchicine was also evaluated. Generally, the demethylthiocolchicine derivatives were considerably less toxic than colchicine in most cell lines. Compounds **7a** with an unmodified side chain and **13a** with a hydrolyzed side chain were not cytotoxic. Among the compounds containing variable side chains, **16a** was active against KB cells and **26a** was active against both KB and MCF-7 cells. Compound **14b**, a salt form of **14a**, showed enhanced cytotoxic effects toward three cell lines compared to its parent compound. The increased cytotoxicity might be due to improved water solubility.

These demethylthiocolchicine derivatives are not very potent as cytotoxic agents (for significant activity of a pure compound, $EC_{50} \leq 4.0$ μg/mL is required). Their weak cytotoxicity may result from the multiple phenolic

Scheme 1. Synthesis of Exhaustive Demethylation of Thiocolchicine Analogues **7a**, **13a**−**26a**, and **14b**[a]

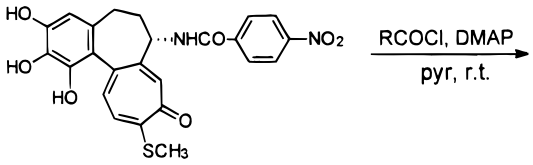


[a] Reagent: (a) $NaSCH_3$, H_2O, (b) 10 equiv of BBr_3/CH_2Cl_2, 0 °C; (c) 2 N HCl, MeOH, reflux; (d) RCOCl, CH_2Cl_2/pyr, room temperature; (e) 4-aminobenzolic acid, CH_2Cl_2, DCC, room temperature; (f) (1S)-(−)-camphanic chloride, CH_2Cl_2/pyr, room temperature; (g) 4 M HCl/dioxane.

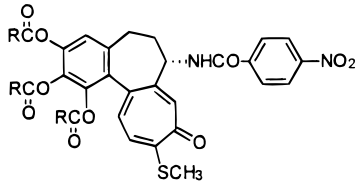
hydroxy groups, making these compounds too hydrophilic to pass through the cell plasma membrane. To improve the lipid solubility, we prepared three esters **27**−**29** and one carbonate **30** by protecting all three hydroxy groups of **16a**. According to the prodrug hypothesis, esters and carbonates should be hydrolyzed intracellularly by esterases to generate the hydroxy groups of the parent compound. As the hydroxy groups were blocked, all prodrugs (**27**−**30**) became inactive against either topoisomerase or tubulin (Table 2). This lack of an antitopoisomerase effect further confirmed that the hydroxy groups are required for enzyme inhibition. Hydrogen bonds formed between the hydroxy groups and the residues of its binding pocket might play an important role in drug−enzyme interactions. An in vitro cytotoxic assay revealed that **27** was about 3 times more potent that its parent compound **16a** in KB cells, with the EC_{50} value of 2.7 μg/mL (Table 3). Carbonate **30** also showed improved cytotoxic activity against three cell lines compared to **16a**. However, the prodrugs containing larger protecting groups (**28** and **29**) did not display cytotoxicity superior to that of their parent compound. Thus, the small protecting groups might be removed easily by esterases in comparison with the large protecting groups.

We also examined the ability of compound **16a** and its prodrug **27** to stabilize the cleavable complex in KB cells. For comparison, etoposide was included as a positive control. Data obtained are presented in Figure 4. As expected, etoposide produced a significant amount of enzyme-linked DNA complexes at a 50 μM drug concentration. In contrast, compound **16a** and **27** did not induce cleavable complex formation compared to the control. This result indicated that the two compounds do not arrest the enzyme at its transition state after DNA strand breakage, and thus, they have a different inhibitory mechanism of action from that of etoposide. The ability of **16a** and **27** to interfere with etoposide-

Scheme 2. Synthesis of Compounds **27**−**30**



16a



27: R = CH_3
28: R = C_2H_5
29: R = $(CH_3)_2CH$
30: R = CH_3O

Table 2. Evaluation of Demethylthiocolchicines as Inhibitors of Tubulin and Topoisomerases in Vitro

