assessed in MOLT-4 cells by culture by determining the incorporation of [^{14}C]formate into [^{14}C]FGAR. After 24-h incubation in the presence of varying concentrations of 1, cells were resuspended in fresh medium [RPMI 1640 (minus folate) plus 10 nM calcium leucovorin, 10% fetal calf serum, and 0.05 mg of gentamycin/mL], and 0.1 mM azaserine was added. After 15-min incubation [^{14}C]formate was added (0.25 μCi/mL; 1 mM final concentration), the cells were incubated an additional 60 min, and [^{14}C]FGAR was determined.[21]

Acknowledgment. We are indebted to Drs. S. R. M. Bushby and L. P. Elwell for the antibacterial screening, to Patricia Parker and Ernest H. Dark for technical support, and to Drs. R. J. Harvey and I. K. Dev for testing against N^5,N^{10}-methylenetetrahydrofolate dehydrogenase. Anticancer testing at Southern Research Institute was coordinated by Dr. R. L. Tuttle and V. C. Knick. We thank Drs. D. A. Brent and B. S. Hurlbert and their staff for the elemental analyses, NMR spectra, and mass spectrum; Mr. Aris Ragouzeous for assistance with the 100-MHz NMR analysis; and Mrs. A. Melton for excellent technical assistance. We are also indebted to Mr. Don Bell of the Burroughs Wellcome Co. Chemical Development Laboratories for repreparing some of the synthetic intermediates. The advice of M. McGuire on the nomenclature and the encouragement and support of Dr. B. Roth and Dr. J. Burchall are appreciated. We acknowledge the assistance of Ms. T. Cozart, S. Paris, J. Appleton, and D. Alston in preparation of the manuscript and thank Mr. A. Jones for proofreading the manuscript.

Registry No. 1, 118252-44-1; 2, 118252-45-2; 3, 118252-46-3; 4, 118252-48-5; 5, 118252-49-6; 6, 118252-50-9; 7, 118252-53-2; 8, 118252-51-0; 9, 123541-74-2; 10, 123541-75-3; 11, 118252-52-1; 12, 123541-76-4; 13, 123541-77-5; 14, 123541-78-6; 15, 123541-79-7; 16, 123565-68-4; GAR-TFase, 9032-02-4; AICAR-TFase, 9032-03-5; FPGS, 63363-84-8; $NCCH_2COOEt$, 105-56-6; $ClCH_2CH_2CH(OEt)_2$, 35573-93-4; $PhNH_2$, 62-53-3; PhNHMe, 100-61-8; 4-$H_2NC_6H_4CO$-Glu(OMe)-OMe, 52407-60-0; 4-$H_2NC_6H_4CO$-Gly-OMe, 5259-86-9; dihydrofolate reductase, 9002-03-3; thymidylate synthase, 9031-61-2; 10-formyltetrahydrofolate synthetase, 9023-66-9; 5,10-methylenetetrahydrofolate dehydrogenase, 9029-14-5; 5,10-methenyltetrahydrofolate cyclohydrolase, 9027-97-8; serine hydroxymethyltransferase, 9029-83-8; methionine synthase, 37290-90-7; purine, 120-73-0.

Antitubulin Effects of Derivatives of 3-Demethylthiocolchicine, Methylthio Ethers of Natural Colchicinoids, and Thioketones Derived from Thiocolchicine. Comparison with Colchicinoids

Anjum Muzaffar,[†] Arnold Brossi,*[,†] Chii M. Lin,[‡] and Ernest Hamel[‡]

Medicinal Chemistry Section, Laboratory of Analytical Chemistry, NIDDK, and Laboratory of Biochemical Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, National Institutes of Health, Bethesda, Maryland 20892. *Received April 10, 1989*

Esterification of the phenolic group in 3-demethylthiocolchicine and exchange of the *N*-acetyl group with other *N*-acyl groups or a *N*-carbalkoxy group afforded many compounds which showed superior activity over the parent drug as inhibitors of tubulin polymerization and of the growth of L1210 murine leukemia cells in culture. A comparison of naturally occuring *Colchicum* alkaloids with thio isosters, obtained by replacing the OMe group at C(10) with a SCH_3 group, showed the thio ethers to be invariably more potent in these assays. The comparison included 3-demethylthiodemecolcine prepared from 3-demethylthiocolchicine by partial synthesis. Thiation of thiocolchicine with Lawesson's reagent afforded novel thiotropolones which exhibited high antitubulin activity. Their structures are fully secured by spectral data. Colchicine and several of its analogues show good antitumor effect in mice infected with P388 lymphocytic leukemia, and all of them show high affinity for tubulin and inhibit tubulin polymerization at low concentration. Consequently, antitubulin assays with this class of compounds can serve as valuable prescreens for the initial evaluation of potential antitumor drugs.

