Antitumor Agents. 141.† Synthesis and Biological Evaluation of Novel Thiocolchicine Analogs: *N*-Acyl-, *N*-Aroyl-, and *N*-(Substituted benzyl)deacetylthiocolchicines as Potent Cytotoxic and Antimitotic Compounds

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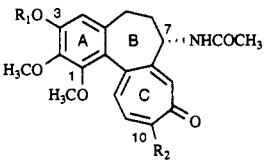
Three series of novel thiocolchicine analogs, *N*-acyl-, *N*-aroyl-, and *N*-(substituted benzyl)-deacetylthiocolchicinoids, have been synthesized and evaluated for their cytotoxicity against various tumor cell lines, especially solid tumor cell lines, and for their inhibitory effects on tubulin polymerization in vitro. Most of these compounds showed strong inhibitory effects on tubulin polymerization comparable to that obtained with thiocolchicine and greater than that obtained with colchicine. Only compounds with a long side chain at the C(7) position, such as **22–24**, did not inhibit tubulin polymerization. Several of the active *N*-aroyldeacetylthiocolchicine analogs had positive optical rotations, in contrast to the negative optical rotation observed with most colchicinoids. This property might be attributed to a reversal of biaryl configuration from the normal *aS* to *aR*. Therefore, the *N*-aroyl analogs were further evaluated by circular dichroism, which readily distinguishes between the *aS* and *aR* biaryl configurations. This latter technique demonstrated that the active *N*-aroyl analogs do have an *aS* configuration despite their positive optical rotations. However, comparison of ^{1}H NMR and UV spectral data of *N*-(substituted benzyl)-deacetylthiocolchicines with those of corresponding *N*-aroyldeacetylthiocolchicines suggested a different biaryl dihedral angle [even though these compounds have the same *aS* biaryl configuration]. The similar tubulin binding properties of these compounds suggest that a biaryl dihedral angle of 53° is not essential for colchicinoid–tubulin interaction. The increased cytotoxicity of *N*-(substituted benzyl)deacetylthiocolchicines compared to the *N*-aroyldeacetylthiocolchicines may be attributed to different lipophilicity, drug uptake, or drug metabolism in the tumor cells. The side chain at the C(7) position affects inhibition of tubulin polymerization and the cytotoxic activity of colchicinoids as a function of its size and its contribution to lipophilicity.

Introduction

Colchicine (1) (Figure 1), the major alkaloid isolated from *Colchicum autumnale*,[2] is a well-known tubulin toxin.[3] The biological effects of 1, such as its antiinflammatory and antitumor properties, probably derive from its tubulin binding activity.[2] Binding of 1 to tubulin prevents microtubule assembly and causes cells treated with the drug to arrest in mitosis.[3] Because of the severe toxicity of 1, many related compounds have been synthesized, with the goal of improving the therapeutic index and enhancing antitumor properties.[4-6]

Studies on the binding of colchicinoids to tubulin have indicated that the configuration and conformation (including the dihedral angle of the biaryl system) composed of the trimethoxyphenyl A ring and the tropolonic C ring are of major importance.[7] It has been proposed that compounds with an *aS* configured biaryl system bind readily to tubulin, while those with an *aR* configured biaryl system bind poorly.[7] In addition, a dihedral angle of −53° may be necessary for optimal binding of colchicinoids to tubulin.[8] Thus, modification of the biaryl configuration or conformation of colchicinoids should influence the binding of 1 analogs to tubulin. It has been shown that



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).

substituents at the C(1) position do affect the biaryl conformation, and these analogs have the expected reduced affinity for tubulin.[9] The side chain at the C(7) position could also change the tubulin affinity for colchicinoids by affecting their biaryl configuration or conformation through steric effects.[10] Changes in the biaryl configuration or conformation are usually accompanied by large changes in optical properties (e.g., optical rotation), and these properties have been attributed mainly to the asymmetry derived from the biaryl system rather than to the asymmetry derived from the C(7) position.[7] Thus, large changes in optical rotation could be indicative of changes in the biaryl conformation or configuration of the colchicinoids.

Previous studies have focused mainly on the potent antileukemic activity of 1 and related compounds synthesized by modifications of 1.[4-6] However, cytotoxicity studies against various solid tumor cell lines are limited. 3-Demethylthiocolchicine (2) (Figure 1) appears to have a broader antitumor spectrum and lower toxicity than 1

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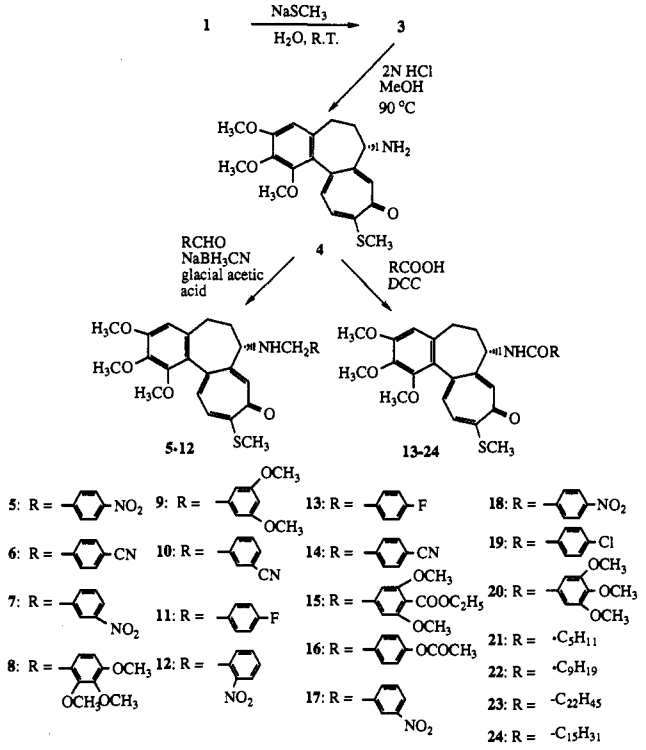
Scheme I. Syntheses of *N*-Acyl-, *N*-Aroyl-, and *N*-(Substituted benzyl)deacetylthiocolchicinoids



and thiocolchicine (**3**) (Figure 1).[3] Consequently, analogs of **2** may prove rewarding in a search for new chemotherapeutic agents, particularly for one directed at the colchicine site of tubulin, which has thus far not been exploited clinically in cancer treatment. In addition, the role of the substituent at the C(7) position of colchicinoids in tubulin binding is not entirely clear.

