pressure transducer coupled to a Beckman R611 polygraph. Right and left femoral veins were catheterized to inject PAF (0.5 μg/kg) or the test compound. Test compounds were administered by intravenous injection (1 mL/kg, dissolved in saline) 3 min before PAF injection. Control animals received only the vehicle. Blood pressure was monitored and percent inhibition of PAF-induced hypotension with respect to controls was calculated. The results were expressed as ID_{50} values, i.e. the dose of the test compound required to inhibit PAF-induced hypotension by 50%. The results were calculated by linear regression from a single experimental curve with no less than four points, each point being the mean of the percent inhibition at a given dose obtained from two or more independent experiences.

Statistics. Statistical analyses of pharmacological data (i.e. IC_{50} and ID_{50} values with their 95% confidence limits) were made by using a standard pharmacology program implemented on an IBM PC.[11]

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Registry No. 1 (I^-), 131729-40-3; 1 (Cl^-), 100488-87-7; **3**, 136408-39-4; **4a**, 136408-61-2; **4b**, 136408-62-3; **4c**, 134472-16-5; **4d**, 136408-63-4; **4e**, 136408-64-5; **4f**, 136408-65-6; **5a**, 136408-66-7; **5b**, 136408-67-8; **5c**, 134472-00-7; **5d**, 136408-68-9; **5e**, 136408-69-0; **5f**, 136408-70-3; **6a**, 136408-71-4; **6b**, 136408-72-5; **6c**, 134472-01-8; **6d**, 136408-73-6; **6e**, 136408-74-7; **6f**, 136408-75-8; **7e**, 136408-76-9; **8e**, 136408-77-0; **9**, 134471-90-2; **10**, 134471-93-5; **11**, 134471-91-3; **12**, 134472-12-1; **13**, 134471-85-5; **14**, 134471-87-7; **15**, 136408-40-7; **16**, 134472-08-5; **17**, 134472-05-2; **18**, 134505-53-6; **19**, 134471-75-3; **20**, 134472-11-0; **21**, 136408-41-8; **22**, 136408-42-9; **23**, 136434-36-1; **24**, 134472-02-9; **25**, 136408-43-0; **26**, 136408-44-1; **27**, 136408-45-2; **28**, 136408-47-4; **29**, 136408-48-5; **30**, 136408-49-6; **31**, 136408-50-9; **32**, 136408-51-0; **33**, 136408-52-1; **34**, 136408-53-2; **35**, 136408-54-3; **36**, 134471-99-1; **37**, 136408-55-4; **38**, 136408-56-5; **39**, 136408-57-6; **40**, 136408-58-7; **41**, 136408-60-1; **43**, 131730-72-8; **44**, 131730-85-3; **45**, 131730-92-2; $HO(CH_2)_2S(CH_2)_2OH$, 111-48-8; $CH_3(CH_2)_{16}NCO$, 7418-01-1; $PhOC(O)O(CH_2)_2S(CH_2)_2OC(O)OPh$, 136434-37-2; pyridine, 110-86-1; 2-picolylamine, 3731-51-9; *n*-octadecylamine, 124-30-1; 1,3-propanediol, 504-63-2.

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Fluorinated Colchicinoids: Antitubulin and Cytotoxic Properties

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The synthesis of B-ring and C-ring trifluoroacetamide-substituted colchicinoids and fluoro-substituted colchicineethylamides is presented. The B-ring trifluoroacetamido-substituted analogues exhibit moderate enhancement of potency compared to the nonfluorinated analogues for tubulin assembly inhibition and cytotoxicity toward two wild type cell lines. The C-ring substituted fluoroethylamides have reduced relative potencies in the same systems due to the strong electron-withdrawing effect of the fluoro derivatives. The fluoro colchicinoids are much more cytotoxic toward drug-resistant cell lines than to the wild type cell lines. Their enhanced potency is probably due to an effect of the fluoro moiety on functions specific to resistant cells and/or their higher hydrophobicity that may result in higher intracellular drug content. This finding may suggest the application of designed fluorinated anticancer drugs to overcome acquired resistance which may develop after several regiments of treatment with a nonfluorinated chemotherapeutic agent.