	% inhibition of DNA		
compd no.	Topo II activity (100 μM)[a]	Topo I activity (100 μM)[b]	tubulin polymerization IC_{50} (μM)[c]
13a	100	0	>40
14a	100	0	>40
14b	100	0	>40
15a	0	0	>40
16a	100	<100	>40
17a	100	0	>40
18a	100	0	>40
19a	100	0	>40
20a	100	0	>40
21a	100	0	>40
22a	100	0	>40
23a	0	0	>40
24a	0	0	>40
25a	100	0	>40
26a	100	100	>40
27	0	0	>40
28	0	0	>40
29	0	0	>40
30	0	0	>40

[a] Measured as ATP-dependent unknotting of P4 DNA compared to enzyme and DNA control reactions. Etoposide (100 μM) completely inhibited the unknotting activity. [b] Measured as ATP-independent relaxation of supercoiled plasmid DNA compared to enzyme and DNA control reaction. Camptothecin at 100 μM served as the positive control. [c] Concentration which inhibits tubulin polymerization by 50% compared to the control.

induced protein-linked DNA complex formation was also examined at a 80 μM drug concentration. In comparison with the positive control, **16a** stimulated the ternary complex adducts induced by etoposide. Therefore, **16a** did not block DNA binding with the enzyme, otherwise DNA would not be available to be trapped by etoposide. Surprisingly, no statistically significant stimulation of etoposide-induced cleavage complex was observed with compound **27**. The different behavior of the two compounds questioned the prodrug hypothesis.

In conclusion, complete demethylation of thiocolchicine in the A ring of colchicine is required for maximal topoisomerase II inhibitory activity in vitro. Generally, demethylthiocolchine analogues are selective topoisomerase II inhibitors without antitubulin activity. The cytotoxicities were improved by side chain modifications and further elevated by protecting the hydroxy groups. In KB cells, demethylthiocolchicines appear inhibit topoisomerase II without producing cleavable protein-linked DNA complexes. The precise molecular mechanism of action merits further investigation.

Experimental Section

Melting points were measured with a Fisher-Johns melting point apparatus without correction. Optical rotations were determined with a DIP-1000 polarimeter. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker AC-300 spectrometer with Me_4Si(TMS) as the internal reference. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. MS was determined by NIH. Thin-layer chromatography (TLC) silica gel plates were purchased from Analtech, Inc. Silica gel (230−400 mesh), from Aldrich, was used for column chromatography. MCI gel was purchased from Supelco.

General Procedures for Exhaustive Demethylation of *N*-Acyldeacetylthiocolchicines (7a and 13a−26a). To a solution of starting material in anhydrous CH_2Cl_2 was added dropwise a 1 M solution of boron tribromide in CH_2Cl_2 (molar ratio of 1:10) under ice cooling. The reaction mixture was stirred at room temperature for 20−24 h. The reaction mixture was cooled in an ice bath, and MeOH (20 mol) was added dropwise. The solution was refluxed for 2 h, and then the solvent was removed in vacuo. The residue was purified by MCI Gel CHP-20P column chromatography using water and MeOH as eluents.

Tridemethylthiocolchicine (7a): yield 61.5% (starting from 108.5 mg of **7**); amorphous; $[\alpha]^{25}_D$ −282.27° (*c* 0.11, MeOH); ^{1}H NMR (pyridine) δ 2.0 (s, 3H, $COCH_3$), 2.1 (s, 3H, SCH_3-10), 1.88−2.9 (m, 4H, H-5,6), 5.29 (m, 1H, H-7), 6.79 (s, 1H, H-4), 6.69 (d, J = 10.4 Hz, 1H, H-11), 7.70 (d, J = 10.3 Hz, 1H, H-12), 9.24 (d, J = 7.1 Hz, 1H, NHCO); CIMS *m/z* 373 M^+. Anal. ($C_{19}H_{19}NSO_5 \cdot 1^3/_4H_2O$) C, H, N.

