Antitumor Agents. 183.† Syntheses, Conformational Analyses, and Antitubulin Activity of Allothiocolchicinoids

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7-*O*-Substituted analogues of allothiocochicine were synthesized and evaluated for their inhibitory effects on tubulin polymerization in vitro. Ketone **6**, a key compound in this study, was derived from thiocolchicone **5** by ring contraction. The structure of **6** was determined from spectral data. Optically active alcohols **7a** and **7b** were obtained by reduction of ketone **6** followed by chemical resolution including a separation of the camphanate diastereomers **8a** and **8b** and basic hydrolysis. The *aR*,7*R* configuration of **8b** was verified by X-ray crystallographic analysis. Almost all compounds had strong inhibitory effects on the tubulin polymerization reaction, with IC_{50} values from 1.7 to 9.0 μM. The camphanates, cyclohexanates, and, most notably, the 7*S*-benzoate ester (**10a)**, were inactive with IC_{50} values >40 μM. Compounds **6** and **7a** also showed potent antitumor activity with GI_{50} values at nM concentration range for most cell lines in NCI's in vitro screening. Generally, the 7*S* enantiomers of colchicinoids with a troplone C-ring showed greater activity than the 7*R* enantiomers. In the current allothiocolchicinoid (with a benzenoid C-ring) study, only small differences occurred between the two active enantiomers of each pair. The acyl esters with a 7*S* configuration were slightly more active than the 7*R* isomers. However, the aroyl ester with a 7*S* configuration was less active than the 7*R* isomer. NMR, optical rotation, and molecular modeling studies revealed two conformers in a solvent-dependent equilibrium for both 7*S* and 7*R* isomers. In polar solvents, the molecular chirality in esters with a 7-*O*-aroyl substituent was reversed from *aS* to *aR* or from *aR* to *aS* at an intensified rate.

Introduction

Colchicine (**1**) (Figure 1) is one of the oldest known alkaloids and was first isolated from *Colchicum autumnale* and *Gloriosa superba*. It is best known for its antimitotic effects [2] and has been investigated as a drug for the treatment of gout, familial Mediterranean fever,[3] and liver cirrhosis.[4] Colchicine has been studied as an anticancer agent; however, because of their toxicity, neither colchicine nor its 10-thiomethyl ether derivative, thiocolchicine (**2**) (Figure 1), has been useful in the treatment of human neoplasms.

The biological effects of colchicine, including pharmacological activity and toxicity, are attributed to its binding to the protein tubulin, the structural subunit of microtubules.[5] The interaction of colchicine site agents with tubulin typically causes the complete disappearance of cellular microtubules. Numerous synthetic and natural colchicine derivatives have been used to study the properties of tubulin and microtubules, to find active antimitotic antitumor agents, and to develop structure−activity information about requirements for binding to the colchicine site. These studies have shown that conformation and configuration of colchicinoids are important facts for drug binding to tubulin. Natural colchicine and active analogues with a phenyltropolone biaryl ring system all have a (−)-*aS*,7*S* configuration,

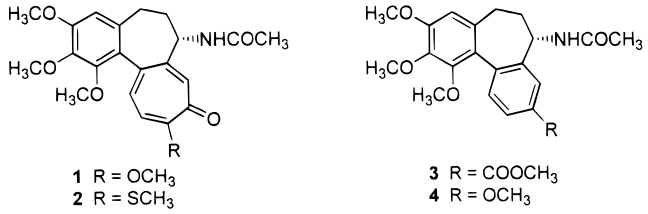

Figure 1. Structures of colchicine (**1**), thiocolchicine (**2**), and allocolchicine derivatives (**3** and **4**).

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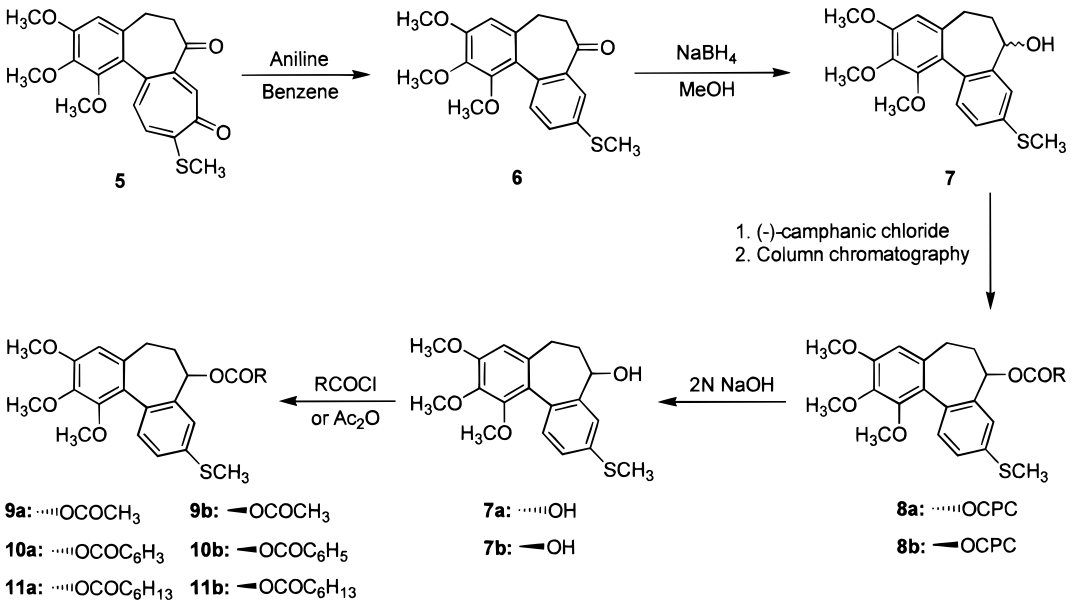
Scheme 1. Synthesis of Allothiocolchicine Derivatives



while the (+)-*aR*,7*R* antipodal isomers bind poorly.[4,6−10] This conclusion regarding the stereoselectivity of the tubulin/colchicinoid interaction was further supported by our recent studies.[6−10]

The structural features of the colchicinoids themselves that control the *aS*−*aR* configuration are not entirely clear. NMR spectral analysis indicated that the C(7) side chain may play a role in the phenyltropolone ring system configuration. The substituents at C(7) of both natural (7*S*)-colchicine and its (7*S*)-analogues and unnatural (7*R*)-colchicine energetically favor an equatorial over an axial orientation. This conformational preference of the C(7) substituents causes (7*S*)-colchicinoids to adopt the *aS* configuration, whereas unnatural (7*R*)-colchicine adopts the *aR* configuration. This finding has been confirmed with many series of colchicine analogues containing a phenyltropolone biaryl ring system.[7−10] However, in allocolchicinoids, where colchicine's tropolonic C ring is replaced with an aromatic phenyl ring, the structural features controlling the *aS*−*aR* configuration are less clear. Two allocolchicinoids allocolchicine (**3**) and *N*-acetylcolchinol *O*-methyl ether (**4**) (Figure 1) have been reported to prefer the *aS* biaryl configuration.[11,12]

In the course of our investigation of novel colchicinoids as potent antimitotic antitumor agents and as tools to elucidate their interaction with tubulin, we synthesized a series of new allothiocolchicinoids. In this study, we report their syntheses, antitubulin activity, antitumor activity, and the effect of the C(7) side chain on the biaryl configuration, including solvent-dependency.

