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Antitumor Agents—CLXXV. Anti-tubulin Action of (+)-Thiocolchicine Prepared by Partial Synthesis[†]

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Abstract—(+)-Thiocolchicine (**2b**) was prepared from (±)-colchicine (**1**) in a five-step reaction sequence that included chromatographic separation of appropriate camphanylated diastereomers. Acid hydrolysis of the (+)-diastereomer, followed by acetylation, yielded the desired product **2b**. (+)-Thiocolchicine has 15-fold lower inhibitory activity against tubulin polymerization than (−)-thiocolchicine, and is 29-fold less potent for inhibiting growth of human Burkitt lymphoma cells. The enantiomer **2a**, prepared from the (−)-camphanylated diastereomer, had potent activity in all assays comparable to that of (−)-thiocolchicine prepared by other methods. These results support the hypothesis that the proper configuration of colchicine-related compounds is an important requirement for their anti-tubulin action. © 1997 Elsevier Science Ltd.

Introduction

When racemic mixtures of molecules containing atomic and/or molecular asymmetry interact with biopolymers (receptors and enzymes), invariably one of the two antipodal isomers (enantiomers) is responsible for most or all of the biological activity.[2,3] For example, colchicine (**1a**) (Fig. 1), a major alkaloid of *Colchicum autumnale* and *Gloriosa superba*, requires the (*S*)-configuration of the acetamido group at C(7) for its interaction with the protein tubulin.[4] The optical rotation of natural colchicinoids exhibits a strong negative Cotton effect at 260 nm, which results from an axial *aS* configuration of the noncoplanar biaryl system. However, a positive Cotton effect at 260 nm was observed after reaction of (±)-colchicine (**1**) (Fig. 1) with tubulin, which was explained by the presence of unbound (+)-colchicine (**1b**) (Fig. 1), the unnatural enantiomer of colchicine.[5] This analysis was later supported with additional data.[6] The importance of the aryl-tropolone configuration for the colchicinoid–tubulin interaction was also shown in studies with the antipodal isomers of deacetamidocolchicines, which clearly showed that only the (*S*)-configured enantiomer bound to tubulin.[7] Almost all colchicine or thiocolchicine derivatives that have tubulin binding ability possess (−)-*aS*-configuration. Recently, we synthesized a series of 7-*O*-thiocolchicine derivatives, and bioassay results with these compounds also confirmed the postulate.[8] In addition, unnatural (+)-colchicine with an (*aR*,7*R*)-configuration was prepared by two different routes and was considerably less potent than the natural (−)-enantiomer in assays measuring inhibition of tubulin polymerization and binding of radiolabeled colchicine.[2] Unnatural (+)-colchicine also was much less toxic than the natural alkaloid when given to mice.[9] These results suggest that interaction between tubulin and colchicinoids containing a tropolonic ring C and a C(7) substituent is enantioselective. To further test this important theory in structure-based drug design, we report herein the results of the antitubulin action of (+)-thiocolchicine (**2b**) (Fig. 1) which also has the unnatural configuration. The pharmacological results in this investigation support the postulate.

This paper deals with the synthesis, resolution and biological evaluation of the novel compound **2b**, which is related to unnatural colchicinoids possessing a biaryl axial *R* configuration and an atomic *R* configuration at the C(7) position, through a different synthetic procedure from (+) colchicine. The high activity[10] of the (*S*)-enantiomer was reconfirmed.

Chemistry

Thiocolchicine (**2a**) (Fig. 1), readily available from colchicine by treatment with sodium methanethiolate,[4] is a potent inhibitor of tubulin polymerization and cell growth, and binds to tubulin more rapidly than colchicine.[10] Compound **2b**, (+)-thiocolchicine, is the enantiomer of thiocolchicine (**2a**). Originally, we