Tridemethyldeacetylthiocolchicine hydrobromide (13a): yield 15.8% (starting from 150.6 mg of **13**); amorphous; $[\alpha]^{25}_D$ −188.9° (*c* 0.12, MeOH); ^{1}H NMR (CD_3OD) δ 1.9−2.3 (m, 4H, H-5,6), 2.49 (s, 3H, SCH_3-10), 4.06 (m, 1H, H-7), 6.32 (s, 1H, H-4), 7.0 (s, 1H, H-8), 7.4 (d, J = 10.6 Hz, 1H, H-11), 7.57 (d, J = 10.5 Hz, 1H, H-12); CIMS *m/z* 366 $(M + 2H)^+$. Anal. ($C_{17}H_{18}NSO_4Br \cdot {}^1/_2C_2H_5OH$) C, H, N.

Tridemethyl-*N*-(4′-aminobenzoyl)deacetylthiocolchicine (14a): yield 40% (starting from 168.7 mg of **14**); amorphous; $[\alpha]^{25}_D$ −85.9° (*c* 0.14, DMSO); ^{1}H NMR (DMSO) δ 2.0−2.1 (m, 4H, H-5,6), 2.40 (s, 3H, SCH_3-10), 4.61 (m, 1H, H-7), 5.67 (s, 2H, NH_2), 6.24 (s, 1H, H-4), 6.54 (d, J = 8.4 Hz, 2H, H-3′,5′), 7.10 (s, 1H, H-8), 7.21 (d, J = 10.4 Hz, 1H, H-11), 7.29 (d, J = 10.7 Hz, 1H, H-12), 7.61 (d, J = 8.6 Hz, 2H, H-2′,6′), 8.43 (d, J = 6.7 Hz, 1H, NHCO), 8.44 (s, 1H, OH-1), 8.56 (s, 1H, OH-2), 9.33 (s, 1H, OH-3); CIMS *m/z* 449 $(M + H)^+$. Anal. ($C_{24}H_{22}N_2SO_5 \cdot {}^1/_2H_2O$) C, H, N.

Tridemethyl-*N*-(4′-camphanoylbenzoyl)-deacetylthiocolchicine (15a): yield 18% (starting from 536 mg of **15**); amorphous; $[\alpha]^{25}_D$ −104.4° (*c* 0.12, MeOH); ^{1}H NMR (DMSO) δ 0.91 (s, 3H, camphanoyl CH_3), 1.05 (s, 6H, camphanoyl CH_3), 1.94−2.11 (m, 8H, H-5,6 and camphanoyl H-4,5), 2.40 (s, 3H, SCH_3-10), 4.65 (m, 1H, H-7), 6.26 (s, 1H, H-4), 7.10 (s, 1H, H-8), 7.23 (d, J = 10.8 Hz, 1H, H-11), 7.30 (d, J = 10.4 Hz, 1H, H-12), 8.86 (d, J = 7.7 Hz, 1H, NHCO); CIMS *m/z* 630 $(M + H)^+$. Anal. ($C_{34}H_{34}N_2SO_8 \cdot {}^1/_2H_2O$) C, H, N.

Tridemethyl-*N*-(4′-nitrobenzoyl)deacetylthiocolchicine (16a): yield 30% (starting from 102 mg of **16**); amorphous; $[\alpha]^{25}_D$ −119.9° (*c* 0.09 MeOH); ^{1}H NMR (DMSO) δ 2.08−2.21 (m, 4H, H-5,6), 2.40 (s, 3H, SCH_3-10), 4.65 (m, 1H, H-7), 6.26 (s, 1H, H-4), 7.09 (s, 1H, H-8), 7.24 (d, J = 10.8 Hz, 1H, H-11), 7.32 (d, J = 10.5 Hz, 1H, H-12), 8.10 (d, J = 8.7 Hz, 2H, H-3′,5′), 8.35 (d, J = 8.8 Hz, 2H, H-2′,6′), 8.48 (s, 1H, OH-1), 8.60 (s, 1H, OH-2), 9.29 (d, J = 7.5 Hz, 1H, NHCO), 9.37 (s, 1H, OH-3); CIMS *m/z* 481 $(M + H)^+$. Anal. ($C_{24}H_{20}N_2SO_7 \cdot 1^3/_4H_2O$) C, H, N.

