Synthesis and Tubulin Binding of Novel C-10 Analogues of Colchicine

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A series of novel C-10 derivatives of colchicine have been prepared and evaluated for inhibition of in vitro microtubule assembly and of [^{3}H]colchicine binding to tubulin. The C-10 substituent of colchicine was replaced by halogens, alkyl and alkoxy groups, and hydrogen. Many of these compounds are available by nucleophilic substitution of 10-fluoro-10-demethoxycolchicine (**9**) without concomitant formation of ring contraction products. Compound **9** is prepared by reaction of (diethylamino)sulfur trifluoride with colchiceine. Unlike most reactions of colchiceine, the colchicine rather than the isocolchicine regiosiomer is the predominant product of this reaction. It was found that modification of the C-10 substituent of colchicine had a relatively minor effect on the potency of the colchicinoids. The electronic nature of the substituent had no significant effect on the efficacy of the compound, indicating that hydrogen bonding or polar interactions between the C-10 substituent of colchicinoids and an amino acid in the colchicine binding site on tubulin are not present in the colchicine-tubulin complex. A decrease in activity was observed with increasing length of the alkyl chain bonded to the C-10 position, but potency was less affected when the alkyl groups were positioned in close proximity to the C-10 carbon of the tropone ring. It is concluded that the steric rather than the electronic properties of the C-10 substituent are the predominant determinants of activity in this series.

Introduction

Colchicine (**1**) belongs to a class of compounds that exert their biological effects through disruption of microtubule dynamics.[1] Colchicine inhibits microtubule assembly as a result of binding to a single high affinity site on the tubulin heterodimer,[2] while the vinca alkaloids disrupt microtubule assembly by associating with tubulin at a separate site,[3] and taxol binds to the intact microtubule, inhibiting its disassembly. The vinca alkaloids and taxol have clinical utility in the treatment of neoplastic diseases,[4] but the toxicity of colchicine has limited its use to the treatment of gout and related inflammatory disease states.[5]

The A and C rings of colchicine appear to comprise the minimum structural features of the molecule necessary for high affinity binding to tubulin.[6] Structure–activity studies indicate that the C-10 substituent of colchicine is involved in its biological activity; however, only a small variety of C-10 analogues of colchicine have been prepared.[2,5] Two aspects of colchicine chemistry have hampered the preparation of novel C-10 analogues. First, treatment of colchicine with basic nucleophiles often results in rearrangement of the tropone ring to benzenoid compounds such as allocolchicine (**3**).[5,7] In addition, the tropolone ether is easily hydrolyzed to colchiceine (**4**), which exists in two tautomeric forms. Alkylation of colchiceine results in the formation of both colchicine and isocolchicine (**2**) isomers. The essentially inactive isocolchicine isomer is the major product (~1:3 colchicine:isocolchicine).

In this work, we have prepared a series of novel C-10 analogues of colchicine and determined their ability to inhibit [^{3}H]colchicine binding to tubulin and to inhibit in vitro microtubule assembly. Many of these compounds may be prepared from 10-fluoro-10-demethoxycolchicine in moderate to good yields using strongly basic nucleophiles without concomitant formation of ring contraction products. In addition, we have found that reaction of colchiceine with (diethylamino)sulfur trifluoride (DAST) results in the preferential formation of the colchicine isomer over the isocolchicine isomer (~3:1 colchicinoid:isocolchicinoid). These procedures should be of general use in the preparation of C-10 analogues of colchicine in higher yields than available with previous methods.

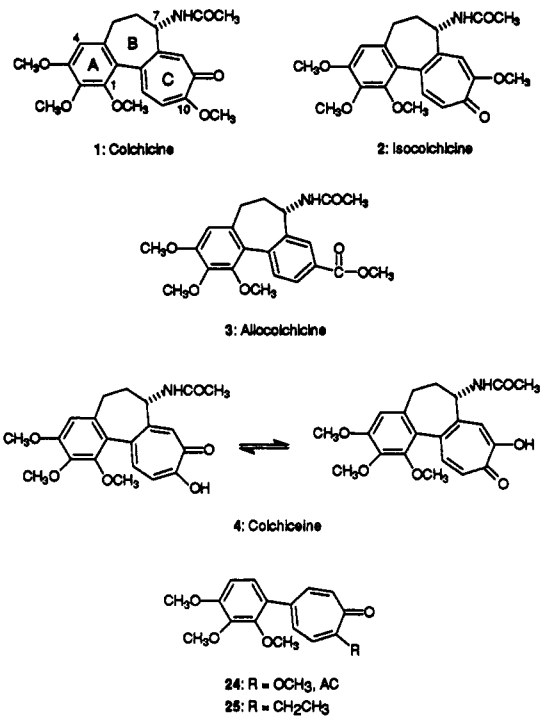

Figure 1.

Variation of the substituent α to the tropone carbonyl had relatively little effect on the potency of the colchicinoids. It is concluded that the colchicine binding site on tubulin can accommodate a wide variety of substituents at the C-10 position of colchicine. In addition, the results from this series of analogues support the hypothesis that the C-10 substituent is not engaged in a hydrogen bonding interaction with an amino acid residue in the colchicine binding site on tubulin.[8]

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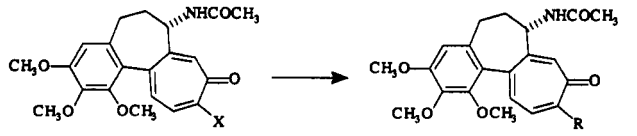
Table I. Synthesis of C-10 Analogues of Colchicine



substrate, X =	product, R =	yield (%)
OH (4)	OTos (5)	89 (22)[a]
OTos	Cl (6)	86
OTos	Br (7)	73
Br	I (8)	70
OH	F (9)	88 (66)
F	CH_3 (10)	52
OH	CH_3 (10)	52 (39)
F	CH_2CH_3 (11)	48
OH	CH_2CH_3 (11)	60 (45)
F	$(CH_2)_2CH_3$ (12)	69
F	$CH(CH_3)_2$ (13)	72
F	$(CH_2)_3CH_3$ (14)	49
F	$CH_2CH(CH_3)_2$ (15)	55
F	$CH(CH_2CH_3)(CH_3)$ (16)[b]	56
F	$C(CH_3)_3$ (17)	60
F	Ph (18)	28
OH	Ph (18)	64 (42)
F	H (19)	70
OTos	OCH_3 (1)	81
OTos	OCH_2CH_3 (20)	87
OTos	$O(CH_2)_2CH_3$ (21)	80
OTos	$OCH(CH_3)_2$ (22)	73

[a] Overall yield; yield of colchicine isomer in parentheses when both regioisomers obtained. [b] Mixture of diastereomers.

Chemistry

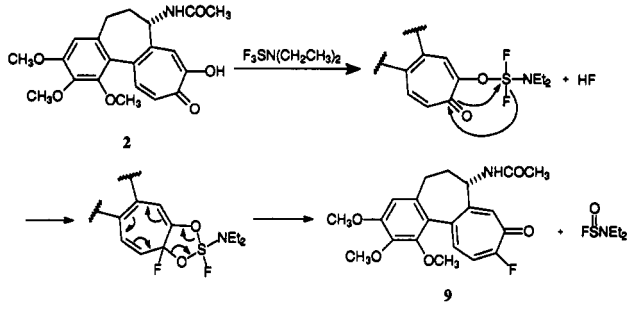
Syntheses of the C-10 analogues of colchicine for this study are outlined in Table I. Several of the colchicine analogues in this study were prepared by nucleophilic substitution of 10-demethoxy-10-tosylcolchicine (**5**), which is prepared by treatment of colchiceine with *p*-toluenesulfonyl chloride.[9] The product ratio of the colchicine isomer to the isocolchicine isomer was 1:3, which is consistent with the data indicating that colchiceine exists in solution primarily as the iso tautomer.[10] Unlike many colchicinoid/isocolchicinoid mixtures, the tosylated isomers are easily separated by radial chromatography.