[†]For part 174, see ref 1.
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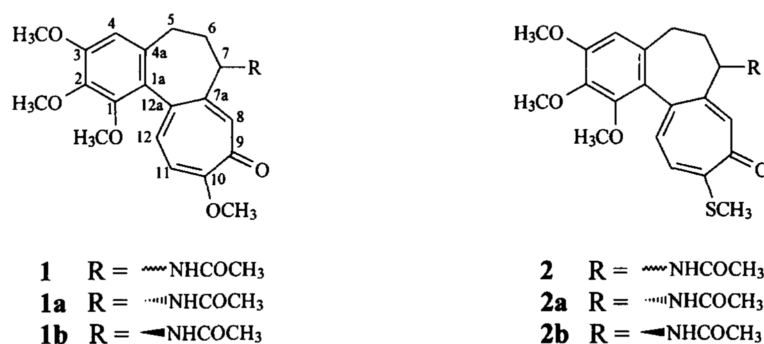


Figure 1. Structures of colchicine and thiocolchicine.

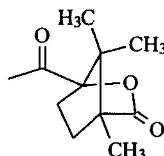
attempted to synthesize **2b** from (−)-colchicine via (+)-colchicine in nine steps.[11] However, the yield of the desired compound (+)-thiocolchicine would be extremely low, because two isomer-resolutions are involved in this route. Furthermore, it is very difficult to resolve (±)-thiocolchicine by the method used for (±)-colchicine due to low solubility. However, although compound **2b** could not be obtained by the route used for the synthesis of (+)-colchicine, it was successfully synthesized by the route shown in Scheme 1, with which only six steps and one resolution.

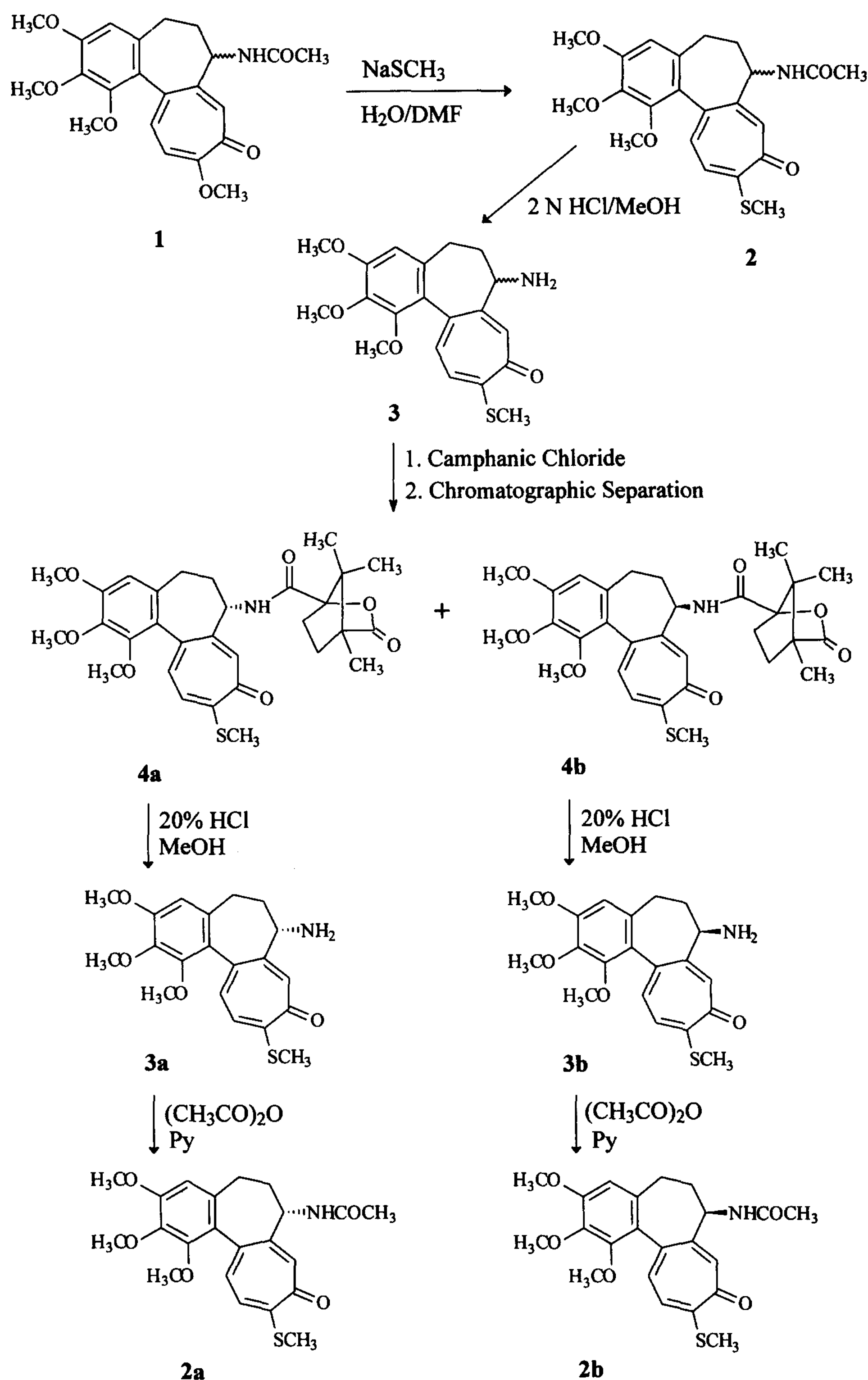
Racemization of (−)-colchicine by reflux in acetic anhydride was accomplished following Bladé–Font's procedure.[12] (±)-Colchicine (**1**) was then reacted with sodium methanethiolate to give (±)-thiocolchicine (**2**). Hydrolysis of (±)-thiocolchicine with 20% HCl yielded (±)-deacetylthiocolchicine (**3**).[13] Both compounds **2** and **3** showed zero optical rotation (Table 1). Chemical resolution of (**3**) was achieved by reaction with camphanic chloride, and separation of the resulting amide diastereomers (**4a** and **4b**) by flash column chromatography; **4b** eluted faster than **4a**. This method was first applied in resolution of racemic *N*-deacetylcolchicine analogues. After crystallization, pure isomers with opposite optical rotations were obtained. Hydrolysis of amides **4a** and **4b** with 20% methanolic HCl gave amines **3a** and **3b**, respectively.[13] The enantiomers **3a**, **3b** and their racemic mixture **3** have identical ^{1}H NMR, MS and TLC data. The optical purity of **4a** and **4b** was established by chromatography on a chiral column.[14] The acetamides **2a** and **2b** were obtained from **3a** and **3b** by acetylation and showed identical ^{1}H NMR, MS and TLC data but opposite optical proper-