Tridemethyl-*N*-(3′-nitrobenzoyl)deacetylthiocolchicine (17a): yield 60% (starting from 845.3 mg of **17**);

Table 3. Inhibition of in Vitro Tumor Cell Growth[a,b] by Selected Demethylthiocolchicines and Prodrugs **27**−**30**

	cytotoxicity EC_{50} (μg/mL)[c]				
compd	KB	A549	MCF-7	CAKI-1	SK-MEL-2
1	0.002	0.022	>0.4 (33−45)[d]	0.4	0.008
7a	>17.8 (0)[e]	>17.8 (0)	13.6	>17.8 (22)	>17.8 (0)
13a	>13.2 (0)	>13.2 (0)	>13.2 (23)	>13.2 (2)	>13.2 (0)
14a	>18.0 (35)	>18.0 (39)	14.8	>18.0 (13)	>18.0 (0)
14b	4.8	9.7	10.7	>19.5 (37)	>19.5 (42)
16a	7.7	>9.6 (36)	>9.6 (38)	>9.6 (20)	>9.6 (0)
26a	9.6	>9.6 (30)	8.6	>9.6 (29)	>9.6 (30)
27	2.7	>0.15 (18−43)	>0.15 (23−48)	>0.3 (12−38)	>0.3 (26−41)
28	12.3	>25.9 (29)	>12.9 (40)	>25.9 (16)	12.9
29	25.5	>27.6 (19)	>27.6 (43)	>27.6 (17)	27.6
30	6.5	24.2	13.1	>26.2 (38)	>26.2 (44)

[a] Data obtained from our in-house in vitro disease-oriented human tumor cell screen. [b] KB, epidermoid carcinoma of the nasopharynx; A549, lung carcinoma; MCF-7, breast adenocarcinoma; CAKl-1, kidney carcinoma; SK-MEL-2, malignant melanoma. [c] EC_{50} is the concentration of compound that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using SRB assay. [d] Plateau dose response observed. [e] Observed percent inhibition in parentheses.

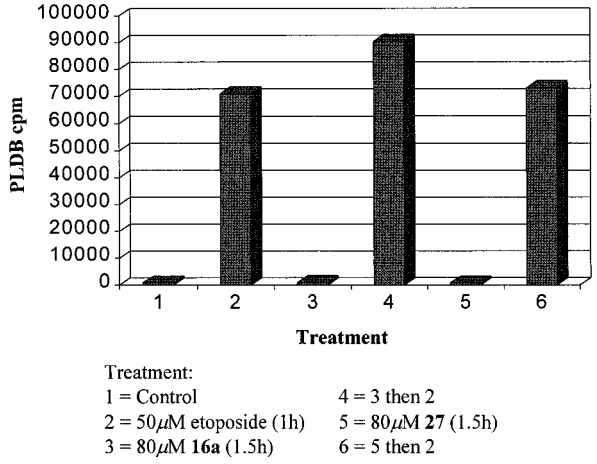


Figure 4. Effects of compounds **16a** and **27** on cellular protein−DNA complexes formation in KB cells.

amorphous; $[\alpha]^{25}_D$ −90.7° (*c* 0.17, DMSO); ^{1}H NMR (DMSO) δ 2.0−2.2 (m, 4H, H-5,6), 2.40 (s, 3H, SCH_3-10), 4.70 (m, 1H, H-7), 6.27(s, 1H, H-4), 7.11 (s, 1H, H-8), 7.25 (d, J = 10.7 Hz, 1H, H-11), 7.33 (d, J = 10.4, 1H, H-12), 7.81 (t, 1H, H-5′), 8.31 (d, J = 7.9 Hz, 1H, H-6′), 8.41 (d, J = 7.9 Hz, 1H, H-4′), 8.75 (s, 1H, H-2′), 9.33 (d, J = 7.6 Hz, 1H, NHCO); CIMS *m*/*z* 480 $(M + H)^+$. Anal. $(C_{24}H_{20}N_2SO_7 \cdot {}^3/_4CH_3CO_2C_2H_5)$ C, H, N.