The 10-chloro (**6**) and 10-bromo (**7**) analogues were prepared by treatment of **5** with gaseous HCl and HBr, respectively. 10-Iodo-10-demethoxycolchicine (**8**) was synthesized from **7** with potassium iodide in a mixture of acetic acid and water. Halogen exchange by treatment of the brominated or tosylated derivatives with sodium iodide in the absence of acid was found to be extremely inefficient. This behavior is consistent with results obtained for halotropones.[11]

Treatment of colchiceine with DAST resulted in 10-fluoro-10-demethoxycolchicine (**9**) and 10-fluoro-10-demethoxyisocolchicine in an overall yield of 88%. Treatment of **7** with KF in refluxing DMSO or acetonitrile was unsuccessful, as was the use of calcium fluoride supported silver fluoride under similar reaction conditions. Surprisingly, the ratio of the 10-fluoro-10-demethoxycolchicine to 10-fluoro-10-demethoxyisocolchicine was 3:1. Direct substitution of the tropolone alcohol by fluorine would dictate that the isocolchicine isomer would be the major product of the reaction.

A possible mechanism to explain the product distribution is illustrated in Scheme I for the reaction of DAST with the isotautomer of colchiceine. DAST may initially react with the tropolone alcohol in the same manner as with simple alcohols or acids. Subsequently, a nucleophilic

Scheme I



displacement of fluorine by sulfur may occur, forming a cyclic intermediate, which upon breakdown yields the regioisomer of the original colchicinoid. A mechanism involving a similar intermediate has been proposed to explain the reaction of DAST with α-hydroxy lactones[12] and the reaction of sulfur tetrafluoride with α-hydroxy acids.[13]

Fluorotropones are known to be very susceptible to nucleophilic substitution.[14] The fluorinated colchicinoid was found to be useful for preparing a variety of C-10 derivatives of colchicine by nucleophilic substitution. In particular, C-10 alkylated colchicinoids (**10–17**) are available from **9**. Treatment of **5**, **6**, or **7** with ethylmagnesium bromide under a variety of catalytic conditions (Ni^{2+}, Cu^{+}, Fe^{3+}) resulted in poor yields, and the product mixture was extremely difficult to purify. In contrast, when **9** was used as the substrate, the 10-alkylcolchicinoid was the major product (Table I). The products were easily purified by radial chromatography and were obtainted in moderate yields.

The C-10 alkyl derivatives of colchicine may also be synthesized by treating **4** with the appropriate organolithium reagent. Both regioisomers are produced, with the colchicine isomer predominating. The product mixture was difficult to separate when the alkyl substituent was small (methyl or ethyl). When the phenyl-substituted product was prepared (**18**), the two isomers were easily separated by radial chromatography.

Colchicide (**19**) has been prepared previously by reductive desulfurization of thiocolchicine with Raney nickel.[15] The original procedure, which was said to produce erratic results, was altered by Dumont et al. to yield 10-demethoxy-8,10,11,12-tetrahydrocolchicine as one of the products.[16] This ketone was oxidized to produce **19** in an overall yield from thiocolchicine of 62%. Treatment of **9** with $NaBH_4$ produced **19** in a 70% yield.

The alkoxy derivatives of colchicine (**20–22**) were prepared by treating **5** with the appropriate alkoxymagnesium bromide or magnesium alkoxide. **9** could also be used as a substrate for this reaction. In general, treatment of tropones bearing a mobile group α to the carbonyl with bases such as sodium alkoxides or hydroxides results in rearrangement of the tropone to a benzene derivative.[17] For example, allocolchicine (**3**) is prepared by reaction of colchicine with sodium methoxide. Our attempts to treat 9-chloro-9-demethoxyisocolchicine with sodium methoxide at low temperatures resulted in significant amounts of ring contraction product. Attempts to hydrolyze 9-demethoxy-9-tosylisocolchicine with 5% NaOH in water/ethanol also resulted in ring contraction product as well as colchiceine. We have found that when the alkoxy-*magnesium* compound is the nucleophile rather than the

Table II. Inhibition of in Vitro Microtubule Assembly and [^{3}H]Colchicine Binding to Tubulin by Colchicine and 10-Substituted Colchicine Derivatives

substituent[b]	I_{50}, μM[c]	percent inhibition[a] 1:1[d]	1:10
OCH_3 (1)	5.1	37.2 (±3.3)	89.7 (±4.5)
F (9)	8.2	11.0 (±2.9)	50.2 (±7.2)
Cl (6)	3.7	57.2 (±3.8)	96.2 (±7.2)
Br (7)	3.3	59.4 (±7.0)	97.4 (±9.8)
I (8)	3.8	74.0 (±5.7)	98.3 (±9.2)
N_3 (23)	4.2	51.1 (±2.2)	95.8 (±5.2)
H (19)	7.9	13.2 (±1.7)	53.7 (±2.5)
CH_3 (10)	4.1	56.6 (±1.5)	96.7 (±9.9)
CH_2CH_3 (11)	4.2	54.9 (±6.8)	96.5 (±9.4)
$CH_2CH_2CH_3$ (12)	3.5	41.7 (±1.5)	89.2 (±5.9)
$CH(CH_3)_2$ (13)	4.2	44.9 (±5.4)	90.0 (±1.9)
$(CH_2)_3CH_3$ (14)	26	13.9 (±7.0)	24.7 (±5.8)
$CH_2CH(CH_3)_2$ (15)	8.5	13.4 (±1.9)	36.7 (±4.6)
$CH(CH_2CH_3)(CH_3)$ (16)	10	19.6 (±5.7)	57.1 (±6.3)
$C(CH_3)_3$ (17)	4.2	21.9 (±6.8)	69.4 (±3.7)
Ph (18)	57	10.6 (±4.3)	25.6 (±5.9)
OCH_2CH_3 (20)	6.1	24.6 (±2.0)	75.0 (±9.0)
$OCH_2CH_2CH_3$ (21)	11	16.6 (±3.0)	54.8 (±5.0)
$OCH(CH_3)_2$ (22)	23	10.1 (±0.6)	50.5 (±4.2)

[a] Percent inhibition of [^{3}H]colchicine binding to tubulin, determined relative to control without added ligand. The values are an average of four independent determinations. The standard error is shown in parentheses. [b] C-10 substituent; compound number is shown in parentheses. [c] I_{50} is the concentration of ligand required to effect a 50% reduction in microtubule polymerization, determined as described under Experimental Procedures. [d] Molar ratio of inhibitor to [^{3}H]colchicine.

sodium alkoxide, the occurrence of benzenoid rearrangement is minimal.

Biological Results and Discussion

The activity of the colchicinoids was assessed by the ability of the analogues to inhibit [^{3}H]colchicine binding to tubulin and to inhibit in vitro microtubule assembly (Table II). Nearly all of the compounds were potent inhibitors of microtubule assembly. The inhibition of microtubule assembly can be correlated with inhibition of [^{3}H]colchicine binding to tubulin, indicating that the observed activity is mediated through binding to the colchicine site on tubulin.

