Antitumor Agents. 139.† Synthesis and Biological Evaluation of Thiocolchicine Analogs 5,6-Dihydro-6(*S*)-(acyloxy)- and 5,6-Dihydro-6(*S*)-[(aroyloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-ones as Novel Cytotoxic and Antimitotic Agents

Li Sun,‡ Andrew T. McPhail,§ Ernest Hamel,∥ Chii M. Lin,∥ Susan B. Hastie,⊥ Jer-Jang Chang,● and Kuo-Hsiung Lee*,‡

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706, Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, Department of Chemistry, State University of New York, Vestal Parkway East, Binghamton, New York 13902-6000, and Department of Laboratory Animal Medicine, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599

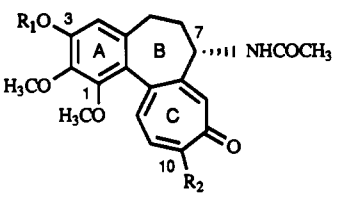
Received October 30, 1992

A series of novel thiocolchicine analogs, 5,6-dihydro-6(*S*)-(acyloxy)- and 5,6-dihydro-6(*S*)-[(aroyloxy)-methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-ones, possessing a six-membered ring B, have been synthesized and evaluated for their cytotoxicity against various tumor cell lines, including solid tumor cell lines, and for their interaction with tubulin. The configuration of the parent alcohol (compound **5**) was established unequivocally as (*aR*,6*S*) by X-ray crystallographic analysis. The side chain at the C(6) position is in a pseudoaxial orientation. The optical properties and ^{1}H NMR data indicated that these compounds have the same conformations in solution as in the solid state. Biological results showed that compounds (**5**, **6**, **14**, **15**, **17**, and **18**) bearing a small side chain at C(6) demonstrate high potency in inhibiting tubulin polymerization and binding of radiolabeled colchicine to tubulin. The most cytotoxic compounds were **14**, **15**, **17**, and **18**, with good activity against several solid tumor cell lines. To explain the strong antitubulin activity of compound **5** (with an *aR* configured biaryl system in contrast to the *aS* configuration previously described for colchicinoids, allocolchicinoids, and steganacin) we speculate that a rapid atropisomerism equilibrium must exist for **5** and its active derivatives. This equilibrium would yield adequate amounts of *aS*-configured conformers that interact strongly with tubulin. Since the optically inactive **18** is also a potent inhibitor of tubulin, the configuration of the side chain of these six-membered ring B analogs cannot be essential for their binding to tubulin. Instead we propose that the size of ring B and of its side chain play important roles in tubulin binding activity by affecting the rotation of the rings A and C along their linking C–C bond axis.

Introduction

Colchicine (**1**) (Figure 1) is a major alkaloid isolated from *Colchicum autumnale*.[2] It has been used in the treatment of acute gout, familial Mediterranean fever, and liver cirrhosis.[2] Compound **1** also has potent experimental antileukemic activity, but it is too toxic for effective clinical use in the treatment of neoplastic diseases.[3] As a result, a large number of analogs of **1** have been synthesized in the hope of developing a useful antitumor agent.[4]

The mechanism of action of **1** is based on its ability to inhibit tubulin polymerization, thereby interfering with formation of the mitotic spindle and causing cells to accumulate in apparent mitotic arrest during the cell cycle.[2] It has been suggested that the biological effects of **1** probably relate to its tubulin binding activity, and the interactions of tubulin with analogs of **1** have been studied extensively.[3,5,6] Structure–activity relationship studies have indicated that stereochemistry plays an important role in the ability of colchicinoids and the closely related allocolchicinoids, which have a six-membered ring C, to bind to tubulin.[7-10] In particular, it has been proposed that **1** and active colchicinoids and allocolchicinoids have an *aS* biaryl configuration [and, usually, a chiral center with *S* configuration at position C(7)] whereas their inactive optical isomers have an *aR* biaryl configuration.[7,10]



1: $R_1 = CH_3$, $R_2 = OCH_3$
2: $R_1 = H$, $R_2 = SCH_3$
3: $R_1 = CH_3$, $R_2 = SCH_3$

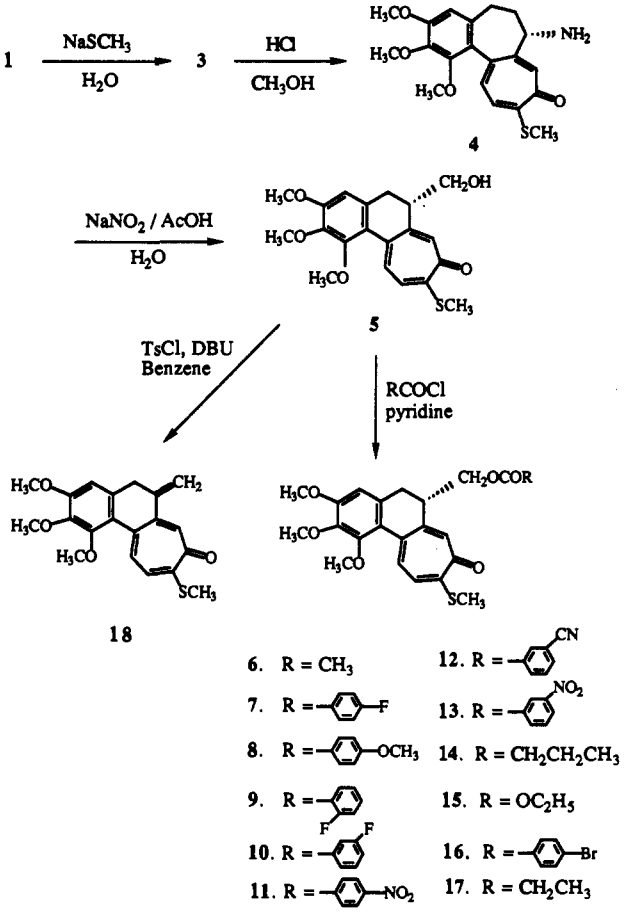
Figure 1. Structures of colchicine (**1**), 3-demethylthiocolchicine (**2**), and thiocolchicine (**3**).

Even though studies on **1** and its analogs have been extensive, there are still aspects that merit further investigation. Most cytotoxicity evaluations of these compounds have used leukemia cell lines. Studies with solid tumor cell lines have been limited. Among analogs of **1**, 3-demethylthiocolchicine (**2**) (Figure 1) is one of the most promising in terms of its antitumor properties. This

* To whom correspondence should be addressed.
† For part 138, see ref 1.
‡ Natural Products Laboratory, School of Pharmacy, University of North Carolina.
§ Department of Chemistry, Duke University.
∥ Laboratory of Molecular Pharmacology, NCI.
⊥ Department of Chemistry, State University of New York.
● School of Medicine, University of North Carolina.

0022-2623/93/1836-0544$04.00/0

Scheme I. Syntheses of 5,6-Dihydro-6(*S*)-(acyloxy)- and 5,6-Dihydro-6(*S*)-[(aroyloxy)methyl]-1,2,3-trimethoxy-9-methylthio-8*H*-cyclohepta[*a*]naphthalen-8-ones



compound showed a better therapeutic index than either 1 or thiocolchicine (3) (Figure 1) with leukemia cell lines and a broad antitumor spectrum against several solid tumor cell lines.[3] Moreover, understanding of the effects of the conformation of colchicinoids on their binding to tubulin is incomplete. Recent studies have demonstrated that the ring B and its side chain affect the rate of binding by restricting the rotation of the biaryl system and causing steric hindrance.[11-13] Such effects are not fully understood.

In this paper we report the synthesis of a series of novel cytotoxic analogs of 3 (compounds 5–18). These agents have a six-membered ring B instead of the usual seven-membered ring B. The biaryl configuration of these new compounds is *aR*, thus differing from the *aS* configuration of 1 and 3. Nevertheless, many of them are potent inhibitors of tubulin polymerization and the binding of radiolabeled 1 to tubulin, and, like 1, cause the accumulation of cells arrested in mitosis.