Table 1. Physicochemical data for thiocolchicine derivatives

Compound	R	Configuration	$[\alpha]^{25}_{D}$	Mp (°C)	R_f
2	$NHCOCH_3$	(±)	0	250–252	0.44[a]
2a	$NHCOCH_3$	(−)-(a*S*,7*S*)	−290°	188-190	0.44[a]
2b	$NHCOCH_3$	(+)-(a*R*,7*R*)	+289°	183–185	0.44[a]
3	NH_2	(±)	0	202–204	0.29[a]
3a	NH_2	(−)-(a*S*,7*S*)	−160.6°	194–195	0.29[a]
3b	NH_2	(+)-(a*R*,7*R*)	+162°	NT[c]	0.29[a]
4a	NHCAM[d]	(+)-(a*S*,7*S*)	−189°	165–167	0.20[b]
4b	NHCAM[d]	(+)-(a*R*,7*R*)	+211°	195–197	0.23[b]

[a]Developing solvents: $CHCl_3$:MeOH (100:8).
[b]Developing solvents:CH_2Cl_2:EtOAc:hexane (1:1:1).
[c]NT: Not tested.
[d]CAM:





Scheme 1. Synthesis of (−)- and (+)-thiocolchicine.

ties. On the other hand, reaction of deacetylthiocolchicine obtained through an identical reaction sequence starting with natural colchicine yielded (−)-camphanamide deacetylthiocolchicine identical with **4a**. Hydrolysis of the resulting compound with 20% methanolic HCl produced deacetylthiocolchicine identical with **3a**. Furthermore, treatment of the newly resulting deacetylthiocolchicine with Ac_2O generated only (−)-thiocolchicine, **2a**. This result verified the reliability of the resolution method. The final isomers are optically pure enantiomers; **2a** has an (*aS*,7*S*)-configuration[4] and, **2b** has an (*aR*,7*R*)-configuration.[4]

Biological Results and Discussion

Compounds **2b**, **4a**, and **4b** were evaluated for their potential inhibitory effects on tubulin polymerization and compared with the previously characterized **2a** and **3a**.[15,16] In addition, the (+)-thiocolchicine (**2b**) was evaluated for its effects on the binding of [^{3}H] colchicine to tubulin and on the proliferation of human Burkitt lymphoma CA46 cells, in comparison with **2a** and **3a**.