In this paper we report the synthesis and biological evaluation of three series of novel thiocolchicine analogs, *N*-acyl-, *N*-aroyl-, and *N*-(substituted benzyl)deacetylthiocolchicinoids, which possess bulky substituents in the side chain at the C(7) position and/or different lipophilicity. These compounds were evaluated for their cytotoxicity against SR leukemia cells and seven solid human tumor cell lines, non-small cell lung cancer A549/ATCC, small cell lung cancer DMS 114, colon carcinoma HCT-15, CNS carcinoma SNB-19, melanoma SK-MEL-28, ovarian carcinoma OVCAR-3, and renal carcinoma RXT-631. The newly synthesized compounds were evaluated for their effects on tubulin polymerization, in comparison to **1** and **3**, and their optical rotation and circular dichroic properties were examined.

Chemistry

As shown in Scheme I, all of the compounds were synthesized from deacetylthiocolchicine (**4**), which was prepared by established procedures.[11,12] Acylation of **4** with various commercially available substituted acids in the presence of DCC afforded *N*-acyl- and *N*-aroyldeacetylthiocolchicinoids.[13] Reductive alkylation of **4** by reaction with substituted benzaldehydes in the presence of sodium cyanoborohydride afforded *N*-(substituted benzyl)deacetylthiocolchicines.[14] Structures of the compounds were confirmed by spectral data and elemental analysis.

Conformational changes in the biaryl systems of *N*-aroyldeacetylthiocolchicinoids are suggested by differences

Table I. Chemical Shifts, Optical Properties, and Inhibition of Tubulin Polymerization of Corresponding *N*-Aroyl- and *N*-(Substituted benzyl)deacetylthiocolchicines

compd	chemical shift, δ (ppm) H-11	chemical shift, δ (ppm) H-12	optical rotation, deg	$[\theta] \times 10^{-4}$, deg cm²/dmol (nm)[a]	ITP[b] IC_{50}, μM
5	7.06	7.24	−179	−2.9(369)	2.8
18	7.18	7.42	+89.5	−3.3(367)	2.1
6	7.05	7.23	−158	−3.2(365)	2.3
14	7.17	7.42	+152	−3.2(368)	1.9
7	7.05	7.24	−212	−3.0(368)	2.8
17	7.09	7.30	−41.5	−3.2(369)	3.4
11	7.05	7.23	−161	−3.0(365)	2.3
13	7.13	7.37	−23.2	−3.4(367)	2.4

[a] [θ] is the term for molar ellipticity. The nm value is the minimum of the peak and is the value at which molar ellipticity was calculated. [b] ITP = inhibition of tubulin polymerization.

in their ^{1}H NMR spectra and optical rotations in comparison with those of the corresponding *N*-(substituted benzyl)- and *N*-acyldeacetylthiocolchicines (as well as normal colchicinoids). The chemical shifts for protons at the C(11) and the C(12) positions of *N*-aroyldeacetylthiocolchicines were shifted downfield relative to both *N*-(substituted benzyl)- and *N*-acyldeacetylthiocolchicines. For the *N*-aroyldeacetylthiocolchicines, **18**, **14**, **17**, and **13**, there were 0.04–0.12 (for H-11) and 0.06–0.18 (for H-12) ppm downshifts from those of the corresponding *N*-(substituted benzyl)deacetylthiocolchicines, **5**, **6**, **7**, and **11**, respectively (Table I). In addition, the optical rotations of these *N*-aroyldeacetylthiocolchicines were more positive compared to the corresponding *N*-(substituted benzyl)-deacetylthiocolchicinoids, which have the large negative optical rotations common to most colchicinoids (Table I). In particular, several *N*-aroyldeacetylthiocolchicinoids (**14** and **18–20**) were found to have positive optical rotations, even though these compounds all strongly inhibited tubulin polymerization (see below, Table II). Since this is opposite to the negative rotation observed with **1** and **3**, these potent new antitubulin agents appeared to have an *aR* biaryl configuration.[7,9] Although unexpected, this is not unprecedented. We recently described the preparation and detailed characterization of a series of six-membered B ring analogs of **3** with *aR* configurations, that were potent antitubulin compounds.[15] The activity of the six-membered B ring agents was thought to require a rapid *aR*–*aS* equilibrium, with the minor *aS* species binding to tubulin.

In order to confirm the *aR* configuration suggested by the positive optical rotations of these *N*-aroyl derivatives, we examined the circular dichroic properties of some of these compounds as well as those of the corresponding *N*-(substituted benzyl)deacetylthiocolchicines, which showed large negative optical rotations (Table I). All eight compounds show large negative bands in the near-UV region of the spectrum (Table I), indicating that the *aS* biaryl conformation of the phenyltropone ring system is retained in these thiocolchicinoids. The molar ellipticities of the *N*-benzyldeacetylthiocolchicinoids are less than or equal to the molar ellipticities of the corresponding *N*-aroyldeacetylthiocolchicinoids. Similarly, in the colchicine series, the molar ellipticities of several N-alkylated colchicinoids are less than the molar ellipticity of colchicine.[16] Thus, the minor differences in the near-UV CD bands of the *N*-aroyl- and *N*-benzyldeacetylthiocolchicinoids may be due to different electronic properties of an amine vs an amide at this position. Alternatively, the aromatic ring at C(7) of these compounds may be positioned differently in the *N*-aroyl- vs the *N*-benzyl-