Chemistry

As shown in Scheme I, the target compounds (5–18) were prepared from deacetylthiocolchicine (4) which was obtained from 1 by literature procedures.[14,15] Demjanov rearrangement converted 4 to 5,6-dihydro-6-(hydroxymethyl)-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (5), which possesses a six-membered ring B and a hydroxymethyl side chain at C(6).[16] Reaction of 5 with various acid chlorides in anhydrous dichloromethane containing pyridine afforded the desired 5,6-dihydro-6-(acyloxy)- and 5,6-dihydro-6-[(aroyloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-ones (6–17). To determine whether or not the asymmetry at C(6) is a requirement for the binding of these compounds to tubulin, we also synthesized 18 by dehydration of 5 with tosyl chloride in the presence of DBU in benzene according to the established procedure.[17]

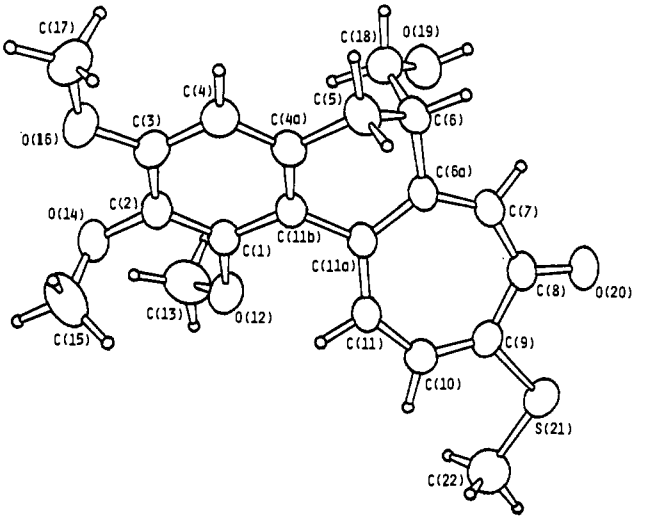


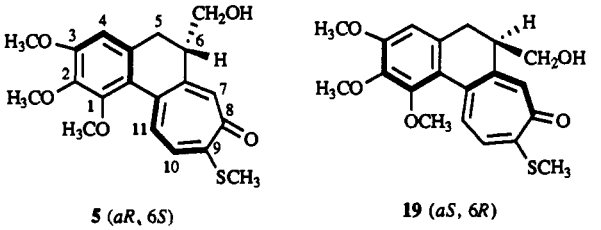
Figure 2. Structure of compound 5 determined by X-ray crystallography.

The structure of 5 was established unequivocally from ^{1}H NMR and IR spectral data, elemental analysis, and X-ray crystallographic analysis (Figure 2) (see Experimental Section). Moreover, by use of the anomalous scattering of X-rays, the absolute stereochemistry at C(6) in 5, and thus also in 6–17, was defined as *S*. A view of the solid-state conformation of 5 is provided in Figure 2. The C4a-C11b-C11a-C6a and C1-C11b-C11a-C11 torsion angles are −28.1(3)° and −32.5(3)°, respectively, and the dihedral angle between the least-squares plane through atoms of the approximately planar phenyl ring A and that through those of the slightly puckered tropolone ring C is 29.5(1)°. The biaryl system in 5 thus has an *aR* configuration, the opposite of that found in 1 and 3 (see ref 18 for an explanation of this assignment). Endocyclic torsion angles in ring B are related by an approximate C_2-symmetry axis passing through the midpoints of the C5–C6 and C11a–C11b bonds, and, with two of the angles being very small [3.5(4)°, 5.7(3)°], this ring has a 1,3-diplanar conformation; the α-(hydroxymethyl) substituent is pseudoaxially oriented while the β-hydrogen atom at C(6) is pseudoequatorially disposed. Large negative optical rotations and negative CD bands are characteristic of normal derivatives of 1, and this has been attributed to their *aS*-configured biaryl systems.[10] In contrast, most of the 5 derivatives have large positive values that probably result primarily from their *aR*-configured biaryl systems (see Experimental Section and Table I). Compound 18, which has a methylene substituent at C(6), has no measurable optical rotation or CD bands (data will be published elsewhere). This observation may be ascribed either to diminished molecular asymmetry or to the presence of an equal amount of *aS*- and *aR*-configured isomers at equilibrium.[8] In the presence of tubulin, the CD spectrum of 18 bound to tubulin resembles that of 3. This result strongly supports the idea that 18 exists in a racemic mixture of the two conformational isomers in solution. The *aS* conformer binds to tubulin, generating the negative CD band. The remaining free drug rapidly reequilibrates to the racemic mixture. In addition, ^{1}H NMR data indicate that the conformation of 5 is the same

Table I. Molar Ellipticities of Some Six-Membered B Ring Analogs[a]

compound	[Θ] deg cm²/dmol[b]
5	1.8×10^4
7	2.1×10^4
10	2.1×10^4
11	1.5×10^4
13	2.2×10^4
18	0

[a] The CD spectra of the compound in EtOH were measured. The CD spectra of these compounds bound to tubulin were also measured (data will be published elsewhere). [b] The molar ellipticities were calculated based on the extinction coefficient determined for compound 5 (λ_{max} = 402 nm).



5 (aR, 6S) 19 (aS, 6R)

Figure 3. Structures of compounds 5 and 19.

in solution as in the solid state. Coupling constants between the proton at C(6) and those at C(5) in 5 and its derivatives range from 2 to 5 Hz, indicating pseudoequatorial protons and pseudoaxial side chains at C(6) in all of these compounds in solution.

Lincoln et al.[13] reported the synthesis of compound 19 (Figure 3), the enantiomer of 5, by a somewhat different procedure than that described here, and Olsson et al.[19] reported crystallographic data for compound 19. These workers, however, did not determine the absolute stereochemistry of compound 19 from the X-ray data. Rather, they inferred it from CD and NMR spectral data, as well as the biological activity of their agent. The NMR data reported[13] for 19 are nearly identical to those we obtained for 5 (see below). Similarly, the bond lengths and angles, torsion angles, and dihedral angle reported for 19[19] differ little from those we obtained for 5. Since Lincoln et al.[13] did not report optical rotatory data for 19, we examined the CD properties of 5 in order to compare with 19. We found that the CD spectrum of 5 was indistinguishable from that reported for 19 (data not presented; cf. ref 13). We therefore conclude that our compound 5 is identical to the compound synthesized by Lincoln et al.,[13] and the stereochemistry of 19 was incorrectly assigned before.

Biological Results

Compounds 1, 3, and 5–18 were examined for cytotoxic effects against six tumor cell lines (murine P388 leukemia and five human solid tumor cell lines: nasopharyngeal carcinoma KB, lung carcinoma A549, colon carcinoma HCT-8, melanoma RPMI-7951, and central nervous system carcinoma TE671) according to the procedures described by Monks et al.[20] Cytotoxicity data are presented as ED_{50} (nM) values and summarized in Table II. All of the compounds were also tested by the methods described previously[21-24] for their inhibitory effect on tubulin polymerization and on the binding of [³H]-colchicine to tubulin, as well as their stimulatory effects on GTP hydrolysis. Data from these assays are presented as IC_{50} (μM) values, percentage inhibition of [³H]colchicine binding to tubulin over the control, and percentage stimulation of GTP hydrolysis over control, respectively, as indicated in Table III.

Table II. Biological Evaluations of 5,6-Dihydro-6(S)-(acyloxy)- and 5,6-Dihydro-6(S)-[(aroyloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-8H-cyclohepta[a]naphthalen-8-ones

	cytotoxicity (ED_{50}, nM)[a]					
compound	KB	A549	HCT-8	P388	RPMI-7951	TE671
1	2	18	175	100	1	1
3	0.04	72	16	5	0.024	0.024
5	144	240	160	144	134	26
6	132	1945	5714	96	144	1320
7	1067	1570	1974	81	926	1249
8	806	I[b]	3284	138	806	2.0
9	110	140	6404	12	40	129
10	110	80	161	141	100	0.02
11	13	4279	5673	10	114	10
12	139	8222	5779	99	629	1534
13	1050	1471	19	1050	1089	936
14	0.13	1372	3037	0.09	0.022	23
15	11	0.001	179	16	0.001	0.001
16	92	16954	9197	110	110	129
17	93	220	162	8	0.093	0.12
18	140	252	196	22	0.084	0.084

[a] ED_{50} value are the drug concentrations required to inhibit the growth of tumor cells by 50%. [b] I = Inactive (ED_{50} value greater than 10 μg/mL).

As shown in Table II, compound 15 was the most potent of the new agents. In three human tumor cell lines (A549, RPMI-7951, and TE671 cells) it was substantially more potent than 3, in P388 murine cells 15 and 3 had comparable (less than 5-fold difference) activity, but in KB and HCT-8 cells 3 was more active. Except for the KB and HCT-8 cell lines, compound 17 and 18 had cytotoxic activity comparable (5-fold difference or less) to that of 3. Compound 14 was essentially equivalent to 3 in three cell lines. Four additional compounds (9–11 and 13) had activity equivalent to that of 3 in one or two cell lines. In addition, we examined compounds 5, 11, and 13 for their effects on the growth of human Burkitt lymphoma cells. IC_{50} values after 36 h of growth for the three agents were 0.06, 0.02, and 0.6 μM, respectively, as compared with a simultaneously obtained value of 0.05 μM for 1. Large numbers of mitotic cells, reaching levels as high as 67%, were seen with all three agents at toxic drug concentrations.