Colchicine (1), a major alkaloid from *Colchicum autumnale*, has antitumor properties but is too toxic to be of value as a clinical antineoplastic agent.[1] The synthesis of analogues obtained by modifying the structure of colchicine has afforded many compounds with improved properties,[2-5] including 3-demethylthiocolchicine (7), which showed broad-spectrum antitumor activity in in vivo systems.[4,5] We decided to further explore the lead provided by 7 and to prepare ester analogues and modified amides of 7. Thiocolchicine (6), studied extensively by Velluz and Muller in France in the early 1950s,[6-9] is a slightly less active antitumor agent than colchicine (1),[4] whereas its 3-demethyl congener 7 has superior antitumor activity.[4,5] Further evaluation of methylthio ether analogues of natural congeners of colchicine (colchicinoids) to assess the significance of this substitution therefore seemed warranted. For this purpose several methylthio ethers prepared earlier were reevaluated. In addition, thiocornigerine (8),[10] prepared here from cornigerine (2),[1] and 3-demethylthiodemecolcine (9), synthesized from thiocolchicine (6), were included in the comparison. Compound 9 is a thioether analogue of 3-demethyldeme-

[†] NIDDK.
[‡] NCI.

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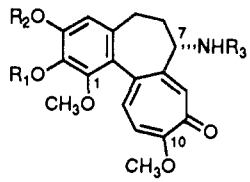
colcine (3) recently detected as a metabolite of demecolcine (4) in liver microsomal preparations.[11] To further assess the importance of the C(10) substituent for antitubulin activity, we included for the comparison also the known *N*-methylamide 17.[12]



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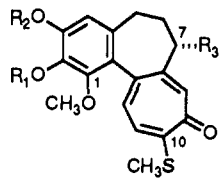
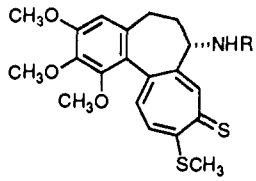
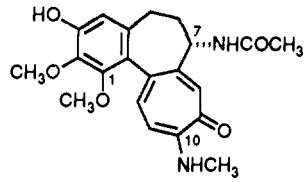
2: $R_1 = R_2 = -CH_2-$, $R_3 = COCH_3$
3: $R_1 = R_3 = CH_3$, $R_2 = H$
4: $R_1 = R_2 = R_3 = CH_3$
5: $R_1 = R_2 = CH_3$, $R_3 = H$



6: $R_1 = R_2 = CH_3$, $R_3 = NHCOCH_3$
7: $R_1 = CH_3$, $R_2 = H$, $R_3 = NHCOCH_3$
8: $R_1 = R_2 = -CH_2-$, $R_3 = NHCOCH_3$
9: $R_1 = R_3 = CH_3$, $R_2 = NH_2$
10: $R_1 = CH_3$, $R_2 = H$, $R_3 = NHCHO$
11: $R_1 = CH_3$, $R_2 = H$, $R_3 = NH_2$
12: $R_1 = CH_3$, $R_2 = PO(OC_2H_5)_2$, $R_3 = NHCOCH_3$
13: $R_1 = CH_3$, $R_2 = H$, $R_3 = NHCOCF_3$
14: $R_1 = CH_3$, $R_2 = H$, $R_3 = N(CH_3)COCF_3$



15: R = $COCH_3$
16: R = $CSCH_3$



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A third set of compounds included in this investigation are novel thioketones **15** and **16** obtained from thiocolchicine (**6**) by reaction with Lawesson's reagent.[13] Their structures are fully secured by spectral data.

Although it was shown that a direct relationship between potency in the P388 system in vivo and antitubulin activity of thiocolchicinoids measured in vitro does not exist,[4] it should be emphasized that all compounds found active in vivo did bind well to tubulin and markedly inhibited tubulin polymerization.[14] This, in our opinion, allows the use of antitubulin assays as covenient and economical prescreens valuable for the preliminary identification of compounds which merit further study.

Chemistry

Esters listed in Table I were obtained from 3-demethylthiocolchicine (**7**)[15] and 3-demethyldeacetylthiocolchicine (**10**)[8] by reaction with chloroformates and acid anhydrides in pyridine solution, followed by the usual workup and chromatography of reaction products on silica gel. Diethyl phosphate **12** was obtained from **7** with diethyl chlorophosphate in methylene chloride in the presence of triethylamine. *N*-Acyl analogues of **7** were prepared from 3-demethyldeacetylthiocolchicine (**11**)[7] with acid anhydrides followed by hydrolysis of the ester intermediates with potassium carbonate in acetone–water. An example describing preparation of *N*-butyryl-3-demethyldeacetylthiocolchicine **24** (COC_3H_7 in **7** instead of Ac) is given. Reaction of deacetylcolchicine (**5**)[16] and demecolcine (**4**)[16] with chloroformates afforded carbonate analogues of the colchicine series listed in Table II. These studies include methylamide **17** prepared by a published procedure.[12] Also listed in Table I are thiocornigerine (**8**),[10] obtained from cornigerine (**2**)[18] on reaction with sodium methanethiolate in water,[19] and 3-demethylthiodemecolcine (**9**), obtained by a reaction sequence elaborated upon earlier for synthesis of demecolcine (**4**) from colchicine (**1**),[16] which proceeded as follows: **11** → trifluoracetamide **13** → trifluoracetamide **14** → **9**. Thioketones **15** and **16** were obtained from **6** with Lawesson's reagent[13] in toluene at room temperature and separated by chromatography on silica gel. Both compounds are amorphous, dark yellow materials and are optically active. The UV maximum of the tropolonic system of thiocolchicine (**6**) at 350 nm (EtOH) is shifted in both compounds to 448 nm, and both compounds generate ions of correct mass on electron impact. Acetamide **15** shows in the IR spectrum the amide carbonyl at 1670 cm^{-1} shifted to 1710 cm^{-1} in thioamide **16**. Conversion of **15** into thioamide **16** was observed by TLC when **15** was treated with Lawesson's reagent at room temperature. Similar treatment of colchicine with Lawesson's reagent gave a complex mixture of products, which has not yet been investigated. All new compounds are fully characterized by spectral data, melting points, and optical rotations, which are listed in Tables I and II.