Results and Discussion

Syntheses of Allothiocolchicone (6), Racemic and Optically Active *Allo*-Alcohols (7, 7a,b), and Their Esters (8a,b−11a,b). Allothiocolchicone (**6**) was prepared by reaction of thiocolchicone (**5**), obtained as described previously,[9] with aniline in 39% yield (Scheme 1). The tropolonic ring contraction might occur through a similar mechanism to that hypothesized by par B. P. Vaterlaus and M. A. Iorio[13,14] to give a stable benzenoid ring compound. This ring contraction did not occur for either a deacetylthiocolchicine with an amine group at C(7) or thiocolchicine with an acetamido substituent at C(7). Therefore, the C(7) ketone group may be an important contributor for the transformation. Use of other amines such as methylamine or butylamine did not form the desired compound. The structure of **6** was verified by UV and ^{1}H, ^{13}C, and 2D NMR data. A strong absorbance at 340 nm is caused by the tropolone moiety and is observed in most colchicinoids, but this absorbance was not present in the *allo*-ketone **6** and its derivatives. Reduction of ketone **6** with sodium borohydride gave alcohol **7**. Reaction of **7** with optically pure (1*S*)-(−)-camphanic acid chloride (Scheme 1) yielded a mixture of camphanate esters **8a** and **8b**, which were separated by flash column chromatography on silica gel to afford the optically active esters **8a** and **8b**. Basic hydrolysis of **8a** and **8b** led to alcohols **7a** and **7b**, respectively; these enantiomers have identical melting points, NMR spectra, and TLC properties, but opposite optical rotations and CD spectra (Table 1). Esterification with acid chlorides or anhydrides yielded optically active esters **9a**−**11a** and **9b**−**11b.**

Solid State Structure of 8b. The complete structure and absolute stereochemistry of **8b** were established unequivocally from NMR, UV, and MS spectral data, elemental analysis, and X-ray crystallographic analysis.[15] A view of the solid-state conformation is presented in Figure 2. The overall absolute configuration was defined

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Table 1. Inhibition of Tubulin Polymerization, [^{3}H]Colchicine Binding to Tubulin, Optical Rotations, and Molar Ellipticities of *Allo*-Congeners

compd	ITP,[a] $IC_{50} \pm SD$ (μM)	ICB,[b] % inhibition	$[\alpha]^{25}_D$ in $CHCl_3$, deg	$[\alpha]^{25}_D$ in MeOH, deg	$[\theta] \times 10^{-3}$ deg cm^2/dmol (nm)[c] CH_2Cl_2	$[\theta] \times 10^{-3}$ deg cm^2/dmol (nm)[c] MeOH
2	2.1 ± 0.4	44	−290		NT[d]	NT
5	2.1 ± 0.09	74	0	0	0	0
6	1.7 ± 0.08	87	0	0	0	0
7	3.3 ± 0.2	−	0	0	0	0
7a	1.8 ± 0.4	95	−143.6	−130.4	−10.5	−9.6
7b	9.0 ± 0.3	28	+150.9	+132.3	+6.3	NT
8a	>40	−	−100	NT	NT	NT
8b	>40	−	+131	NT	NT	NT
9a	2.1 ± 0.4	84	−146.9	−123.7	NT	NT
9b	3.1 ± 0.5	62	+140.8	+122.2	NT	NT
10a	>40	18	−12.6	+12	−9.1	−2.4
10b	2.7 ± 0.4	44	+13.2	−12.2	+6.6	NT
11a	>40	−	−123.6	−122.2	−0.23	NT
11b	>40	−	+116.1	+96	+0.21	NT

[a] Inhibition of tubulin polymerization, The tubulin concentration was 10 mM (1.0 mg/mL). [b] Inhibition of [^{3}H]colchicine binding to tubulin. Inhibitor and [^{3}H]colchicine at 5.0 mM and tubulin at 1.0 mM (0.1 mg/mL). Incubation 10 min at 37 °C. [c] $[\theta]$ is the term for molar ellipticity. The nm value is 280 and is the value at which molar ellipticities were calculated. [d] Not tested.

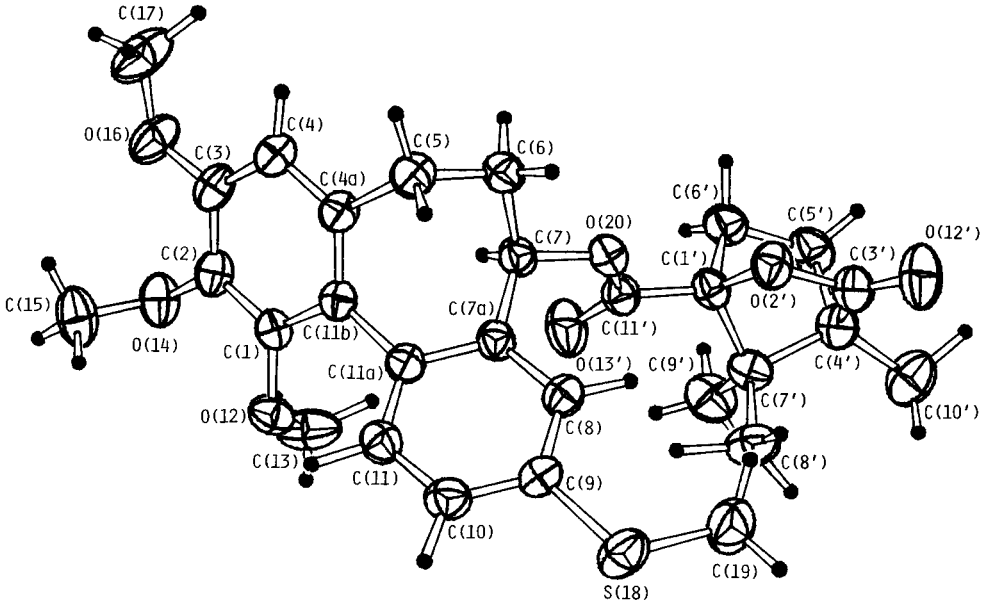

Figure 2. ORTEP diagram (40% probability ellipsoids) showing the crystallographic atom numbering scheme and solid-state conformation of **8b**; small filled circles represent hydrogen atoms.

by that of the (1′*S*)-camphanoyl moiety. Endocyclic torsion angles characterizing the conformation of seven-membered ring B [$\omega_{4a,5}$ 71.6(4), $\omega_{5,6}$ −43.8(4), $\omega_{6,7}$ −45.0(4), $\omega_{7,7a}$ 77.5(4), $\omega_{7a,11a}$ −2.9(5), $\omega_{11a,11b}$ −52.1(4), $\omega_{11b,4a}$ 3.3(5)°] are related by an approximate C_2-symmetry axis passing through C6 and the midpoint of the C11a−C11b bond; thus, the ring has a distorted twist-boat form with the C7 substituent in a pseudoequatorial orientation. Furthermore, the C4a−C11b−C11a−C7a and C11−C11a−C11b−C1 torsion angles of −52.1(4)° and −56.2(5)°, respectively, and the dihedral angle of 54.9° between the least-squares planes through the A and C ring atoms indicate that **8b** has an *aR*-biaryl configuration. The *aR*,7*R* absolute configuration determined for **8b** also establishes those of its analogues **7b**, **9b**−**11b**. Consequently, compound **8a** and its analogues **7a**, **9a**−**11a** should have an *aS*,7*S*-configuration.