Table II. Biological Evaluation of *N*-(Substituted benzyl)-, *N*-Aroyl-, and *N*-Acyldeacetylthiocolchicine Analogs

	cytotoxicity (log GI_{50})[a]								
compd	SR	A549/ATCC	DMS114	HCT-15	SNB-19	SK-MEL-5	OVCAR-3	RXF-631	ITP IC_{50}, μM[b]
1	–[c]	<–7.20	–6.95	<–7.20	<–7.20	<–7.20	<–7.20		4.2 ± 0.1
3									2.1 ± 0.07
5	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	2.8 ± 0.5
6	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	2.3 ± 0.2
7	<–4.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	2.8 ± 0.1
8	<–8.00	–7.60	<–8.00	<–8.00	–7.18	<–8.00	<–8.00	–7.80	2.5 ± 0.2
9	<–8.00	–7.43	–7.78	<–8.00	–7.17	<–8.00	–7.84	<–8.00	3.4 ± 0.2
10	<–8.12	<–8.12	<–8.12	<–8.12	<–8.12	<–8.12	<–8.12	<–8.12	2.5 ± 0.2
11	<–8.12	<–8.12	<–8.12	<–8.12	<–8.12	<–8.12	<–8.12	<–8.12	2.3 ± 0.1
12	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	3.2 ± 0.3
13	–7.78	–7.31	–7.57	–7.37	–7.54	–7.52	–7.74	–7.57	2.4 ± 0.2
14	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	1.9 ± 0.1
15		–6.37	–7.58	–6.35	–6.49	–7.34	–7.49	–6.63	ND[d]
16	–8.11	>–7.00	–7.84	>–7.00	–7.67	–7.71	–7.82	–7.56	2.1 ± 0.3
17	–8.07	–7.48	–7.78	–7.57	–7.57	–7.74	–7.72	–7.78	3.4 ± 0.2
18	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	2.1[e]
19	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	2.3 ± 0.1
20	<–8.00	7.77	<–8.00	–7.35	–7.58	<–8.00	<–8.00	–7.47	3.0 ± 0.1
21	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	<–8.00	2.3 ± 0.1
22									>40
23									>40
24									>40

[a] The cytotoxicity data were provided by the NCI. GI_{50} (M) is the concentration which caused 50% inhibition of tumor cell growth. [b] Concentrations which inhibit tubulin polymerization (ITP) by 50% compared to the control (±standard deviation; at least three independent determinations performed). [c] Not tested. [d] Not determined. Specimen poorly soluble in dimethyl sulfoxide. [e] Three values of 2.1 μM obtained.

deacetylthiocolchicinoids, resulting in different chemical shifts of the C(11) and C(12) protons due to either steric or electronic effects and in slightly different characteristics of the near-UV CD bands. The similar UV spectra of corresponding *N*-(substituted benzyl)- [e.g., **5**: λ_{max}^{EtOH} 382 (log ϵ 3.59), 258 (log ϵ 3.79), and 204 (log ϵ 4.01)] and *N*-aroyldeacetylthiocolchicines [e.g., **18**: λ_{max}^{EtOH} 385 (log ϵ 3.77), 257 (log ϵ 4.02), and 205 (log ϵ 4.18)] indicated that the differences in the chemical shifts of H-11 and H-12 are the results of the steric effects instead of electronic effects. Thus, these different chemical shifts suggested different biaryl dihedral angles (due to different shielding effects of the A ring on H-11 and H-12).[17]

Biological Results

All of the compounds were evaluated for inhibition of the polymerization of purified tubulin[18] according to the previous method.[15,19] The data are presented in terms of IC_{50} values (μM), which are the drug concentrations required to inhibit the extent of the polymerization reaction by 50%. In addition, the compounds were examined by the National Cancer Institute drug screening program, for their in vitro cytotoxicity against human SR leukemia cells and seven solid human tumor cell lines (non-small cell lung cancer A549/ATCC, small cell lung cancer DMS 114, colon carcinoma HCT-15, CNS carcinoma SNB-19, melanoma SK-MEL-28, ovarian carcinoma OVCAR-3, and renal carcinoma RXT-631).[20] The cytotoxicity results are presented in terms of GI_{50} values (M), which are the drug concentrations required to inhibit cell growth by 50%.

As a group, the *N*-(substituted benzyl)deacetylthiocolchicines were somewhat more potent than the *N*-aroyldeacetylthiocolchicines in the cytotoxicity assay. The *N*-aroyl compounds **13**, **15**, **16**, and **17** showed the least cytotoxicity. However, when compounds with similar substituents at C(7) are compared, this difference is not always notable. Compounds **7** and **11** were more cytotoxic than **17** and **13** in most cell lines, respectively, while compounds **5** and **18** as well as **6** and **14** had GI_{50} values of less than 10 nM in all eight human tumor cell lines.

Virtually all of the newly synthesized compounds are potent inhibitors of tubulin polymerization, with activities that differ little from that of **3**. The only exceptions were three derivatives with long-chain *N*-alkyl substituents (compounds **22**, **23**, and **24**) and possibly one *N*-aroyl derivative (**15**). Compound **15**, however, could not be evaluated because of poor solubility. Among the active compounds, one agent (**14**; IC_{50} = 1.9 μM) appeared to be more active than **3** (IC_{50} = 2.1 μM), although the difference is not statistically significant. The least active of the new agents was compound **9** (IC_{50} = 3.4 μM). The IC_{50} values obtained differ little when the *N*-aroyldeacetylthiocolchicine series is compared with the *N*-(substituted benzyl)-deacetylthiocolchicine series (compare the IC_{50} values of compound **5** and **18**, **6** and **14**, **7** and **17**, and **11** and **13**). These similar biological activities support the conclusion from the circular dichroic comparison: these compounds all have an *aS* biaryl configuration.

Discussion and Conclusions

Compounds with a substituted benzyl moiety (**5–12**) in the side chain at the C(7) position have large negative optical rotations similar to most **1** derivatives synthesized previously, indicating that the biaryl configurations of these compounds are not significantly changed. Interestingly, we found that *N*-aroyldeacetyl compounds (**13–20**) having both carbonyl and substituted phenyl moieties in the side chain showed greatly decreased negative or even positive optical rotations in contrast to those of compounds **5–12**, suggesting an *aR* biaryl configuration. However, the similar circular dichroic and tubulin binding properties of these *N*-aroyldeacetylthiocolchicines compared with those of corresponding *N*-(substituted benzyl)-deacetylthiocolchicines strongly indicate that the former retain the *aS* configuration. Alternatively, ^1H NMR and UV spectral data suggest a biaryl dihedral angle different from 53° for *N*-aroyldeacetylthiocolchicinoids. Thus, the similar inhibitory effects on tubulin polymerization of the *N*-aroyl- and *N*-(substituted benzyl)deacetylthiocolchicines suggest that a dihedral angle of 53° is not essential for colchicinoid–tubulin interactions. This conclusion was

further supported by our recent study on a series of six-membered B ring analogs of **3** which have a dihedral angle of 30°, but are potent inhibitors of tubulin polymerization.[15] In addition, similar tubulin binding potencies as well as cytotoxicity within each group (amine or amide derivatives) indicated that the electronic properties of the side chain do not have a large effect on the biological activities.