As anticipated, compound **2b**, which has the *aR*,7*R* configuration of (+)-colchicine (**1b**), was substantially less active than (−)-thiocolchicine (**2a**), with the *aS*,7*S* configuration, in all assays. It was 15-fold less active as an inhibitor of tubulin polymerization, fivefold less active as an inhibitor of [^{3}H]colchicine binding, and 29-fold less active as an inhibitor of Burkitt cell growth. The amine **3a** was about half as active as (−)-thiocolchicine as an inhibitor of polymerization, equivalent as an inhibitor of [^{3}H]colchicine binding, and about eightfold less active as an inhibitor of Burkitt cell growth.

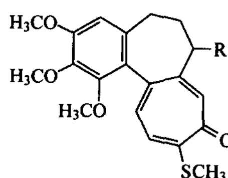
The two camphanic acid amides had widely divergent activities. The 7*R* enantiomer was essentially inactive, while the 7*S* enantiomer had significant inhibitory effect on tubulin polymerization, although it was about fourfold less potent than (−)-thiocolchicine. We had previously separated camphanate esters of deacetamidothiocolchicin-7-ol and evaluated their effects on tubulin polymerization.[8] The 7*S* ester was about twice as inhibitory as the 7*S* amide, and the 7*R* ester was at least twice as active as the 7*R* amide. (The oxygen isostere of **2a** is more active than **2a**, and the 7*S* enantiomer of deacetamidothiocolchicin-7-ol is more active then **3a**.[8])

In summary, the findings with the thiocolchicine enantiomers are in complete accord with the previous conclusion that the major explanation for the activity of 7*S* colchicinoids versus the poor activity of 7*R* colchicinoids derives from the biaryl *aS* configuration preferred by the former and the *aR* configuration preferred by the latter.[5–7] The similar optical properties of (−)-thiocolchicine and (−)-colchicine and of (+)-thiocolchicine and (+)-colchicine (cf. Table 2 and ref. 11) imply that the thiocolchicines and colchicines have equivalent biaryl conformation. Our confidence in this assumption is further strengthened by our recent findings with a series of 7-*O*-acylated thiocolchicine analogues.[8] In this work, too, optimal activity occurred universally with the 7*S* enantiomers.

Experimental

Chemistry. Melting points were measured with a Fisher–Johns melting point apparatus without correction. Optical rotations were determined with a DIP-1000 polarimeter. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker AC-300 spectrometer with Me_4Si (TMS) as the internal reference and $CDCl_3$ as solvent. Elemental analyses were determined by Atlantic Microlab Inc., Norcross, GA. MS was determined by NIH. Thin-layer chromatographic (TLC) silica gel plates were purchased from Analtech, Inc. Silica gel

Table 2. Biological activities of thiocolchicine derivatives



Compound and Configuration	R	Inhibition of Tubulin Polymerization[a] IC_{50} (μM)±SD	Inhibition of [^{3}H]colchicine Binding[b] (%) 1:1[d]	Inhibition of [^{3}H]colchicine Binding[b] (%) 10:1[e]	Inhibition of Burkitt Lymphoma Cell Growth[c] IC_{50} (nM)
2a (−)-(a*S*,7*S*)	$NHCOCH_3$	0.65±0.03	45		4.5
2b (+)-(a*R*,7*R*)	$NHCOCH_3$	9.7±0.50	9	43	130
3a (−)-(a*S*,7*S*)	NH_2	1.3±0.08	49		38
4a (−)-(a*S*,7*S*)	NHCAM	2.7±0.10			
4b (+)-(a*R*,7*R*)	NHCAM	>40			