Biological Evaluation

All compounds listed in Tables I and II were examined for inhibitory effects on the polymerization of purified tubulin,[20] with modifications as described below. The data are presented in terms of IC_{50} values (μM) representing the drug concentration required to inhibit the extent of the reaction by 50%. Most of the compounds were also evaluated for their ability to inhibit the binding of radiolabeled colchicine to tubulin. Without exception, significant inhibition of colchicine binding was observed (data not presented), provided appropriate drug concentrations were used, thus confirming that all analogues were acting at the colchicine site. Finally, we examined all the most potent inhibitors of tubulin polymerization ($IC_{50} \leq 2.0$ μM) and colchicine itself for their cytotoxic effects on L1210 murine leukemia cells in culture. These studies were undertaken to verify potential antitumor activity in vivo. We wanted to determine, in particular, whether any polymerization inhibitors would show limited cytotoxicity and represent a poor choice for further study in animal tumor systems. These data are presented in Tables I and II and demonstrate that all compounds examined were cytotoxic, with IC_{50} values for cell growth ranging from 8 to 70 nM.

Conclusions

All compounds examined here had significant activity as inhibitors of tubulin polymerization, with most of them being superior to colchicine itself. Considering first demethylation at position C(3), four sets of compounds were available for comparison (**1** vs **32**, **6** vs **7**, **30** vs **9**, and **31** vs **11**). In the first three cases, all representing compounds bearing substituents on the amino group at position C(7), the data demonstrated that 3-demethylation resulted in only small losses of activity in the polymerization assay. Demethylation of the amine **31** to **11**, however, resulted

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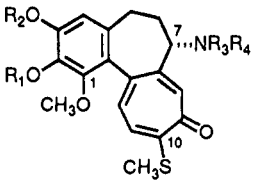
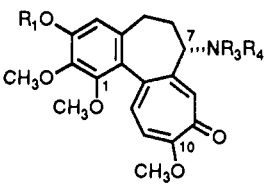
Table I. Biological Activities of Thiocolchicinoids