Colchicide (19) was originally reported to be a potent inhibitor of [^{3}H]colchicine binding to tubulin.[18] In a subsequent paper, 19 was reported to be essentially inactive in inhibiting [^{3}H]colchicine binding to tubulin and in the P388 in vivo mouse leukemia screen.[16] We have found that 19 is a reasonably potent inhibitor of [^{3}H]colchicine binding to tubulin and of in vitro assembly of microtubules. It is unclear why 19 was inactive in the evaluations of Dumont et al.[16] It should be noted that the Dumont et al. studies were performed with the hydrochloride salt of 19, while our data were obtained with unmodified 19.

Several points should be noted from the data in Table II. First, the electronic nature of the C-10 substituent appears to be of little importance in the efficacy of the ligand. The 10-ethyl-10-demethoxycolchicine/colchicine (11/1) and 10-demethoxy-10-propylcolchicine/10-ethoxy-10-demethoxycolchicine (12/20) pairs are virtually identical in their activity in inhibiting microtubule polymerization, and the alkyl derivatives are in fact more potent in inhibiting [^{3}H]colchicine binding to tubulin. This observation is also true for the 10-fluoro-10-demethoxycolchicine/colchicide (9/19) pair, in which the electronic nature of the substituent is vastly different yet the potencies in both assays are the same. These data indicate that interactions between tubulin and the C-10 substituent of colchicine are not mediated by polar interactions. They furthermore support our previous suggestion based on spectroscopic data that the C-10 substituent of colchicine is not engaged in a hydrogen-bonding interaction with tubulin.[8]

In general, the size of the substituent appears to be the more important determinant of activity in the series. The two analogues in which the C-10 substituent is relatively small (9 and 19) are slightly less potent that those analogues with larger substituents. Surprisingly, the bulk of the substituent appears to be less important in the vicinity of the C ring carbons than at a greater distance. In the butyl series it is seen that the *tert*-butyl analogue of colchicine (17) is nearly as potent as colchicine in both assays, while the *n*-butyl analogue (14) is much less active. This relationship, however, does not hold for the propoxides (21 and 22). We attribute the differences in the propoxide vs the butyl series to the differences in the structures of the substituents rather than electronic differences, which should have been evident in the other analogues. The alkoxy substituents occupy a slightly different space within the colchicine binding site as a result of the different bond lengths and angles of the oxygen vs the methylene. The different space occupied by the alkoxy vs the alkyl substituents may cause them to interact with different portions of the interior of the colchicine binding site.

These results correlate with previous structure–activity studies of C-10 analogues of colchicine. Analysis of a series of thiocolchicine and 10-amino-10-demethoxycolchicine derivatives showe that potency significantly decreased with increasing size of the alkyl substituent on the heteroatom.[19,20]

We have previously prepared a smaller series of C ring analogues of 2-methoxy-5-(2,3,4-trimethoxyphenyl)tropone (AC, 24), the bicyclic analogue of colchicine.[21] In contrast to the results shown here for the colchicine series, the potency of the AC analogues was highly dependent on the electronic nature of the substituent. For example, the ethyl derivative of AC (25) was found to be 10-fold less potent than AC in inhibiting microtubule assembly. These findings are particularly intriguing as thermodynamic studies of colchicine, AC, and allocolchicine binding to tubulin show that for this series, the B ring of the molecule makes no contribution to the enthalpy of binding.[22] Moreover, the differences in the affinity constants for AC and colchicine binding to tubulin may be explained by the differences in their binding entropies.[5]

Conclusions

The compound 10-fluoro-10-demethoxycolchicine (9) is a suitable substrate for the preparation of a variety of C-10 analogues of colchicine. It is particularly useful in semisynthesis of colchicinoids due to the preferential formation of the biologically active regioisomer. Use of magnesium alkoxides as nucleophiles for substitution at the C-10 position of colchicine results in high yields of the tropone derivative and essentially no benzenoid rearrangement product. This method should be useful for the preparation of isotopically-substituted colchicinoids.

In the colchicine series, potency is a function of the size of the substituent at the C-10 carbon and the placement of bulky groups in relation to the C ring. The electronic nature of the substituent has no apparent effect on the potency of the colchicinoid, indicating that a hydrogen

bond or polar interactions are not occurring between tubulin and the C-10 substituent of colchicine. At this time the reasons for the differences in the structure–activity relationships in the colchicine and AC series are unclear. Insight into the problem may arise from thermodynamic measurements of C ring derivatives of the two types of molecules binding to tubulin.

Experimental Procedures

Syntheses. General. Melting points were determined with an Electrothermal IA6304 open-capillary melting point apparatus and are uncorrected. Infrared spectra were measured in $CHCl_3$ on a Perkin-Elmer 1420 ratio recording infrared spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM 360 spectrometer in $CDCl_3$ (unless otherwise indicated) with either $CHCl_3$ or TMS as the internal standard. NMR data are reported as follows: chemical shift, multiplicity, integration, proton assignment, and coupling. Low-resolution mass spectra were obtained on a Hewlett-Packard 5995 gas chromatograph/mass spectrometer. High-resolution mass spectra were recorded on a VG 70-70 spectrometer at an ionization voltage of 16 eV. Optical rotations were measured with Perkin-Elmer Model 243B polarimeter in $CHCl_3$ at the concentrations specified.

Column chromatography was performed using Baker silica gel (60–200 mesh). Radial chromatography was performed on a Chromatotron (Harrison Research, Inc.) using Merck silica gel PF-254 with $CaSO_4 \cdot {}^1/_2H_2O$. Silica gel plates (Merck F_{245}, 0.25-mm thickness) were used for thin-layer chromatography. Colchicine was purchased from the U.S. Biochemical Corp. and was found to be >95% pure by thin-layer chromatography, ^1H NMR, and ^{13}C NMR. The Grignard reagents used were purchased from Aldrich Chemical Co. in Sure Seal bottles and were used directly. All compounds (**5–23**) migrated as a single spot by TLC analysis.

Colchiceine (**4**) was prepared from colchicine by mild acidic hydrolysis according to a literature procedure.[23] Compounds **5** and **23** were prepared as described previously.[9]

10-Chloro-10-demethoxycolchicine (6) and 10-Bromo-10-demethoxycolchicine (7). Compound **5** (0.20 g, 0.37 mmol) was dissolved in 5 mL of dry THF. Dry HCl(g) or HBr(g) was bubbled into the solution for 2 h. The solution was then poured into 30 mL of cold water and extracted three times with 30-mL portions of CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and evaporated to yield a yellow oil (**6**) or an orange-yellow oil (**7**). The product was purified by radial chromatography using 95:5 CH_2Cl_2–MeOH to produce a yellow solid in a yield of 86% (**6**) or 73% (**7**). Compound **6**: mp 117–118 °C; $[\alpha]^{25}_D$ −226° (*c* 1.0); ^1H NMR δ 7.84 (d, 1 H, H-11, J = 10.8 Hz), 7.54 (s, 1 H, H-8), 7.17 (d, 1 H, H-12, J = 10.5 Hz), 7.16 (br, 1 H, NH), 6.54 (s, 1 H, H-4), 4.63 (p, 1 H, H-7), 3.94 (s, 3 H, 3-OCH_3), 3.91 (s, 3 H, 2-OCH_3), 3.68 (s, 3 H, 1-OCH_3), 2.57 (dd, 1 H, H-5), 2.40 (m, 1 H, H-5), 2.28 (m, 1 H, H-6), 2.00 (s, 3 H, $COCH_3$), 1.87 (m, 1 H, H-6); ^{13}C NMR δ 179.8, 170.1, 154.2, 152.1, 151.2, 146.7, 143.9, 141.7, 135.7, 134.2, 133.7, 132.6, 124.8, 107.0, 61.7, 61.3, 56.1, 52.3, 36.1, 29.8, 23.0; IR 3460, 3330, 3020–2850, 1682, 1621 cm^{-1}; LRMS m/z 403 (M^+), 405 (M + 2), 377, 375, 318, 316; HRMS calcd for $C_{21}H_{22}O_5NCl$ 403.119, found 403.122. Compound **7**: mp 138–139 °C; $[\alpha]^{25}_D$ −242° (*c* 1.0); ^1H NMR δ 8.13 (d, 1 H, H-11, J = 10.2 Hz), 7.54 (s, 1 H, H-8), 7.32 (d, 1 H, NH), 7.08 (d, 1 H, H-12, J = 10.5 Hz), 6.54 (s, 1 H, H-4), 4.64 (p, 1 H, H-7), 3.94 (s, 3 H, 3-OCH_3), 3.91 (s, 3 H, 2-OCH_3), 3.69 (s, 3 H, 1-OCH_3), 2.56 (dd, 1 H, H-5), 2.40 (m, 1 H, H-5), 2.27 (m, 1 H, H-6), 2.01 (s, 3 H, $COCH_3$), 1.88 (m, 1 H, H-6); ^{13}C NMR δ 180.2, 169.9, 154.0, 151.8, 151.0, 143.9, 141.8, 140.9, 139.1, 134.1, 133.9, 131.6, 124.9, 107.5, 61.7, 61.3, 56.1, 52.2, 36.3, 29.8, 23.0; IR 3458, 3330 (br), 3020–2850, 1682, 1621 cm^{-1}; LRMS m/z 447 (M^+), 449 (M + 2), 421, 419, 406, 404, 390, 388, 362, 360; HRMS calcd for $C_{21}H_{22}O_5NBr$ 447.068, found 447.071.