Even though the *N*-aroyl and *N*-(substituted benzyl)-deacetylthiocolchicinoids have comparable inhibitory activity on tubulin polymerization, these two series of compounds have different cytotoxicity. *N*-(Substituted benzyl)deacetylthiocolchicinoids as a group showed slightly higher cytotoxicity than *N*-aroyldeacetylthiocolchicinoids. This may result from different lipophilicity and drug uptake of these compounds and/or metabolism by tumor cells. As shown in a previous study, compounds with an amide moiety in the side chain are usually less lipophilic than the corresponding compounds with an amine side chain.[21] In addition, compounds with an amide side chain may be hydrolyzed in the tumor cells by various enzymes, such as amidases. However, in the compounds tested, no clear trend in the cytotoxicity was seen with either the substitution pattern or type of substituent on the phenyl ring in the side chain. For example, similar cytotoxicities were seen for compounds **5**, **7**, and **12**, which have para, meta, and ortho nitro groups on the phenyl ring, respectively. Potencies of compounds **5**, **6**, and **11** (amine derivatives) or **18**, **14**, and **19** (amide derivatives) with nitro, cyano, and halide groups were similar. Slightly decreased cytotoxicities were seen with compounds having multiple methoxy substituents (**8**, **9**, **15**, and **20**).

Compounds **22–24** possessing side chains of nine or more carbons at the C(7) position were inactive as inhibitors of tubulin polymerization, perhaps because the large side chain may interfere with a favorable interaction between the colchicinoid and tubulin, such as the interaction of the A and C rings with their binding domains on tubulin. We conclude that the side chain at the C(7) position affects inhibition of tubulin polymerization and the cytotoxic activity of colchicinoids as a function of its size and its contribution to lipophilicity. Further synthetic and biological studies will be done to confirm and clarify these conclusions.

Experimental Section

Chemistry. Melting points were measured with a Fisher-Johns melting point apparatus without correction. Optical rotations were determined with a Rudolph Research Autopol III polarimeter. IR spectra were recorded on a Perkin-Elmer Model 1320 spectrometer. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker AC-300 spectrometer with Me_4Si (TMS) as the internal reference. Circular dichroic spectra were obtained on a JASCO Model J-20 ORD/UV-5 spectropolarimeter equipped with a Sproul Scientific SS15-2CD modification. Solutions in ethanol (30–50 μM) were observed at ambient temperature (23 °C). Spectra and baselines were recorded in duplicate. Elemental analyses were determined 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 used for column chromatography. Colchicine was purchased from Aldrich, Inc.

General Procedure for Synthesizing *N*-(Substituted Benzyl)deacetylthiocolchicines (5–12). A mixture of **4**, the corresponding aldehyde, and sodium cyanoborohydride (molar ratio of 1:1.5:1.2) in anhydrous CH_2Cl_2 containing glacial acetic acid (a few drops) was stirred at room temperature for 2 h. The reaction mixture was poured into water, neutralized with 10% $NaHCO_3$ to a pH of 7, and extracted three times with $CHCl_3$. The $CHCl_3$ extract was washed with brine, dried over anhydrous Na_2SO_4, and concentration. The residue was chromatographed on preparative TLC plates using $CHCl_3$–MeOH (95:5) as eluant.

***N*-(4′-Nitrobenzyl)deacetylthiocolchicine (5)**: yield 49% (starting with 160.2 mg of **4**); amorphous; $[\alpha]^{20}_D$ −179° (*c* 0.53, $CHCl_3$); IR (KBr) 3320 (NH), 2930 and 2830 (aliphatic CH), and 1600 (CO, tropolone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.20–2.48 (m, 4 H, H-5, 6), 2.46 (s, 3 H, SCH_3-10), 3.42–3.51 (dd, *J* = 10.9, 6.27 Hz, 1 H, H-7), 3.57 (d, *J* = 14.0 Hz, 1 H, NCH_2-7), 3.59 (s, 3 H, OCH_3-1), 3.81 (d, *J* = 14.0, 1 H, NCH_2-7), 3.91 (s, 6 H, OCH_3-2, 3), 6.53 (s, 1 H, H-4), 7.06 (d, *J* = 10.4 Hz, 1 H, H-11), 7.24 (d, *J* = 10.4 Hz, 1 H, H-12), 7.44 (d, *J* = 8.5 Hz, 2 H, H-2′,6′), 7.67 (s, 1 H, H-8), and 8.11 (d, *J* = 8.5 Hz, 2 H, H-3′,5′).

Anal. ($C_{27}H_{28}O_6N_2S\cdot H_2O$) C, H, N, S.

***N*-(4′-Cyanobenzyl)deacetylthiocolchicine (6)**: yield 65% (starting with 234.5 mg of **4**); amorphous; $[\alpha]^{20}_D$ −158° (*c* 0.60, $CHCl_3$); IR (KBr) 3315 (NH), 2915 and 2830 (aliphatic CH), 2220 (CN), and 1600 (CO, tropolone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.68–2.53 (m, 4 H, H-5, 6), 2.45 (s, 3 H, SCH_3-10), 3.43 (dd, *J* = 11.0, 6.36 Hz, 1 H, H-7), 3.51 (d, *J* = 14.0 Hz, 1 H, NCH_2-7), 3.58 (s, 3 H, OCH_3-1), 3.76 (d, *J* = 14.0 Hz, 1 H, NCH_2-7), 3.91 (s, 6 H, OCH_3-2, 3), 6.53 (s, 1 H, H-4), 7.05 (d, *J* = 10.4 Hz, 1 H, H-11), 7.23 (d, *J* = 10.4 Hz, 1 H, H-12), 7.38 (d, *J* = 8.06 Hz, 2 H, H-2′,6′), 7.54 (d, *J* = 8.06 Hz, 2 H, H-3′5′), and 7.67 (s, 1 H, H-8).