compound	R_1	R_2	R_3	R_4	mp, °C (ref)	$[\alpha]^{22-25}_D$, (ref) deg	ITP[a] IC_{50}, μM (±SD)	CT[b] IC_{50}, nM	Tox,[c] mg/kg
thiocolchicine (**6**)	CH_3	CH_3	H	$COCH_3$	(1, 6)	(1, 6)	1.3 (±0.04)	8	1.0 (4)
3-demethylthiocolchicine (**7**)	CH_3	H	H	$COCH_3$	(6, 15)	(6, 15)	2.0 (±0.1)	30	11.3 (4)
thiocornigerine (**8**)		$-CH_2-$	H	$COCH_3$	(10)	(10)	1.5 (±0.05)	10	NT[e]
3-demethylthiodemecolcine (**9**)	CH_3	H	H	CH_3	197–198	−175	2.3 (±0.1)	–	NT[e]
N-formyl-3-demethyldeacetylthiocolchicine (**10**)	CH_3	H	H	CHO	284–287	−263	1.7 (±0.3)	40	NT[e]
3-demethyldeacetylthiocolchicine (**11**)	CH_3	H	H	H	(7)	(7)	32 (±4)	–	NT[e]
3-(diethoxyphosphonyl)-3-demethylthiocolchicine (**12**)	CH_3	$PO(OC_2H_5)_2$	H	$COCH_3$	111	−198	4.1 (±0.4)	–	NT[e]
N-(trifluoroacetyl)-3-demethyldeacetylthiocolchicine (**13**)	CH_3	H	H	$COCF_3$	175–177	−246	1.4 (±0.2)	10	NT[e]
N-(trifluoroacetyl)-3-demethylthiodemecolcine (**14**)	CH_3	H	$COCF_3$	CH_3	197–198	−175	1.4 (±0.1)	7	NT[e]
3-(butoxycarbonyl)-3-demethylthiocolchicine (**18**)	CH_3	$COOC_4H_9$	H	$COCH_3$	212–215	−111	1.4 (±0.3)	30	NT[e]
3-(ethoxycarbonyl)-3-demethylthiocolchicine (**19**)	CH_3	$COOC_2H_5$	H	$COCH_3$	249	−128	1.3 (±0.1)	40	NT[e]
3-(butoxycarbonyl)-*N*-formyl-3-demethyldeacetylthiocolchicine (**20**)	CH_3	$COOC_4H_9$	H	CHO	213–214	−158	2.1 (±0.06)	–	NT[e]
3-butyryl-3-demethylthiocolchicine (**21**)	CH_3	COC_3H_7	H	$COCH_3$	232	−139	1.4 (±0.2)	30	NT[e]
3-(ethoxyacetyl)-3-demethylthiocolchicine (**22**)	CH_3	$COCH_2OC_2H_5$	H	$COCH_3$	247	−116	1.6 (±0.2)	30	NT[e]
N,*O*-dibutyryl-3-demethyldeacetylthiocolchicine (**23**)	CH_3	COC_3H_7	H	COC_3H_7	192	−146	2.0 (±0.7)	70	NT[e]
N-butyryl-3-demethyldeacetylthiocolchicine (**24**)	CH_3	H	H	COC_3H_7	153–155	−302	1.7 (±0.4)	60	NT[e]
N-butyryl-3-acetyl-3-demethyldeacetylthiocolchicine (**25**)	CH_3	$COCH_3$	H	COC_3H_7	142–144	−147	1.7 (±0.4)	70	NT[e]
N-(ethoxyacetyl)-3-demethyldeacetylthiocolchicine (**26**)	CH_3	H	H	$COCH_2OC_2H_5$	150–151	−225	2.2 (±0.2)	–	NT[e]
N,*O*-bis(butoxycarbonyl)-3-demethyldeacetylthiocolchicine (**27**)	CH_3	$COOC_4H_9$	H	$COOC_4H_9$	108–110	−129	3.1 (±0.2)	–	NT[e]
3-acetyl-3-demethylthiocolchicine (**28**)	CH_3	$COCH_3$	H	$COCH_3$	182	−188	2.1 (±0.06)	–	NT[e]
3-benzoyl-3-demethylthiocolchicine (**29**)	CH_3	COC_6H_5	H	$COCH_3$	amorphous	−111	4.4 (±0.5)	–	NT[e]
thiodemecolcine (**30**)	CH_3	CH_3	H	CH_3	(4, 8)	(4, 8)	1.3 (±0.2)	20	NT[e]
deacetylthiocolchicine (**31**)	CH_3	CH_3	H	H			2.0 (±0.2)	40	NT[e]
9-thiodeoxothiocolchicine (**15**)	–	–	–	–	–	*d*	1.6 (±0.2)	30	NT[e]
9-thiodeoxo-7-(thioacetamido)thiocolchicine (**16**)	–	–	–	–	–	*d*	1.4 (±0.01)	40	NT[e]
10-demethoxy-10-(methylamino)-3-demethylcolchicine (**17**)	–	–	–	–	(12)		2.3 (±0.4)	–	NT[e]

[a] ITP = inhibition of tubulin polymerization. [b] CT = cytotoxicity in murine leukemia cells. [c] Tox = acute toxicity in mice after im injection of drug. [d] Compounds are optically active (negative rotation), but the exact rotation could not be measured due to rapid mutarotation. [e] NT = not tested.

Table II. Biological Activities of Colchicinoids



compound	R_1	R_2	R_3	mp, °C (ref)	$[\alpha]^{22-25}_D$, (ref) deg	ITP[a] IC_{50}, μM (±SD)	CT[b] IC_{50}, nM	Tox,[c] mg/kg
colchicine (**1**)	CH_3	H	$COCH_3$	(1)	(1)	2.4 (±0.08)	40	3.0 (2)
cornigerine (**2**)	see formula **2**			(1)	(1)	2.1 (±0.2)	–	9.7 (3)
demecolcine (**4**)	CH_3	H	CH_3	(16)	(16)	2.4 (±0.1)	–	237 (2)
deacetylcolchicine (**5**)	CH_3	H	H	(16)	(16)	3.0 (±0.2)	–	28 (2)
3-demethylcolchicine (**32**)	H	H	$COCH_3$	(2)	(2)	2.9 (±0.5)	–	26 (2)
N-(ethoxycarbonyl)-deacetylcolchicine (**33**)	CH_3	H	$COOC_2H_5$	214–215	−173	1.4 (±0.1)	30	114 (3)
N-(butoxycarbonyl)-deacetylcolchicine (**34**)	CH_3	H	$COOC_4H_9$	188–189	−170	1.4 (±0.08)	20	NT[d]
N-(butoxycarbonyl)-demecolcine (**35**)	CH_3	CH_3	$COOC_4H_9$	167–169	−214	2.0 (±0.2)	70	NT[d]

[a] ITP = inhibition of tubulin polymerization. [b] CT = cytotoxicity in murine leukemia cells. [c] Tox = acute toxicity in mice after im injection of drug. [d] NT = not tested.