10-Iodo-10-demethoxycolchicine (8). Compound **7** (0.02 g, 0.045 mmol) was treated with 20 equiv of KI (0.15 g, 0.90 mmol) in a mixture of 1 mL of acetic acid containing 0.1 mL of water. The mixture was stirred for 5 h at room temperature. The reaction mixture was then diluted with water and extracted three times with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and evaporated to yield a yellow solid. This product was purified by radial chromatography (95:5) CH_2Cl_2–MeOH and obtained in a yield of 70%. Compound **8**: mp 127–128 °C; $[\alpha]^{25}_D$ −265° (*c* 1.0); ^1H NMR δ 8.45 (d, 1 H, H-11, J = 10.5 Hz), 7.38 (s, 1 H, H-8), 6.89 (d, 1 H, H-12, J = 10.5 Hz), 6.86 (d, 1 H, NH), 6.53 (s, 1 H, H-4), 4.63 (p, 1 H, H-7), 3.94 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 2.55 (m, 1 H, H-5), 2.40 (m, 1 H, H-5), 2.24 (m, 1 H, H-6), 2.01 (s, 3 H, $COCH_3$), 1.84 (m, 1 H, H-6); ^{13}C NMR δ 182.2, 169.6, 154.1, 151.3, 150.5, 146.1, 144.1, 141.9, 134.5, 134.0, 128.7, 125.1, 125.0, 107.5, 61.7, 61.4, 56.1, 52.0, 36.4, 29.9, 23.0; IR 3458, 3330 (br), 3020–2850, 1680, 1615 cm^{-1}; LRMS m/z 495 (M^+), 467, 408, 368; HRMS calcd for $C_{21}H_{22}O_5NI$ 495.054, found 495.059.

10-Fluoro-10-demethoxycolchicine (9) and 9-Fluoro-9-demethoxyisocolchicine. DAST (0.25 mL, 1.2 equiv) was dissolved in 2 mL of dry CH_2Cl_2. This solution was placed on ice, and 0.66 g (1.7 mmol) of **4** dissolved in 2 mL of CH_2Cl_2 was added. The mixture was allowed to warm to room temperature and was stirred for 24 h. The solution was then extracted with water three times. The CH_2Cl_2 layer was dried over Na_2SO_4 and evaporated to yield a yellow oil. TLC analysis (60:40 acetone–petroleum ether) of this product showed two spots corresponding to 9-fluoro-9-demethoxyisocolchicine and **9** with R_f values of 0.43 and 0.33, respectively. The products were separated by radial chromatography (60:40 acetone–petroleum ether). The overall yield of product was 88% with a 3:1 ratio of the colchicine derivative to the isocolchicine derivative. Compound **9**: mp 102–103 °C; $[\alpha]^{25}_D$ −217° (*c* 1.0); ^1H NMR δ 8.15 (d, 1 H, NH), 7.74 [d, 1 H, H-8, J = 9.1 Hz (F–H)], 7.42 and 7.37 [dd, 1 H, H-11, J = 19 Hz (F–H) and 10.5 Hz (H–H)], 7.31 and 7.28 [dd, 1 H, H-12, J = 4.4 Hz (F–H) and 10.5 Hz (H–H)], 6.56 (s, 1 H, H-4), 4.67 (m, 1 H, H-7), 3.95 (s, 3 H, 3-OCH_3), 3.92 (s, 3 H, 2-OCH_3), 3.68 (s, 3 H, 1-OCH_3), 2.58 (m, 1 H, H-5), 2.36 (m, 2 H, H-5 and H-6), 1.99 (s, 3 H, $COCH_3$), 1.94 (m, 1 H, H-6); ^{13}C NMR δ 177.4, 177.2, 170.0, 166.0, 163.1, 156.6, 154.1, 153.7, 151.1, 142.2, 142.3, 141.7, 134.3, 134.1, 134.0, 125.0, 120.1, 119.8, 107.4, 61.6, 61.3, 56.1, 52.7, 36.2, 29.7, 22.7; IR 3455, 3330, 3040–2850, 1680, 1628 cm^{-1}; LRMS m/z 387 (M^+), 359, 344, 328, 316, 300; HRMS calcd for $C_{21}H_{22}O_5NF$ 387.1481, found 387.1454. **9-Demethoxy-9-fluoroisocolchicine**: mp 118–119 °C; $[\alpha]^{25}_D$ −267° (*c* 1.0); ^1H NMR δ 8.06 (d, 1 H, NH), 7.75 [d, 1 H, H-8, J = 23 Hz (F–H)], 7.60 [d, 1 H, H-12, J = 12.8 Hz (H–H)], 7.30 and 7.26 [dd, 1 H, H-11, J = 12.8 Hz (H–H) and 9.2 Hz (F–H)], 6.61 (s, 1 H, H-4), 4.62 (p, 1 H, H-7), 3.95 (s, 3 H, 3-OCH_3), 3.93 (s, 3 H, 2-OCH_3), 3.72 (s, 3 H, 1-OCH_3), 2.55 (m, 1 H, H-5), 2.34 (m, 2 H, H-5 and H-6), 2.10 (m, 1 H, H-6), 2.06 (s, 3 H, $COCH_3$); ^{13}C NMR δ 177.3, 177.1, 170.5, 166.1, 163.3, 154.2, 150.8, 144.2, 144.1, 142.6, 141.6, 139.6, 139.5, 136.7, 136.5, 135.0, 125.2, 118.0, 117.7, 107.6, 61.6, 61.3, 56.1, 53.7, 52.2, 38.0, 29.8, 29.2, 22.7; IR 3458, 3330, 3040–2850, 1670, 1620 cm^{-1}; LRMS m/z 387 (M^+), 359, 344, 328, 316, 300; HRMS calcd for $C_{21}H_{22}O_5NF$ 387.1481, found 387.1520.