Anal. ($C_{28}H_{28}O_4N_2S\cdot H_2O$) C, H, N, S.

***N*-(3′-Nitrobenzyl)deacetylthiocolchicine (7)**: yield 55% (starting with 171.6 mg of **4**); amorphous; $[\alpha]^{20}_D$ −212° (*c* 0.57, $CHCl_3$); IR (KBr) 3300 (NH), 2920 and 2820 (aliphatic CH), 1600 (CO, tropolone), and 1525 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.19–2.53 (m, 4 H, H-5, 6), 2.46 (s, 3 H, SCH_3-10), 3.44 (dd, *J* = 10.9, 6.24 Hz, 1 H, H-7), 3.55 (d, *J* = 13.8 Hz, 1 H, NCH_2-7), 3.58 (s, 3 H, OCH_3-1), 3.83 (d, *J* = 13.8 Hz, 1 H, NCH_2-7), 3.90 (s, 6 H, OCH_3-2, 3), 6.53 (s, 1 H, H-4), 7.05 (d, *J* = 10.4 Hz, 1 H, H-11), 7.24 (d, *J* = 10.4 Hz, 1 H, H-12), 7.43 (t, *J* = 7.73 Hz, 1 H, H-5′), 7.64 (d, *J* = 7.73 Hz, 1 H, H-6′), 7.65 (s, 1 H, H-8), 8.04 (d, *J* = 7.73 Hz, 1 H, H-4′), and 8.07 (s, 1 H, H-2′).

Anal. ($C_{27}H_{28}O_6N_2S$) C, H, N, S.

***N*-(2′,3′,4′-Trimethoxybenzyl)deacetylthiocolchicine (8)**: yield 64% (starting with 222.0 mg of **4**); amorphous; $[\alpha]^{20}_D$ −145° (*c* 0.62, $CHCl_3$); IR (KBr) 3320 (NH), 2925 and 2825 (aliphatic CH), 1600 (CO, tropolone), and 1270 (C–O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.13–2.45 (m, 4 H, H-5, 6), 2.45 (s, 3 H, SCH_3-10), 3.28 (d, *J* = 12.4 Hz, 1 H, NCH_2-7), 3.44 (dd, *J* = 10.9, 6.20 Hz, 1 H, H-7), 3.63 (s, 3 H, OCH_3-1), 3.65 (d, *J* = 12.4 Hz, 1 H, NCH_2-7), 3.82 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), and 3.93 (s, 3 H, OCH_3), 6.53 (s, 1 H, H-4), 6.53 (d, *J* = 8.4 Hz, 1 H, H-5′), 6.84 (d, *J* = 8.4 Hz, 1 H, H-6′), 7.04 (d, *J* = 10.4 Hz, 1 H, H-11), 7.23 (d, *J* = 10.4 Hz, 1 H, H-12), and 7.77 (s, 1 H, H-8).

Anal. ($C_{30}H_{35}O_7NS$) C, H, N, S.

***N*-(3′,5′-Dimethoxybenzyl)deacetylthiocolchicine (9)**: yield 79% (starting with 176.0 mg of **4**); amorphous; $[\alpha]^{20}_D$ −183° (*c* 0.67, $CHCl_3$); IR (KBr) 3325 (NH), 2925 and 2985 (aliphatic CH), and 1600 (CO, tropolone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.67–2.50 (m, 4 H, H-5,6), 2.45 (s, 3 H, SCH_3-10), 3.36 (d, *J* = 13.2 Hz, 1 H, NCH_2-7), 3.44 (dd, *J* = 10.9, 6.20 Hz, 1 H, H-7), 3.54 (s, 3 H, OCH_3-1), 3.68 (d, *J* = 13.2 Hz, 1 H, NCH_2-7), 3.74 (s, 6 H, OCH_3-3′,5′), 3.91 (s, 6 H, OCH_3-2,3), 6.28 (t, *J* = 2.21 Hz, 1 H, H-4′), 6.39 (s, 2 H, H-2′,6′), 6.52 (s, 1 H, H-4), 7.04 (d, *J* = 10.3 Hz, 1 H, H-11), 7.23 (d, *J* = 10.3 Hz, 1 H, H-12), and 7.74 (s, 1 H, H-8).

Anal. ($C_{29}H_{33}O_6NS$) C, H, N, S.

***N*-(3′-Cyanobenzyl)deacetylthiocolchicine (10)**: yield 77% (starting with 151.0 mg of **4**); amorphous; $[\alpha]^{20}_D$ −257° (*c* 0.51, $CHCl_3$); IR (KBr) 3315 (NH), 2925 and 2825 (aliphatic CH), 2220 (CN), and 1600 (CO, tropolone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.69–2.64 (m, 4 H, H-5, 6), 2.46 (s, 3 H, SCH_3-10), 3.42 (dd, *J* = 10.7, 6.02 Hz, 1 H, H-7), 3.48 (d, *J* = 13.8 Hz, 1 H, NCH_2-7), 3.59 (s, 3 H, OCH_3-1), 3.75 (d, *J* = 13.8 Hz, 1 H, NCH_2-7), 3.91 (s, 6 H, OCH_3-2,3), 6.55 (s, 1 H, H-4), 7.07 (d, *J* = 10.4 Hz, 1 H, H-11), 7.25 (d, *J* = 10.4 Hz, 1 H, H-12), 7.36 (t, *J* = 7.98 Hz, 1 H, H-5′), 7.48 (d, *J* = 7.98 Hz, 1 H, H-4′), 7.53 (s, 1 H, H-2′), 7.54 (d, *J* = 7.98 Hz, 1 H, H-6′), and 7.65 (s, 1 H, H-8).

Anal. ($C_{28}H_{28}O_4N_2S$) C, H, N, S.