Conformational Analyses in Solution. NMR Analysis. Detailed analysis of NMR spectra of optically active *allo*-congeners, including the alcohols and their esters, demonstrated that these compounds exist in two conformers in a solvent-dependent equilibrium. This was not the case with the tropolonic thiocolchicone and its derivatives, despite having the same substituents at C(7).[9]

Comparable solvent-dependent conformational equilibria have been reported by Hastie *et al.*[16] and other groups for several colchicine analogues.[4,12,17,18] Generally, in the *aS* configuration of colchicine, the coupling constant between H−C(7) and Ha−C(6) is 5.5−6.5 Hz, while

(15) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

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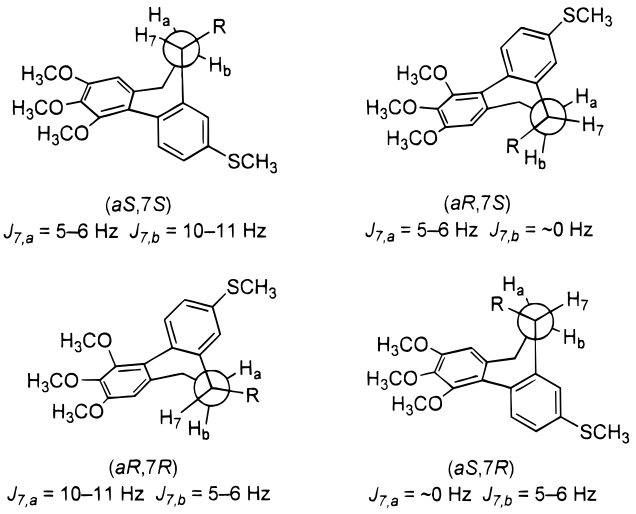

Figure 3. Coupling constants for allocolchicinoids in different configurations.

the coupling constant between H−C(7) and Hb-C(6) is 11−12.5 Hz.[4,12,16,17] Although colchicine does not have an *aR* configuration, (−)-isocolchicine and some synthetic colchicine analogues do exist in this configuration.[16,17] In the *aR* configuration of colchicinoids, the dihedral angle between H−C(7) and Hb−C(6) is about 90° resulting in a coupling constant of zero, while the coupling constant between H−C(7) and Ha−C(6) remains 6−7.5 Hz.[4,10,16,17]

In the ^{1}H NMR spectra of compounds **7b**−**11b**, which have a 7*R* configuration, two conformers are seen in the ^{1}H NMR spectrum. The H−C(7) signal appears as a double doublet with coupling constants of about 7 and 11 Hz and as a doublet with a coupling constant of about 5 Hz. When the biphenyl system of a 7*R* derivative is arranged in an *aR* configuration in a Dreiding model (Figure 3), H−C(7) and Ha,b−C(6) are oriented at about 180° and 40°. Such angles are consistent with coupling constants of 11 and 7 Hz when fitted onto the Karplus plot[19] and are similar to those observed in the solid-state (vide supra), where the dihedral angles are 166(3)° and 46(3)°, respectively. When the biphenyl system is arranged in an *aS* configuration, the H-7 is equatorially positioned, and the dihedral angles between these protons are about 90° and 60° (Figure 3). Such angles are consistent with the observed coupling constants of about 0 and 5 Hz, respectively. Thus, in the 7*R allo*-series, the conformer with a H−C(7) double doublet can be assigned an *aR* configuration, and the conformer with a H−C(7) doublet has an *aS* configuration.

For 7*S* congeners (**7a**−**11a**), the ^{1}H NMR behavior is similar to that of the 7*R* analogues. In the ^{1}H NMR spectra of compounds **7a**−**11a**, which have a 7*S* configuration, two conformers are also seen in the ^{1}H NMR spectrum. The H−C(7) for one conformer appears as a double doublet with coupling constants of about 5 and 11 Hz. These data are consistent with an *aS* configuration of the colchicinoid skeleton. On the other hand, the H−C(7) signal for the other conformer occurs as a doublet with a coupling constant about 5.5 Hz, consistent with an *aR* configuration of the colchicinoid skeleton (Figure 3). These data are identical to those obtained with the 7*R* analogues.

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Table 2. Ratio of Conformers in Different Solvents

solvent		7	8a,b	9a,b	10a,b	11a,b
$CDCl_3$	major	10	10	10	10	10
$CDCl_3$	minor	1	1	0.5	3	1.2
MeOH-d_4	major	10	10	10	10	10
MeOH-d_4	minor	0	2.2	1.3	7	2.0

The ratio of the two conformers in solution depends on the solvent employed. In $CHCl_3$, *aS* is the major conformer for all 7*S allo*-congeners; *aR* is the minor conformer. As the polarity of the solvent increases, the *aS*/*aR* ratio decreases. For the 7*R* analogues, *aR* predominates, but the *aS*/*aR* ratio increases as the solvent polarity increases (Table 2).

The solvent dependency of this conformational equilibration also varies with the C(7) substitution. In general, the H−C(7) proton signal for the alcohols (**7** and **7a,b**) and the C(7) *O*-acylated compounds (**8a,b**; **9a,b**; **11a,b**) does not change significantly on switching solvents from $CDCl_3$ to MeOH-d_4. However, this is not the case for compounds with an *O*-aroyl side chain at C(7) (**10a,b**). In $CDCl_3$, H−C(7) in these *O*-aroyl compounds gives two ^{1}H NMR signals, indicative of an *aS*/*aR* ratio of about 10:3 in 7*S* compounds and 3:10 in 7*R* isomers, respectively (Table 2). In contrast, in MeOH-d_4 the *aS*/*aR* ratio is about 10:7 in 7*S* compounds and 7:10 in 7*R* isomers (Table 2), indicating that the conformational equilibrium has reversed toward the *aR* isomer in 7*S* compounds and toward the *aS* isomer in 7*R* compounds, respectively.

Compounds with different C(7) substituents also show solvent-dependent optical rotations. For the alcohols (**7a,b**) and compounds with a C(7) *O*-acyl substituent (**9a,b,** and **11a,b**), the optical rotations were very similar in $CHCl_3$ and in MeOH. However, for compounds with an *O*-aroyl substituent at C(7) (**10a,b**), the optical rotations changed significantly with solvent (Table 1). The (7*S*)-compound **10a** had a negative rotation in $CHCl_3$, but a positive value in MeOH. Correspondingly, **10b**, the (7*R*)-compound, had a positive rotation in $CHCl_3$ that shifted to a negative value in MeOH. These data suggest a conformational conversion of *aS* to *aR* in 7*S*-*O*-aroyl esters and of *aR* to *aS* in 7*R*-*O*-aroyl esters, respectively, in MeOH.

The ^{1}H NMR signals for the A and C rings as well as the signals for the three A-ring methoxy groups and the C-ring methylthio group also changed in compounds with different C(7) substituents. The C(7) side chains containing an aromatic group may have a different orientation from the C(7) side chains containing an aliphatic group, resulting in different chemical shifts of H-4, H-8, H-10, and H-11 due to either steric or electronic effects.

Molecular Modeling Analysis. The conformational conversion of a*S* to a*R* in 7*S*-*O*-aroyl esters and of a*R* to a*S* in 7*R*-*O*-aroyl esters in polar solvents (e.g., MeOH or DMSO), as indicated by the NMR analysis, led us to use computational methods to study the conformational preference in more detail. Systematic conformation search and molecular dynamics (MD) simulation of compound **10b**, a 7*R* benzoyl ester, were performed to study the conformational preference in a vacuum and in polar solvent (water), respectively.