General Procedure for the Synthesis of C-10 Alkyl Derivatives. Compound **9** (15 mg, 0.039 mmol) was dissolved in 2 mL of dry THF. This solution was placed in an ice bath, and 3 equiv of the appropriate Grignard reagent in 2 mL of THF was added *slowly*. The reaction was monitored by TLC (conditions are indicated under the specific compounds). If the reaction was incomplete after 1 h of stirring, an additional 1 equiv of Grignard reagent was added and the stirring was continued for 30 min. When the reaction was complete the solution was diluted with water, acidified with 10% HCl, and extracted with CH_2Cl_2 three times. The organic extracts were combined, dried over Na_2SO_4, and evaporated.

10-Demethoxy-10-methylcolchicine (10). This compound was synthesized as described above using methylmagnesium bromide. The reaction was monitored by TLC using 50:50 acetone–petroleum ether. R_f values for the starting material and product were 0.30 and 0.19, respectively. The product was purified by radial chromatography (50:50 acetone–petroleum ether) and was obtained as a yellow solid in a yield of 52%. Compound **10**: mp 112–113 °C; $[\alpha]^{25}_D$ −269° (*c* 0.6); ^1H NMR δ 7.41 (d, 1 H, H-12, J = 9.7 Hz), 7.29 (s, 1 H, H-8), 7.22 (d, 1 H, H-11, J = 9.7 Hz), 6.79 (d, 1 H, NH), 6.53 (s, 1 H, H-4), 4.62 (p, 1 H, H-7), 3.95 (s, 3 H, 3-OCH_3), 3.91 (s, 3 H, 2-OCH_3), 3.67 (s, 3 H, 3-OCH_3), 2.54 (m, 1 H, H-5), 2.44 (m, 1 H, H-5), 2.33 (s, 3 H, 10-CH_3), 2.24 (m, 1 H, H-6), 2.01 (s, 3 H, $COCH_3$), 1.81 (m,

1 H, H-6); ^{13}C NMR δ 186.5, 169.5, 153.7, 151.4, 150.8, 150.1, 141.8, 141.2, 135.8, 135.23, 134.1, 133.1, 125.6, 107.4, 61.6, 61.4, 56.1, 52.0, 36.6, 29.9, 23.0, 22.3; IR 3450, 3270 (br), 3030–2850, 1670, 1615 cm^{-1}; LRMS 383 (M$^+$), 355, 340, 312, 296; HRMS calcd for $C_{22}H_{25}O_5N$ 383.1731, found 383.1696.

10-Ethyl-10-demethoxycolchicine (11). This compound was synthesized as described above using ethylmagnesium bromide, and the reaction was monitored by TLC using 95:5 $CHCl_3$–MeOH. The R_f values of the starting material and product were 0.32 and 0.39, respectively. The product was purified by radial chromatography (95:5 $CHCl_3$–MeOH) and was obtained as a yellow solid in a yield of 48%. Compound 11: mp 115–116 °C; $[\alpha]^{25}_D$ −220° (c 1.0), ^{1}H NMR δ 7.31 (d, 1 H, H-12, J = 9.8 Hz), 7.22 (s, 1 H, H-8), 7.19 (d, 1 H, H-11, J = 9.8 Hz), 6.51 (s, 1 H, H-4), 6.44 (d, 1 H, NH), 4.61 (p, 1 H, H-7), 3.93 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.66 (s, 3 H, 1-OCH_3), 2.71 (m, 2 H, 10-CH_2CH_3, J = 7.7 Hz), 2.47 (m, 2 H, H-5), 2.21 (m, 1 H, H-6), 2.00 (s, 3 H, $COCH_3$), 1.77 (m, 1 H, H-6), 1.23 (t, 3 H, 10-CH_2CH_3); ^{13}C NMR δ 186.2, 169.4, 155.8, 153.6, 151.6, 149.6, 141.7, 140.9, 135.8, 134.1, 133.8, 133.5, 125.6, 107.4, 61.5, 61.4, 56.1, 51.9, 36.7, 29.9, 28.0, 23.1, 13.0; IR 3450, 3270 (br), 3030–2850, 1670, 1615 cm^{-1}; LRMS m/z 397 (M$^+$), 369, 310; HRMS calcd for $C_{23}H_{27}O_5N$ 397.1888, found 397.1887.

10-Demethoxy-10-propylcolchicine (12). This compound was synthesized as described above using n-propylmagnesium chloride. The reaction was monitored by TLC using 95:5 CH_2Cl_2–MeOH, and the R_f values for the starting material and product were 0.34 and 0.43, respectively. The product was purified by radial chromatography (95:5 CH_2Cl_2–MeOH) and was obtained as a yellow solid in a yield of 69%. Compound 12: mp 104–105 °C; $[\alpha]^{25}_D$ −179° (c 0.9); ^{1}H NMR δ 7.35 [d (broad at base), 2 H, H-12 and NH], 7.19 (d, 1 H, H-11, J = 9.8 Hz), 6.52 (s, 1 H, H-4), 4.65 (p, 1 H, H-7), 3.94 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.67 (s, 3 H, 1-OCH_3), 2.65 (m, 2 H, 10-$CH_2CH_2CH_3$), 2.52 (m, 1 H, H-5), 2.44 (m, 1 H, H-5), 2.23 (m, 1 H, H-6), 2.00 (s, 3 H, $COCH_3$), 1.83 (m, 1 H, H-6), 1.65 (m, 2 H, 10-$CH_2CH_2CH_3$), 1.01 (t, 1 H, 10-$CH_2CH_2CH_3$); ^{13}C NMR δ 186.1, 169.5, 154.2, 153.5, 151.2, 150.0, 141.5, 141.1, 137.6, 135.6, 134.7, 133.5, 125.4, 107.2, 61.4, 61.2, 56.0, 51.8, 37.0, 36.3, 29.8, 22.8, 21.8, 14.0; LRMS m/z 411 (M$^+$), 383, 324; HRMS calcd for $C_{24}H_{29}O_5N$ 411.2044, found 411.2032.

10-Demethoxy-10-isopropylcolchicine (13). This compound was synthesized as described above using isopropylmagnesium chloride. The reaction was monitored by TLC (95:5 CH_2Cl_2–MeOH) with the R_f values of the starting material and product being 0.34 and 0.43, respectively. The product was purified by radial chromatography (95:5 CH_2Cl_2–MeOH) and was obtained in a yield of 72%. Compound 13: mp 112–113 °C; $[\alpha]^{25}_D$ −183° (c 1.0); ^{1}H NMR δ 7.38 (d, 1 H, NH), 7.34 (s, 1 H, H-8), 7.29 (d, 1 H, H-12, J = 9.9 Hz), 7.24 (d, 1 H, H-11, J = 9.8 Hz), 6.52 (s, 1 H, H-4), 4.62 (p, 1 H, H-7), 3.94 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.68 (s, 3 H, 1-OCH_3), 3.47 [m, 1 H, 10-$CH(CH_3)_3$, J = 6.7 Hz], 2.47 (m, 2 H, H-5), 2.22 (m, 1 H, H-6), 2.00 (s, 3 H, $COCH_3$), 1.82 (m, 1 H, H-6), 1.23 [dd, 6 H, 10-CH (CH_3)$_2$, J = 6.6 Hz]; ^{13}C NMR δ 186.1, 169.7, 159.7, 153.6, 151.4, 150.0, 141.7, 141.2, 135.9, 134.2, 133.8, 132.1, 125.6, 107.3, 61.6, 61.3, 56.1, 51.9, 36.4, 30.2, 29.9, 23.0, 22.5, 22.1; IR 3440, 3270 (br), 3020–2850, 1670, 1613; LRMS m/z 411 (M$^+$), 383, 324, 309; HRMS calcd for $C_{24}H_{29}O_5N$ 411.2044, found 411.2093.