Introduction

Colchicine (**1a**, Chart I) is a potent drug that interferes with microtubule assembly both in vitro and in vivo.[1] It forms a complex with tubulin which inhibits the microtubule assembly process.[2] Two partial binding sites on tubulin were observed for the colchicine molecule: one for the trimethoxyphenyl A-ring and one for the 2-methoxytropone C-ring.[3] The role of ring B in the binding process is still unclear.[4] NMR studies utilizing ^{13}C-labeled colchicine established the existence of a second low-affinity binding site.[5] ^{13}C and ^{1}H NMR studies of drug-biological substrate binding have several major drawbacks, among them buffer or medium background signals, substrate signals, and low sensitivity in the case of ^{13}C. On the other hand, ^{19}F NMR proved to be an effective tool for such studies,[6] and metabolites of fluorinated drugs in body fluids can be detected by NMR at concentrations as low as 10 μM.[7] We decided to explore the feasibility of using colchicinoids with fluorinated substituents for studies exploring the binding of cholchicine to tubulin,[8] cells, and tissues. The synthesis of colchicine analogues has afforded many compounds with improved cytotoxic properties; among them are several trifluoroacetamido-substituted analogues.[9,10]

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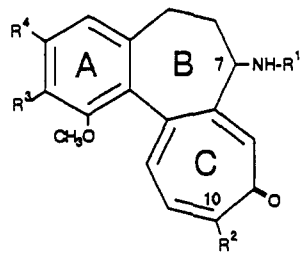
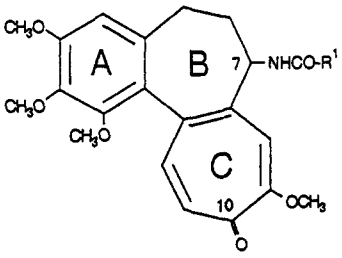
Chart I



1a: $R^1 = COCH_3$, $R^2 = R^3 = R^4 = OCH_3$
1b: $R^1 = COCF_3$, $R^2 = R^3 = R^4 = OCH_3$
2a: $R^1 = COCH_3$, $R^2 = R^4 = OCH_3$, $R^3 = OH$
2b: $R^1 = COCF_3$, $R^2 = R^4 = OCH_3$, $R^3 = OH$
3a: $R^1 = COCH_3$, $R^2 = NH_2$, $R^3 = R^4 = OCH_3$
3b: $R^1 = COCF_3$, $R^2 = NH_2$, $R^3 = R^4 = OCH_3$
4a: $R^1 = COCH_3$, $R^2 = SCH_3$, $R^3 = OCH_3$, $R^4 = OH$
4b: $R^1 = COCF_3$, $R^2 = SCH_3$, $R^3 = OCH_3$, $R^4 = OH$
5a: $R^1 = COCH_3$, $R^2 = OH$, $R^3 = R^4 = OCH_3$
5b: $R^1 = COCF_3$, $R^2 = OH$, $R^3 = R^4 = OCH_3$
7: $R^1 = COCH_3$, $R^2 = NHCOCF_3$, $R^3 = R^4 = OCH_3$
8: $R^1 = COCF_3$, $R^2 = NHCOCF_3$, $R^3 = R^4 = OCH_3$
9a: $R^1 = COCH_3$, $R^2 = NHCH_2CH_3$, $R^3 = R^4 = OCH_3$
9b: $R^1 = COCH_3$, $R^2 = NHCH_2CH_2F$, $R^3 = R^4 = OCH_3$
9c: $R^1 = COCH_3$, $R^2 = NHCH_2CF_3$, $R^3 = R^4 = OCH_3$
10: $R^1 = COCF_3$, $R^2 = NHCH_2CF_3$, $R^3 = R^4 = OCH_3$
11: $R^1 = H$, $R^2 = R^3 = R^4 = OCH_3$
12: $R^1 = H$, $R^2 = NH_2$, $R^3 = R^4 = OCH_3$



6a: $R^1 = CH_3$
6b: $R^1 = CF_3$

In this study, we present a series of colchicine analogues with substituents containing fluoro atoms attached to either the B- or C-ring of colchicine, or both. The affinity of these compounds to tubulin, their potency as inhibitors of tubulin assembly and cell proliferation, and their cytotoxic effect on several resistant cell lines is being reported. We have also synthesized the comparable nonfluorinated analogues and a comparison is made between them and the fluorinated ones.

Chemistry

Trifluoroacetamides **1b**, **5b**, and **6b** from deacetylisocolchicine were prepared from *N*-deacetylcolchiceine[11] [^{19}F NMR ($CDCl_3$, δ) 86.25, 86.15, and 86.20 (3 F, s), respectively]. 2-Demethylcolchicine **2a** and the (trifluoroacetyl)deacetyl analogue **2b** were repeatedly made by selective ether cleavage of **1a** and **1b**, respectively, with concentrated sulfuric acid at 50–60 °C.[12] Reacting **1a** with ammonium hydroxide solution yields **3a**.[13] Compound **4a** was prepared from 3-demethylcolchicine[14] and **4b** from 3-demethyldeacetylthiocolchicine.[15] Compound **5a** was prepared by a mild acidic hydrolysis of **1a**.[16]