The novel structure of compounds 5–18, with the six-membered ring B, led us to evaluate several aspects of their interactions with purified bovine brain tubulin in comparison with 1 and 3. Our findings are summarized in Table III. First, we examined these compounds for their effects on the glutamate-induced polymerization of tubulin,[25] including in the reaction sequence a tubulin–drug preincubation prior to addition of the GTP required for polymerization to occur. This preincubation enhances the inhibitory effect (i.e., lowers the IC_{50} value obtained) of compounds, such as colchicinoids,[24,26] that bind slowly to tubulin. The parent alcohol 5, all compounds with small alkyl ester groups (i.e., 6, 14, 15, and 17), and compound 18, with an sp^2 center introduced at position C(6), had IC_{50} values intermediate between those of 1 and 3 (except for 14, which was slightly less inhibitory than 1). Compound 18, with the sp^2 center, was the most potent inhibitor (IC_{50} of 2.4 μM) of all these new agents and only slightly less inhibitory than 3 (IC_{50} of 2.1 μM), which in our hands is the most potent inhibitor of tubulin polymerization binding in the colchicine site we have yet examined. No simple structure–activity relationship was observed with the series of compounds possessing aroyl moieties in the side chain. Most of them had little or no effect on the polymerization reaction, with IC_{50} values greater than 40

Table III. Interaction of Six-Membered Ring B Analogs of Colchicine with Tubulin

compound	inhibition of tubulin polymerization: $IC_{50} \pm SD^e$ (μM) +preincubation[a]	−preincubation[b]	inhibition of colchicine binding:[c] % inhibition	stimulation of GTP hydrolysis:[d] % control
1	4.2 ± 0.1		26	235
3	2.1 ± 0.07	4.9 ± 0.3	61	233
5	3.6 ± 0.1	3.7[f]	53	228
6	3.4 ± 0.1		56	232
7	>40		0	177
8	>40		0	175
9	3.0 ± 0.4	3.8 ± 0.4	91	233
10	>40		0	201
11	6.6 ± 0.2		78	176
12	>40		0	118
13	>40		0	109
14	4.5 ± 0.2		46	222
15	3.7 ± 0.1	4.3 ± 0.3	60	226
16	>40		2	225
17	3.4 ± 0.3		83	236
18	2.4 ± 0.1	2.8 ± 0.4	91	233
20	2.9 ± 0.2	3.2 ± 0.06	97	

[a] Drug and tubulin were preincubated for 15 min at 37 °C prior to addition of the GTP required for polymerization. [b] All components, including GTP, were mixed prior to incubation of the reaction mixtures at 37 °C. [c] Triplicate reaction mixtures contained 0.1 mg/mL (1.0 μM) tubulin, 5 μM [^{3}H]colchicine, the indicated compund at 5 μM, 10% (v/v) dimethyl sulfoxide, and a series of components to stabilize tubulin (1.0 M monosodium glutamate, 1.0 mM $MgCl_2$, 0.1 mM GTP, 0.5 mg/mL albumin, and 0.1 M glucose-1-phosphate) as decribed elsewhere.[29] Incubation was for 10 min at 37 °C, at which point about half the maximal colchicine binding occurs in the control reaction mixtures. In the control samples an average of 0.29 mol of colchicine was bound per mol of tubulin. [d] Reaction mixtures contained 1.0 mg/mL (10 μM) tubulin, 1.0 M monosodium glutamate (pH 6.6 with HCl), 1.0 mM $MgCl_2$, 50 μM [8-^{14}C]GTP, the indicated compound at 50 μM, and 5% (v/v) dimethyl sulfoxide. Incubation was for 30 min at 37 °C. In the control reaction mixture 20 nmol/mL of [8-^{14}C]GTP was formed. [e] SD, standard deviation. At least three experiments were performed with each compound. [f] A value of 3.7 μM was obtained in all three experiments.

μM (compound 7, 8, 10, 12, 13, and 16). Two of these agents, however, had significant activity. Compound 11, with a nitro group para to the carbonyl group, had an IC_{50} value of 6.6 μM (cf. the inactive 13 with a *m*-nitro group); and compound 9, with an *o*-fluorine atom, was the second most inhibitory agent in the six-membered ring B series, with an IC_{50} value of 3.0 μM (cf. the inactive 7 and 10 with fluorine atoms para and meta to the carbonyl group).

The size of ring B and it side chain can affect the rate of binding of 1 analogs to tubulin.[11,12] In general, the smaller the side chain, the faster the colchicinoid binds to the protein. Moreover, Lincoln et al.[13] studied the interaction of 19 (5) with tubulin by spectroscopic methods and found that it bound within seconds to tubulin even at 0 °C. It was therefore of interest to determine whether the six-membered ring B compounds represent another class of agents that might bind rapidly to tubulin. We therefore determined IC_{50} values for inhibition of tubulin polymerization without a preincubation with representative members of the series (compounds 5, 9, 15, and 18; see Table III) in comparison with 3, which is known to bind relatively slowly to tubulin,[27,28] and combretastatin A-4 (20) (Figure 4), which binds rapidly.[29]

Only with 3 was a large increase in the apparent IC_{50} value observed when the preincubation was omitted. The value is more than doubled, from 2.1 to 4.9 μM. In fact, 3 went from being the most active agent with a preincubation to the least active without a preincubation among the six compounds studied under both reaction conditions. With compound 5 the change in IC_{50} value was minimal, in agreement with the finding of Lincoln et al.[13] that this compound binds rapidly to tubulin. Only small increases were observed in the IC_{50} values obtained with 20, and with compounds 15 and 18, suggesting that the latter two agents, like 20,[29] bind rapidly to tubulin. A slightly larger increase in IC_{50} value, from 3.0 to 3.8 μM, was observed with compound 9, suggesting that a bulkier side chain in the six-membered ring B series may also reduce the rate of binding of these compounds to tubulin, as with seven-membered ring B colchicinoids.[11,12]

We also examined the ability of the six-membered ring B series of compounds to inhibit the binding of radiolabeled 1 to tubulin. Data obtained when the inhibitor and radiolabeled 1 were present in equimolar concentrations (5.0 μM) with 1.0 μM tubulin are presented in Table III. The extent of inhibition obtained in this assay is not subject to straightforward interpretation because of the relatively slow binding reaction of the radiolabeled 1 to the protein.[5,6] Extent of inhibition obtained with any compound reflects not only relative affinities of inhibitor and 1 for tubulin, but also their relative rates of binding to the protein and the relative dissociation rates of the protein–ligand complexes. All compounds which had inhibited tubulin polymerization also inhibited the binding of 1 to tubulin. Compounds 5, 6, 14, and 15 were less inhibitory than 3, while compounds 11, 17, and especially 9 and 18 were more potent. The latter two compounds were almost as inhibitory as 20,[30] a structurally simple colchicine site compound that binds rapidly to tubulin and inhibits [^{3}H]-colchicine binding more potently than any other compound we have yet examined.[29] Six compounds (7, 8, 10, 12, 13, and 16) that did not inhibit tubulin polymerization also failed to inhibit [^{3}H]colchicine binding at 5.0 μM. These six compounds did inhibit radiolabeled 1 binding when their concentration was raised to 50 μM (data not presented).

Most drugs that bind in the colchicine site of tubulin uncouple GTP hydrolysis from the normal polymerization reaction, so that there is an apparent stimulation of net hydrolysis, particularly after long incubation times, even though polymer formation is completely suppressed.[5] All the six-membered ring B analogs (except possibly compound 13) also stimulate net GTP hydrolysis, when present in a 5-fold molar excess over tubulin (Table III). Since this experiment was performed only a single time (an equivalent result, 231% of control, was obtained in a second