N-(4'-Fluorobenzyl)deacetylthiocolchicine (11): yield 64% (starting with 166.8 mg of 4); amorphous; $[\alpha]^{20}_D$ −161° (c 0.54, $CHCl_3$); IR (KBr) 3320 (NH), 2925 and 2825 (aliphatic CH), and 1600 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.14–2.51 (m, 4 H, H-5,6), 2.45 (s, 3 H, SCH_3-10), 3.40 (d, J = 9.87 Hz, 1 H, NCH_2-7), 3.43 (dd, J = 13.8, 6.25 Hz, 1 H, H-7), 3.57 (s, 3 H, OCH_3-1), 3.67 (d, J = 9.87 Hz, 1 H, NCH_2-7), 3.91 (s, 6 H, OCH_3-2,3), 6.53 (s, 1 H, H-4), 6.91 (t, J = 8.51 Hz, 2 H, H-3',5'), 7.05 (d, J = 10.2 Hz, 1 H, H-11), 7.23 (d, J = 10.2 Hz, 1 H, H-12), 7.24 (dd, J = 12.5, 8.04 Hz, 2 H, H-2',6'), and 7.73 (s, 1 H, H-8).

Anal. ($C_{27}H_{28}O_4NSF$) C, H, N, S.

N-(2'-Nitrobenzyl)deacetylthiocolchicine (12): yield 32% (starting with 170.2 mg of 4); amorphous; $[\alpha]^{20}_D$ −225° (c 0.50, $CHCl_3$); IR (KBr) 3320 (NH), 2920 and 2820 (aliphatic CH), and 1600 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.69–2.52 (m, 4 H, H-5,6), 2.46 (s, 3 H, SCH_3-10), 3.45 (dd, J = 10.8, 6.26 Hz, 1 H, H-7), 3.63 (s, 3 H, OCH_3-1), 3.78 (m, 2 H, NCH_2-7), 3.92 (s, 6 H, OCH_3-2,3), 6.53 (s, 1 H, H-4), 7.06 (d, J = 10.4 Hz, 1 H, H-11), 7.24 (d, J = 10.4 Hz, 1 H, H-12), 7.37 (dt, J = 8.42, 1.87 Hz, 1 H, H-4'), 7.57 (m, 2 H, H-5',6'), 7.77 (s, 1 H, H-8), and 7.88 (d, J = 8.42 Hz, 1 H, H-3').

Anal. ($C_{27}H_{28}O_6N_2S \cdot {}^1/_4H_2O$) C, H, N, S.

General Procedures for Synthesizing N-Acyl and N-Aroyldeacetylthiocolchicines (13–21). A mixture of 4 and the corresponding acid in anhydrous CH_2Cl_2 containing DCC (molar ratio of 1:1.2:1.2) was stirred at room temperature for 24 h. The reaction mixture was filtered, and the filtrate was washed with EtOAc. After concentration, the residue was poured into water and extracted with EtOAc three times. The EtOAc extract was washed with 10% $NaHCO_3$ and brine until pH = 7, dried over anhydrous Na_2SO_4, and concentrated to give an oil residue. This residue was chromatographed on preparative TLC plates using $CHCl_3$–MeOH (97:3) as eluant. Crystallization from CH_2Cl_2–Et_2O afforded the N-acyl- and N-aroyldeacetylthiocolchicine.

N-(4'-Fluorobenzoyl)deacetylthiocolchicine (13): yield 72% (starting with 148.7 mg of 4); crystallization from $CHCl_3$–MeOH–Et_2O afforded light yellow crystals; mp 279–280 °C dec; $[\alpha]^{20}_D$ −23.2° (c 0.84, $CHCl_3$); IR (KBr) 3325 (NH), 2940 (aliphatic CH), 1655 (CO, amide), and 1600 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.13–2.58 (m, 4 H, H-5,6), 2.45 (s, 3 H, SCH_3-10), 3.75 (s, 3 H, OCH_3-1), 3.91 (s, 3 H, OCH_3-2), 3.97 (s, 3 H, OCH_3-3), 4.93 (m, 1 H, H-7), 6.56 (s, 1 H, H-4), 6.84 (d, J = 8.53 Hz, 1 H, H-3' or H-5'), 6.86 (d, J = 8.53 Hz, 1 H, H-5' or H-3'), 7.13 (d, J = 10.5 Hz, 1 H, H-11), 7.37 (d, J = 10.5 Hz, 1 H, H-12), 7.63 (s, 1 H, H-8), 7.87 (d, J = 8.53 Hz, 1 H, H-2' or H-6'), 7.90 (d, J = 8.53 Hz, 1 H, H-6' or H-2'), and 8.61 (d, J = 7.19 Hz, 1 H, NH-7).

Anal. ($C_{27}H_{26}O_5NSF \cdot H_2O$) C, H, N, S.

N-(4'-Cyanobenzoyl)deacetylthiocolchicine (14): yield 62% (starting with 69.4 mg of 4); crystallization from $CHCl_3$–MeOH–Et_2O gave light yellow fine needle crystals; mp 281–282 °C dec; $[\alpha]^{20}_D$ +151.6° (c 0.57, $CHCl_3$); IR (KBr) 3220 (NH), 2925 and 2820 (aliphatic CH), 2220 (CN), 1655 (CO, amide), and 1595 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.05–2.60 (m, 4 H, H-5,6), 2.57 (s, 3 H, SCH_3-10), 3.77 (s, 3 H, OCH_3-1), 3.92 (s, 3 H, OCH_3-2), 3.98 (s, 3 H, OCH_3-3), 4.95 (m, 1 H, H-7), 6.57 (s, 1 H, H-4), 7.17 (d, J = 10.6 Hz, 1 H, H-11), 7.42 (d, J = 10.6 Hz, 1 H, H-12), 7.63 (s, 1 H, H-8), 7.50 (d, J = 8.00 Hz, 2 H, H-3',5'), 8.02 (d, J = 8.00 Hz, 2 H, H-2',6'), and 9.05 (d, J = 7.85 Hz, 1 H, NH-7).

Anal. ($C_{28}H_{26}O_5N_2S$) C, H, N, S.