Tridemethyl-*N*-(2′-nitrobenzoyl)deacetylthiocolchicine (18a): yield 58.7% (starting from 511 mg of **18**); amorphous; $[\alpha]^{25}_D$ −293.6° (*c* 0.24, MeOH); ^{1}H NMR (DMSO) δ 1.87−2.18 (m, 4H, H-5,6), 2.42 (s, 3H, SCH_3-10), 4.61 (m, 1H, H-7), 6.28 (s, 1H, H-4), 7.17 (s, 1H, H-8), 7.25 (d, J = 10.6 Hz, 1H, H-11), 7.31 (d, J = 10.4 Hz, 1H, H-12), 7.60 (d, J = 7.3 Hz, 1H, H-6′), 7.72 (t, 1H, H-5′), 7.84 (t, 1H, H-4′), 8.08 (d, J = 8.0 Hz, 1H, H-3′), 8.49 (broad, 1H, OH-1), 8.63 (s, 1H, OH-2), 9.31 (d, J = 7.7 Hz, 1H, NHCO), 9.38 (s, 1H, OH-3); CIMS *m*/*z* 480 $(M + H)^+$. Anal. $(C_{24}H_{20}N_2SO_7 \cdot {}^1/_2H_2O)$ C, H, N.

Tridemethyl-*N*-(3′,5′-dinitrobenzoyl)deacetylthiocolchicine (19a): yield 76.3% (starting from 658.2 mg of **19**); amorphous; $[\alpha]^{25}_D$ −38.7° (*c* 0.7 MeOH); ^{1}H NMR (DMSO) δ 2.0−2.2 (m, 4H, H-5,6), 2.40 (s, 3H, SCH_3-10), 4.72 (m, 1H, H-7), 6.27 (s, 1H, H-4), 7.11 (s, 1H, H-8), 7.25 (d, J = 10.7 Hz, 1H, H-11), 7.32 (d, J = 10.5 Hz, 1H, H-12), 8.48 (s, 1H, OH-1), 8.63 (s, 1H, OH-2), 8.97 (s, 1H, H-4′), 9.11 (s, 2H, H-2′,6′), 9.37 (s, 1H, OH-3), 9.63 (d, J = 7.5 Hz, 1H, NHCO); CIMS *m*/*z* 525 $(M + H)^+$. Anal. $(C_{24}H_{19}N_3SO_9 \cdot 1{}^1/_2H_2O)$ C, H, N.

Tridemethyl-*N*-(4′-fluorobenzoyl)deacetylthiocolchicine (20a): yield 78.5% (starting from 545.6 mg of **20**); amorphous; $[\alpha]^{25}_D$ −203.7° (*c* 0.07 MeOH); ^{1}H NMR (DMSO) δ 1.9−2.2 (m, 4H, H-5,6), 2.08 (s, 3H, SCH_3-10), 4.67 (m, 1H, H-7), 6.26 (s, 1H, H-4), 7.10 (s, 1H, H-8), 7.23 (d, J = 10.7 Hz, 1H, H-11), 7.31 (d, J = 11.2 Hz, 1H, H-12), 7.35 (d, J = 8.9 Hz, 2H, H-3′,5′), 7.95 (d, J = 8.3 Hz, 2H, H-2′,6′), 8.96 (d, J = 7.6 Hz, 1H, NHCO); CIMS *m*/*z* 453 $(M + H)^+$. Anal. $(C_{24}H_{20}NSO_5F \cdot {}^1/_2H_2O)$ C, H, N.