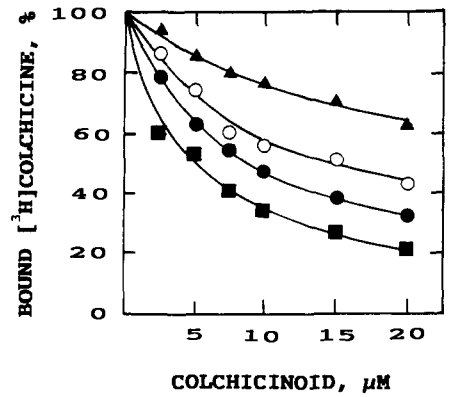


Figure 1. Competition between colchicine and several fluoro colchicinoids for the colchicine binding site on tubulin. Tubulin (1.0 mg/mL) was premixed with 8 μM [^{3}H]colchicine at 4 °C. Various concentrations of drug were added to 300-μL aliquots of the mixture and incubated for 45 min at 37 °C. The colchicine binding assay was performed according to the method of Borisy.[20] The drugs presented are colchicine (**1a**) (●), **1b** (■), **9b**, (○), and **9c** (▲).

In an attempt to prepare C-ring analogues, we have synthesized the 10-trifluoroacetamide analogues of **1a** and **1b**. The desired products **7** and **8** were obtained by treating the corresponding amides **3a** and **12** with trifluoroacetic anhydride. Unfortunately, the compounds were found to be unstable in aqueous solutions. Mild hydrolysis of **7** in aqueous methanol afforded **3a**, whereas heating it with 2 N sodium hydroxide gave (−)-colchiceine (**5a**), also obtained by similar hydrolysis of **3a**.[13] When similar mild hydrolysis was carried out with bis(trifluoroacetamide) **8**, amide **3b** was obtained. All the compounds are optically active and their structures are supported by mass, NMR, and UV spectra, and their purity was assessed by TLC.

In a continuous search for a stable 10-fluoro derivative, we synthesized the 2′-fluorinated analogues (**9b** and **9c**) of ethylcolchiceinamide (**9a**) by replacing the 10-methoxy group with the appropriate fluoroethylamine. These colchicine analogues are stable in aqueous solutions including cell medium. THe "double" fluoro substituted (on ring B and C) analogue (**10**) was synthesized from **1b** by the same procedure.

Biological Evaluation

All compounds were examined for inhibitory effects on polymerization of purified tubulin. Samples of tubulin (1 mg/mL) were incubated with two different concentrations of each drug (2 and 5 μM) prior to the initiation of the assembly by GTP or taxol (Table I). The difference between the absorbance of the assembled polymer, at steady state, in the absence and presence of drug, represented the extent of inhibition induced by the drug (Table I). All the compounds were evaluated for their ability to

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Table I. Biological Activities of Colchicinoids

	% ITP[a]				ED$_{50}$[b] (10^{-7} M)						
	GTP		taxol								
compd	2 μM	5 μM	2 μM	5 μM	J774.2	J7.COL-100	J7.VBL-1	P388	P388.ADR	t_R[c]	log P[d]
1a	37	70	27	61	0.6	~2000	170	0.12	4.7	3.9	0.60
1b	40	74	27	62	0.1	140	8	0.04	0.4	5.4	1.72
2a	20	27	15	35	3	1400	625	0.95	12.2	3.0	0.48
2b	24	35	17	47	0.4	105	50	0.4	1.2	3.8	1.67
3a	36	70	33	66	0.2	700	150	0.13	4	4.0	0.30
3b	48	82	42	79	0.1	45	13	0.13	0.6	5.7	2.32
4a	51	89	34	61	0.2	310	45	0.14	4	3.8	1.43
4b	52	98	35	68	0.13	45	7.5	0.06	0.38	5.3	2.61
5a	24[e]	50[e]	NT[f]	NT[f]	31	>1000	>500	12	100	NT[f]	1.20
5b	22[e]	44[e]	NT[f]	NT[f]	30	49	200	30	60	NT[f]	1.82
6a	7[e]	13[e]	NT[f]	NT[f]	56	NT[f]	>500	150	150	3.9	1.00
6b	5[e]	10[e]	NT[f]	NT[f]	39	NT[f]	>200	15	130	5.9	2.08
9a	43	85	32	67	0.1	150	19	0.05	3.2	7.5	2.34
9b	30	53	23	50	.1.5	320	48	0.23	4.7	5.1	1.70
9c	20	36	11	24	1.9	500	170	0.51	9	7.4	1.45
10	30	45	15	35	2.0	88	29	0.5	0.9	10.6	1.36

[a] ITP = inhibition of tubulin polymerization. Tubulin samples (1 mg/mL) were incubated with 2 or 5 μM of each drug, and polymerization was induced by the introduction of 1 mM GTP or 10 μM taxol. [b] ED$_{50}$ = drug concentration that inhibits cell division by 50% after 72 h. [c] t_R = retention time (min) of colchicinoids eluted on HPLC RP-18 column, MeOH/0.01 M sodium acetate, pH 5.5 = 65:35 at 1 mL/min. [d] log P = log of partition coefficient octanol/water. The concentration of the drug in each phase was determined by HPLC as described for the t_R measurements. [e] The concentrations of **5a**, **5b**, **6a**, and **6b** are 50 and 100 μM for the left and right panels, respectively. [f] NT = not tested.