in a much larger loss of inhibitory activity in the polymerization assay, perhaps indicating increased importance for the substituent at position C(3) when constraints of atropisomerism are reduced.[21]

In the thiocolchicinoid series examined here, esterification of the C(3) phenolic group with alkyl acids generally led to complete or partial restoration of the particularly potent inhibition of tubulin polymerization observed with thiocolchicine (**6**) (i.e., **18**, **19**, **21**, **22**). A small yet significant loss of antitubulin activity did occur, however, with 3-benzoyl-3-demethylthiocolchicine (**29**) and with the diethyl phosphate ester (**12**) of 3-demethylthiocolchicine. This is reminiscent of the poor activity of colchicoside,[22] which bears a sugar substituent at position C(3), and suggests that introduction of a bulky or a polar group at C(3) of colchicinoids is disadvantageous.

Considering 3-demethylthiocolchicine (**7**) as the model compound, alterations at the nitrogen atom at position C(7) either enhanced (**10**, **13**, **14**, **24**) or reduced (**9**, **11**, **26**) inhibition of polymerization. Since only a few agents are presently available, it is premature to generalize. Similar observations have been reported for N-substituted colchicine analogues.[3,23]

It is worth emphasizing that in every case in which we could compare a compound with an SCH_3 group to one with an OCH_3 group at position C(10) the thioether was the more potent inhibitor of tubulin polymerization (**6** vs **1**, **8** vs **2**, **7** vs **32**, **31** vs **5**, **30** vs **4**). This agrees with earlier results in which inhibition of colchicine binding was evaluated.[4,10] In addition, results obtained with 3-demethylcolchicinemethylamide (**17**) suggest that $NHCH_3$ substituents at C(10) would yield compounds of only slightly greater inhibitory activity than those with an OCH_3 group. This is consistent with other reports.[24,25]

The potency of the thiocolchicinoids as inhibitors of tubulin polymerization prompted us to synthesize colchicine analogues with additional sulfur substituents. Thus far two of these (**15** and **16**) have been completely characterized chemically and examined for inhibitory effects of tubulin polymerization. Both are significantly more inhibitory than colchicine, but they appear to be slightly less active than thiocolchicine.

Following confirmation that many of the compounds described here were potent inhibitors of tubulin polymerization, we decided that an initial evaluation of their cytotoxic properties was required. All compounds which inhibited polymerization with IC_{50} values of 2.0 μM or less were compared to colchicine for their effects on the growth of L1210 murine leukemia cells in culture. In this series of experiments, the IC_{50} value obtained for colchicine was 40 nM, while that for thiocolchicine was 5-fold lower (8 nM). Despite being more like thiocolchicine than colchicine as inhibitors of tubulin polymerization, most of the thiocolchicinoids were more comparable to colchicine in terms of their cytotoxicity (i.e., **7**, **10**, **18**, **19**, **21**, **22–25**, **30**, **31**). The compounds with two (**15**) and three (**16**) sulfur atoms were also more comparable to colchicine than to thiocolchicine as cytotoxic agents. The only agents similar to thiocolchicine in their toxicity for L1210 cells were thiocornigerine (**8**) and two analogues (**13**, **14**) with a trifluoroacetyl group on the nitrogen substituent at position C(7).

These in vitro biological evaluations detect activity in terms of the ultimate target of the drugs, the protein tubulin. Their correlation with whole-animal antitumor studies is imperfect. As noted in the introduction, thiocolchicine, despite its more potent inhibition of tubulin polymerization and cell growth as compared to that of colchicine, appears to have reduced antitumor properties. In contrast, 3-demethylthiocolchicine, which differs little from colchicine in its in vitro behavior, has superior activity against murine neoplasms. Thus the potent thiocolchicinoids, whose synthesis and initial evaluation are described here, all have potential as antineoplastic compounds.

Although only limited animal toxicity data (LD_{50} in mice) on colchicinoids and thio congeners are available (**1**, **6**, **7**, **33**), a comparison with respective cytotoxicity data reported here shows that in most cases they are reasonably well-correlated. If reduced cytotoxicity in L1210 murine leukemia cells indicates reduced animal toxicity (permitting an estimate of therapeutic indices), the following potent inhibitors of tubulin polymerization may possess considerably better indices than that of colchicine (**1**): Compounds **10**, **16**, **19**, **23–25**, and **35**.

Experimental Section

Chemistry: Melting points (uncorrected) were determined with a Fisher-Johns apparatus. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter in chloroform with the concentration specified at temperature range 22–25 °C. IR spectra were recorded on a Beckman IR 4230 spectrometer. The proton nuclear magnetic resonance (^1H NMR) spectra were determined in $CDCl_3$ using a JEOL JNM-FX 100 spectrometer with Me_4Si as the internal reference. Electron-impact mass spectra were obtained with a V. G. Micromass 7070F mass spectrometer (70 eV, source temperature 210 °C). Thin-layer chromatography plates (silica gel) were purchased from Analtech, Inc., Newark, DE, and Merck 60 (230–400 mesh) from Aldrich was used for column chromatography. The solvent system used for TLC analysis was $CHCl_3$–MeOH (9:1) and $CHCl_3$–MeOH–NH_4OH (9:0.9:0.1). Lawessons's reagent was purchased from Fluka, Ronkonkoma, NY.