Tridemethyl-*N*-(4′-chlorobenzoyl)deacetylthiocolchicine (21a): yield 36% (starting from 100 mg of **21**); amorphous; $[\alpha]^{25}_D$ −41.7° (*c* 0.57, acetone); ^{1}H NMR (DMSO) δ 2.0−2.2 (m, 4H, H-5,6), 2.40 (s, 3H, SCH_3-10), 4.65 (m, 1H, H-7), 6.25 (s, 1H, H-4), 7.10 (s, 1H, H-8), 7.23 (d, J = 10.7 Hz, 1H, H-11), 7.31 (d, J = 10.4 Hz, 1H, H-12), 7.57 (d, J = 8.5 Hz, 2H, H-3′,5′), 7.90 (d, J = 8.6 Hz, 2H, H-2′,6′), 8.47 (s, 1H, OH-1), 8.50 (s, 1H, OH-2), 9.0 (d, J = 7.6 Hz, 1H, NHCO), 9.36 (s, 1H, OH-3); CIMS *m*/*z* 470 M^+. Anal. $(C_{24}H_{20}NSO_5Cl)$ C, H, N.

Tridemethyl-*N*-hexanoyldeacetylthiocolchicine (22a): yield 39.3% (starting from 330 mg of **22**); amorphous; $[\alpha]^{25}_D$ −270.8° (*c* 0.06 MeOH); ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, hexanoyl-CH_3), 1.27−1.89 (m, 8H, hexanoyl-CH_2), 2.0−2.1(m, 4H, H-5,6), 2.45 (s, 3H, SCH_3-10), 4.50 (m, 1H, H-7), 6.29 (s, 1H, H-4), 7.16 (s, 1H, H-8), 7.33 (d, J = 10.5 Hz, 1H, H-11), 7.48 (d, J = 10.4 Hz, 1H, H-12); CIMS *m*/*z* 429 $(M + H)^+$. Anal. $(C_{23}H_{27}NSO_5)$ C, H, N.

Tridemethyl-*N*-adamantyldeacetylthiocolchicine (23a): yield 75.4% (starting from 215.8 mg of **23**); amorphous; $[\alpha]^{25}_D$ −176.8° (*c* 0.11 DMSO); ^{1}H NMR (DMSO) δ 1.66 (s, 6H, adamantyl CH_2), 1.77 (s, 6H, adamantyl CH_2), 1.98 (s, 3H, adamantyl CH), 1.9−2.1 (m, 4H, H-5,6), 2.40 (s, 3H, SCH_3-10), 4.41 (m, 1H, H-7), 6.22 (s, 1H, H-4), 7.02 (s, 1H, H-8), 7.20 (d, J = 10.7 Hz, 1H, H-11), 7.26 (d, J = 10.4 Hz, 1H, H-12); CIMS *m*/*z* 493 $(M + H)^+$. Anal. $(C_{28}H_{31}NSO_5 \cdot {}^1/_2CH_3CO_2C_2H_5)$ C, H, N.

Tridemethyl-*N*-camphanoyldeacetylthiocolchicine (24a): yield 39.4% (starting from 60.8 mg of **24**); amorphous; $[\alpha]^{25}_D$ −210.4° (*c* 0.11, DMSO); ^{1}H NMR (DMSO) δ 0.8 (s, 3H, camphanoyl CH_3), 0.90 (s, 6H, camphanoyl CH_3), 0.99 (camphanoyl H-4,5), 1.54−1.95 (m, 4H, H-5,6), 2.40 (s, 3H, SCH_3-10), 4.62 (m, 1H, H-7), 6.22 (s, 1H, H-4), 7.09 (s, 1H, H-8), 7.22 (d, J = 10.6 Hz, 1H, H-11), 7.27 (d, J = 10.4 Hz, 1H, H-12), 8.73 (d, J = 8.3 Hz, 1H, NHCO); CIMS *m*/*z* 512 $(M + H)^+$. Anal. $(C_{27}H_{29}NSO_7 \cdot {}^3/_4H_2O)$ C, H, N.