compete with [^{3}H]colchicine for the colchicine binding site on tubulin and were found to act at the same binding site as colchicine (Figure 1). The extent of colchicine displacement by the drugs was correlated to the extent of assembly inhibition induced by them. As tubulin assembly inhibitors, 7-(trifluoroacetamido)colchicinoids exhibited a moderate increase in potency compared to their nonfluorinated analogues while the 10-fluoro colchicinoids displayed a marked decrease in potency in proportion to the number of fluorine atoms (CH_3 > CH_2F > CF_3). Colchiceine (**5a**) and isocolchicine (**6a**) are, practically, inactive analogues of colchicine, and their fluorinated analogues (**5b** and **6b**) are inactive as well. In addition, cells treated with various fluorocolchicinoids were subjected to immunofluorescence studies. The cells were treated with a mouse antibody to tubulin and a second antibody, fluorescein-conjugated rabbit anti-mouse antibody. No microtubule filaments were observed in the cells (data not presented).

We have examined the effect of the fluorinated and nonfluorinated colchicinoids on two different cell lines, and murine macrophagelike J774.2 and the P388 mouse lymphocytic leukemia cell line. The cytotoxicity of the drugs (ED$_{50}$) followed a similar trend as was observed for the inhibition of tubulin assembly. Minor elevation in potency was observed for the 7-(trifluoroacetamido) analogues compare to that observed for the nonfluorinated analogues, while the 10-colchiceinamide analogues present marked decrease in cytotoxicity as the number of fluoro atoms increases. The same trend prevailed for both cell lines.

To gain a better comprehension of the cytotoxic properties of the fluorinated compounds we decided to test them against drug-resistant variants of J774.2 and P388. The two variants of J774.2 are cell lines resistant to microtubule disrupting agents (colchicine, J7.COL-100, and vinblastine, J7.VBL-1), while the variant of P388 is resistant to doxorubicin (P388.ADR), a cell-proliferation inhibitor which acts by a different mechanism. All B-ring fluoro analogues (except **5b** and **6b**, which are practically inactive colchicinoids) exhibited a marked increase in cytotoxicity relative to their nonfluorinated analogues (e.g. **1b** and **3b** are ~14 and ~16 times more cytotoxic against J7.COL-100 than 1a and 3a, respectively). The differences were larger than those observed for the nonresistant cell lines (e.g. **1b** and **3b** are only 6 and 2 times more cytotoxic against J774.2 than **1a** and **3a**, respectively). Moreover, the fluoroanalogues on ring C (**9b** and **9c**), although less cytotoxic than the nonfluorinated analogue (**9a**), presented the same trend as the B-ring fluoroanalogues. Their relative cytotoxicity toward drug-resistant cells was higher than to the wild type ones. A clue for a possible explanation can be found in the hydrophobic behavior of the compounds (t_R and log P) summarized in Table I. It can be clearly observed that all the fluoro analogues which are more potent than their nonfluorinated counterparts have stronger hydrophobic properties.

Discussion and Conclusions

7-(Trifluoroacetamido) derivatives of colchicine examined here (except **5b** and **6b**) had significant activity as inhibitors of tubulin assembly, with most of them being superior to colchicine itself and their nonfluorinated analogues. On the other hand, introduction of fluoro substituent to ethylcolchiceinamide (**9b** and **9c**) induced a decrease in potency of the colchicinoid which depends on the number of fluorine atoms introduced (CH_3 > CH_2F > CF_3). The same trend in activity was observed for the action of fluorocolchicinoids on J774.2 and P388 cell lines. This behavior conforms to the observations of Quinn and Beisler[10] that colchicinoids bearing a fluoro substituent on the 7-amide linkage have augmented inhibitory effect on tubulin assembly due to the electron-withdrawing capability of the halogen, and strong electron-withdrawing groups substituted at the 10-position of the tropolone ring diminish that activity. These results and the observation that 7-(trifluoroacetamido) substitution on colchiceine and isocolchicine did not augment the apparent potency of these inactive drugs affirm the conclusion that the effect of the fluoro moiety on the potency of the colchicinoid as tubulin assembly inhibitors stemmed mostly from the electronic effect on the NH–CO bond which is involved in the binding of tubulin. These observations exclude nonspecific toxic effect of the fluorine atoms, and any contribution to the cytotoxicity of the small amount of trifluoroacetic acid (e.g. less than 8% of **1b** after incubation at 37 °C in serum for 72 h).