10-*n*-Butyl-10-demethoxycolchicine (14). This compound was synthesized as described above utilizing n-butylmagnesium bromide, and the reaction was monitored by TLC using 95:5 CH_2Cl_2–MeOH. The R_f values of the starting material and product were 0.34 and 0.45, respectively. The product was purified by radial chromatography (95:5 CH_2Cl_2–MeOH) and was obtained as a yellow solid in a yield of 49%. Compound 14: mp 109–110 °C; $[\alpha]^{25}_D$ −179° (c 0.9); ^{1}H NMR δ 7.31 (d, 1 H, H-12, J = 9.9 Hz), 7.29 (s, 1 H, H-8), 7.16 (d, 1 H, H-11, J = 9.8 Hz), 6.90 (br, 1 H, NH), 6.51 (s, 1 H, H-4), 4.62 (p, 1 H, H-7), 3.93 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.67 (s, 3 H, 1-OCH_3), 2.68 [m, 2 H, 10-CH_2 $(CH_2)_2CH_3$], 2.48 (m, 2 H, H-5), 2.22 (m, 1 H, H-6), 1.99 (s, 3 H, $COCH_3$), 1.80 (m, 1 H, H-6), 1.60 (m, 2 H, 10-$CH_2CH_2CH_2CH_3$), 1.41 (m, 2 H, 10-$CH_2CH_2CH_2CH_3$), 0.95 (t, 3 H, 10-$CH_2CH_2CH_2CH_3$); ^{13}C NMR δ 186.2, 169.7, 154.6, 153.6, 151.4, 150.2, 141.7, 141.2, 135.8, 134.8, 134.2, 133.6, 125.6, 107.3, 61.6, 61.4, 56.1, 51.9, 36.4, 34.8, 30.9, 29.9, 23.0, 22.8, 14.0; IR 3450, 3280 (br), 3020–2850, 1670, 1615 cm^{-1}; LRMS m/z 425 (M$^+$), 397, 366, 338, 295; HRMS calcd for $C_{25}H_{31}O_5N$ 425.2200, found 425.2187.

10-Isobutyl-10-demethoxycolchicine (15). This compound was synthesized as described above using isobutylmagnesium bromide, and the reaction was monitored by TLC using 95:5 CH_2Cl_2–MeOH. The R_f values for the starting material and product were 0.34 and 0.45, respectively. The product was purified by radial chromatography (95:5 CH_2Cl_2–MeOH) and was obtained as a yellow solid in a yield of 55%. Compound 15: mp 111–112 °C; $[\alpha]^{25}_D$ −200° (c 1.0); ^{1}H NMR δ 7.69 (d, 1 H, -NH), 7.44 (s, 1 H, H-8), 7.32 (d, 1 H, H-12, J = 9.8 Hz), 7.19 (d, 1 H, H-11, J = 9.7 Hz), 6.53 (s, 1 H, H-4), 4.66 (p, 1 H, H-7), 3.94 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.67 (s, 3 H, 1-OCH_3), 2.52 [complex multiplet, 4 H, H-5 and 10-$CH_2CH(CH_3)_2$], 2.23 (m, 1 H, H-6), 2.02 [m, 1 H, 10-$CH_2CH(CH_3)_2$], 2.00 (s, 3 H, $COCH_3$), 1.84 (m, 1 H, H-6), 0.95 (dd, 6 H, 10-$CH_2CH(CH_3)_2$]; ^{13}C NMR δ 186.3, 169.8, 153.6, 153.4, 151.4, 150.5, 141.7, 141.5, 135.9, 135.6, 134.2, 133.8, 125.5, 107.3, 61.6, 61.4, 56.1, 51.9, 44.4, 36.3, 30.0, 27.6, 22.9, 22.8, 22.7; LRMS m/z 425 (M$^+$), 397, 366, 338; HRMS calcd for $C_{25}H_{31}O_5N$ 425.2200, found 425.2231.

10-*sec*-Butyl-10-demethoxycolchicine (16). This compound was synthesized as described above utilizing *sec*-butylmagnesium bromide, and the reaction was monitored by TLC using 95:5 CH_2Cl_2–MeOH. The R_f values for the product and starting material were 0.45 and 0.34, respectively. The product was purified by radial chromatography (95:5 CH_2Cl_2–MeOH) and was obtained as a yellow solid in 56% yield. Compound 16: mp 108–109 °C; $[\alpha]^{25}_D$ −205° (c 1.0); ^{1}H NMR (acetone-d_6) δ 7.73 (d, 1 H, -NH), 7.23 (d, 1 H, H-12, J = 9.8 Hz), 7.08 (s, 1 H, H-8), 7.06 (d, 1 H, H-11, J = 9.7 Hz), 6.70 (s, 1 H, H-4), 4.45 (p, 1 H, H-7), 3.85 (s, 3 H, 3-OCH_3), 3.80 (s, 3 H, 2-OCH_3), 3.58 (s, 3 H, 1-OCH_3), 3.18 [m, 1 H, 10-$CH(CH_2CH_3)(CH_3)$], 2.59 (m, 1 H, H-5), 2.35 (m, 1 H, H-5), 2.10 (m, 1 H, H-6), 1.85 (s and m, 4 H, $COCH_3$ and H-6), 1.61 [m, 1 H, 10-$CH(CH_2CH_3)(CH_3)$], 1.46 [m, 1 H, 10-$CH(CH_2CH_3)(CH_3)$], 1.12 [dd, 3 H, 10-$CH(CH_2CH_3)(CH_3)$], 0.82 [dt, 3 H, 10-$CH(CH_2CH_3)(CH_3)$]. This compound as prepared consists of two diastereomers. Thus, there are two signals for some of the protons and carbons of this compound. ^{13}C NMR δ 186.2, 169.7, 158.8, 153.6, 151.4, 150.1, 150.0, 141.7, 141.2, 141.1, 135.9, 134.2, 133.8, 133.7, 132.9, 132.8, 125.5, 107.3, 61.6, 61.5, 61.4, 56.1, 51.8, 51.7, 37.0, 36.6, 36.5, 36.4, 29.9, 29.6, 29.3, 23.0, 20.1, 19.8, 12.2, 12.0; IR 3450, 3290 (br), 3020–2850, 1670, 1618; LRMS m/z 425 (M$^+$), 397, 366, 338; HRMS calcd for $C_{25}H_{31}O_5N$ 425.2200, found 425.2183.

10-*tert*-Butyl-10-demethoxycolchicine (17). This compound was synthesized as described above utilizing *tert*-butylmagnesium bromide, and the reaction was monitored by TLC using 95:5 CH_2Cl_2–MeOH. The R_f values for the product and starting material were 0.48 and 0.34, respectively. The product was purified by radial chromatography (95:5 CH_2Cl_2–MeOH) and was obtained as a yellow solid in a yield of 60%. Compound 17: mp 128–129 °C; $[\alpha]^{25}_D$ −197° (c 1.1); ^{1}H NMR δ 7.38 (d, 1 H, H-12, J = 9.9 Hz), 7.19 (d, 1 H, H-11, J = 9.8 Hz), 7.12 (br, 1 H, H-4), 7.07 (s, 1 H, H-8), 6.49 (s, 1 H, H-4), 4.59 (m, 1 H, H-7), 3.93 (s, 3 H, 3-OCH_3), 3.89 (s, 3 H, 2-OCH_3), 3.68 (s, 3 H, 1-OCH_3), 2.45 (m, 2 H, H-5), 2.18 (m, 1 H, H-6), 2.01 (s, 3 H, $COCH_3$), 1.73 (m, 1 H, H-6), 1.42 [s, 9 H, 10-C(CH_3)$_3$]; ^{13}C NMR δ 187.9, 159.7, 159.7, 153.5, 151.5, 148.5, 141.7, 140.5, 135.0, 134.3, 132.6, 131.9, 125.3, 107.4, 61.6, 61.4, 56.0, 51.2, 38.0, 36.6, 30.0, 29.6, 23.0; IR 3450, 3290 (br), 3020–2850, 1670, 1619 cm^{-1}; LRMS m/z 425 (M$^+$), 397, 338, 323; HRMS calcd for $C_{25}H_{31}O_5N$ 425.2200, found 425.2236.