Compound **10b** contains eight degrees of freedom: three rotatable bonds connecting three methoxy groups to ring A, one rotatable bond connecting a methanethioyl ether group to C(9) of ring C, three rotatable bonds in the C(7) side chain, and one flippable ring corner in ring

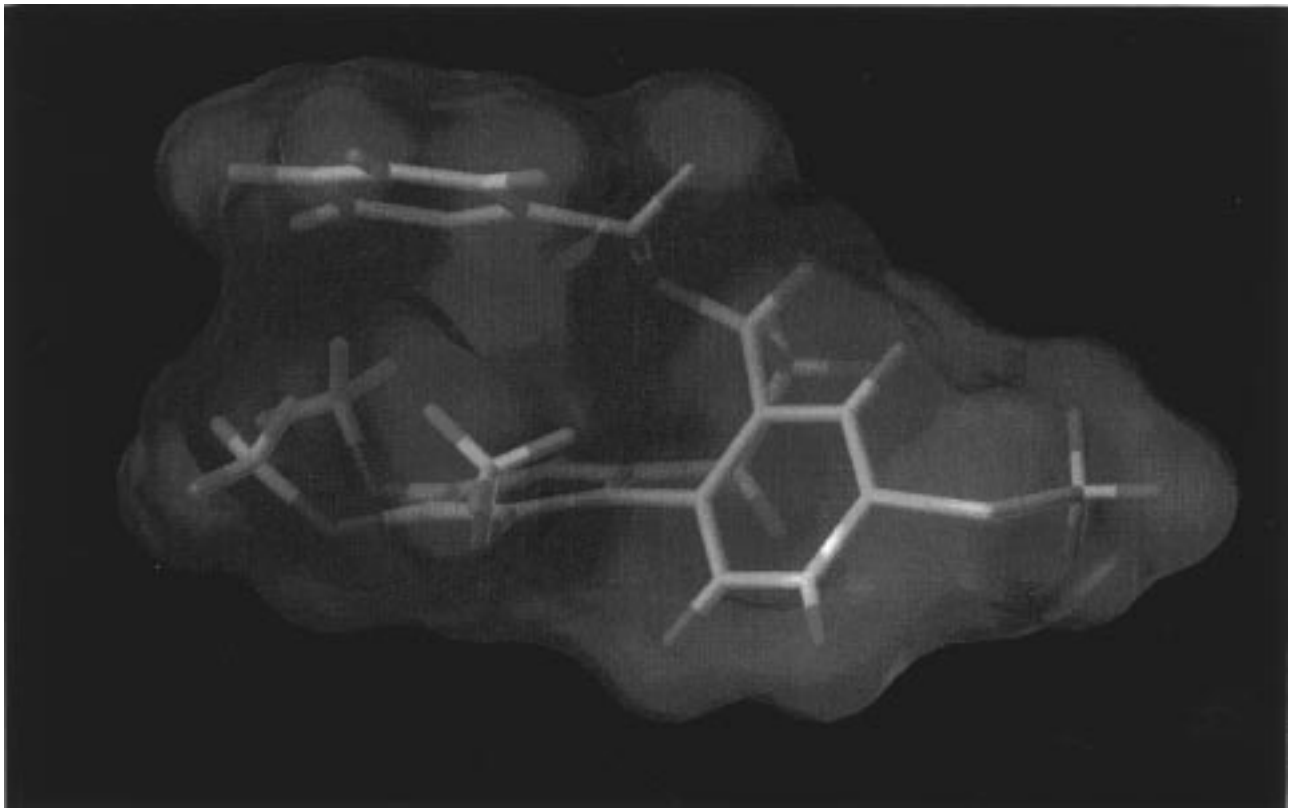

Figure 4. Conformation of compound **10b** after 200 ps MD simulation in water, surrounded by the solvent accessible surface (in green).

B. A systematic conformation search using the SYBYL molecular modeling package found three lowest energy conformations: an a*S*,7*R* configuration with an axially oriented benzoyl side chain close to ring A (conformation 1), an a*S*,7*R* configuration with an axially oriented benzoyl side chain close to ring C (conformation 2), and an a*R*,7*R* configuration with an equatorial benzoyl side chain (conformation 3). The conformational energies of these configurations are 8.83, 13.66, and 12.86 kcal/mol, respectively, suggesting that the a*S*,7*R* configuration (conformation 1) is energetically more stable than the a*R*,7*R* configuration (conformation 3) by about 4 kcal/mol. This result differs from our previous systematic conformational search (unpublished data) and NMR study,[4] in which the a*S*,7*S* configuration with an equatorial acetamido side chain at C(7) for colchicine is more stable than the a*R*,7*S* configuration.

To further study the conformational preference of compound **10b** in a polar solvent, MD simulation in water was performed. Using the SIGMA program package,[20] we did three independent molecular dynamics simulations, starting from the three lowest energy conformations determined in a vacuum, given above as conformations 1, 2, and 3. After 200 ps MD simulation, conformations 1 and 2 converged to a common conformation similar to conformation 1. This is shown in Figure 4. Within the same MD simulation time, no obvious conformational change was observed for conformation 3; compound **10b** retained the benzoyl side chain in the equatorial position. These results indicated that the energy barrier for conformational change between conformation 1 and conformation 3 is higher than the conformational change from conformation 2 to conformation 1. Thus, longer simulation time or raising simulation temperature may be required to overcome the larger energy barrier and observe the conformational change. From Figure 4, the benzoyl ring in the side chain is stacked over ring A and, together with the three methoxy groups, forms an almost perfect hydrophobic pocket. Thus, a hydrophobic effect may contribute to stabilize the a*S*,7*R* configuration of compound **10b**.

Inhibition of Tubulin Polymerization, Tubulin Binding, and Antitumor Activity. The newly synthesized compounds were evaluated for inhibitory effects on tubulin polymerization and colchicine binding to tubulin. The results are shown in Table 1. Relative to thiocolchicine (**2**), a potent antimitotic drug,[5,10] the *allo*-ketone (**6**), alcohols (**7,7a**), acetates (**9a,b**), and 7*R* benzoate (**10b**) had strong inhibitory effects on the polymerization reaction, with IC_{50} values ranging from 1.7 to 3.3 μM. Compound **7b** showed weaker inhibition with an IC_{50} value of 9.0 μM. The camphanate esters (**8a** and **8b**), the cyclohexanate esters (**11a** and **11b**), and, most notably, the 7*S* benzoate ester **10a** had negligible effects on polymerization, yielding IC_{50} values greater than 40 μM. For the most potent compounds, inhibition of the binding of [^{3}H]colchicine to tubulin was quantitatively consistent with their inhibitory effects on tubulin assembly.

In the previous thiocolchicinoid ester study, the 7*S* enantiomers all showed greater activity than the 7*R* enantiomers.[9] In contrast, in the current allothiocolchicinoid study, there was only a small difference between the two active acetate enantiomers (**9a**, **9b**), and with the benzoate esters (**10a**, **10b**), the 7*R* isomer was at least

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Table 3. Cytotoxicity of Colchicine 1, *Allo*-Ketone 6, and *Allo*-Alcohol 7a in Human Tumor Cell Lines

	cytotoxicity (log GI_{50})[a]								
compd	leukemia	NSCL[b]	colon	CNS[c]	melanoma	ovarian	renal	prostate	breast
1	−7.74	−6.91	−7.47	−6.77	−6.95	−6.13	−6.52	−6.35	−6.77
6	<−8.00	<−8.00	<−8.00	−6.90	−7.13	−6.86	−6.88	<−8.00	−7.33
7a	<−8.00	<−8.00	<−8.00	−7.32	−7.49	−7.43	−6.73	<−8.00	−7.33

[a] The cytotoxicity data were provided by the NCI. GI_{50} (M) is the concentration that caused 50% inhibition of tumor cell growth. [b] Non-small cell lung cancer. [c] Central nervous system cancer.

15-fold more inhibitory in the assembly reaction than the 7*S* isomer. Although solution conformer states do not necessarily include the active or active states, it is interesting that the more potent 7*R O*-aroyl derivative **10b** adopts a predominant a*S* biaryl configuration in polar solvents, such as water, as shown from the NMR data. Analogously, the alcohols and, especially, the acetates probably adopt both the *aS* or *aR* conformation in aqueous solution, consistent with the comparable biological activity observed in both the 7*S* and 7*R* isomers and with the NMR findings. This phenomenon would account for the substantial loss of chiral specificity in this series of allocolchicine analogues, as compared with the substantially greater activity of (−)-7*S*-colchicine versus (+)-7*R*-colchicine.