Treating the resistant cell lines with the colchicinoids revealed a profound enhancement in cytotoxicity for the

Table II. Fold Change in Activity between Fluorinated and Nonfluorinated Colchicinoids (ED_{50} of Nonfluorinated/ED_{50} of Fluorinated)

	ED_{50} of **a**/ED_{50} of **b**				
compd	J774.2	J7.COL-100	J7.VBL-1	P388	P388.ADR
1a/1b	6.0	14.3	21.3	3.0	11.8
2a/2b	7.5	13.2	12.5	2.4	10.2
3a/3b	2.0	15.6	11.5	1.0	6.7
4a/4b	1.5	6.9	6.0	2.3	10.5
9a/9b	0.07	0.47	0.40	0.22	0.68
9a/9c	0.05	0.30	0.11	0.10	0.36

fluorinated analogues. This intensified potency is prominent in the J7.COL-100 cell line, which would be assumed less sensitive to colchicine analogues. The enhanced sensitivity for the fluorinated analogues is also eminent for the other two resistant cell lines (J7.VBL-1 and P388.ADR). Table II displays the changes in potency between several fluoro colchicinoids and their nonfluoro analogues. The results clearly demonstrate that all fluoro-substituted analogues display ~2–8 fold increase in cytotoxicity whether the substitution is on the B- or C-ring. It is evident from these results that the intensified cytotoxic effect of the fluoro colchicinoid on the resistant cell lines may derives from an effect of the fluoro moiety which is not related to the colchicinoid–tubulin binding capability of the drug. Additional explanation can be inferred from the hydrophobic properties of the compounds. It is clear from Table I that the cytotoxic activity of the drug correlates with its hydrophobic nature. Changes in membrane fluidity and drug permeability are among several considered rationalization for the multidrug-resistance phenomenon.[17] Higher hydrophobicity may elevate the intracellular concentration of the drug. Drug solubility in cell membrane may be enhanced, thus changing membrane fluidity and the membrane-anchored drug-transport systems.

In conclusion, we have synthesized B-ring- and C-ring-fluorinated analogues of colchicinoids. The B-ring fluoro analogues exhibited moderate enhancement of potency compared to the nonfluorinated analogues for tubulin-assembly inhibition and cytotoxicity toward two wild type cell lines. The results of the C-ring fluoro analogues presented a reduction in the relative potencies in the same systems. Fluoro colchicinoids are potent cytotoxic agents toward multidrug-resistant cell lines. This latest finding may suggest the application of designed fluorinated anticancer drugs to overcome acquired resistance, which may develop after several regiments of a nonfluorinated chemotherapeutic agent. Fluorinated colchicinoids also can be used in ^{19}F NMR studies to illuminate several aspects of the mechanism of acquired drug resistance. Studies along this line are currently being performed in our laboratory.

Experimental Section

General Methods. Melting points (uncorrected) were determined with a Fisher-Jones apparatus. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter in chloroform at room temperature. The UV spectra ($CHCl_3$) were measured on a Hewlett-Packard 8450 A and for tubulin-assembly studies on Uvikon 930 UV/VIS spectrophotometers. Electron-impact mass spectra were obtained with a V. G. Micromass 7070F mass spectrometer (70 eV, source temperature 210 °C). NMR measurements were performed on a Varian VXR 300S spectrometer interfaced with a Sun-3/60 computer and equipped with a 5-mm multinuclear probe. The ^1H and ^{19}F chemical shifts, in $CDCl_3$ as solvent, are reported relative to Me_4Si and hexafluorobenzene (0 ppm), respectively. Silica gel 60 (230–400 mesh) purchased from Fluka was used for column chromatography. The solvent system used for TLC analysis was $CHCl_3$/MeOH = 9:1 and $CHCl_3$/MeOH/NH_4OH = 90:9:1. HPLC analysis was carried out using Merck-Hitachi integrated system and LiChroCART RP-18 250-4 mm or 250-10 mm columns.

Chemistry. 2-Demethyl-*N*-(trifluoroacetyl)deacetylcolchicine (2b). Trifluoroacetamide **1b** (105 mg, 0.23 mmol) was heated to 50–60 °C with concentrated H_2SO_4 (1 mL) and stirred at this temperature for 5 h. The reaction mixture was poured on ice and neutralized with saturated $NaHCO_3$ solution to pH 7. The aqueous layer was extracted with methylene chloride. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The crude extract was chromatographed on silica gel using CH_2Cl_2/MeOH (97:3) as eluent to give **2b** as yellow crystals from CH_2Cl_2/hexane (36 mg, 0.08 mmol, 37%): mp 271–273 °C; $[\alpha]_D$ −201° [c = 0.11, $CHCl_3$/MeOH (7:3)], ^1H NMR ($CDCl_3$, δ) 3.65 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 4.02 (3 H, s, OCH_3), 4.73 (1 H, m, H-7), 6.54 (1 H, s, H-4), 6.88 (1 H, d, J = 10.4 Hz, Ar-H), 7.38 (1 H, d J = 10.4 Hz, Ar-H), 7.40 (1 H, s, Ar-H), 8.30 (1 H, bd, J = 7.0 Hz, NH); ^{19}F NMR ($CDCl_3$, δ) 86.1 (3 F, s); EIMS m/e 439 (M^+).