Synthesis of C-10 Alkyl Derivatives from Colchiceine. Colchiceine (0.050 g, 0.13 mmol) was dissolved in 4 mL of dry THF. The solution was placed on ice, and 5 equiv of the appropriate alkyllithium was added. The reaction mixture was allowed to stir on ice for ~30 min. The solution was then poured into cold water (15 mL), neutralized with 10% HCl, and extracted three times with CH_2Cl_2. The organic extracts were dried with Na_2SO_4 and evaporated. The overall yield of both regioisomers was 64% using PhLi, 52% using MeLi, and 60% using EtLi. In each case, the colchicine isomer was the predominant product (~3:1 colchicinoid:isocolchicinoid). The spectra of **10** and **11** prepared in this manner were identical to the spectra of the product prepared as described above. Compound 18: mp 141–

142 °C; $[\alpha]^{25}_D$ −180° (c 1.1); ^{1}H NMR δ 7.48 (dd, 2 H, phenyl ring, J = 8.1 Hz, 1.6 Hz), 7.40 (s, 1 H, H-8), 7.24–7.37 (m, 4 H, phenyl ring and H-12), 7.19 (d, 1 H, H-11), 7.09 (br, 1 H, NH), 6.45 (s, 1 H, H-4), 4.56 (p, 1 H, H-7), 3.86 (s, 3 H, 3-OCH_3), 3.82 (s, 3 H, 2-OCH_3), 3.65 (s, 3 H, 1-OCH_3), 2.44 (m, 2 H, H-5), 2.11 (m, 1 H, H-6), 1.87 (s, 3 H, $COCH_3$), 1.78 (m, 1 H, H-6); ^{13}C NMR δ 179.3, 169.9, 153.8, 151.4, 150.9, 142.5, 141.7, 139.6, 136.7, 135.7, 135.6, 134.3, 129.2, 128.5, 128.1, 127.4, 125.7, 125.2, 107.4, 61.6, 61.3, 56.1, 51.9, 36.3, 30.0, 22.9.

10-Demethoxycolchicine (19). To a solution of **9** (15 mg, 0.04 mmol) in DMSO (2 mL) was added slowly a slight excess of sodium borohydride (1.2 equiv) dissolved in 2 mL of DMSO. The solution was stirred for 30 min at room temperature. After completion of the reaction, the mixture was diluted with water and extracted three times with CH_2Cl_2. The organic extracts were combined and extracted three times with water. The organic layer was then dried over Na_2SO_4 and evaporated to yield a light yellow solid. This solid was purified by radial chromatography (95:5 EtOAc–MeOH) and obtained in a yield of 70%. Compound **19**: mp 119–120 °C; $[\alpha]^{25}_D$ −196° (c 1.0) [lit. mp 105–120 °C; $[\alpha]^{27}_D$ −196° (c 0.47, $CHCl_3$);[15] $[\alpha]^{24}_D$ −166° (c 0.53, $CHCl_3$)[18]]; ^{1}H NMR δ 7.75 (d, 1 H, NH), 7.40 (d, 1 H, H-8, J = 2.8 Hz), 7.32–7.24 (m, 2 H, H-10 and H-11), 7.09 (dd, 1 H, H-12, J = 11.7 Hz and 2.5 Hz), 6.53 (s, 1 H, H-4), 4.62 (p, 1 H, H-7), 3.94 (s, 3 H, 3-OCH_3), 3.91 (s, 3 H, 2-OCH_3), 3.70 (s, 3 H, 1-OCH_3), 2.56 (m, 1 H, H-5), 2.45 (m, 1 H, H-5), 2.26 (m, 1 H, H-6), 1.97 (s, 3 H, $COCH_3$), 1.89 (m, 1 H, H-6); ^{13}C NMR δ 187.5, 169.7, 153.9, 152.1, 151.3, 144.2, 141.7, 140.4, 136.9, 136.5, 135.4, 134.1, 125.4, 107.3, 61.7, 61.4, 56.1, 52.3, 36.1, 29.8, 22.9; IR 3450, 3270 (br), 3020–2850, 1670, 1622 cm^{-1}; LRMS m/z 341, 282; HRMS calcd for $C_{21}H_{23}O_5N$ 369.1593, found 369.1568.

General Procedure for the Synthesis of C-10 Alkoxy Derivatives. To generate the alkoxymagnesium bromide, a solution of methylmagnesium bromide (0.74 mmol) in THF (3 mL) was treated with excess of the desired alcohol. A solution of **4** (0.020 g, 0.037 mmol) in THF (2 mL) was then treated with 10 equiv of the magnesium alkoxide. The reaction mixture was monitored by TLC utilizing 95:5 EtOAc–MeOH. The R_f value for the tosylated derivative was 0.45 while the R_f values for the ethoxy, propoxy, and isopropoxy derivatives were 0.20, 0.22, and 0.23, respectively. The typical reaction time was 5 h. When the reaction appeared complete by TLC, the mixture was acidified with acetic acid and extracted with CH_2Cl_2 three times. The organic extracts were combined, dried over Na_2SO_4, and evaporated. All of the alkoxy derivatives were purified by radial chromatography using 95:5 EtOAc–MeOH.

10-Ethoxy-10-demethoxycolchicine (20). This compound was prepared as described above and was obtained as an off-white solid in a yield of 87%. Compound **20**: mp 138–139 °C; $[\alpha]^{25}_D$ −113° (c 1.9); ^{1}H NMR δ 7.76 (d, 1 H, NH), 7.56 (s, 1 H, H-8), 7.32 (d, 1 H, H-12, J = 10.9 Hz), 6.87 (d, 1 H, H-11, J = 10.9 Hz), 6.54 (s, 1 H, H-4), 4.56 (p, 1 H, H-7), 4.25 (q, 2 H, 10-OCH_2CH_3, J = 7.0 Hz), 3.95 (s, 3 H, 3-OCH_3), 3.91 (s, 3 H, 2-OCH_3), 3.66 (s, 3 H, 1-OCH_3), 2.53 (m, 1 H, H-5), 2.33 (m, 2 H, H-5 and H-6), 1.98 (s, 3 H, $COCH_3$), 1.90 (m, 1 H, H-6), 1.56 (t, 3 H, 10-OCH_2CH_3); ^{13}C NMR δ 179.6, 169.9, 163.5, 153.4, 151.6, 151.2, 141.7, 136.4, 135.3, 134.2, 130.5, 125.7, 113.3, 107.3, 65.0, 61.5, 61.3, 56.1, 52.4, 36.6, 29.9, 22.9, 14.3; IR 3440, 3270 (br), 3020–2850, 1670, 1615 cm^{-1}; LRMS m/z 413 (M^+), 398, 385, 370, 356, 354, 326; HRMS calcd for $C_{23}H_{27}O_6N$ 413.1867, found 413.1839.