[a]Tubulin polymerization was evaluated as described in ref 15. A minimum of two independent experiments was performed with each compound. The IC_{50} value is defined as the concentration that inhibits by 50% the extent of assembly after 20 min at 30 °C.
[b]The binding of [^{3}H]colchicine (5.0 μM) to tubulin (1.0 μM, 0.1 mg/mL) was measured as dscribed in ref 10.This was incubated for10 min at 37 °C. The values shown in the table represent the averages of two independent experiments, each performed with triplicate samples.
[c]Human Burkitt lymphoma line CA46 was cultured in 5 mL suspension culture for 24 h at 37 °C under a 5% CO_2 atmosphere as described for another cell line in ref 16. The IC_{50} value represents the concentration that inhibits increase in cell number relative to the control without drug by 50%. Averages obtained in two independent experiments are presented.
[d]Compound (inhibitor) concentration was 5.0 μM.
[e]Compound concentration was 50 μM.

(23–400 mesh) from Aldrich, Inc. was used for column chromatography.

Starting material. (±)-Colchicine was prepared according to the procedure of R. Dumond.[11] 68% yield, mp 273–275 °C.

(±)-Thiocolchicine (2). (±)-Colchicine (**1**) (1.75g, 4.39 mmol) was dissolved in 100 mL of MeOH:DMF (1:1) at 70–80 °C. After the solution was cooled to rt, sodium methanethiolate (2.8 g, 40 mmol) was added. The reaction mixture was stirred at rt overnight. Water (20 mL) was added, and the reaction mixture was extracted with CH_2Cl_2. The CH_2Cl_2 fraction was dried over Na_2SO_4 and concentrated. Crystallization of the residue from Et_2O–acetone gave 1.3 g of **2** (71% yield). Mp 250–252 °C; $[\alpha]^{25}_D$ 0° (*c* 0.22; MeOH); ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H, $COCH_3$), 2.10–2.40 (m, 4H, H-5,6), 2.45 (s, 3H, SCH_3), 3.60 (s, 3H, OCH_3-1), 3.82 (s, 3H, OCH_3-2), 3.89 (s, 3H, OCH_3-3), 4.62 (m, 1H, H-7), 6.48 (s, 1H, H-4), 6.92 (br, 1H, NH-7), 7.14 (d, *J* = 10.5 Hz, 1H, H-11), 7.38 (d, *J* = 10.5 Hz, 1H, H-12), 7.54 (s, 1H, H-8). Anal. calcd for ($C_{22}H_{25}NO_5S$): C, 63.60, H, 6.06, N, 3.37, S, 7.72; found: C, 63.71, H, 6.15, N, 3.42, S, 7.79.

(±)-Deacetylthiocolchicine (3). A solution of 1.3 g (3.13 mmol) of **2** in MeOH (50 mL) and 2 N HCl (25 mL) was heated at 85–90 °C with stirring for 2.5 days. After the reaction mixture was cooled, the solution was neutralized with saturated $NaHCO_3$ solution, extracted with CH_2Cl_2, and washed with brine. The extract was dried over Na_2SO_4 and evaporated to give 1.25 g of crude **3**. Flash chromatography (CH_2Cl_2: EtOAc:MeOH, 170:3:2) and crystallization from CH_2Cl_2/MeOH gave 1.04 g of pure **3** in an 89% yield; mp 202–204 °C; $[\alpha]^{25}_D$ 0° (*c* 0.455; MeOH); ^{1}H NMR ($CDCl_3$) δ 2.28–2.41 (m, 4H, H-5,6), 2.43 (s, 3H, SCH_3), 3.66 (s, 3H, OCH_3-1), 3.73 (m, 1H, H-7), 3.91 (s, 6H, OCH_3-2,3), 6.54 (s, 1H, H-4), 7.03 (d, *J* = 10.5 Hz, 1H, H-11), 7.19 (d, *J* = 10.5 Hz, 1H, H-12), 7.58 (s, 1H, H-8). Anal. calcd for ($C_{20}H_{23}NO_4S$): C, 64.32, H, 6.21, N, 3.75, S, 8.58; found: C, 64.38, H, 6.27, N, 3.83, S, 8.50.