***N,O*-Dibutyryl-3-demethyldeacetylthiocolchicine (23).** A mixture of 3-demethyldeacetylthiocolchicine (**11**, 66 mg, 0.18 mmol) and butyric anhydride (0.1 mL, 0.7 mmol) in pyridine (1 mL) was stirred at room temperature for 2 h. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2 (15 mL), washed successively with 20% HCl, H_2O, and brine, dried over anhydrous Na_2SO_4, and concentrated to give crude product (80 mg). This was passed through silica gel and afforded 75 mg of amorphous material. Crystallization from acetone yielded **23** as light yellow crystals (60 mg, 66%): mp 192 °C; $[\alpha]_D$ −146° (*c* 0.46, $CHCl_3$); IR ($CHCl_3$) 1760, 1680 (CO), 1620 cm^{-1}; ^1H NMR δ 0.93 (3 H, t, $NCOCH_2CH_2CH_3$), 1.08 (3 H, t, $OCOCH_2CH_2CH_3$), 1.65 (2 H, m, $NCOCH_2CH_2$), 1.82 (2 H, m, $OCOCH_2CH_2$), 2.20 (2 H, t, $NCOCH_2$), 2.44 (3 H, s, SMe), 2.60 (2 H, t, $OCOCH_2$), 3.66 (3 H, s, 1-OMe), 3.92 (3 H, s, 2-OMe), 4.63 (1 H, m, C_7H), 6.67 (1 H, s, ArH), 7.05 (1 H, d, ArH), 7.21 (1 H, s, ArH), 7.28 (1 H, d, ArH); EIMS, *m/e* 499 (M^+), 466, 429, 401, 358, 342, 314 (100).

***N*-Butyryl-3-demethyldeacetylthiocolchicine (24).** *N,O*-Dibutyryl-3-demethyldeacetylthiocolchicine (**23**, 55 mg, 0.11 mmol) was dissolved in acetone (2 mL) and stirred with a solution of K_2CO_3 (60 mg) in H_2O (1 mL) at 60 °C for 24 h. The reaction mixture was poured onto chloroform, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated. Crystallization from CH_2Cl_2–Et_2O afforded **24** as yellow, fine crystals (20 mg, 42%): mp 153–155 °C; $[\alpha]_D$ −302° (*c* 0.13, $CHCl_3$); IR ($CHCl_3$) 3520 (OH), 1670 (CO), 1610 cm^{-1}; ^1H NMR δ 0.92 (3 H, t, CH_2CH_3), 1.63 (2 H, m, $COCH_2CH_2CH_3$), 2.19 (2 H, m, $COCH_2CH_2CH_3$), 2.43 (3 H, s, SMe), 2.65 (3 H, s, OMe), 4.01 (3

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H, s, OMe), 4.66 (1 H, m, C_7H), 6.14 (1 H, b s, NH), 6.58 (1 H, s, ArH), 7.04 (1 H, d, ArH), 7.20 (1 H, s, ArH), 7.26 (1 H, d, ArH); EIMS m/e 429 (M^+), 401, 396, 382, 358, 314 (100).

3-(Diethoxyphosphoryl)-3-demethylthiocolchicine (12). 3-Demethylthiocolchicine (7, 100 mg, 0.24 mmol) was dissolved in methylene chloride (3 mL) and triethylamine (0.5 mL), and diethyl chlorophosphate (0.2 mL, 1.24 mmol) was added under ice cooling. The reaction mixture was stirred at room temperature for 3 h, diluted with methylene chloride, and washed with 20% HCl, H_2O, and brine. Crystallization of the dried concentrated extract from ethyl acetate yielded orange crystals (63 mg, 50%): mp 111 °C; $[\alpha]_D$ -198° (c 0.16, $CHCl_3$); IR ($CHCl_3$) 3220 (NH), 1665 (CO), 1280 (PO), 1150 cm^{-1}; ^1H NMR δ 1.40 (6 H, t, OCH_2CH_3), 2.10 (3 H, s, NAc), 2.55 (3 H, s, SMe), 3.64 (3 H, s, OMe), 3.99 (3 H, s, OMe), 4.30 (4 H, m, OCH_2), 4.68 (1 H, m, C_7H), 7.00 (1 H, s, ArH) 7.46 (1 H, d, ArH), 7.65 (1 H, d, ArH), 8.30 (1 H, s, NH); EIMS m/e 537 (M^+), 509, 504, 478, 450 (100), 435, 422, 401.