10-Demethoxy-10-propoxycolchicine (21). This compound was synthesized as described above and was obtained in a yield of 80%. Compound **21**: mp 116–117 °C; $[\alpha]^{25}_D$ −115° (c 1.1); ^{1}H NMR δ 7.51 (s, 1 H, H-8), 7.41 (br, 1 H, NH), 7.30 (d, 1 H, H-12, J = 10.9, Hz), 6.85 (d, 1 H, H-11, J = 10.9 Hz), 6.54 (s, 1 H, H-4), 4.65 (p, 1 H, H-7), 4.10 (q, 2 H, 10-$OCH_2CH_2CH_3$), 3.94 (s, 3 H, 3-OCH_3), 3.91 (s, 3 H, 2-OCH_3), 3.65 (s, 3 H, 1-OCH_3), 2.52 (m, 1 H, H-5), 2.40 (m, 1 H, H-5), 2.29 (m, 1 H, H-6), 1.98 (s, 3 H, $COCH_3$), 1.97–1.79 (m, 2 H, 10-$OCH_2CH_2CH_3$), 1.07 (t, 3 H, 10-$OCH_2CH_2CH_3$); ^{13}C NMR δ 179.6, 170.0, 163.7, 153.4, 151.2, 141.7, 136.2, 135.3, 134.2, 130.6, 125.8, 113.3, 107.4, 70.9, 61.5, 61.4, 56.1, 52.3, 36.7, 29.9, 22.9, 22.0, 10.4; IR 3440, 3270 (br), 3020–2850, 1670, 1610 cm^{-1}; LRMS m/z 427 (M^+), 399, 398, 384, 368, 356, 340; HRMS calcd for $C_{24}H_{29}O_6N$ 427.1993, found 427.1969.

10-Demethoxy-10-isopropoxycolchicine (22). This compound was synthesized as described above and was obtained in a yield of 73%. Compound **22**: mp 114–115 °C; $[\alpha]^{25}_D$ −130° (c 1.0); ^{1}H NMR δ 7.46 (s, 1 H, H-8), 7.29 (d, 1 H, H-12, J = 10.9 Hz), 7.15 (d, 1 H, NH), 6.87 (d, 1 H, H-11, J = 11.0 Hz), 6.53 (s, 1 H, H-4), 4.74 [m, 1 H, 10-$OCH(CH_3)_2$], 4.64 (p, 1 H, H-7), 3.94 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.66 (s, 3 H, 1-OCH_3), 2.50 (m, 1 H, H-5), 2.40 (m, 1 H, H-5), 2.27 (m, 1 H, H-6), 1.99 (s, 3 H, -$COCH_3$), 1.85 (m, 1 H, H-6), 1.48 [dd, 6 H, 10-$OCH(CH_3)_2$]; ^{13}C NMR δ 180.0, 169.7, 162.8, 153.4, 151.2, 150.7, 141.7, 136.1, 135.1, 134.2, 130.6, 125.8, 114.6, 107.3, 71.8, 61.5, 61.3, 56.1, 52.2, 36.8, 30.9, 23.0, 21.7, 21.6; IR 3440, 3270 (br), 3020–2850, 1670, 1610 cm^{-1}; LRMS m/z 427 (M^+), 399, 385, 357, 342, 314; HRMS calcd for $C_{24}H_{29}O_6N$ 427.1993, found 427.2021.

Colchicine (1). Colchicine was prepared as described above using methoxymagnesium bromide. The product was separated from small amounts of starting material by radial chromatography using 95:5 EtOAc–MeOH and was obtained in a yield of 85%. The ^{1}H NMR, ^{13}C NMR, and IR spectra agreed with that obtained for an authentic sample of this compound. Alternatively, **5** could be reacted with magnesium methoxide to yield **1**. The magnesium methoxide was generated by refluxing a solution of magnesium (1 g) in 20 mL of MeOH containing 10 mg of iodine. Approximately 10 equiv of the magnesium methoxide suspension was added to a solution of **5** in MeOH. The yield of **1** was 78%.

General. Pipes, EGTA, DTE, GTP (Type II-S), and Sephadex G-50 (fine) were obtained from Sigma Chemical Co. [^{3}H]-Colchicine was purchased from Dupont-New England Nuclear Research Products. Scintillation counting was performed on a Beckman LS 7500 scintillation spectrometer. An IEC Centra-7R refrigerated table top centrifuge with a 216 rotor was used in the experiments with tubulin. Microtubule assembly experiments were performed in PME buffer (PME buffer = 0.1 M Pipes, 1 mM $MgSO_4$, 2 mM EGTA, pH 6.90 at 23 °C). All other experiments were performed in PMEG buffer (PME buffer containing 0.1 mM GTP).

Ligand concentrations were determined from the following extinction coefficients (units are M^{-1} cm^{-1}) at the absorption maxima in aqueous solution: colchicine (**1**), $\epsilon_{352} = 1.69 \times 10^4$; compound **7**, $\epsilon_{354} = 1.10 \times 10^4$; compound **8**, $\epsilon_{360} = 8.08 \times 10^3$; compound **9**, $\epsilon_{378} = 1.18 \times 10^4$; compound **10**, $\epsilon_{356} = 1.59 \times 10^4$; compound **11**, $\epsilon_{344} = 9.23 \times 10^3$; compound **12**, $\epsilon_{344} = 9.19 \times 10^3$; compound **13**, $\epsilon_{344} = 1.08 \times 10^4$; compound **14**, $\epsilon_{344} = 1.32 \times 10^4$; compound **15**, $\epsilon_{346} = 1.09 \times 10^4$; compound **16**, $\epsilon_{346} = 1.15 \times 10^4$; compound **17**, $\epsilon_{346} = 1.30 \times 10^4$; compound **18**, $\epsilon_{368} = 1.20 \times 10^4$; compound **19**, $\epsilon_{336} = 8.53 \times 10^3$; compound **20**, $\epsilon_{354} = 1.55 \times 10^4$; compound **21**, $\epsilon_{354} = 1.64 \times 10^4$; compound **22**, $\epsilon_{352} = 1.59 \times 10^4$.

Inhibition of Microtubule Assembly. Microtubule protein at a concentration of 2 mg mL^{-1} was incubated with the appropriate ligand at the desired concentrations in PME buffer for 20 min at room temperature. GTP was then added to achieve a final concentration of 1 mM. Assembly was initiated by warming the solution to 37 °C, and the polymerization process was monitored by observing the change in turbidity of the solution at 400 nm on a Beckman (Model 25) UV–visible spectrophotometer containing a thermostated cell holder. Polymerization data were digitized using UN-PLOT-IT (Silk Scientific Inc.) and were transferred to an IBM PC/AT for subsequent manipulation. The I_{50} (ligand concentration required to produce a 50% inhibition of microtubule assembly relative to a control without added ligand) was determined by interpolation from a plot of percent inhibiton vs ligand concentration. The percent inhibition was determined from the plateau absorbance values after polymerization of the microtubule protein.

Inhibition of [^{3}H]Colchicine Binding to Tubulin. Solutions containing 5 μM (or 50 μM) of the ligand to be tested, 5 μM tubulin, and 5 μM [^{3}H]colchicine in PMEG buffer were incubated at 37 °C for 1.5 h. The reaction was quenched by placing the solutions on ice. The ligand–protein complex was then separated from unbound ligand by rapid gel filtration according to the method of Penefsky.[24] Aliquots of the protein–ligand solutions were applied to prepared 1 mL of G-50 columns and centrifuged at 900 rpm for 2 min. The column effluent was analyzed for tubulin-bound [^{3}H]colchicine by scintillation spectrometry. Negligible amounts of [^{3}H]colchicine were found in the column effluent when control experiments were performed in which [^{3}H]-

colchicine in the absence of tubulin was applied to the columns. The percent inhibition of [^{3}H]colchicine binding was calculated relative to a control without added ligand. Assays at each ligand concentration were performed in quadruplicate, and an average value was calculated.

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