(7*S*)-camphanamide (4a) and (7*R*)-camphanamide (4b). To a solution of **3** (107.2 mg, 0.29 mmol) in anhydrous pyridine (2 mL) was added 93.25 mg (0.43 mmol) of (*S*)-(−)-camphanic chloride. The reaction mixture was stirred at rt overnight. After evaporation of solvent, the residue was diluted with water and extracted with EtOAc. The extract was dried over Na_2SO_4 and concentrated. The residue was separated by using flash chromatography (CH_2Cl_2:hexane: EtOAc, 1:1:0.5) with **4b** (R_f value of 0.23) eluting faster than **4a** (R_f value of 0.20) (CH_2Cl_2:EtOAc:hexane, 1:1:1). Crystallization from EtOAc/hexane gave 63.6 mg of pure **4b** as yellow needles in a 40% yield and 35.0 mg of **4a** as white fine needles in a 22% yield.

(7*S*)-camphanamide (4a). Melting point 165–167 °C; $[\alpha]^{25}_D$ −189° (*c* 0.41; MeOH), ^{1}H NMR ($CDCl_3$) δ 0.89, 1.03, 1.11 (s, each 3H, camphanoyl CH_3), 1.65, 1.92 (m, each 2H, camphanoyl CH_2), 2.20, 2.55 (m, each 2H, H-5,6), 2.42 (s, 3H, SCH_3), 3.67 (s, 3H, OCH_3-1), 3.92 (s, 3H, OCH_3-2), 3.96 (s, 3H, OCH_3-3), 4.65 (m, 1H, H-7), 6.54 (s, 1H, H-4), 7.06 (d, *J* = 10.5 Hz, 1H, H-11), 7.13 (d, *J* = 7.5 Hz, 1H, NH-7), 7.22 (s, 1H, H-8), 7.29 (d, *J* = 10.5 Hz, 1H, H-12); CIMS *m/z* 554 $(M+H)^+$. Anal. calcd for ($C_{30}H_{35}O_7NS$): C, 65.08, H, 6.37, N, 2.53, S, 5.79; found: C, 65.12, H, 6.45, N, 2.45, S, 5.82.

(7*R*)-camphanamide (4b). Mp 195–197 °C; $[\alpha]^{25}_D$ +211° (*c* 0.36; MeOH); ^{1}H NMR ($CDCl_3$) (1.01, 1.09, 1.12 (s, each 3H, camphanoyl CH_3), 1.65, 1.92 (m, each 2H, camphanoyl CH_2), 2.22, 2.60 (m, each 2H, H-5,6), 2.44 (s, 3H, SCH_3), 3.68 (s, 3H, OCH_3-1), 3.91 (s, 3H, OCH_3-2), 3.94 (s, 3H, OCH_3-3), 4.61 (m, 1H, H-7), 6.56 (s, 1H, H-4), 6.99 (d, *J* = 6.8 Hz, 1H, NH-7), 7.06 (d, *J* = 10.0 Hz, 1H, H-11), 7.20 (s, 1H, H-8), 7.29 (d, *J* = 10.0 Hz, 1H, H-12); CIMS *m/z* 554 $(M+H)^+$. Anal. calcd for ($C_{30}H_{35}O_7NS$): C, 65.08, H, 6.37, N, 2.53, S, 5.79; found: C, 64.94, H, 6.34, N, 2.50, S, 5.70.

(+)-Deacetylthiocolchicine (3b). A solution of **4b** (36.5 mg, 0.07 mmol) in 20% methanolic HCl was heated at 90 °C with stirring for 72 h. After cooling, the reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The extract was washed with brine and dried over anhydrous Na_2SO_4. After evaporation of solvent, the residue (25.4 mg) was separated on a silica gel column giving 4.9 mg of pure compound **3b**: yield 20%; amorphous; $[\alpha]^{25}_D$ +162° (*c* 0.35; MeOH); ^{1}H NMR δ 2.46 (s, 3H, SCH_3), 2.28–2.55 (m, 4H, H-5,6), 3.65 (s, 3H, OCH_3-1), 3.73 (m, 1H, H-7), 3.95 (s, 6H, OCH_3-2,3), 6.55 (s, 1H, H-4), 7.04 (d, *J* = 10.4 Hz, 1H, H-11), 7.19 (d, *J* = 10.4 Hz, 1H, H-12), 7.54 (s, 1H, H-8); CIMS *m/z* 374 $(M+H)^+$. Anal. calcd for ($C_{20}H_{23}NO_4S$): C, 64.32, H, 6.21, N, 3.75, S, 8.58; found: C, 64.41, H, 6.18, N, 3.79, S, 8.52.