***N*-(Trifluoroacetyl)-3-demethyldeacetylthiocolchicine (13).** To a mixture of 3-demethyldeacetylthiocolchicine (11, 50 mg, 0.14 mmol) and anhydrous Na_2CO_3 (147 mg, 1.4 mmol) in ether (20 mL) was added trifluoroacetic anhydride (0.2 mL, 1.4 mmol) at 0 °C. The reaction mixture was kept stirred at room temperature for 2.5 h and then poured onto chloroform. The chloroform layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and concentrated, and the crude product was chromatographed on silica gel using 2% MeOH in $CHCl_3$ as eluant. The first four fractions contained a single yellow compound, which was crystallized from hexane–Et_2O to give 13 as a yellow powder (40 mg, 63%): mp 175–177 °C; $[\alpha]_D$ -245° (c 0.1, $CHCl_3$); IR ($CHCl_3$) 3510 (OH), 1725 (CO), 1610 cm^{-1}; ^1H NMR δ 2.44 (3 H, s, SMe), 3.65 (3 H, s, 2-OMe), 4.02 (3 H, s, 1-OMe), 4.74 (1 H, m, C_7H), 6.61 (1 H, s, ArH) 7.10 (1 H, d, ArH), 7.32 (1 H, d, ArH), 7.33 (1 H, s, ArH), 8.58 (1 H, b s, NH); EIMS m/e 455 (M^+), 427 (100), 408, 394, 365, 342, 314, 299, 283, 267, 252.

***N*-(Trifluoroacetyl)-3-demethylthiodemecolcine (14).** A solution of *N*-(trifluoroacetyl)-3-demethyldeacetylthiocolchicine (13, 268 mg, 0.59 mmol) in acetone (15 mL) was stirred with anhydrous K_2CO_3 (122 mg, 0.88 mmol) and methyl iodide (0.4 mL) at room temperature for 2 days. The reaction mixture was poured onto chloroform and washed with H_2O until neutral and finally with brine. The chloroform layer was dried over anhydrous Na_2SO_4, concentrated, and flash chromatographed on silica gel using $CHCl_3$–MeOH (99:1) as eluant. Crystallization from CH_2Cl_2 afforded 14 as yellow crystals (220 mg, 80%): mp 197–198 °C; $[\alpha]_D$ -175° (c 0.4, $CHCl_3$); ^1H NMR δ 2.44 (3 H, s, SCH_3), 3.66 (3 H, s, OCH_3), 3.91 (3 H, s, NCH_3), 3.95 (3 H, s, OCH_3), 4.71 (1 H, m, C_7H), 6.55 (1 H, s, ArH), 7.09 (1 H, d, J = 10.8 Hz, ArH), 7.33 (1 H, d, J = 10.8 Hz, ArH), 8.07 (1 H, d, ArH); EIMS m/e 469 (M^+), 441 (100), 422, 408, 366, 328, 313.

3-Demethylthiodemecolcine (9). To a solution of *N*-(trifluoroacetyl)-3-demethylthiodemecolcine (14, 200 mg, 0.42 mmol) in acetone (10 mL) and H_2O (10 mL) was added anhydrous K_2CO_3 (87 mg, 0.63 mmol). The reaction mixture was heated to 60 °C and stirred continuously at this temperature for $1^1/_2$ days (monitored by TLC). It was poured onto $CHCl_3$ and washed with water and then with brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was flash chromatographed on silica gel using $CHCl_3$ and $CHCl_3$–MeOH (96:4) as eluant to afford pure 3-demethylthiodemecolcine (9), which was crystallized from CH_2Cl_2–Et_2O as yellow crystals (80 mg, 52%): mp 197–198 °C; $[\alpha]_D$ -191° (c 0.4, $CHCl_3$); IR ($CHCl_3$) 1575 (CO) cm^{-1}; ^1H NMR δ 2.36 (3 H, s, SMe), 3.64 (3 H, s, NMe), 3.91 (3 H, s, OMe), 3.92 (3 H, s, OMe), 4.14 (1 H, s, C_7H), 6.55 (1 H, s, ArH), 7.05 (1 H, d, ArH), 7.28 (1 H, d, ArH), 7.58 (1 H, s, ArH); EIMS m/e 373 (M^+, 100), 345, 328, 313, 298, 281, 266.

Thiocornigerine (8) from Cornigerine (2). Cornigerine (2, 38 mg, 0.09 mmol) was dissolved in 4 mL of 50% methanol and sodium methanethiolate (66 mg, 0.9 mmol) was added. The solution was stirred at room temperature for 8 h and left standing overnight in a freezer. After addition of 1 mL of acetic acid and 6 mL of H_2O the reaction product was extracted with chloroform. The product obtained after filtration through silica gel, evaporation of solvent, and crystallization from acetone had mp 143 °C (lit.[10] mp 140 °C) and $[\alpha]^{20}_D$ -157° (c, 0.45 $CHCl_3$) [lit.[10] $[\alpha]_D$ -155° (c 0.65, $CHCl_3$)].