Compounds **6** and **7a** were tested in the NCI's in vitro disease-oriented antitumor screen.[21,22] This assay involves determinations of a test agent's effect on growth parameters against a panel of approximately 60 human tumor cell lines, which consist largely of solid tumors and a few leukemia cell lines. The cytotoxic effects of each compound are obtained as GI_{50}, which represents the molar drug concentrations required to cause half growth inhibition and total growth inhibition, respectively. The results are expressed in Table 3 as log GI_{50} values for each panel of cell lines and compared with colchicine (**1**). Compounds **6** and **7a** showed strong inhibitory effects against a variety of tumor cell lines, including leukemia, colon, CNS, melanoma, ovarian, renal, prostate, breast, and small cell lung cancer cell lines, with values in the low micromolar to nanomolar concentration range.

In conclusion, the 7-*O*-allothiocolchicine derivatives, particularly those with an *O*-aroyl group, are exceptions to the generalization that colchicinoids, thiocolchicinoids, and allocolchicinoids with a 7*S* or 7*R* configuration energetically favor an *aS* or *aR* biaryl configuration, respectively, to maintain the C(7) side chain in an equatorial orientation. Since these allothiocolchicines readily assume both biaryl configurations, members of this family of compounds have little chiral specificity in solution and in their interaction with tubulin. In some cases, the 7*R* enantiomers are more active than the 7*S* enantiomers and favor an *aS* biaryl configuration, especially in polar solvents.

Experimental Section

All chemicals were reagent grade and were used without further purification. Melting points are uncorrected. Optical rotations were measured in $CHCl_3$ at 25 °C. The ^1H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, with TMS as the internal reference; s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad. UV spectra were recorded in CH_2Cl_2. CD were obtained in CH_2Cl_2 or MeOH (20−30 mM). 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, Inc. was used for column chromatography.

Computational Details. The systematic search module in the SYBYL program package was used to do the systematic conformation search. The standard TRIPOS force field was used for the conformational energy calculation. Atomic charge distribution was neglected. During the search process, each rotatable bond was increased by 60° at every step and the flippable ring corner was flipped up or down. The SIGMA molecular dynamics program package was used to do the MD simulation work. However, the standard Tripos force field parameters for the bond, angle, torsion and van der Waals interactions were used for compound **10b**, and the charge distribution on the compound **10b** was calculated by the AM1 method. The periodic boundary conditions were used to keep the whole system in a ca. 20'20'20 Å box of SPC water.[23] The cutoff radius for the nonbond interaction was 10 Å. The whole system was kept at the simulation condition of 298 K and 1 atm.

Deaminodeoxycolchinol-7-one Methylthio Ether (6). Thiocolchicone (**5**) (1488 mg, 4.0 mmol), prepared according to the procedure reported previously,[9] was dissolved in 50% aniline solution in benzene (60 mL) and refluxed with stirring. The reaction was monitored by TLC. After the reaction was complete, the mixture was concentrated, and the residue was chromatographed on a flash column using hexanes−EtOAc (10:1.2) as eluant. The combined product eluate was concentrated to give a colorless solid, which was crystallized from CH_2Cl_2/MeOH and recrystallized from CH_2Cl_2 to yield colorless prisms (**6**) 537 mg (39% yield). The reaction in aniline−benzene at other concentrations led to low yield. Mp 165−166 °C; $[\alpha]^{25}_D$ 0° (*c* 0.35, $CHCl_3$); UV λ_{max} CH_2Cl_2 286 (log ϵ 4.36), 251 (log ϵ 4.27), 231 (log ϵ 4.38); ^1H NMR in $CDCl_3$: δ 2.55 (s, 3H, SCH_3), 2.60−3.10 (m, 4H, H-5,6), 3.52 (s, 3H, OCH_3-1), 3.90 (s, 3H, OCH_3-3), 3.91 (s, 3H, OCH_3-2), 6.61 (s, 1H, H-4), 7.38 (d, J = 2.0 Hz, 1H, H-8), 7.40 (dd, J = 8.9, 2.0 Hz, 1H, H-10), 7.51 (d, J = 8.9 Hz, 1H, H-11); in MeOH-d_4: δ 2.51 (s, 3H, SCH_3), 2.65−3.04 (m, 4H, H-5,6), 3.47 (s, 3H, OCH_3-1), 3.85 (s, 3H, OCH_3-3), 3.88 (s, 3H, OCH_3-2), 6.68 (s, 1H, H-4), 7.31 (d, J = 2 Hz, 1H, H-8) 7.38 (dd, J = 8.3, 2.0 Hz, 1H, H-10), 7.46 (d, J = 8.3 Hz, 1H, H-11). ^{13}C NMR ($CDCl_3$) δ 15.4 (SCH_3), 30.0 (C-5), 47.9 (C-6), 56.0 (OCH_3-3), 60.9 (OCH_3-1), 61.1 (OCH_3-2), 107.1 (C-4), 123.9 (C-1a), 124.6 (C-8), 128.6 (C-4a), 128.6 (C-11), 131.6 (C-10), 135.7 (C-7a), 138.2 (C-9), 139.8 (C-11a), 141.6 (C-2), 152.2 (C-1), 153.1 (C-3), 206.5 (C-7). CIMS *m/z* 362 $[M + H + NH_3]^+$. Anal. Calcd for $C_{19}H_{20}O_4S$ (344.40): C 66.26, H 5.85, S 9.31. Found: C 66.38, H 5.96, S 9.47.

(±)-Deaminodeoxycolchinol-7-ol 9-Methylthio Ether (7). To a solution of *allo*-ketone (**6**) (688 mg, 2.0 mmol) in CH_2Cl_2/MeOH was added $NaBH_4$ (303 mg, 8.0 m mol) at −78 °C, and the solution was stirred at −78 °C to 0 °C overnight. The reaction mixture was acidified with 50% acetic acid and then extracted with CH_2Cl_2 (4 × 10 mL). The combined

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organic phases were washed with saturated NaCl, dried over Na_2SO_4, and concentrated to give a residue, which was crystallized from Et_2O/CH_2Cl_2 and recrystallized twice from Et_2O/CH_2Cl_2 to yield pure **7** as colorless prisms 630 mg (91% yield); mp 177 °C; $[\alpha]^{25}_D$ 0° (*c* 0.31, $CHCl_3$); UV λ_{max} CH_2Cl_2 283 (log ϵ 4.48), 230 (log ϵ 4.38); ^{1}H NMR ($CDCl_3$): for major conformer: δ 1.87−2.62 (m, 4H, H-5,6), 2.57 (s, 3H, SCH_3), 3.62 (s, 3H, OCH_3-1), 3.92 (s, 3H, OCH_3-2), 3.91 (s, 3H, OCH_3-3), 4.62 (dd, *J* = 7.0, 10.8 Hz, 1H, H-7), 6.60 (s, 1H, H-4), 7.23 (dd, *J* = 2.1, 8.0 Hz, 1H, H-10), 7.40 (d, *J* = 8.0 Hz, 1H, H-11), 7.60 (d, *J* = 2.1 Hz, 1H, H-8); for minor conformer δ 1.87−2.62 (m, 4H, H-5,6), 2.55 (s, 3H, SCH_3), 3.61 (s, 3H, OCH_3-1), 3.91 (s, 3H, OCH_3-3), 3.92 (s, 3H, OCH_3-2), 4.72 (d, *J* = 5.0, Hz, 1H, H-7), 6.66 (s, 1H, H-4), 7.23 (dd, *J* = 2.1, 8.0 Hz, 1H, H-10), 7.40 (d, *J* = 8.0 Hz, 1H, H-11), 7.60 (d, *J* = 2.1 Hz, 1H, H-8); ^{1}H NMR (MeOH-d_4): δ 1.92−2.62 (m, 4H, H-5,6), 2.62 (s, 3H, SCH_3), 3.63 (s, 3H, OCH_3-1), 3.97 (s, 3H, OCH_3-3), 3.99 (s, 3H, OCH_3-2), 4.58 (dd, *J* = 7.0, 10.8 Hz, 1H, H-7), 6.75 (s, 1H, H-4), 7.28 (d, *J* = 8.0 Hz, 1H, H-10), 7.43 (d, *J* = 8.0 Hz, 1H, H-11), 7.66 (d, *J* = 2.1 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ 15.6 (SCH_3), 30.1 (C-5), 41.3 (C-6), 56.0 (OCH_3-1), 60.9 (OCH_3-3) 61.1 (OCH_3-2), 69.9 (C-7), 107.5 (C-4), 120.5 (C-8), 124.1 (C-1a), 124.2 (C-10), 129.8 (C-4a), 130.2 (C-11), 135.6 (C-9), 137.3 (C-7a), 141.0 (C-11a), 142.2 (C-2), 150.8 (C-1), 152.6 (C-3). CIMS *m*/*z* 364 $[M + H + NH_3]^+$. Anal. Calcd for $C_{19}H_{22}O_4S$ (346.44): C 65.87, H 6.40, S 9.25. Found: C 65.93, H 6.35, S 9.35.