Tridemethyl-*N*-(4′-bromomethylenebenzoyl)-deacetylthiocolchicine (25a): yield 90.7% (starting from 353 mg of **25**); amorphous; $[\alpha]^{25}_D$ −57.8° (*c* 0.1, acetone); ^{1}H NMR (CD_3OD) δ 1.9−2.2 (m, 4H, H-5,6), 2.40 (s, 3H, SCH_3-10), 4.68 (m, 1H, H-7), 4.75 (s, 2H, CH_2Br-4′), 6.28 (s, 1H, H-4), 7.11 (s, 1H, H-8), 7.23 (d, J = 10.7 Hz, 1H, H-11), 7.32 (d, J = 10.4 Hz, 1H, H-12), 7.56 (d, J = 8.1 Hz, 2H, H-3′,5′), 7.85 (d, J = 8.6 Hz, 2H, H-2′,6′), 8.97 (d, J = 7.7 Hz, 1H, NHCO); CIMS *m*/*z* 530 $(M + 2H)^+$. Anal. $(C_{25}H_{22}NSO_5Br \cdot {}^1/_2H_2O)$ C, H, N.

Tridemethyl-*N*-(3′,4′,5′-trihydroxylbenzoyl)deacetylthiocolchicine (26a): yield 23% (starting from 165 mg of **26**); amorphous; $[\alpha]^{25}_D$ −189.1° (*c* 0.07, MeOH); ^{1}H NMR (CD_3OD) δ 2.0−2.36 (m, 4H, H-5,6), 2.45 (s, 3H, SCH_3-10), 4.73 (m, 1H, H-7), 6.33 (s, 1H, H-4), 6.89 (s, 2H, H-2′,6′), 7.28 (s, 1H, H-8), 7.37 (d, J = 10.7 Hz, 1H, H-11), 7.53 (d, J = 10.6 Hz, 1H, H-12); CIMS *m*/*z* 483 $(M + H)^+$. Anal. $(C_{24}H_{21}N_3SO_8 \cdot 2{}^1/_4H_2O)$ C, H, N.

Tridemethyl-*N*-(4′-aminobenzoyl)deacetylthiocolchicine hydrochloride (14b): A solution of **14a** (50 mg) in MeOH was allowed to react with 4 M HCl in dioxane for 1 min with TLC monitoring. The reaction mixture was concentrated to give a pure powder: yield 100.4%; $[\alpha]^{25}_D$ −176.8° (*c* 0.1, CH_3OH); ^1H NMR (CH_3OH) spectrum was identical with that of **14a**. Anal. ($C_{24}H_{23}N_2SO_5Cl\cdot{}^3/_4CHCl_3$) C, H, N.

General Procedures for Synthesizing 1,2,3-*O*-Acyl-1,2,3 -demethyl-*N*-(4′-nitrobenzoyl)deactylthiocolchicines 27−30. To a solution of **16a** in dry THF was added pyridine (0.1 mL), DMAP (0.5 equiv), and appropriate acyl chlorides or methyl chloroformate (10−12 equiv). The mixture was allowed to stir for 4−8 h at room temperature, and the volatiles were evaporated in vacuo. The residue was diluted with water and extracted three times with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous $NaSO_4$, and concentrated in vacuo. The residue was chromatographed on a preparative TLC plate.

1,2,3-*O*-Acetyl-1,2,3-demethyl-*N*-(4′-nitrobenzoyl)-deacetylthiocolchicine (27): yield 46.6% (starting from 52 mg of **16a**); amorphous; $[\alpha]^{25}_D$ −47.6° (*c* 0.1, DMSO); ^1H NMR (DMSO) δ 2.12−2.22 (m, 4H, H-5,6), 2.9 (s, 3H, CH_3CO_2-1), 2.31 (s, 3H, CH_3CO_2-2), 2.34 (s, 3H, CH_3CO_2-3), 2.43 (s, 3H, SCH_3-10), 4.59 (m, 1H, H-7), 6.99 (d, J = 10.3 Hz, 1H, H-11), 7.09 (s, 1H, H-4), 7.26 (s, 1H, H-8), 7.31 (d, J = 10.6 Hz, 1H, H-12), 8.11 (d, J = 8.7 Hz, 1H, H-3′,5′), 8.37 (d, J = 8.7 Hz, 1H, H-2′,6′), 9.30 (d, J = 7.2 Hz, 1H, NHCO); CIMS *m*/*z* 606 M^+. Anal. ($C_{30}H_{26}N_2SO_{10}$) C, H, N.