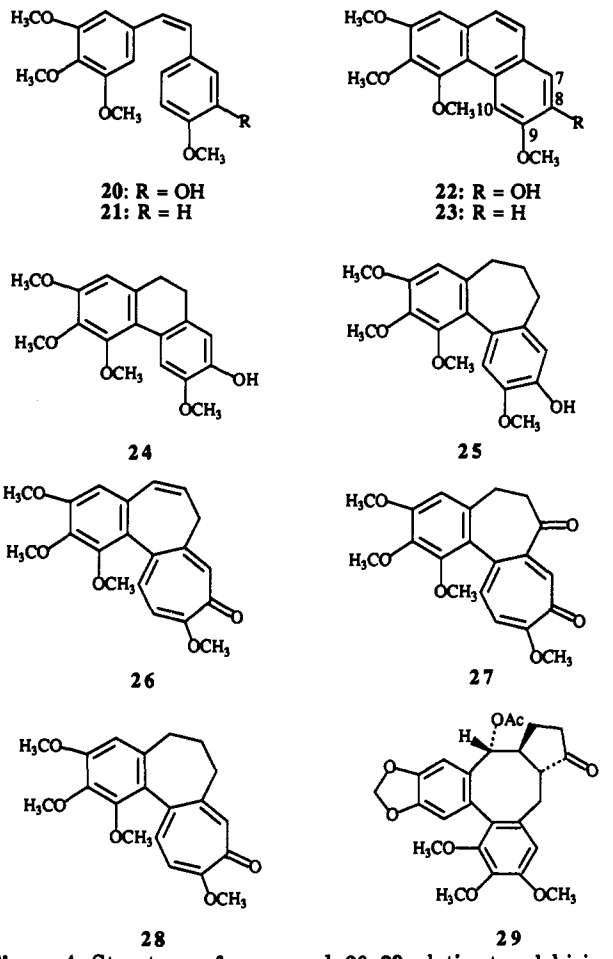


Figure 4. Structures of compounds 20–29 relating to colchicine.

experiment with compound 18), we cannot be certain that the differences observed with this series of compounds are significant. In general, the more active compounds showed stimulation equivalent to that obtained with 1, while the less active compounds were less stimulatory. With the latter agents, the drug concentration of 50 μM was probably subsaturating. In contrast, 1 results in near-maximal stimulation of GTPase activity with 10 μM tubulin (data not presented).

Discussion and Conclusions

In the six-membered ring B series described here, all of the most cytotoxic agents (compounds 5, 9, 11, 14, 15, and 17) were effective inhibitors of tubulin polymerization, although there was otherwise not a complete correlation between antitubulin effects and cytotoxicity. Several good inhibitors of tubulin polymerization (compounds 6 and 18 and 3 itself) were among the less potent cytotoxic agents, and five compounds (7, 8, 10, 12, and 13) with only weak interactions with tubulin (more readily demonstrated at high concentrations in the GTPase and [^{3}H]colchicine binding assays rather than in the polymerization assay) had definite cytotoxic activity. In the case of compound 13 we documented that its cytotoxic activity was associated with the accumulation of cells arrested in mitosis, strongly indicating that the intracellular target of the drug was tubulin. The basis for these discrepancies is at present unknown, but the five compounds with feeble antitubulin activities could be readily converted to compound 5 by either extracellular or intracellular esterases.

In terms of inhibition of tubulin polymerization, there are several interesting aspects to the structure–activity relationships that we have examined here. Although we have not prepared analogs of 3 in which the only change is conversion of the ring B from seven-membered to six-membered, it appears that such a change results in only a small loss of inhibitory activity (compound 6 is probably the closest analog of 3 in terms of size of the ring B side chain). This small change can be contrasted to the virtual elimination of inhibitory activity which occurs when 20 and its almost equally active tetramethoxy analog 21 (Figure 4) have a six-membered ring B introduced when they are converted to the phenanthrenes (22 and 23) (Figure 4).[31,32] In addition, the other three isomers of 23, with the methoxy group at positions C(7), C(8), or C(9), had little effect on tubulin polymerization.[32] In the case of 20, there was still further loss of activity when the C(5)–C(6) double bond of the phenanthrene 22 (IC_{50} value of 100 μM) was reduced to yield the dihydrophenanthrene (24) (IC_{50} value > 100 μM) (Figure 4).[31,33] However, when the reduced six-membered ring B of 24 was expanded to a seven-membered ring (25 in Figure 4), essentially creating an allocolchicinoid, significant inhibition of tubulin polymerization again occurred.[31,33] Thus it appears that enlargement of ring C from six to seven atoms has a major impact on an acceptable size for ring B, and vice versa as well.

Furthermore, the good inhibitory activity of 18 on tubulin polymerization indicated that the *S* configuration of the side chain at C(6) is not essential for the binding of these compounds to tubulin. Rather, introduction of an sp^2 center in the six-membered ring B resulted in about a 50% enhancement of inhibitory activity (cf. the IC_{50} value of 2.4 μM for 18 with the value of 3.6 μM for 5). Similar enhancement occurs in colchicinoids. 5,6-Dihydrodeacetamidocolchicine (26) (Figure 4) and 7-oxodeacetamidocolchicine (27) (Figure 4) are better inhibitors of tubulin polymerization than 1 and deacetamidocolchicine (28) (Figure 4).[34,35] In allocolchicinoids, in contrast, which have a seven-membered ring B and six-membered ring C, introduction of an sp^2 center in ring B neither enhances nor reduces inhibitory activity.[34]

Of greatest interest is that compound 5 is a potent inhibitor of tubulin polymerization and binds rapidly to tubulin, despite having an *aR* biaryl configuration opposite to the *aS* configuration of colchicinoids,[7] allocolchicinoids,[36] and the related antimitotic natural product steganacin (29) (Figure 4).[37,38] However, based on the strong antitubulin activity of compound 28, which has no optical activity and presumably contains equal amounts an *aS* and *aR* configured isomers, it was suggested[7] that an atropisomerism equilibrium between *aS* and *aR* configured colchicinoids occurs in solution. The CD spectrum of 18 bound to tubulin further supports this suggestion (data will be published elsewhere). Thus it would be the minor (*aS*,6*S*) conformer of 5 and its derivatives that would be the active species. This would require an extremely rapid equilibrium between the (*aR*,6*S*) and (*aS*,6*S*) conformations to account for the rapid interaction of these compounds with tubulin as documented here and previously.[13] The binding of the (*aS*,6*S*) species to tubulin and hence its removal from solution should accelerate its formation from the (*aR*,6*S*) conformer. The minimally active derivatives (compounds 7, 8, 10, 12, 13, and 16), which all have bulky substituents at position C(6), probably are unable to undergo rapid conversion between the *aR* and *aS* conformations. Similar substituents in both 1[39] and

3 (manuscript in preparation) at position C(7) have little effect on the ability of these compounds to inhibit tubulin polymerization.

The enhanced activities of compound 18 relative to compound 5 and of compounds 26 and 27 relative to compound 1 and 28 (see above) are pertinent to these speculations. While introduction of the sp^2 center should make these agents more planar, like compound 28 they, too, should contain equivalent amounts of *aR* and *aS* conformers in rapid equilibrium. Their enhanced activities suggest, however, that the optimal dihedral angle for binding to tubulin is relatively small. The inactivity of phenanthrenes 22 and 23, however, indicates that the planar conformations themselves have negligible affinity for tubulin.

In conclusion, the six-membered ring B analogs described here represent a class of compounds that bind rapidly in the colchicine site of tubulin and, by inhibiting its polymerization, cause cells to accumulate in the M phase of the cell cycle. In a cytotoxicity evaluation, with comparison to 1 and 3, compound 15 was more active in three human solid tumor cell lines, and compounds 14, 17, and 18 had activity comparable to that of 3 in at least three cell lines. This class of agent merits further synthetic work for the selection of optimally active analogs and further investigation of their potential as clinically useful antitumor drugs.

Experimental Section

Chemistry. Melting points were measured with a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were determined with a Rudolph Research Autopol III polarimeter. IR spectra were recorded on a Perkin-Elmer 1320 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AC-300 spectrometer. The chemical shift is presented in terms of ppm with Me_4Si as internal reference. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Thin-Layer chromatography (TLC) silica gel plates were purchased from Analtech, Inc. Silica gel (230–400 mesh) from Aldrich Inc. was utilized for column chromatography. Colchicine was purchased from Aldrich, Inc. The thiocolchicine used in the tubulin studies was from Roussel-Uclaf, and combretastatin A-4 was a generous gift of Dr. G. R. Pettit, Arizona State University.

5,6-Dihydro-6(*S*)-(hydroxymethyl)-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (5). To deacetylthiocolchicine (42.7 mg, 0.11 mmol) in water (4 mL) was added sodium nitrite (10.9 mg, 0.15 mmol) in water (1 mL) containing glacial acetic acid (2 drops). The reaction mixture was stirred at room temperature for 24 h and extracted with $CHCl_3$ (30 mL) three times. The extract was washed with 5% $NaHCO_3$ solution, water, and brine until pH = 7, dried over anhydrous Na_2SO_4, and concentrated. Purification of the residue with preparative TLC plates with $CHCl_3$–MeOH (95:5) as eluant furnished pure product (18.1 mg). Crystallization from CH_2Cl_2–Et_2O afforded yellow crystals: yield 42%; mp 201–203 °C; $[\alpha]^{20}_D$ +116° (*c* 0.86, $CHCl_3$); IR (KBr) 3400 (OH), 2970, 2930, and 2840 (aliphatic CH), and 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3 H, SCH_3-9). 2.94 (br, 2 H, H-5), 3.03 (m, 1 H, H-6), 3.41 (dd, *J* = 10.7, 7.91 Hz, 1 H, $HOCH_2$-6), 3.59 (dd, *J* = 10.7, 6.38 Hz, 1 H, $HOCH_2$-6), 3.68 (s, 3 H, OCH_3-1), 3.91 (s, 6 H, OCH_3-2,3), 6.60 (s, 1 H, H-4), 7.05 (d, *J* = 10.7 Hz, 1 H, H-10), 7.17 (s, 1 H, H-7), and 7.92 (d, *J* = 10.7 Hz, 1 H, H-11). Anal. ($C_{20}H_{22}O_5S \cdot {}^3/_4H_2O$) C, H, S.