9-Thiodeoxothiocolchicine (15) and 9-Thiodeoxo-7-(thioacetamido)thiocolchicine (16). To a solution of thiocolchicine (6, 500 mg, 1.2 mmol) in toluene (15 mL) was added Lawesson's reagent (244 mg, 0.6 mmol) and the reaction mixture was stirred at room temperature for 4 h under nitrogen. Solvent was evaporated under high vacuum and the crude product was flash chromatographed on silica gel with CH_2Cl_2–MeOH (98:2) as eluant. Major compound 15 was obtained as a dark yellow, amorphous powder (200 mg, 0.46 mmol, 39%): UV ($CHCl_3$) 317, 446 nm; IR ($CHCl_3$) 1670 (CO, amide) cm^{-1}; ^1H NMR δ 2.04 (3 H, s, $COCH_3$), 2.53 (3 H, s, SMe), 3.70 (3 H, s, OMe), 3.93 (3 H, s, OMe), 3.97 (3 H, s, OMe), 4.65 (1 H, m, C_7H), 6.04 (1 H, d, NH), 6.53 (1 H, s, ArH), 7.14 (1 H, d, ArH), 7.44 (1 H, d, ArH), 8.49 (1 H, s, ArH); EIMS m/e 431 (M^+), 416 (100), 399, 387, 374, 355, 328, 313, 297. The specific rotation ($CHCl_3$, MeOH) could not be determined since mutarotation occurred.

The less polar, minor band was collected and rechromatographed on silica gel using CH_2Cl_2–MeOH (99.8:0.2) as eluant to give thioamide 16 as an amorphous dark yellow material (40 mg, 8%): UV ($CHCl_3$) 317, 448 nm; IR ($CHCl_3$) 1710 (CS, amide) cm^{-1}; ^1H NMR δ 2.52 (3 H, s, SMe), 2.57 (3 H, s, $CSCH_3$), 3.76 (3 H, s, OMe), 3.91 (3 H, s, OMe), 3.94 (3 H, s, OMe), 5.03 (1 H, m, C_7H), 6.56 (1 H, s, ArH), 7.16 (1 H, d, ArH), 7.51 (1 H, d, ArH), 8.41 (1 H, s, ArH), 8.51 (1 H, d, NH); EIMS m/e 447 (M^+), 432, 413, 402, 372, (100), 357, 341, 328; measuring the optical rotation revealed mutarotation.

Biological Studies. Inhibition of the polymerization of electrophoretically homogeneous bovine brain tubulin was evaluated as described previously.[20] In brief, 0.24 mL of reaction mixtures were prepared containing 0.25 mg of tubulin, 1.0 M monosodium glutamate (pH 6.6 with HCl), and, if present, appropriate drug concentrations (chosen from the following standard concentrations: 1, 2, 3, 4, 5, 7.5, 10, 15, 20, 25, 30, 40, 50, 75, and 100 μM). The reaction mixtures also contained 10 μL of dimethyl sulfoxide, the solvent for the drugs. (Note that the glutamate and drug concentrations refer to the final reaction volume of 0.25 mL.) The reaction mixtures were incubated at 37 °C for 15 min to allow slow binding drugs like colchicine to bind to the tubulin. The reaction mixtures were then chilled on ice, 10 μL of 10 mM GTP (final concentration, 0.4 mM) was added to each mixture, and they were transferred to cuvettes in Gilford spectrophotometers held at 0 °C by electronic temperature controllers (four instruments with a total capacity for 16 samples were used in each experiment). The temperature controllers were then set at 37 °C (75-s jump from 0 to 37 °C), and the polymerization reaction was followed turbidimetrically for 20 min. Polymer formation was confirmed by evaluation of depolymerization at 0 °C. Extent of inhibition of polymerization at 20 min in drug-treated samples was always calculated by comparing them to a pair of drug-free samples in each experimental set. IC_{50} values were obtained by graphical analysis of the data, and at least three independent determinations were performed with each drug.

Inhibition of the growth of L1210 murine leukemia cells was performed as described previously.[20] Each culture flask was innoculated with 10^5 cells, incubated for 24 h at 37 °C, and counted in a Coulter counter to determine cell number. IC_{50} values, determined in at least two independent experiments, indicate the drug concentration, determined graphically, which inhibited 50% of the increase in cell number as compared to cultures without drug.

Registry No. 1, 64-86-8; 2, 6877-25-4; 4, 477-30-5; 5, 3476-50-4; 6, 2730-71-4; 7, 87424-25-7; 8, 101246-61-1; 9, 123643-49-2; 10, 113092-25-4; 11, 97043-09-9; 12, 123643-50-5; 13, 123643-51-6; 14, 123643-52-7; 15, 123643-53-8; 16, 123643-54-9; 17, 77409-66-6; 18, 123643-55-0; 19, 123417-77-6; 20, 123643-56-1; 21, 123417-79-8; 22, 123417-78-7; 23, 123643-57-2; 24, 123643-58-3; 25, 123643-59-4; 26, 123643-60-7; 27, 123643-61-8; 28, 2730-86-1; 29, 123417-80-1; 30, 76129-11-8; 31, 2731-16-0; 32, 7336-33-6; 33, 123643-62-9; 34, 123643-63-0; 35, 123643-64-1.