1,2,3-*O*-Propionyl-1,2,3-demethyl-*N*-(4′-nitrobenzoyl)-deacetylthiocolchicine (28): yield 35.3% (starting from 62.8 mg of **16a**); amorphous; $[\alpha]^{25}_D$ +55° (*c* 0.85, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.17−1.39 (m, 9H, C**H**$_3$CH$_2$CO$_2$-1,2,3), 2.14−2.27 (m, 4H, H-5,6), 2.44 (s, 3H, SCH_3-10), 2.51−2.69 (m, 6H, CH_3C**H**$_2$-CO_2-1,2,3), 5.02 (m, 1H, H-7), 7.02 (d, J = 10.3 Hz, 1H, H-11), 7.05 (s, 1H, H-4), 7.13 (d, J = 10.6 Hz, 1H, H-12), 7.58 (s, 1H, H-8), 8.08 (d, J = 8.7 Hz, 2H, H-3′,5′), 8.16 (d, J = 8.7 Hz, 2H, H-2′,6′); CIMS *m*/*z* 649 $(M + H)^+$. Anal. ($C_{33}H_{32}N_2SO_{10}$) C, H, N.

1,2,3-*O*-Isobutyryl-1,2,3-demethyl-*N*-(4′-nitrobenzoyl)-deacetylthiocolchicine (29): yield 68.8% (starting from 58.2 mg of **16a**); amorphous; $[\alpha]^{25}_D$ +30.8° (*c* 0.42, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.15−1.38 (m, 18H, (C**H**$_3$)$_2$CHCO$_2$-1,2,3), 2.43 (s, 3H, SCH_3-10), 2.48−2.5 (m, 4H, H-5,6), 2.64−2.83 (m, 3H, (CH$_3$)$_2$C**H**CO$_2$-1,2,3), 5.02 (m, 1H, H-7), 7.01 (d, J = 10.6 Hz, 1H, H-11), 7.06 (s, 1H, H-4), 7.12 (d, J = 10.3 Hz, 1H, H-12), 7.53 (s, 1H, H-8), 8.07 (d, J = 8.8 Hz, 2H, H-3′,5′), 8.15 (d, J = 8.8 Hz, 2H, H-2′,6′); CIMS *m*/*z* 691 $(M + H)^+$. Anal. ($C_{36}H_{38}N_2SO_{10}$) C, H, N.

1,2,3-Methoxylcarbonyl-*N*-(4′-nitrobenzoyl)deacetylthiocolchicine (30): yield 11.8% (starting from 106 mg of **16a**); amorphous; $[\alpha]^{25}_D$ −50° (*c* 0.12,DMSO); ^1H NMR (DMSO) δ 2.09−2.9 (m, 4H, H-5,6), 2.43 (s, 3H, SCH_3-10), 3.81 (s, 3H, CH_3OCO-1), 3.89 (s, 3H, CH_3OCO-2), 3.90 (s, 3H, CH_3OCO-3), 4.60 (m, 1H, H-7), 7.06 (d, J = 10.2 Hz, 1H, H-11), 7.11 (s, 1H, H-4), 7.32 (d, J = 10.6 Hz, 1H, H-12), 7.49 (s, 1H, H-8), 8.12 (d, J = 8.7 Hz, 2H, H-3′,5′), 8.37 (d, J = 8.8 Hz, 2H, H-2′,6′), 9.32 (d, J = 7.2 Hz, NHCO); CIMS *m*/*z* 655 $(M + H)^+$. Anal. ($C_{30}H_{26}N_2SO_{13}\cdot{}^3/_4CH_3CO_2C_2H_5$) C, H, N.

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