General Procedures for the Synthesis of 5,6-Dihydro-6-(*S*)-(acyloxy)- and 5,6-Dihydro-6(*S*)-[(aroyloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-ones (6–17). To 5 in anhydrous dichloromethane was added the corresponding acid chloride in pyridine. The reaction mixture was stirred at room temperature for 6–10 h and extracted with chloroform. The $CHCl_3$ extract was washed with a 2 N HCl solution, water, and brine until pH = 7, dried over anhydrous Na_2SO_4, and concentrated to give an oil residue. Separation of this residue on preparative TLC with $CHCl_3$–MeOH (97:3) as eluant afforded the pure product.

5,6-Dihydro-6(*S*)-[(acetyloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (6): yield 74%; amorphous; $[\alpha]^{20}_D$ +125° (*c* 0.48, $CHCl_3$); IR (KBr) 2940 and 2840 (aliphatic CH), 1735 (CO, ester), and 1605 (CO, topolone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3 H, CH_3OCOCH_2-6), 2.45 (s, 3 H, SCH_3-9), 2.78 (dd, *J* = 15.3, 4.45 Hz, 1 H, H-5), 2.98 (dd, *J* = 15.3, 2.21 Hz, 1 H, H-5), 3.15 (m, 1 H, H-6), 3.69 (s, 3 H, OCH_3-1), 3.91 (s, 3 H, OCH_3-2), 3.92 (s, 3 H, OCH_3-3), 3.83 (dd, *J* = 11.0, 7.13 Hz, 1 H, CH_3COOCH_2-6), 4.08 (dd, *J* = 11.0, 8.02 Hz, 1 H, CH_3COOCH_2-6), 6.55 (s, 1 H, H-4), 7.05 (d, *J* = 10.6 Hz, 1 H, H-10), 7.14 (s, 1 H, H-7), and 7.93 (d, *J* = 10.6 Hz, 1 H, H-11). Anal. ($C_{22}H_{24}O_6S$) C, H, S.

5,6-Dihydro-6(*S*)-[[(4′-fluorobenzoyl)oxy]methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (7): yield 97%; amorphous, $[\alpha]^{20}_D$ +107° (*c* 0.41, $CHCl_3$); IR (KBr) 2925 and 2825 (aliphatic CH), 1705 (CO, ester), and 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H, SCH_3-9), 2.85 (dd, *J* = 15.2, 2.52 Hz, 1 H, H-5), 3.05 (dd, *J* = 15.2, 4.75 Hz, 1 H, H-5), 3.31 (m, 1 H, H-6), 3.71 (s, 3 H, OCH_3-1), 3.90 (s, 3 H, OCH_3-2), 3.92 (s, 3 H, OCH_3-3), 4.41 (dd, *J* = 11.1, 7.28 Hz, 1 H, CH_2-6), 4.21 (dd, *J* = 11.1, 8.02 Hz, 1 H, CH_2-6), 6.56 (s, 1 H, H-4), 7.06 (d, *J* = 10.7 Hz, 1 H, H-10), 7.12 (dt, *J* = 8.70, 1.86 Hz, 2 H, H-3′,5′), 7.24 (s, 1 H, H-7), 7.95 (d, *J* = 10.7 Hz, 1 H, H-11), and 8.01 (ddd, *J* = 8.70, 5.28, 2.11 Hz, 2H, H-2′,6′). Anal. ($C_{27}H_{25}O_6SF \cdot {}^3/_4H_2O$) C, H, S.

5,6-Dihydro-6(*S*)-[[(4′-methoxybenzoyl)oxy]methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (8): yield 96%; amorphous; $[\alpha]^{20}_D$ +80° (*c* 0.44, $CHCl_3$); IR (KBr) 2920 and 2830 (aliphatic CH), 1695 (CO, ester), and 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H, SCH_3-9), 2.84 (dd, *J* = 15.3, 2.09 Hz, 1 H, H-5), 3.03 (dd, *J* = 15.3, 4.41 Hz, 1 H, H-5), 3.32 (m, 1 H, H-6), 3.70 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 4.12 (dd, *J* = 11.1, 6.66 Hz, 1 H, CH_2-6), 4.17 (dd, *J* = 11.1, 7.26 Hz, 1 H, CH_2-6), 6.56 (s, 1 H, H-4), 6.93 (d, *J* = 5.88 Hz, 2 H, H-3′,5′), 7.05 (d, *J* = 10.8 Hz, 1 H, H-10), 7.27 (s, 1 H, H-7), 7.95 (d, *J* = 10.8 Hz, 1 H, H-11), and 7.96 (d, *J* = 5.88 Hz, 2 H, H-2′6′). Anal. ($C_{28}H_{28}O_7S \cdot {}^3/_4H_2O$) C, H, S.

5,6-Dihydro-6(*S*)-[[(2′-fluorobenzoyl)oxy]methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (9): yield 8.5%; amorphous; $[\alpha]^{20}_D$ +136° (*c* 0.50, $CHCl_3$); IR (KBr) 2930 and 2820 (aliphatic CH), 1710 (CO, ester), and 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H, SCH_3-9), 2.93 (dd, *J* = 15.3, 2.55 Hz, 1 H, H-5), 3.01 (dd, *J* = 15.3, 4.26 Hz, 1 H, H-5), 3.33 (m, 1 H, H-6), 3.70 (s, 3 H, OCH_3-1), 3.89 (s, 3 H, OCH_3-2), 3.92 (s, 3 H, OCH_3-3), 4.05 (dd, *J* = 10.9, 8.43 Hz, 1 H, CH_2-6), 4.33 (dd, *J* = 10.9, 7.26 Hz, 1 H, CH_2-6), 6.59 (s, 1 H, H-4), 7.05 (d, *J* = 10.7 Hz, 1 H, H-10), 7.14 (dd, *J* = 10.4, 8.79 Hz, 1 H, H-3′), 7.23 (dd, *J* = 15.1, 4.8 Hz, 1 H, H-5′), 7.26 (s, 1 H, H-7), 7.52 (m, 1 H, H-4′), 7.90 (ddd, *J* = 15.1, 5.09, 1.17 Hz, 1 H, H-6′), and 7.95 (d, *J* = 10.7 Hz, 1 H, H-11). Anal. ($C_{27}H_{25}O_6SF \cdot H_2O$) C, H, S.

5,6-Dihydro-6(*S*)-[[(3′-fluorobenzoyl)oxy]methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (10): yield 99%; amorphous $[\alpha]^{20}_D$ +93° (*c* 0.50, $CHCl_3$); IR (KBr) 2930 and 2820 (aliphatic CH), 1710 (CO, ester), and 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H, SCH_3-9), 2.87 (dd, *J* = 15.2, 2.15 Hz, 1 H, H-5), 3.04 (dd, *J* = 15.2, 4.37 Hz, 1 H, H-5), 3.33 (m, 1 H, H-6), 3.71 (s, 3 H, OCH_3-1), 3.89 (s, 3 H, OCH_3-2), 3.91 (s, 3 H, OCH_3-3), 4.12 (dd, *J* = 10.9, 7.61 Hz, 1 H, CH_2-6), 4.28 (dd, *J* = 10.9, 7.57 Hz, 1 H, CH_2-6), 6.57 (s, 1 H, H-4), 7.06 (d, *J* = 10.7 Hz, 1 H, H-10), 7.23 (s, 1 H, H-7), 7.27 (m, 1 H, H-4′), 7.43 (dt, *J* = 8.02, 5.90 Hz, 1 H, H-5′), 7.65 (d, *J* = 8.99 Hz, 1 H, H-2′), 7.79 (d, *J* = 8.02 Hz, 1 H, H-6′), and 7.95 (d, *J* = 10.7 Hz, 1 H, H-11). Anal. ($C_{27}H_{25}O_6SF \cdot H_2O$) C, H, S.