N-(4'-((Ethoxycarbonyl)oxy)-3',5'-dimethoxybenzoyl)deacetylthiocolchicine (15): yield 78% (starting with 57.4 mg of 4); mp 226–228 °C; $[\alpha]^{20}_D$ +7.83° (c 0.69, $CHCl_3$); IR (KBr) 3320 (NH), 2915 and 2840 (aliphatic CH), 1760 (CO, ester), and 1600 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (t, J = 7.14 Hz, 3 H, $OCOOCH_2CH_3$-4'), 2.04–2.63 (m, 4 H, H-5,6), 2.43 (s, 3 H, SCH_3-10), 3.70 (s, 6 H, OCH_3), 3.72 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.96 (s, 3 H, OCH_3), 4.25 (q, J = 7.14 Hz, 2 H, $OCOOCH_2CH_3$-4'), 4.87 (m, 1 H, H-7), 6.54 (s, 1 H, H-4), 7.12 (d, J = 10.6 Hz, 1 H, H-11), 7.21 (s, 2 H, H-2',6'), 7.36 (d, J = 10.6 Hz, 1 H, H-12), 7.69 (s, 1 H, H-8), and 8.79 (d, J = 6.24 Hz, 1 H, NH-7).

Anal. ($C_{32}H_{35}O_{10}NS \cdot {}^1/_2H_2O$) C, H, N, S.

N-(4'-Acetoxybenzoyl)deacetylthiocolchicine (16): yield 78% (starting with 53.5 mg of 4); amorphous; $[\alpha]^{20}_D$ −36.3° (c 0.57, $CHCl_3$); IR (KBr) 3320 (NH), 2930 and 2859 (aliphatic CH), 1755 (CO, ester), 1650 (CO, amide), and 1600 (CO topolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (t, J = 6.80 Hz, 3 H, H_3CCH_2OCOO-4'), 2.11–2.55 (m, 4 H, H-5,6), 2.42 (s, 3 H, SCH_3-10), 3.73 (s, 3 H, OCH_3-1), 3.89 (s, 3 H, OCH_3-2), 3.95 (s, 3 H, OCH_3-3), 4.24 (q, J = 6.80 Hz, 2 H, H_3CCH_2OCOO-4'), 4.91 (m, 1 H, H-7), 6.55 (s, 1 H, H-4), 6.97 (d, J = 8.46 Hz, 2 H, H-3',5'), 7.11 (d, J = 10.6 Hz, 1 H, H-11), 7.35 (d, J = 10.6 Hz, 1 H, H-12), 7.64 (s, 1 H, H-8), 7.90 (d, J = 8.4 Hz, 2 H, H-2',6'), and 8.41 (d, J = 7.14 Hz, 1 H, NH-7).

Anal. ($C_{29}H_{29}O_7NS \cdot {}^3/_2H_2O$) C, H, N, S.

N-(3'-Nitrobenzoyl)deacetylthiocolchicine (17): yield 100% (starting with 289.3 mg of 4); crystallization from CH_2Cl_2–Et_2O afforded light yellow fine needle crystals; mp 305–308 °C dec; $[\alpha]^{20}_D$ −41.5° (c 0.55, $CHCl_3$); IR (KBr) 3320 (NH), 2915 and 2840 (aliphatic CH), 1660 (CO, amide), and 1625 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.05–2.56 (m, 4 H, H-5,6), 2.37 (s, 3 H, SCH_3-10), 3.61 (s, 3 H, OCH_3-1), 3.81 (s, 3 H, OCH_3-2), 3.87 (s, 3 H, OCH_3-3), 4.75 (m, 1 H, H-7), 6.51 (s, 1 H, H-4), 7.09 (d, J = 10.5 Hz, 1 H, H-11), 7.17 (s, 1 H, H-8), 7.30 (d, J = 10.5 Hz, 1 H, H-12), 7.50 (t, J = 8.0 Hz, 1 H, H-5'), 8.10 (d, J = 8.00 Hz, 1 H, H-6'), 8.22 (dd, J = 8.00, 1.76 Hz, 1 H, H-4'), 8.66 (t, J = 1.76 Hz, 1 H, H-2'), and 9.81 (d, J = 7.16 Hz, 1 H, NH-7).

Anal. ($C_{27}H_{26}O_7N_2S$) C, H, N, S.

N-(4'-Nitrobenzoyl)deacetylthiocolchicine (18): yield 82% (starting with 180.0 mg of 4); mp 285–287 °C dec; $[\alpha]^{20}_D$ +89.5° (c 0.86, $CHCl_3$); IR (KBr) 3240 (NH, amide), 2940 and 2840 (aliphatic CH), 1670 (CO, amide), and 1600 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.16–2.60 (m, 4 H, H-5,6), 2.49 (s, 3 H, SCH_3-10), 3.77 (s, 3 H, OCH_3-1), 3.92 (s, 3 H, OCH_3-2), 3.98 (s, 3 H, OCH_3-3), 4.95 (m, 1 H, H-7), 6.57 (s, 1 H, H-4), 7.18 (d, J = 10.5 Hz, 1 H, H-11), 7.42 (d, J = 10.5 Hz, 1 H, H-12), 7.67 (s, 1 H, H-8), 8.01 (d, J = 9.04 Hz, 2 H, H-2',6'), 8.05 (d, J = 9.04 Hz, 2 H, H-3',5'), and 9.15 (d, J = 7.43 Hz, 1 H, NH-7).

Anal. ($C_{27}H_{26}O_7N_2S \cdot {}^1/_4H_2O$) C, H, N, S.

N-(4'-Chlorobenzoyl)deacetylthiocolchicine (19): yield 92% (starting with 205.5 mg of 4); mp 291–293 °C dec; $[\alpha]^{20}_D$ +59.4° (c 1.09, $CHCl_3$); IR (KBr) 3320 (NH), 2915 and 2845 (aliphatic CH), 1660 (CO, amide), and 1620 (CO, tropolone); ^1H NMR ($CDCl_3$) δ 2.12–2.58 (m, 4 H, H-5,6), 2.54 (s, 3 H, SCH_3-10), 3.76 (s, 3 H, OCH_3-1), 3.92 (s, 3 H, OCH_3-2), 3.97 (s, 3 H, OCH_3-3), 4.92 (m, 1 H, H-7), 6.56 (s, 1 H, H-4), 7.13 (d, J = 10.4 Hz, 1 H, H-11), 7.17 (d, J = 8.27 Hz, 2 H, H-2',5'), 7.37 (d, J = 10.4 Hz, 1 H, H-12), 7.59 (s, 1 H, H-8), 7.81 (d, J = 8.27 Hz, 2 H, H-2',6'), and 8.45 (br, 1 H, NH-7).

