ANTITUMOR AGENTS. 192+. ANTITUBULIN EFFECT AND CYTOTOXICITY OF C(7)-OXYGENATED ALLOCOLCHICINOIDS

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This paper is dedicated to the memory of Dr Miroslav Protiva, a close friend of Arnold Brossi, who died in Prague, Czech Republic on March 7th 1998.

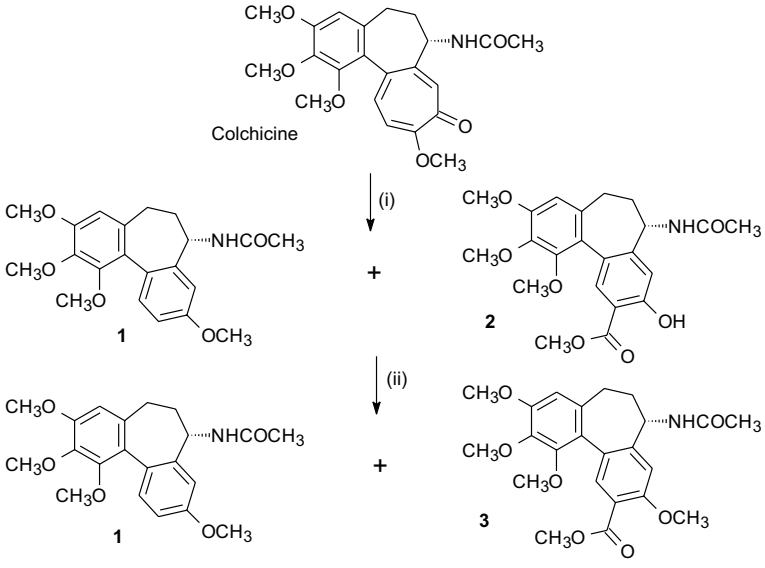
Two allocolchicinoids **6** and **8**, prepared from colchicine, together with allo compounds **9**–**11**, made from **6** by reduction and regiodemethylation, were evaluated for antitubulin and antitumor activities. Structures of **6**, **8**, and **10** were confirmed by X-ray crystallographic analysis. Compounds **6**, **8**, and **9** have high tubulin binding affinity and display potent inhibitory activities against tubulin polymerization and solid human tumor cell lines. Particularly, drug-resistant KB cell lines, including KB-7d, KB-VCR, and KB-CPT, do not show marked resistance to these compounds.

Key words: Colchicinoids; Colchicine; Allocolchicine; Antimitosis; Antitumor agents.

The family of allocolchicinoids in which the tropolone ring C of colchicine has been converted into a benzenoid ring is represented in nature by several examples[2]. *N*-Acetylcolchinol methyl ether (**1**, Scheme 1) obtained

+ For Part 191, see ref.[1].

from colchicine[3] played an important role in establishing the structure of colchicine and also is a representative of this group. Allo compound **1**, prepared here by Iorio's procedure[4], has the same α*S*,7*S* absolute configuration as natural colchicine[5], and has been proven to be a valuable internal standard because of its potent activity in assays measuring interactions with tubulin[6–8].



(i) 30% H_2O_2, 90 °C; (ii) 1. CH_3I, K_2CO_3, Et_3N, 2. chromatography

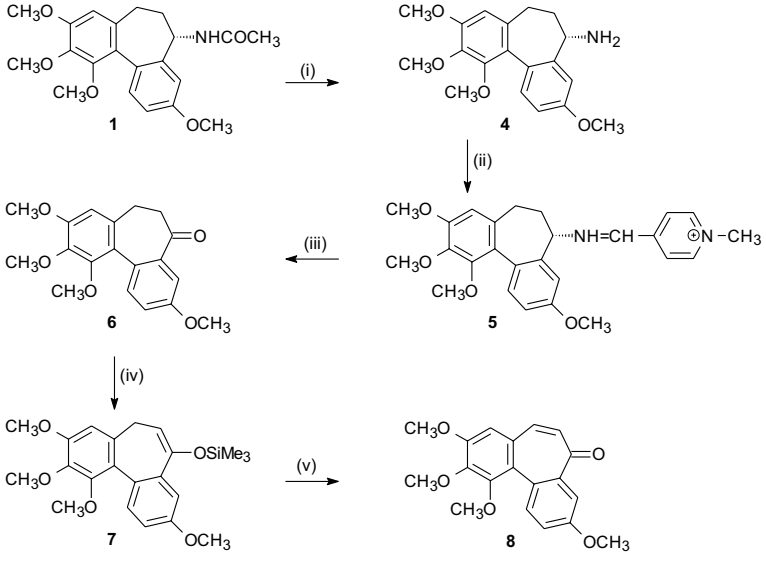
SCHEME 1

Allocolchicinoids, when assayed *in vivo* in the p388 lymphocytic leukemia assay in mice, were found to be much less potent then expected[9]. However, they continue to attract attention since they are available by total synthesis using modern methods to construct the biphenyl backbone in these compounds[10]. The Suzuki cross coupling recently regarded to be a superior method, may well be of practical significance in such attempts[11].

Results of recent studies have shown that the acetamido group in thiocolchicine[12] and in methyl thioether analog of **1** (ref.[13]), can be replaced by an oxygen functionality without weakening their interaction with tubulin. It seemed, therefore, of interest to prepare C(7)-oxygenated analogs of **1** and to measure their effects on tubulin and the growth of several human tumor cell lines.