5,6-Dihydro-6(*S*)-[[(4′-nitrobenzoyl)oxy]methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (11): yield 89%; amorphous; $[\alpha]^{20}_D$ +40° (*c* 0.51, $CHCl_3$); IR (KBr) 2920 and 2820 (aliphatic CH), 1715 (CO, ester), and 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H, SCH_3-9), 2.86 (dd, *J* = 15.3, 2.03 Hz, 1 H, H-5), 3.09 (dd, *J* = 15.3, 4.45 Hz, 1 H, H-5), 3.34 (m, 1 H, H-6), 3.72 (s, 3 H, OCH_3-1), 3.91 (s, 3 H, OCH_3-2), 3.93 (s, 3 H, OCH_3-3), 4.21 (dd, *J* = 11.0, 7.02 Hz, 1 H, CH_2-6), 4.28 (dd, *J* = 11.0, 7.86 Hz, 1 H, CH_2-6), 6.58 (s, 1 H, H-4), 7.07

(d, J = 10.8 Hz, 1 H, H-10), 7.22 (s, 1 H, H-7), 7.96 (d, J = 10.8 Hz, 1 H, H-11), 8.17 (d, J = 8.74 Hz, 2 H, H-2′,6′), and 8.29 (d, J = 8.74 Hz, 2 H, H-3′,5′). Anal. ($C_{27}H_{25}O_8NS$) C, H, N, S.

5,6-Dihydro-6(*S*)-[[(3′-cyanobenzoyl)oxy]methyl]-1,2,3-trimethoxy-9-(methoxythio)-8*H*-cyclohepta[*a*]naphthalen-8-one (12): yield 100%; amorphous; $[\alpha]^{20}_D$ +115° (c 0.56, $CHCl_3$); IR (KBr) 2930 and 2820 (aliphatic CH), 2220 (CN), 1715 (CO, ester), and 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H, SCH_3-9), 2.86 (dd, J = 15.3, 2.40 Hz, 1 H, H-5), 3.06 (dd, J = 15.3, 4.55 Hz, 1 H, H-5), 3.33 (m, 1 H, H-6), 3.72 (s, 3 H, OCH_3-1), 3.91 (s, 3 H, OCH_3-2), 3.92 (s, 3 H, OCH_3-3), 4.15 (dd, J = 11.1, 7.37 Hz, 1 H, CH_2-6), 4.31 (dd, J = 11.1, 7.88 Hz, 1 H, CH_2-6), 6.57 (s, 1 H, H-4), 7.06 (d, J = 10.8 Hz, 1 H, H-10), 7.21 (s, 1 H, H-7), 7.61 (t, J = 7.80 Hz, 1 H, H-5′), 7.84 (td, J = 7.97, 1.33 Hz, 1 H, H-6′ or H-4′), 7.96 (d, J = 10.8 Hz, 1 H, H-11), 8.22 (td, J = 7.80, 1.33 Hz, 1 H, H-4′ or H-6′), and 8.27 (t, J = 1.33 Hz, 1 H, H-2′). Anal. ($C_{28}H_{25}O_8NS$) C, H, N, S.

5,6-Dihydro-6(*S*)-[[(3′-nitrobenzoyl)oxy]methyl]-1,2,3-trimethoxy-9-(methoxythio)-8*H*-cyclohepta[*a*]naphthalen-8-one (13): yield 97%; amorphous; $[\alpha]^{20}_D$ +128° (c 0.52, $CHCl_3$); *IR* (KBr) *2930 and 2830* (aliphatic CH), *1720 (CO, ester)*, and 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H, SCH_3-9), 2.89 (bd, J = 15.0 Hz, 1 H, H-5), 3.08 (dd, J = 15.0, 4.16 Hz, 1 H, H-5), 3.36 (br, 1 H, H-6), 3.73 (s, 3 H, OCH_3-1), 3.91 (s, 3 H, OCH_3-2), 3.93 (s, 3 H, OCH_3-3), 4.15 (dd, J = 10.9, 7.59 Hz, 1 H, CH_2-6), 4.37 (dd, J = 10.9, 8.09 Hz, 1 H, CH_2-6), 6.60 (s, 1 H, H-4), 7.07 (d, J = 10.6 Hz, 1 H, H-10), 7.21 (s, 1 H, H-7), 7.68 (t, J = 7.96 Hz, 1 H, H-5′), 7.97 (d, J = 10.6 Hz, 1 H, H-11), 8.33 (d, J = 7.96 Hz, 1 H, H-6′), 8.42 (d, J = 7.96 Hz, 1 H, H-4′), and 8.81 (s, 1 H, H-2′). Anal. ($C_{27}H_{25}O_8NS$) C, H, N, S.

5,6-Dihydro-6(*S*)-[(butyryloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (14): yield 63%; amorphous; $[\alpha]^{20}_D$ +115° (c 0.52, $CHCl_3$); IR (KBr) 2970, 2940, and 2840 (aliphatic CH), 1735 (CO, ester), 1605 (CO, tropolone); ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7.35 Hz, 3 H, CH_3-$CH_2CH_2COOCH_2$-6), 1.64 (m, 2H, $CH_3CH_2CH_2COOCH_2$-6), 2.27 (t, J = 7.28 Hz, 2 H, $CH_3CH_2CH_2COOCH_2$-6), 2.44 (s, 3 H, SCH_3-9), 2.77 (dd, J = 15.2, 2.56 Hz, 1 H, H-5), 2.97 (dd, J = 15.2, 4.46 Hz, 1 H, H-5), 3.16 (m, 1 H, H-6), 3.69 (s, 3 H, OCH_3-1), 3.84 (dd, J = 10.9, 7.39 Hz, 1 H, $CH_3CH_2CH_2COOCH_2$-6), 3.90 (s, 3 H, OCH_3-2), 3.92 (s, 3 H, OCH_3-3), 4.05 (dd, J = 10.9, 7.79 Hz, 1 H, $CH_3CH_2CH_2COOCH_2$-6), 6.54 (s, 1 H, H-4), 7.05 (d, J = 10.8 Hz, 1 H, H-10), 7.14 (s, 1 H, H-7), and 7.93 (d, J = 10.8 Hz, 1 H, H-11). Anal. ($C_{24}H_{28}O_6S$) C, H, S.

5,6-Dihydro-6(*S*)-[[(ethoxycarbonyl)oxy]methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (15): yield 57%; amorphous; $[\alpha]^{20}_D$ +134° (c 0.36, $CHCl_3$); IR (KBr) 2980, 2940, and 2840 (aliphatic CH), 1745 (CO, ester), and 1605 (CO, tropolone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7.17 Hz, 3 H, $CH_3CH_2OCOOCH_2CH_3$-6), 2.44 (s, 3 H, SCH_3-9), 2.84 (dd, J = 15.3, 2.45 Hz, 1 H, H-5), 2.95 (dd, J = 15.3, 4.18 Hz, 1 H, H-5), 3.21 (m, 1 H, H-6), 3.69 (s, 3 H, OCH_3-1), 3.90 (s, 3 H, OCH_3-2), 3.92 (s, 3 H, OCH_3-3), 3.81–4.19 (m, 4 H, CH_3CH_2-$OCOOCH_2$-6), 6.58 (s, 1 H, H-4), 7.04 (d, J = 10.7 Hz, 1 H, H-10), 7.17 (s, 1 H, H-7), and 7.92 (d, J = 10.7 Hz, 1 H, H-11). Anal. ($C_{23}H_{26}O_7S$) C, H, S.

5,6-Dihydro-6(*S*)-[[(4′-bromobenzoyl)oxy]methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (16): yield 100%; amorphous $[\alpha]^{20}_D$ +71° (c 0.62, $CHCl_3$); $CHCl_3$); IR (KBr) 2930 and 2830 (aliphatic CH), 1710 (CO, ester), and 1590 (CO, tropolone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H, SCH_3-9), 2.86 (dd, J = 15.0, 2.29 Hz, 1 H, H-5), 3.06 (dd, J = 15.0, 4.53 Hz, 1 H, H-5), 3.32 (m, 1 H, H-6), 3.71 (s, 3 H, OCH_3-1), 3.90 (s, 3 H, OCH_3-2), 3.92 (s, 3 H, OCH_3-3), 4.15–4.22 (m, 2 H, CH_2-6), 6.56 (s, 1 H, H-4), 7.06 (d, J = 10.8 Hz, 1 H, H-10). 7.23 (s, 1 H, H-7), 7.59 (d, J = 8.36 Hz, 2 H, H-3′,5′), 7.86 (d, J = 8.36 Hz, 2 H, H-2′,6′), and 7.96 (d, J = 10.8 Hz, 1 H, H-11). Anal. ($C_{27}H_{25}O_5SBr \cdot {}^1/_2H_2O$) C, H, S.