10-(Trifluoroacetyl)colchiceinamide (7). To a solution of colchiceinamide (**3a**, 75 mg, 0.19 mmol) in ether were added Na_2CO_3 anhydrous (202 mg) and trifluoroacetic anhydride (0.4 mL) under ice cooling. The reaction mixture was stirred at room temperature for 1 h, diluted with $CHCl_3$ (5 mL), washed with water and than brine, dried over Na_2SO_4, concentrated, and chromatographed on silica gel. Pure trifluoroacetamide **7** was eluted with $CHCl_3$/MeOH = 99:1. Trituration of the etherial solution with ligroin gave a yellow powder (60 mg): mp 154–160 °C; $[\alpha]_D$ −206° (c = 0.25, $CHCl_3$); UV_{max} ($CHCl_3$) 254, 354, 382 nm; ^1H NMR ($CDCl_3$, δ) 2.02 (3 H, s, $COCH_3$), 3.65 (3 H, s, OMe), 3.90 (3 H, s, OMe), 3.94 (3 H, s, OMe), 4.62 (1 H, m, H-7), 6.21 (1 H, bd, J = 6.6, NH), 6.54 (1 H, s, H-4), 7.45 (1 H, d, J = 10.5 Hz, Ar-H), 7.52 (1 H, s, Ar-H), 8.88 (1 H, d, J = 10.5 Hz, Ar-H); ^{19}F NMR ($CDCl_3$, δ) 85.84 (3 F, s); EIMS m/e 480 (M^+); Anal. ($C_{23}H_{23}N_2O_6F_3$) C, H, N, F.

Hydrolysis of Trifluoroacetamide 7 into Colchiceinamide (3a). Trifluoroacetamide **7** (20 mg, 0.042 mmol) in MeOH (1 mL) and H_2O (1 mL) was stirred at room temperature for 20 h. Solvent was evaporated and the residue crystallized from CH_2Cl_2/Et_2O to afford **3a** (9 mg), identical with an authentic sample.

Hydrolysis of Trifluoroacetamide 7 into Colchiceine (5a). Trifluoroacetamide **7** (25 mg, 0.052 mmol) in 1 N NaOH (1 mL) was stirred at 90 °C for 24 h. The reaction mixture was acidified with 5% HCl to pH 5 and the precipitate was filtered and washed with H_2O to give colchiceine (12 mg): mp 169–172 °C; $[\alpha]_D$ −260° (c = 0.2, $CHCl_3$); EIMS m/e 385 (M+). The material is identical in every respect with an authentic sample.

Deacetylcolchiceinamide (12). Deacetylcolchicine[8] (**11**, 150 mg, 0.42 mmol) was stirred with 30% NH_4OH (6 mL) at room temperature for 48 h. The reaction mixture was extracted three times with chloroform (10 mL); the extract was washed with brine and dried over Na_2SO_4, to afford on evaporation an amorphous material. Chromatographic purification on silica gel using $CHCl_3$/MeOH = 99:1 as the eluant gave **12** as an amorphous product: $[\alpha]_D$ −186° (c = 0.12, $CHCl_3$); UV_{max} ($CHCl_3$) 251, 355, and 376 nm; ^1H NMR ($CDCl_3$, δ) 3.53 (3 H, s, OCH_3), 3.70 (1 H, m, H-7), 3.82 (6 H, s, 2 OCH_3), 5.79 (2 H, bs, NH_2), 6.45 (1 H, s, H-4), 6.76 (1 H, d, J = 10.8 Hz, Ar-H), 7.15 (1 H, d, J = 10.8 Hz, Ar-H), 7.62 (1 H, s, Ar-H); EIMS m/e 342 (M+).

***N*,10-Bis(trifluoroacetyl)deacetylcolchiceinamide (8).** Amide **12** (10 mg, 0.03 mmol) was converted into **8** by the procedure used for the preparation of **7**. Crystallization from *i*-Pr_2O/petroleum ether afforded yellow crystals (15 mg): mp 218–219 °C; $[\alpha]_D$ −129° (c = 0.26, $CHCl_3$); UV_{max} ($CHCl_3$) 254, 356, and 381 nm; ^1H NMR ($CDCl_3$, δ) 3.67 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 4.72 (1 H, m, H-7), 6.57 (1 H, s, H-4), 7.52 (1 H, s, Ar-H), 7.53 (1 H, d, J = 10.7 Hz, Ar-H), 7.78 (1 H, d, NH), 8.93 (1 H, d, J = 10.7 Hz, Ar-H); ^{19}F NMR ($CDCl_3$, δ) 86.17 (3 F, s, 10-NHCOCF$_3$), 86.22 (3 F, s, 7-NHCOCF$_3$); EIMS m/e 534 (M+). Anal. ($C_{23}H_{20}N_2O_6F_6$) C, H, N.