General Procedure for Synthesizing Enantiomeric Esters (8a,b−11a,b). To a solution of the corresponding alcohol, **7a** or **7b**, in dry pyridine was added an appropriate acyl or aroyl chloride or anhydride (1.5−2.0 equiv) at 0 °C or room temperature. The mixture was stirred and allowed to stand overnight, and the volatile solvents were removed *in vacuo*. The residue was diluted with CH_2Cl_2 or Et_2O, and filtered, and the filtrate was washed with CH_2Cl_2 or Et_2O several times. After concentration in vacuo, the residue was chromatographed on a preparative TLC plate or flash column or purified by crystallization.

(−)-Camphanate (8a). Yield 25%. Resolved from (±)-camphanoyl deacetamidothiocolchicinol methylthio ether (**8**) mixture first by flash column chromatography using CH_2Cl_2/$CHCl_3$ (1:1) as eluant and then by preparative TLC with hexane/EtOAc (2:1) as eluant; colorless oil; $[\alpha]^{25}_D$ −100° (*c* 0.30, $CHCl_3$); ^{1}H NMR in $CDCl_3$, for major conformer: δ 1.01, 1.08, 1.15 (s, each 3H, camphanoyl 3 × CH_3), 1.69−2.65 (m, 8H, camphanoyl and H-5,6), 2.55 (s, 3H, SCH_3), 3.62 (s, 3H, OCH_3-1), 3.92 (s, 3H, OCH_3-3), 3.93 (s, 3H, OCH_3-2), 5.72 (dd, *J* = 7.0, 10.9 Hz, 1H, H-7), 6.60 (s, 1H, H-4), 7.25 (dd, *J* = 8.2, 2.0 Hz, 1H, H-10), 7.40 (d, *J* = 2.0 Hz, 1H, H-8), 7.43 (d, *J* = 8.2 Hz, 1H, H-11); for minor conformer: δ 0.57, 0.82, 0.99 (s, each 3H, camphanoyl 3 × CH_3), 1.69−2.65 (m, 8H, camphanoyl and H-5,6), 2.55 (s, 3H, SCH_3), 3.74 (s, 3H, OCH_3-1), 3.86 (s, 3H, OCH_3-3), 3.90 (s, 3H, OCH_3-2), 5.91 (d, *J* = 5.8 Hz, 1H, H-7), 6.53 (s, 1H, H-4), 7.17 (d, *J* = 2.0 Hz, 1H, H-8), 7.31 (dd, *J* = 8.2, 2.0 Hz, 1H, H-10), 7.54 (d, *J* = 8.2 Hz, 1H, H-11); in MeOH-d_4: for major conformer: δ 0.98, 1.09, 1.10 (s, each 3H, camphanoyl 3 × CH_3), 1.63−2.62 (m, 8H, camphanoyl and H-5,6), 2.51 (s, 3H, SCH_3-9), 3.55 (s, 3H, OCH_3-1), 3.87 (s, 3H, OCH_3-3), 3.88 (s, 3H, OCH_3-2), 5.65 (dd, *J* = 7.0, 11.0 Hz, 1H, H-7), 6.64 (s, 1H, H-4), 7.21 (dd, *J* = 8.0, 2.0 Hz, 1H, H-10), 7.33 (d, *J* = 2.0 Hz, 1H, H-8), 7.38 (d, *J* = 8.0 Hz, 1H, H-11); for minor conformer: δ 0.52, 0.80, 0.95 (s, each 3H, camphanoyl 3 × CH_3), 1.63−2.62 (m, 8H, camphanoyl and H-5,6), 2.51 (s, 3H, SCH_3-9), 3.68 (s, 3H, OCH_3-1), 3.83 (s, 3H, OCH_3-3), 3.84 (s, 3H, OCH_3-2), 5.89 (d, *J* = 5.9, Hz, 1H, H-7), 6.57 (s, 1H, H-4), 7.16 (d, *J* = 2.0 Hz, 1H, H-8), 7.28 (dd, *J* = 8.0, 2.0 Hz, 1H, H-10), 7.47 (d, *J* = 8.0 Hz, 1H, H-11). CIMS *m*/*z* 544 $[M + H + NH_3]^+$. Anal. Calcd for $C_{29}H_{34}O_7S$ (526.64): C 66.14, H 6.51, S 6.09. Found: C 66.11, H 6.61, S 6.10.

(+)-Camphanate (8b). Yield 21%. Resolved from (±)-camphanoyldeacetamidothiocolchicinol methylthio ether (**8**) mixture first by flash column chromatography after the **8a** fraction, using $CHCl_3/CH_2Cl_2$ (1:1) as eluant, and then by preparative TLC with hexane/EtOAc (2:1) as eluant. Crystallization from CH_2Cl_2 gave a white crystal; mp 166−168 °C; $[\alpha]^{25}_D$ +131° (*c* 0.34, $CHCl_3$); ^{1}H NMR in $CDCl_3$: for major conformer: δ 1.02, 1.10, 1.15 (s, each 3H, camphanoyl 3 × CH_3), 1.70−2.67 (m, 8H, camphanoyl and H-5,6), 2.54 (s, 3H, SCH_3), 3.64 (s, 3H, OCH_3-1), 3.92 (s, 3H, OCH_3-3), 3.93 (s, 3H, OCH_3-2), 5.74 (dd, *J* = 7.2, 10.9 Hz, 1H, H-7), 6.59 (s, 1H, H-4), 7.25 (dd, *J* = 8.2, 2.0 Hz, 1H, H-10), 7.35 (d, *J* = 2.0 Hz, 1H, H-8), 7.43 (d, *J* = 8.2 Hz, 1H, H-11); for minor conformer: δ 0.64, 0.82, 1.01 (s, each 3H, camphanoyl 3 × CH_3), 1.70−2.67 (m, 8H, camphanoyl and H-5,6), 2.55 (s, 3H, SCH_3), 3.70 (s, 3H, OCH_3-1), 3.9 (s, 3H, OCH_3-3), 3.90 (s, 3H, OCH_3-2), 6.02 (d, *J* = 5.6 Hz, 1H, H-7), 6.59 (s, 1H, H-4), 7.21 (d, *J* = 2.0 Hz, 1H, H-8), 7.31 (dd, *J* = 8.2, 2.0 Hz, 1H, H-10), 7.51 (d, *J* = 8.2 Hz, 1H, H-11); in MeOH-d_4: for major conformer: δ 0.96, 1.10, 1.11 (s, each 3H, camphanoyl 3 × CH_3), 1.65−2.61 (m, 4H, camphanoyl H-4,5), 2.50 (s, 3H, SCH_3-9), 3.56 (s, 3H, OCH_3-1), 3.88 (s, 3H, OCH_3-3), 3.89 (s, 3H, OCH_3-2), 5.66 (dd, *J* = 7.0, 11.0 Hz, 1H, H-7), 6.65 (s, 1H, H-4), 7.22 (dd, *J* = 8.0, 2.0 Hz, 1H, H-10), 7.27 (br, s, 1H, H-8), 7.39 (d, *J* = 8.0 Hz, 1H, H-11); for minor conformer: δ 0.65, 0.79, 0.96 (s, each 3H, camphanoyl 3 × CH_3), 1.65−2.61 (m, 8H, camphanoyl and H-5,6), 2.51 (s, 3H, SCH_3-9), 3.67 (s, 3H, OCH_3-1), 3.85 (s, 3H, OCH_3-3), 3.86 (s, 3H, OCH_3-2), 5.97 (d, *J* = 5.5, Hz, 1H, H-7), 6.64 (s, 1H, H-4), 7.26 (s, 1H, H-8), 7.27 (dd, *J* = 8.0, 2.0 Hz, 1H, H-10), 7.45 (d, *J* = 8.0 Hz, 1H, H-11). CIMS *m*/*z* 544 $[M + H + NH_3]^+$. Anal. Calcd for $C_{29}H_{34}O_7S$ (526.64) C 66.14, H 6.51, S 6.09. Found: C 66.17, H 6.56, S 6.12.