5,6-Dihydro-6(*S*)-[(propyloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cyclohepta[*a*]naphthalen-8-one (17): yield 67%; amorphous; $[\alpha]^{20}_D$ +79° (c 0.46; $CHCl_3$); IR (KBr) 2940 and *2840 (aliphatic CH)*, *1730 (CO, ester)*, and 1600 *(CO,* tropolone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.11 (t, J = 7.53 Hz, 3 H, $CH_3CH_2COOCH_2$-6), 2.30 (q, J = 7.53 Hz, 2 H, $CH_3CH_2COOCH_2$-6), 2.44 (s, 3 H, SCH_3-9), 2.78 (dd, J = 15.3, 2.29 Hz, 1 H, H-5), 2.98 (dd, J = 15.3, 4.49 Hz, 1 H, H-5), 3.16 (m, 1 H, H-6), 3.70 (s, 3 H, OCH_3-1), 3.84 (dd, J = 11.0, 8.0 Hz, 1 H, CH_2-6), 3.91 (s, 3 H, OCH_3-2), 3.92 (s, 3 H, OCH_3-3), 4.07 (dd, J = 11.0, 7.7 Hz, 1 H, CH_2-6), 6.54 (s, 1 H, H-4), 7.05 (d, J = 10.7 Hz, 1 H, H-10), 7.14 (s, 1 H, H-7), and 7.94 (d, J = 10.7 Hz, 1 H, H-11). Anal. ($C_{23}H_{26}O_6S$) C, H, S.

5,6-Dihydro-6(*S*)-(methylene)-1,2,3-trimethoxy-9-(methoxythio)-8*H*-cyclohepta[*a*]naphthalen-8-one (18). To a benzene solution of **5** (10.6 mg, 0.03 mmol) and DBU (9.4 mg, 0.062 mmol) was added tosyl chloride (11.8 mg, 0.062 mmol) in one portion. The reaction mixture was stirred at 0 °C for 1 h, then at room temperature for 10 h, evaporated, extracted with dichloromethane (10 mL), and washed with a 2 N HCl solution, water, 5% $NaHCO_3$, and brine until pH = 7. The organic layer was dried over anhydrous sodium sulfate and evaporated under vacuum. The separation of the residue on chromatography TLC yielded 7.8 mg of product: yield 77%; crystallization from EtOAc afforded yellow crystals: mp 165–166 °C; IR (KBr) 2920 and 2830 (aliphatic CH), 1590 and 1550 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3 H, SCH_3-9), 3.44 (s, 2 H, H-5), 3.69 (s, 3 H, OCH_3-1), 3.91 (s, 6 H, OCH_3-2,3), 5.21 (s, 1 H, CH_2-6), 5.50 (s, 1 H, CH_2-6), 6.59 (s, *1 H, H-4*), *7.06 (d, J = 10.5 Hz, 1 H, H-10)*, *7.40* (s, *1 H, H-7*), and 7.94 (d, J = 10.5 Hz, 1 H, H-11). Anal. ($C_{20}H_{20}O_4S$) C, H, S.

Determination of the Absolute Stereochemistry of Compound 5 by X-ray Crystallographic Analysis. Crystal data: $C_{20}H_{22}O_5S$, M = 374.46, monoclinic, space group $P2_1$, a = 11.130(1) Å, b = 8.776(1) Å, c = 9.400(1) Å, β = 95.59(1)° (at 25 °C, from 25 orientation reflections, 35° < θ < 40°), V = 913.8(3) Å^3, Z = 2, D_{calcd} = 1.361 g cm^{-3}, μ(Cu Kα radiation, λ = 1.5418 Å) = 17.7 cm^{-1}; crystal dimensions: 0.14 × 0.40 × 0.50 mm.

Preliminary unit-cell parameters and space group information were derived from oscillation and Weisseberg photographs. Intensity data (+h,+k,±l; θ = 75°) were recorded on an Enraf-Nonius CAD-4 diffractometer [Cu Kα radiation, graphite monochromator; ω-2θ scans, scanwidth (0.80 ± 0.14 tan θ)°]; the intensities of four reference reflections; remeasured every 2 h during data collection, showed no significant variation (<1%). The intensity data were corrected for the usual Lorentz and polarization effects; an empirical absorption correction (T_{max}/T_{min} = 1.00:0.66, derived from the φ-dependency of the intensities of several reflections with χ ca. 90°) was also applied.

The crystal structure was solved by direct methods (MULTAN11/82). Approximate coordinates for all non-hydrogen atoms were derived from an E-map. Several rounds of full-matrix least-squares adjustment of positional and thermal parameters of these atoms (at first isotropic and then anisotropic) to convergence were followed by the evaluation of a difference Fourier synthesis, which yielded hydrogen atom positions. In the next series of least-squares calculations, hydrogen atoms were incorporated at their calculated positions and latterly an extinction correction (g) was also introduced as a variable. The parameter refinement converged at $R = [\Sigma||F_o| - |F_c||/\Sigma|F_o|] = 0.0358$ [$R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0.0514$]. The absolute stereochemsitry was established at this stage by introduction of the introduction of the imaginary contributions to the anomalous dispersion corrections into the structure-factor calculations. For parameters corresponding to the absolute stereochemistry represented by structural **5**, R was 0.0379 while R_w was 0.0557 whereas for those of the minor image the values were R' = 0.0407, R'_w = 0.0600. The differences indicate that structure **5** correctly represents the absolute stereochemistry. This determination is significant at the 0.005 level if R'_w/R_w = (0.0600/0.0557 = 1.0772) equals or exceeds 1.0024.[40] Assignment of this absolute stereochemistry was corroborated by measuring the relative intensities of 39 Friedel pairs of enantiomer-sensitive reflections with I > 20.0$\sigma(I)$ and for which the calculated values differed by >25%. In all cases, the sense of the difference between the measured values was in accord with that calculated (see supplementary material). Continuation of the least-squares refinement of parameters for the correct enantiomer converged (max shift/esd = 0.01) at R = 0.035 [R_w = 0.050, GOF = $[\Sigma w\Delta^2/(N_{observation} - N_{parameters})]^{1/2} = 1.89$, $g = 1.90(6) \times 10^{-6}$]. A final difference Fourier synthesis contained no unusual features ($\Delta\rho$ e/Å^3: max 0.22; min −0.22).

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure

Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from ref 41. In the least-squares iterations $\sum w\Delta^2$ [$w = 1/\sigma 2(|F_o|)$; $\Delta = (|F_o| - |F_c|)$] was minimized.

Acknowledgment. This work was supported by grant CA 17625 from the National Cancer Institute (K.H.L.).

Supplementary Material Available: Tables of fractional atomic coordinates and temperature factor parameters, bond lengths, bond angles, torison angles, and relative intensities for 39 pairs of enantiomer-sensitive for **5** (10 pages). Ordering information is given on any current masthead page.

References

(1) Li, L. P.; Wang, H. K.; Chang, J. J.; McPhail, A. T.; Terada, H.; Konoshima, T.; Kokumai, M.; Kozuka, M.; Estes, J. R.; Lee, K. H. Antitumor Agents 138. Rotenoids and Isoflavones as Cytotoxic Constituents from *Amorpha fruticosa*. *J. Natl. Prod.*, in press.

(2) Capraro, H. G.; Brossi, A. Tropolonic Colchicum Alkaloids. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1984; Vol. 23, pp 1–70.

(3) Brossi, A.; Yeh, H. J. C.; Chrzanowska, M.; Wolff, J.; Hamel, E.; Lin, C. M.; Quinn, F.; Suffness, M.; Silverton, J. Colchicine and its Analogues: Recent Findings. *Med. Res. Rev.* **1988**, *8*, 77–94.

(4) Brossi, A. Bioactive Alkaloids. 4. Results of Recent Investigations with Colchicine and Physostigmine. *J. Med. Chem.* **1990**, *33*, 2311–2315.

(5) Hamel, E. Interactions of Tubulin with Small Ligands. In *Microtubule Proteins*; Avila, J., Ed.; CRC Press: Boca Raton, FL, 1990; pp 189–191.

(6) Hastie, S. B. Interactions of Colchicine with Tubulin. *Pharmacol. Ther.* **1991**, *51*, 377–401.

(7) Yeh, H. J. C.; Chrzanowska, M.; Brossi, A. The Importance of the Phenyl-tropolone '*aS*' Configuration in Colchicine's Binding to Tubulin. *FEBS Lett.* **1988**, *229*, 82–86.

(8) Brossi, A. Optically Active Alkaloids and Unnatural Enantiomers: Some Recent Developments. *Actual. Chim. Ther.* **1989**, *16*, 13–35.