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Hydrolysis of Bis(trifluoroacetamide) 8 to *N*-(Trifluoroacetyl)deacetylcolchiceinamide (3b). Bis(trifluoroacetamide) 8 (100 mg, 0.187 mmol) was dissolved in methanol (2 mL) and water (1 mL) and stirred at room temperature for 36 h. Solvent was evaporated and the residue chromatographed on silica gel using $CHCl_3$/MeOH = 98:2 as a solvent to give yellow crystals (60 mg). An analytical sample was obtained on crystallization from EtOAc/Et_2O: mp 247–248 °C; $[\alpha]_D$ −120° (*c* = 0.2, $CHCl_3$); UV_{max} ($CHCl_3$) 258, 354, and 376 nm; ^1H NMR ($CDCl_3$, δ) 3.63 (3 H, s, OCH_3), 3.91 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 4.85 (1 H, m, H-7), 6.00 (2 H, bs, NH_2), 6.56 (1 H, s, H-4), 6.93 (1 H, d, *J* = 11.0 Hz, Ar-H), 7.39 (1 H, d, *J* = 11.0 Hz, Ar-H), 7.49 (1 H, s, Ar-H), 8.81 (1 H, bs, NH); ^{19}F NMR ($CDCl_3$, δ) 86.10 (3 F, s); EIMS *m/e* 438 (M+). Anal. ($C_{21}H_{21}N_2O_5F_3$) C, H, N: calcd, 6.39; found, 5.92.

10-Ethylcolchiceinamide (9a), 10-(2-Fluoroethyl)colchiceinamide (9b), and 10-(2,2,2-Trichloroethyl)colchiceinamide (9c). All compounds were synthesized according to the same procedure. The reactions were carried out in a pressurized flask. Colchicine (200 mg, 0.5 mmol) was dissolved in dry acetonitrile (1 mL). An excess amount (20 mmol) of ethylamine, 2-fluoroethylamine, or 2,2,2-trifluoroethylamine, respectively, was added. The reaction flasks were heated to 80 °C for 1 week when the yield of the product reached 75–80% (as followed by HPLC). Solvent was evaporated and the residue chromatographed on silica gel using $CHCl_3$/MeOH = 98:2 as a solvent. The collected product was further purified by HPLC on a semipreparative RP-18 column using MeOH/H_2O = 52:48 as solvent. The data for each product is as follows. **9a**: mp 164–168 °C; $[\alpha]_D$ −102° (*c* = 0.75, $CHCl_3$); UV_{max} ($CHCl_3$) 253, 358, 378, and 414 nm; ^1H NMR ($CDCl_3$, δ) 1.38 (3 H, t, *J* = 14.4, $NHCH_2CH_3$), 1.96 (3 H, s, $COCH_3$), 3.39 (2 H, m, $NHCH_2$), 3.59 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 3.91 (3 H, S, OCH_3), 4.67 (1 H, m, H-7), 6.50 (1 H, s, H-4), 6.60 (1 H, d, *J* = 11.1 Hz, Ar-H), 7.12 (1 H, bt, NH-10), 7.25 (1 H, s, Ar-H), 7.43 (1 H, d, *J* = 11.1 Hz, Ar-H), 7.54 (1 H, s, Ar-H), 8.65 (1 H, bd, NH-7). Anal. ($C_{23}H_{28}N_2O_5$) C, H, N. **9b**: mp 144–150 °C; $[\alpha]_D$ −127° (*c* = 0.47, $CHCl_3$); UV_{max} ($CHCl_3$) 256, 356, and 409 nm; ^1H NMR ($CDCl_3$, δ) 2.02 (3 H, s, $COCH_3$), 3.62 (3 H, s, OCH_3), 3.41 (2 H, m, $NHCH_2$), 3.90 (3 H, s, OCH_3), 3.94 (3 H, s, OCH_3), 4.72 (2 H, dt, *J* = 47.0, 5.0 Hz, CH_2F), 6.53 (1 H, s, H-4), 6.61 (1 H, d, *J* = 11.1, Ar-H), 7.32 (1 H, s, Ar-H), 7.41 (1 H, d, *J* = 11.1, Ar-H); ^{19}F NMR ($CDCl_3$, δ) 116.35 (1 F, tt, *J* = 27.5, 45.8 Hz). Anal. ($C_{23}H_{27}N_2O_5F$) C, H, N, F. **9c**: mp 156–160 °C; $[\alpha]_D$ −129° (*c* = 0.55, $CHCl_3$); UV_{max} ($CHCl_3$) 252, 355, 379, and 404 nm; ^1H NMR ($CDCl_3$, δ) 2.00 (3 H, s, $COCH_3$), 3.60 (3 H, s, OCH_3), 3.89 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 4.01 (2 H, m, $NHCH_2$), 4.69 (1 H, m, H-7), 6.53 (1 H, s, H-4), 6.67 (1 H, bd, NH-7), 6.71 (1 H, d, *J* = 10.8 Hz, Ar-H), 7.33 (1 H, bt, NH-10), 7.43 (1 H, d, *J* = 10.8, Ar-H), 7.44 (1 H, s, Ar-H); ^{19}F NMR ($CDCl_3$, δ) 90.74 (3 F, t, *J* = 8.5 Hz); ^{13}C NMR ($CDCl_3$, δ) 43.68 (1 C, q, J_{C-F} = 33 Hz, $NHCH_2$), 126.28 (1 C, q, J_{C-F} = 278 Hz, CF_3). Anal. ($C_{23}H_{25}N_2O_5F_3$) C, H, N, F.