Anal. ($C_{27}H_{26}O_5NSCl$) C, H, N, S, Cl.

N-(3',4',5'-Trimethoxybenzoyl)deacetylthiocolchicine (20): yield 79% (starting with 242.3 mg of 4); amorphous; $[\alpha]^{20}_D$ +32.6° (c 0.54, $CHCl_3$); IR (KBr) 3320 (NH), 2920 and 2820 (aliphatic CH), 1640 (CO, amide), and 1580 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.05–2.62 (m, 4 H, H-5,6), 2.43 (s, 3 H, SCH_3-10), 3.70 (s, 6 H, OCH_3), 3.73 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 4.90 (m, 1 H, H-7), 6.53 (s, 1 H, H-4), 7.13 (d, J = 10.6 Hz, 1 H, H-11), 7.24 (s, 2 H, H-2',6'), 7.37 (d, J = 10.6 Hz, 1 H, H-12), 7.74 (s, 1 H, H-8), and 8.78 (d, J = 6.89 Hz, 1 H, NH-7).

Anal. ($C_{30}H_{33}O_8NS \cdot H_2O$) C, H, N, S.

N-Hexanoyldeacetylthiocolchicine (21): yield 78% (starting with 202.1 mg of 4); amorphous; $[\alpha]^{20}_D$ −282° (c 0.24, $CHCl_3$); IR (KBr) 3260 (NH, amide), 2930 and 2860 (aliphatic CH), 1675 (CO, amide), and 1605 (CO, tropolone) cm^{-1}; ^1H NMR ($CHCl_3$) δ 1.25 (m, 11 H, $C_5H_{11}CONH$-7), 2.42 (s, 3 H, SCH_3-10), 3.64 (s, 3 H, OCH_3-1), 3.88 (s, 3 H, OCH_3-2), 3.90 (s, 3 H, OCH_3-3), 4.65 (m, 1 H, H-7), 6.37 (d, J = 7.60 Hz, 1 H, NH-7), 6.51 (s, 1 H, H-4), 7.04 (d, J = 10.4 Hz, 1 H, H-11), 7.24 (s, 1 H, H-8), and 7.28 (d, J = 10.4 Hz, 1 H, H-12).

Anal. ($C_{26}H_{33}O_5NS$) C, H, N, S.

N-Decanoyldeacetylthiocolchicine (22): yield 63% (starting with 255.2 mg of 4); amorphous; $[\alpha]^{20}_D$ −244° (c 0.32, $CHCl_3$); IR (KBr) 3300 (NH, amide), 2930 and 2860 (aliphatic CH), 1650 (CO, amide), and 1605 (CO, tropolone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, J = 6.2 Hz, 3 H, $CH_3(CH_2)_8CONH$-7), 1.24 (br, 16 H, $CH_3(CH_2)_8CONH$-7), 1.72–2.57 (m, 4 H, CH_2-5,6), 2.43 (s, 3 H, SCH_3-10), 3.66 (s, 3 H, OCH_3-1), 3.90 (s, 3 H, OCH_3-2), 3.94 (s, 3 H, OCH_3-3), 4.67 (m, 1 H, H-7), 6.30 (d, J = 7.24 Hz, 1 H,

NH-7), 6.53 (s, 1 H, H-4), 7.04 (d, J = 10.5 Hz, 1 H, H-11), 7.23 (s, 1 H, H-8), and 7.28 (d, J = 10.5 Hz, 1 H, H-12).

Anal. ($C_{30}H_{41}O_5NS \cdot {}^1/_2H_2O$) C, H, N, S.

***N*-Tricosanoyldeacetylthiocolchicine (23)**: yield 68% (starting with 215.4 mg of 4); amorphous; $[\alpha]^{20}_D$ −139° (*c* 0.87, $CHCl_3$); IR (KBr) 3300 (NH, amide), 2930 and 2860 (aliphatic CH), 1650 (CO, amide), and 1605 (CO, tropolone) cm^{-1}; ^1H NMR δ 1.25 (br, 43 H, $C_{21}H_{43}CH_2CONH$-7), 1.85 (m, 4 H, H-5,6), 2.23 (t, J = 7.53 Hz, 2 H, $C_{21}H_{43}CH_2CONH$-7), 2.41 (s, 3 H, SCH_3-10), 3.67 (s, 3 H, OCH_3-1), 3.90 (s, 3 H, OCH_3-2), 3.95 (s, 3 H, OCH_3-3), 4.70 (m, 1 H, H-7), 6.53 (s, 1 H, H-4), 7.07 (d, J = 10.4 Hz, 1 H, H-11), 7.14 (br, 1 H, NH-7), 7.31 (d, J = 10.4 Hz, 1 H, H-12), and 7.37 (s, 1 H, H-8).

Anal. ($C_{43}H_{67}O_5NS$) C, H, N, S.

***N*-Palmitoyldeacetylthiocolchicine (24)**: yield 100% (starting with 202.9 and mg of 4); amorphous; $[\alpha]^{20}_D$ −182° (*c* 0.50, $CHCl_3$); IR (KBr) 3300 (NH, amide), 2980 and 2840 (aliphatic CH), 1650 (CO, amide), and 1605 (CO, tropolone); ^1H NMR ($CDCl_3$) δ 1.25 (br, 27 H, $H_{27}C_{13}H_4C_2CONH$-7), 1.63 (br, 4 H, $H_{27}C_{13}H_4C_2CONH$-7), 1.76–2.57 (m, 4 H, H-5,6), 2.43 (s, 3 H, SCH_3-10), 3.67 (s, 3 H, OCH_3-1), 3.90 (s, 3 H, OCH_3-2), 3.94 (s, 3 H, OCH_3-3), 4.67 (m, 1 H, H-7), 6.36 (d, J = 7.24 Hz, 1 H, NH-7), 6.53 (s, 1 H, H-4), 7.05 (d, J = 10.5 Hz, 1 H, H-11), 7.24 (s, 1 H, H-8), and 7.28 (d, J = 10.5 Hz, 1 H, H-12).

Anal. ($C_{36}H_{53}O_5NS \cdot {}^1/_2H_2O$) C, H, N, S.

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