(9) Kerekes, P.; Brossi, A. Esters of 1-*O*-Demethylthiocolchicines: Formation of Isomers in Chloroform Solution. *Helv. Chim. Acta.* **1985**, *68*, 571–580.

(10) Boye, O.; Brossi, A. Tropolonic *Colchicum* Alkaloids and Allo Congeners. In *The Alkaloids*, Brossi, A., Ed.; Academic Press, Inc. **1992**; Vol. 41, pp 125–176.

(11) Bhattacharyya, B.; Howard, S. N.; Brossi, A.; Sharma, P. N.; Wolff, J. Ring B Regulation of Colchicine Binding Kinetics and Fluorescence. *Proc. Natl. Acad. Sci.* U.S.A. **1986**, *83*, 2052–2055.

(12) Banerjee, A.; Barnes, L. D.; Luduena, R. F.; The Role of the B-ring of Colchicine in the Stability of the Colchicine-tubulin Complex. *Biochim. Biophys. Acta* **1987**, *913*, 138–144.

(13) Lincoln, P.; Nordh, J.; Deinum, J.; Amgstrom, J.; Nordan, B. Conformation of Thiocolchicine and Two B-ring-modified Analogues Bound to Tubulin Studied with Optical Spectroscopy. *Biochemistry* **1991**, *30*, 1179–1187.

(14) Velluz, L.; Muller, G. N° 155. The Thiocolchicine. *Bull. Soc. Chim.* **1954**, 755–757.

(15) Velluz, L.; Muller, G. N° 224. The Thiocolchicine II-Products of Hydrolysis, Reduction, and Oxidation, with Example of Asymmetric Sulfur. *Bull. Soc. Chim. Fr.* **1954**, 1072–1074.

(16) Kotani, R. Demjanov Rearrangement of 1-Methylcyclohexanemethylamine. *J. Org. Chem.* **1965**, *30*, 350–354.

(17) Wolfe, M. S.; Lee, Y.; Barttett, W. J. Borcherding, D. R.; Borchardt, R. T. 4'-Modified Analogous of Aristeromycin and Neplanocin A. Synthesis and Inhibitory Activity Toward *S*-Adenosyl-L-homocysteine Hydrolase. *J. Med. Chem.* **1992**, *35*, 1782–1791.

(18) The *aS* configuration of natural colchicine with a counterclockwise arrangement of the aromatic rings is derived by applying the "steering-wheel rule" as described in *The Alkaloids* **1992**, *41*, 126. By applying this rule, compound **5** should have an *aR* configuration.

(19) Olsson, C.; Lincoln, P.; Delnum, J. Structure of a New Colchicine Derivative, 5,6-Dihydro-6-hydroxymethyl-1,2,3-trimethoxy-9-methylthio-8*H*-cyclohepta[*a*]naphthalen-8-one. *Acta Crystallogr. Sect. C.: Cryst. Struct. Commun.* **1989**, *C45*, 1558–1561.

(20) Monks, A.; Scudiero, D.; Skahan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a High-flux Anticancer Drug Screen Utilizing a Diverse Panel of Human Tumor Cell Lines in Culture. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.

(21) Hamel, E.; Lin, C. M. Separation of Active Tubulin and Microtubule-associated Proteins by Ultracentrifugation and Isolation and a Component Causing the Formation of Microtubule Bundles. *Biochemistry* **1984**, *23*, 4173–4184.

(22) Borisy, G. G. A Rapid Method for Quantitative Determination of Microtubule Protein Using DEAE-cellulose Filters. *Anal. Biochem.* **1972**, *50*, 373–385.

(23) Hamel, E.; Lin, C. M. Guanosine 5'-*O*-(3-thiotriphosphate), a Potent Nucleotide Inhibitor Microtubule Assembly. *J. Biol. Chem.* **1984**, *259*, 11060–11069.

(24) Muzaffar, A.; Brossi, A.; Lin, C. M.; Hamel, E. Antitubulin of Derivatives of 3-Dimethylthiocolchicine, Methylthio Ethers of Natural Colchicinoids, and Thioketones Derived from Thiocolchicine. Comparison with Colchicinoids. *J. Med. Chem.* **1990**, *33*, 567–571.

(25) Hamel, E.; Lin, C. M. Glutamate-induced Polymerization of Tubulin: Characteristics of the Reaction and Application to the Large-scale Purification of Tubulin. *Arch. Biochem. Biophys.* **1981**, *209*, 29–40.

(26) Hamel, E.; Ho, H. H.; Kang, G.-J.; Lin, C. M. Cornigerine, a Potent Antimitotic Colchicum Alkaloid of Unusual Structure. *Biochem. Pharmacol.* **1988**, *37*, 2445–2449.

(27) Chabin, R. M.; Hastie, S. B. Association of Thiocolchicine with Tubulin. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 544–550.

(28) Kang, G-J.; Getahun, Z.; Muzaffar, A.; Brossi, A.; Hamel, E. N-Acetylcolchinol *O*-Methyl Ether and Thiocolchincine, Potent Analogs of Colchicine Modified in the C ring: Evaluation of the Mechanistic Basis for their Enhanced Biological Properties. *J. Biol. Chem.* **1990**, *265*, 10255–10259.

(29) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. Antimitotic Natural Products Combretastatin A-4 and Combretastatin A-2: Studies on the Mechanism of their Inhibition of the Binding of Colchicine to Tubulin. *Biochemistry* **1989**, *28*, 6984–6991.

(30) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S. Isolation and Structure of the Strong Cell Growth and Tubulin Inhibitor Combretastatin A-4. *Experientia* **1989**, *45*, 209–211.

(31) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. Interactions of Tubulin with Potent Natural and Synthetic Analogs of the Antimitotic Agent Combretastatin: a Structure-Activity Study. *Mol. Pharmacol.* **1988**, *34*, 200–208.

(32) Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H.-M.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Analogues of (*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-Trimethoxyphenyl)ethene as Potential Cytotoxic and Antimitotic Agents. *J. Med. Chem.* **1992**, *35*, 2293–2306.

(33) Huber, S. K.; Werbovetz, K. A.; Obaza-Nutaitis, J.; Lehnert, E. K.; Macdonald, T. L. Tubulin Binding of Conformationally Restricted Bis-aryl Compounds. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 243–246.

(34) Boye, O.; Itoh, Y.; Brossi, A.; Hamel, E. Deaminocolchinyl Methyl Ether: Synthesis from 2,3,4,4'-Tetramethoxybiphenyl-carbaldehyde. Comparison of Antitubulin Effects of Deaminocolchinyl Methyl Ether and Dehydro Analogs. *Helv. Chim. Acta* **1989**, *72*, 1690–1696.

(35) Banwell, M. G.; Petters, S. C.; Greenwood, R. J.; Mackay, M. F.; Hamel, E.; Lin, C. M. Semisynthesis, X-ray Crystal Structures and Tubulin Binding-Properties of 7-Oxodeacetamidocolchicine and 7-Oxodeacetamidoisocolchicine. *Aust. J. Chem.* **1992**, *45*, 1577–1588.

(36) Brossi, A.; Boye, O.; Muzaffar, A.; Yeh, H. J. C.; Toome, V.; Wegrzynski, B.; George, C. *aS*, 7*S*-Absolute Configuration of Natural (−)-Colchicine and Allocongeners. *FEBS Lett.* **1990**, *262*, 5–7.

(37) Tomioka, K.; Ishiguro, T.; Koga, K. First Asymmetric Total Synthesis of (+)-Steganacin. Determination of Absolute Stereochemistry. *Tetrahedron Lett.* **1980**, *21*, 2973–2976.

(38) Taafrout, M.; Rouessac, F.; Robin, J.-P. Araliangine, New Bisbenzocyclooctadienolactonic Lignane from *Steganotaenia aralia*, Hochst. *Tetrahedron Lett.* **1983**, *24*, 197–200.

(39) Brossi, A.; Sharma, P. N.; Atwell, L.; Jacobson, A. E.; Iorio, M. A.; Molinari, M.; Chignell, C. F. Biological Effects of Modified Colchicines. 2. Evaluation of Catecholic Colchicines, Colchifolines, Colchicide, and Novel *N*-Acyl- and *N*-Aroyldeacetylcolchicines. *J. Med. Chem.* **1983**, *26*, 1365–1369.

(40) W. C. Hamilton. Significance Tests on the Crystallographic *R* Factor. *Acta Crystallogr.* **1965**, *18*, 502–510.

(41) Ibers, J. A., Hamilton, W. C., Eds. *International Tables for X-Ray Crystallography*; The Kynoch Press, Birmingham, England, 1974; Vol. IV.