(−)-Deaminodeoxycolchinol-7-ol 9-Methylthio Ether (7a). To a solution of **8a** (158 mg, 0.3 mmol) in a mixture of MeOH/CH_2Cl_2 (5 mL) was added 2 N NaOH (1.5 mL) at −78 °C. The solution was stirred at −78 °C for 0.5 h and then at rt for 2 h and monitored by TLC. The reaction mixture was extracted with CH_2Cl_2 (10 mL × 3), and the combined CH_2Cl_2 layer was washed with H_2O (10 mL × 3). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to give crude **7a**. Crystallization of **7a** afforded pure white solid 105 mg, yield 100%; mp 131−133 °C; $[\alpha]^{25}_D$ −143.6° (*c* 0.27, $CHCl_3$); UV λ_{max} CH_2Cl_2 282 (log ϵ 4.36), 229 (log ϵ 4.25); ^{1}H NMR spectra were identical with those of **7**. CIMS *m*/*z* 364 $[M + H + NH_3]^+$. Anal. Calcd for $C_{19}H_{22}O_4S$ (346.44): C 65.87, H 6.40, S 9.25. Found: C 65.91, H 6.33, S 9.37.

(+)-Deaminodeoxycolchinol-7-ol 9-Methylthio Ether (7b). Preparation procedure was the same as for **7a**, starting with 105 mg (0.2 mmol) of **8b**. Crystallization from Et_2O/CH_2Cl_2 afforded white powder 63 mg (yield 92%); mp 129.5−131 °C; $[\alpha]^{25}_D$ +150.9° (*c* 0.25, $CHCl_3$); λ_{max} CH_2Cl_2 282 (log ϵ 4.41), 2.29 (log ϵ 4.31). ^{1}H NMR spectra were identical with those of **7**. CIMS *m*/*z* 364 $(M + H + NH_3)^+$. Anal. for $C_{19}H_{22}O_4S$ (346.44): C 65.87, H 6.40, S 9.25. Found: C 65.80, H 6.44, S 9.40.

(−)-Acetate (9a). Yield 79% (starting with 26 mg of **7a**); mp 107−108 °C; $[\alpha]^{25}_D$ −146.9° (*c* 0.51, $CHCl_3$); ^{1}H NMR in $CDCl_3$: for the major conformer δ 1.94−2.64 (m, 4H, H-5.6), 2.17 (s, 3H, acetyl CH_3), 2.55 (s, 3H, SCH_3), 3.58 (s, 3H, OCH_3-1), 3.91 (s, 3H, OCH_3-3), 3.93 (s, 3H, OCH_3-2), 5.58 (dd, *J* = 7.0, 10.8 Hz, 1H, H-7), 6.59 (s, 1H, H-4), 7.23 (dd, *J* = 1.8, 8.0 Hz, 1H, H-10), 7.35 (d, *J* = 1.8 Hz, 1H, H-8), 7.43 (d, *J* = 8.0 Hz, 1H, H-11); for the minor conformer δ 1.94−2.64 (m, 4H, H-5.6), 2.17 (s, 3H, acetyl CH_3), 2.54 (s, 3H, SCH_3), 3.56 (s, 3H, OCH_3-1), 3.91 (s, 3H, OCH_3-3), 3.93 (s, 3H, OCH_3-2), 5.77 (d, *J* = 5.0 Hz, 1H, H-7), 6.59 (s, 1H, H-4), 7.19 (d, *J* = 1.8 Hz, 1H, H-8), 7.26 (dd, *J* = 1.8, 8.0 Hz, 1H, H-10), 7.48 (d, *J* = 8.0 Hz, 1H, H-11); in MeOH-d_4: for the major conformer δ 1.97−2.60 (m, 4H, H-5.6), 2.16 (s, 3H, acetyl CH_3), 2.53 (s, 3H, SCH_3), 3.53 (s, 3H, OCH_3-1), 3.89 (s, 3H, OCH_3-3), 3.91 (s, 3H, OCH_3-2), 5.51 (dd, *J* = 7.5, 11 Hz, 1H, H-7), 6.72 (s, 1H, H-4), 7.23 (dd, *J* = 1.8, 8.0 Hz, 1H, H-10), 7.29 (br, s, 1H, H-8), 7.39 (d, *J* = 8.0 Hz, 1H, H-11); for the minor conformer δ 1.97−2.60 (m, 4H, H-5.6), 2.15 (s, 3H, acetyl CH_3), 2.52 (s, 3H, SCH_3), 3.52 (s, 3H, OCH_3-1), 3.87 (s, 3H, OCH_3-3), 3.90 (s, 3H, OCH_3-2), 5.73 (d, *J* = 5.0 Hz, 1H, H-7), 6.71 (s, 1H, H-4), 7.21 (d, *J* = 1.8 Hz, 1H, H-8), 7.28 (dd, *J* = 1.8, 8.0 Hz, 1H, H-10), 7.45 (d, *J* = 8.0 Hz, 1H, H-11). CIMS *m*/*z*: 406 $(M + H + NH_3)^+$. Anal. Calcd for $C_{21}H_{24}O_5S$ (388.47): C 64.91, H 6.23, S 8.25. Found: C 64.95, H 6.18, S 8.20.

(+)-Acetate (9b). Yield 76% (starting with 16 mg of **7b**); mp 108−109 °C; $[\alpha]^{25}_D$ +140.8° (*c* 0.41, $CHCl_3$); ^1H NMR spectra were identical with those of **9a**. CIMS *m*/*z* 406 $(M + H + NH_3)^+$. Anal. Calcd for $C_{21}H_{24}O_5S$ (388.47): C 64.91, H 6.23, S 8.25. Found: C 64.98, H 6.20, S 8.32.