***N*-(Trifluoroacetyl)deacetyl-10-(trifluoroethyl)colchiceinamide (10).** Compound **1b** (100 mg, 0.22 mmol) was dissolved in dry acetonitrile (1 mL). An excess amount (20 mmol) of 2,2,2-trifluoroethylamine was added. The procedure for the preparation of compounds 9 was followed: mp 153–157 °C; $[\alpha]_D$ −85° (*c* = 0.08, $CHCl_3$); UV_{max} ($CHCl_3$) 247, 357, and 405 nm; ^1H NMR ($CDCl_3$, δ) 3.62 (3 H, s, OCH_3), 3.91 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 4.04 (2 H, m, $NHCH_2$), 4.75 (1 H, m, H-7), 6.56 (1 H, s, H-4), 6.88 (1 H, d, *J* = 11.2 Hz, Ar-H), 7.37 (1 H, bt, NH-10), 7.48 (1 H, s, Ar-H), 7.4–9.7 (1 H, d, *J* = 11.2 Hz, Ar-H); ^{19}F NMR ($CDCl_3$, δ) 87.23 (3 F, S, $NHCOCF_3$), 91.83 (3 F, t, *J* = 11.6 Hz, $NHCH_2CF_3$). Anal. ($C_{23}H_{22}N_2O_5F_6$) C, H, N, F.

Biological Studies. Tubulin Preparation and Assembly Measurements. Tubulin was prepared from calf brains by three cycles of polymerization–depolymerization according to a procedure modified from Shelanski et al.[18] Tubulin stocks were stored at −20 °C in MES buffer consisting of 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES), 2 mM [ethylenebis(oxyethylenenitrilo)]tetraacetate (EGTA), 0.5 mM MgCl, and 6 M glycerol, pH = 6.7. Stock aliquots were diluted 1:1 with glycerol-free MES buffer, and the working sample was clarified by centrifugration at 25000 rpm for 15 min at 4 °C prior to use. Tubulin samples (1 mg/mL) were incubated at 35 °C with or without drug for 45 min prior to the addition of GTP (1 mM) or taxol (10 μM). This incubation period allows for the formation of drug–tubulin complex which, at least for colchicine, is essential for the inhibition of tubulin assembly. The change in microtubule mass concentration was monitored spectrophotometrically as a change in the absorbance at 350 nm, with time. Extent of inhibition of polymerization in drug-treated samples was calculated by comparing their polymer mass at steady state to that of the drug-free sample.

Cytotoxic Effects of Colchicinoids. The synthesized compounds were tested as cell-growth inhibitors, on two different cell types. One type was mouse macrophagelike cell line J774.2 (wild type) and two of its resistant variants, J7.COL-100 and J7.VBL-1, which are maintained in 100 μM colchicine and 1 μM vinblastine, respectively (courtesy of Dr. S. B. Horwitz, Albert Einstein College of Medicine, NY). The second cell type was the P388 mouse lymphocytic leukemia cell line and its doxorubicin resistant variant P388.ADR (courtesy of Dr. A. Ramu, The Hebrew University-Hasassah Medical School, Jerusalem, Israel). Cells were incubated with or without the drugs in triplicate per concentration, for 72 h (before the control samples reached confluency), and the number of living cells was determined.[19]

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Registry No. **1a**, 64-86-8; **1b**, 26195-65-3; **2a**, 7336-36-9; **2b**, 86436-46-6; **3a**, 3123-89-5; **3b**, 136570-05-3; **4a**, 87424-25-7; **4b**, 123643-51-6; **5a**, 477-27-0; **5b**, 71295-34-6; **6a**, 518-12-7; **6b**, 71324-48-6; **7**, 136570-06-4; **8**, 136570-07-5; **9a**, 67620-24-0; **9b**, 136570-08-6; **9b**, 136570-09-7; **10**, 136570-10-0; **11**, 3476-50-4; **12**, 114537-83-6.

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