(−)-Deacetylthiocolchicine (3a). This compound was prepared in an analogous manner as **3b** from **4a** (100 mg, 0.181 mmol). Purification gave 6.75 mg of **3a** as a solid: yield 10%; mp 194–195 °C; $[\alpha]^{25}_D$ −160.6° (*c* 0.31; MeOH); CIMS *m/z* 374 $(M+H)^+$. ^{1}H NMR ($CDCl_3$) was identical with that of **3b**. Anal. calcd for ($C_{20}H_{23}NO_4S$): C, 64.32, H, 6.21, N, 3.75, S, 8.58; found: C, 64.27, H, 6.35, N, 3.68, S, 8.47.

(+)-Thiocolchicine (2b). To a solution of **3b** (5.0 mg, 0.013 mmol) in anhydrous pyridine was added Ac_2O (anhydrous) (2.5 mL). This reaction mixture was stirred at rt for 4 h. After evaporation in vacuo, the residue was extracted with EtOAc and concentrated. The residue was chromatographed on a preparative TLC plate and gave 5.38 mg of pure **2b** as a solid: yield 97.1%; mp 183–185 °C $[\alpha]^{25}_D$ +289° (*c* 0.31; MeOH); ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H, $COCH_3$), 2.32, 2.55 (m, each 2H, H-5,6), 2.47 (s, 3H, SCH_3), 3.67 (s, 3H, OCH_3-1), 3.92 (s, 3H, OCH_3-2), 3.95 (s,

3H, OCH_3-3), 4.68 (m, 1H, H-7), 6.55 (s, 1H, H-4), 6.88 (d, J = 7.0 Hz, 1H, NH-7), 7.13 (d, J = 10.5 Hz, 1H, H-11), 7.36 (d, J = 10.5Hz, 1H, H-12), 7.43 (s, 1H, H-8). Anal. calcd for ($C_{22}H_{25}NO_5S$): C, 63.60, H, 6.06, N, 3.37, S, 7.72; found: C, 63.54, H, 6.15, N, 3.28, S, 7.61.

(−)-**Thiocolchicine** (**2a**). This compound was prepared from **3a** (5.0 mg, 0.013 mmol) by using the same procedure as for **2b**. After purification by chromatography, 5.19 mg of **2a** was obtained in a 93.5% yield; mp 188–190 °C $[\alpha]^{25}_{D}$ −290° (c 0.22; MeOH); ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H, $COCH_3$), 2.35, 2.55 (m, each 2H, H-5,6), 2.45 (s, 3H, SCH_3), 3.67 (s, 3H, OCH_3-1), 3.91 (s, 3H, OCH_3-2), 3.95 (s, 3H, OCH_3-3), 4.68 (m, 1H, H-7), 6.54 (s, 1H, H-4), 7.09, (d, J = 10.5 Hz, 1H, H-11), 7.33 (d, J = 10.5 Hz, 1H, H-12), 7.04 (s, 1H, H-8) 7.40 (d, J = 8.5 Hz, 1H, NH-7). CIMS m/z 415 $(M+H)^+$. Anal calcd for ($C_{22}H_{l}NO_5S$): C, 63.60, H, 6.06, N, 3.37, S, 7.72; found: C, 63.48, H, 6.21, N, 3.31, S, 7.65. These data are identical with those of material prepared from natural colchicine.

Biological assays. The tubulin polymerization,[15] [^{3}H]colchicine binding,[10] and the cell growth[16] assays were all performed as described previously. The human Burkitt lymphoma CA46 line was provided by Dr P. O'Connor, National Cancer Institute.

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