(−)-Benzoate (10a). Yield 94% (starting with 12 mg of **7a**); mp 134−136 °C; $[\alpha]^{25}_D$ −12.6° (*c* 0.30, $CHCl_3$); UV λ_{max} CH_2Cl_2 282 (log ϵ 4.34), 230.5 (log ϵ 4.51). ^1H NMR in $CDCl_3$: for the major conformer: δ 2.15−2.75 (m, 4H, H-5,6), 2.51 (s, 3H, SCH_3), 3.61 (s, 3H, OCH_3-1), 3.93 (s, 3H, OCH_3-3), 3.95 (s, 3H, OCH_3-2), 5.84 (dd, *J* = 7.2, 10.8 Hz, 1H, H-7), 6.63 (s, 1H, H-4), 7.48 (d, *J* = 8.0 Hz, 1H, H-10), 7.51 (d, *J* = 8.0 Hz, 1H, H-11), 7.46 (s, 1H, H-8), 7.28 (m, 2H, benzoyl H-3′,5′), 7.62 (t, *J* = 7.0 Hz, 1H, benzoyl H-4′), 8.15 (d, *J* = 7.0 Hz, 2H, benzoyl H-2′,6′); for the minor conformer δ 2.15−2.75 (m, 4H, H-5,6), 2.56 (s, 3H, SCH_3), 3.23 (s, 3H, OCH_3-1), 3.90 (s, 3H, OCH_3-3), 3.95 (s, 3H, OCH_3-2), 6.07 (d, *J* = 5.1 Hz, 1H, H-7), 6.71 (s, 1H, H-4), 7.23 (s, 1H, H-8), 7.28 (m, 2H, benzoyl H-3′,5′), 7.41 (t, *J* = 7.0 Hz, 1H, benzoyl H-4′), 7.52 (d, *J* = 7.0 Hz, 2H, benzoyl H-2′,6′), 7.48 (d, *J* = 8.0 Hz, 1H, H-10), 7.53 (d, *J* = 8.0 Hz, 1H, H-11); in MeOH-d_4: for the major conformer: δ 2.17−2.71 (m, 4H, H-5,6), 2.45 (s, 3H, SCH_3), 3.57 (s, 3H, OCH_3-1), 3.90 (s, 3H, OCH_3-3), 3.92 (s, 3H, OCH_3-2), 5.73 (dd, *J* = 7.1, 11.0 Hz, 1H, H-7), 6.78 (s, 1H, H-4), 7.30 (d, *J* = 8.0 Hz, 1H, H-10), 7.54 (d, *J* = 8.0 Hz, 1H, H-11), 7.34 (s, 1H, H-8), 7.26 (m, 2H, benzoyl H-3′,5′), 7.67 (t, *J* = 7.0 Hz, 1H, benzoyl H-4′), 8.12 (d, *J* = 7.0 Hz, 2H, benzoyl H-2′,6′); for the minor conformer δ 2.17−2.70 (m, 4H, H-5,6), 2.55 (s, 3H, SCH_3), 3.09 (s, 3H, OCH_3-1), 3.82 (s, 3H, OCH_3-3), 3.95 (s, 3H, OCH_3-2), 5.99 (d, *J* = 5.0 Hz, 1H, H-7), 6.85 (s, 1H, H-4), 7.23−7.71 (H-8, 10, 11, 2′-6′-H). CIMS *m*/*z* 468 $[M + H + NH_3]^+$. Anal. Calcd for $C_{26}H_{26}O_5S$ (450.54): C 69.63, H 5.82, S 7.12. Found: C 69.62, H 5.85, S 7.10.

(+)-7-*O*-Benzoate (10b). Yield 87% (starting with 12 mg of **7b**); mp 136−137 °C; $[\alpha]^{25}_D$ +13.2° (*c* 0.32, $CHCl_3$); UV λ_{max} CH_2Cl_2 282 (log ϵ 4.51), 231 (log ϵ 4.54). ^1H NMR spectra were identical with those of **10a**. Anal. Calcd for $C_{26}H_{26}O_5S$ (450.54): C 69.63, H 5.82, S 7.12. Found: C 69.78, H 5.75, S 7.16.

(−)-Cyclohexanyl Carbonate (11a). Yield 95% (starting with 13 mg of **7a**); oil; $[\alpha]^{25}_D$ −123.6° (*c* 0.30, $CHCl_3$); UV λ_{max} CH_2Cl_2 282.2 (log ϵ 4.25), 229.2 (log ϵ 4.14). ^1H NMR in $CDCl_3$: for the major conformer δ 1.18−2.04 (m, 13H, cyclohexanyl and H-5,6), 2.55 (s, 3H, SCH_3), 3.58 (s, 3H, OCH_3-1), 3.91 (s, 3H, OCH_3-3), 3.92 (s, 3H, OCH_3-2), 5.56 (dd, *J* = 6.80, 11.0 Hz, 1H, H-7), 6.58 (s, 1H, H-4), 7.22 (dd, *J* = 1.8, 8.1 Hz, 1H, H-10), 7.33 (d, *J* = 1.8 Hz, 1H, H-8), 7.43 (d, *J* = 8.1 Hz, 1H, H-11); for the minor conformer: δ 1.18−2.04 (m, 13H, cyclohexanyl and H-5,6), 2.54 (s, 3H, SCH_3), 3.62 (s, 3H, OCH_3-1), 3.90 (s, 3H, OCH_3-3), 3.92 (s, 3H, OCH_3-2), 5.80 (d, *J* = 5.5 Hz, 1H, H-7), 6.58 (s, 1H, H-4), 7.17 (d, *J* = 2.0 Hz, 1H, H-8), 7.28 (dd, *J* = 8.0, 2.0 Hz, 1H, H-10), 7.50 (d, *J* = 8.0 Hz, 1H, H-11); in MeOH-d_4: for the major conformer δ 1.07−2.54 (m, 13H, cyclohexanyl and H-5,6), 2.51 (s, 3H, SCH_3), 3.52 (s, 3H, OCH_3-1), 3.87 (s, 3H, OCH_3-3), 3.89 (s, 3H, OCH_3-2), 5.48 (dd, *J* = 6.6, 11.1 Hz, 1H, H-7), 6.68 (s, 1H, H-4), 7.21 (dd, *J* = 1.5, 8.1 Hz, 1H, H-10), 7.26 (s, 1H, H-8), 7.37 (d, *J* = 8.1 Hz, 1H, H-11); for the minor conformer: δ 1.07−2.54 (m, 13H, cyclohexanyl and H-5,6), 2.48 (s, 3H, SCH_3), 3.58 (s, 3H, OCH_3-1), 3.86 (s, 3H, OCH_3-3), 3.87 (s, 3H, OCH_3-2), 5.74 (d, *J* = 5.2 Hz, 1H, H-7), 6.67 (s, 1H, H-4), 7.18 (s, 1H, H-8), 7.28 (dd, *J* = 8.1, 1.5 Hz, 1H, H-10), 7.45 (d, *J* = 8.1 Hz, 1H, H-11). Anal. Calcd for $C_{26}H_{32}O_5S$ (456.59): C 68.39, H 7.06, S 7.02. Found: C 68.34, H 7.10, S 7.08.

(+)-Cyclohexanyl Carbonate(11b). Yield 94% (starting with 9 mg of **7b**); oil; $[\alpha]^{25}_D$ +116.1° (*c* 0.30, $CHCl_3$); UV λ_{max} CH_2Cl_2 282.2 (log ϵ 4.28), 229.6 (log ϵ 4.19); ^1H NMR spectra were identical with those of **11a**.

Tubulin Polymerization Assay. The tubulin polymerization assay was performed as described previously.[9,10] A 30% difference in IC_{50} values represents a reproducible difference in the relative activity of two agents.

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Supporting Information Available: Mass and ^1H NMR spectra in $CDCl_3$ and MeOH-d_4 for all compounds together with ^{13}C NMR spectra for compound **6** and X-ray data for compound **10b** (33 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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