Compound **1**, used as the starting material to prepare the desired compounds, was prepared from colchicine by a modification of Iorio's procedure[4]. In an attempt to resolve the separation problem, we removed impurities and isolated 1 and **2** as a mixture. Then, we estimated that by blocking the hydroxy group of **2**, the protected product **3** should have different chromatographic behavior from that of **1** and could be easily separated. Thus, we used iodomethane in the presence of K_2CO_3 and Et_4NF as catalysts to protect the hydroxy group of **2** as a methoxy group and leave **1** unattacked. This method allowed a much better separation of by-product **2** and gave **1** in a 33% yield (Scheme 1).

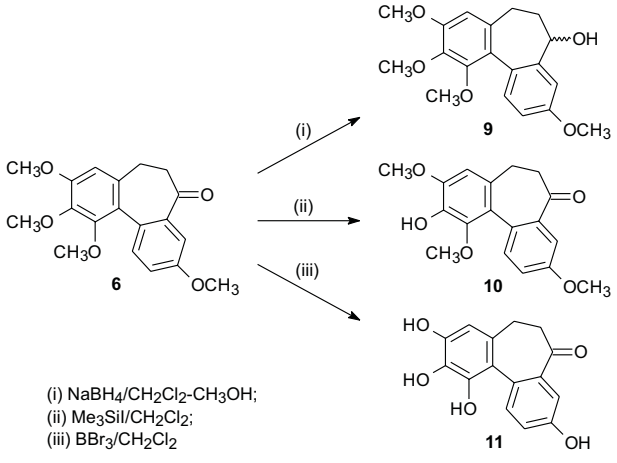
Hydrolysis of the amide group under acidic conditions gave **4**. Ketone **6**, already obtained by total synthesis[14], was prepared from **4** by a Schiff base equilibration followed by hydrolysis, a procedure successfully used in the thiocolchicine series[13]. Unsaturated ketone **8**, also obtained by total synthesis[14], could not be achieved by dehydrogenation of **6** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone or SeO_2, but was finally accomplished by the formation of trimethylsilyl ether **7** using lithium diisopropylamide/trimethylsilyl chloride, which was then oxidized with palladium acetate in acetonitrile[15] (Scheme 2).



(i) 2N HCl/CH_3OH; (ii) 4-formyl-1-methylpyridinium benzenesulfonate/CH_2Cl_2-DMF; (iii) 1. DBU, 2. oxalic acid/H_2O; (iv) 1. lithium diisopropylamine/THF, 2. Me_3SiCl; (v) $Pd(OAc)_2/CH_3CN$

SCHEME 2

Reducing the ketone **6** with sodium borohydride yielded racemic alcohol **9**. Concentrated sulfuric acid was previously used to cleave the C(2) methyl group of colchicine and thiocolchicine[15,17]. In order to improve regioselectivity and to avoid the difficulty in handling concentrated H_2SO_4, we used Me_3SiI, a mild ether-cleavage reagent[18], to selectively remove only the C(2) methyl group from **6** to give **10** in a 54% yield. Exhaustive demethylation was performed with excess BBr_3 as reported earlier in the thiocolchicine series[19], and yielding tetraphenol **11** (Scheme 3). Ketones **6** and **8**, prepared here by partial synthesis from natural colchicine gave physical data which were identical with those reported for the synthetic compounds.



SCHEME 3

The structures of **6**, **8**, and **10** have been confirmed by X-ray crystallographic analysis. A view of the solid-state conformation of **6** is provided in Fig. 1. The C(4a)–C(11b)–C(11a)–C(7a) and C(1)–C(11b)–C(11a)–C(11) torsion angles are –47.5(2) and –46.4(2)°, respectively. A dihedral angle of –47.2° is found between the least-square plane through atoms of the approximately planar phenyl A ring and those of the slightly puckered tropolone C ring. Compound **8** with a dihedral angle of –38.4° is illustrated in Fig. 2. By comparing the three torsion angles C(11b)–C(4a)–C(5)–C(6), C(5)–C(6)–C(7)–O(18), and O(18)–C(7)–C(7a)–C(8) in both compounds, we found that all three angles in **8** were invariably less than those in **9** (24.6(9)° in **11** and 71.2(1)° in **9**; 144.6(6)° in **11** and 166.3(2)° in **9**; 37.9(6)° in **11** and 50.3(3)° in **6**, respectively). Therefore, the overall structural conformation of **8** is less twisted due to the additional double bond in

the B ring. This conformational change may contribute to its reduced dihedral angle. The conformation of **10** as shown in Fig. 3 is similar to that of **9** in terms of bond length and torsion angle. The hydroxyl hydrogen at C(2) forms bifurcated hydrogen bonds with the methoxy oxygen at C(3) (intramolecular) and the carbonyl oxygen at C(7) (intermolecular).

All of the newly synthesized compounds were evaluated for interaction with purified bovine brain tubulin in comparison with **1**. Our findings are summarized in Table I. First, we examined the ability of C(7)-oxygenated allo compounds to inhibit the binding of radiolabeled colchicine to

TABLE I
The inhibitory effects of allocolchicinoids on tubulin polymerization and on [^{3}H]colchicine binding to tubulin

Compound	ITP[a] IC_{50}(μM ± SD)	Binding[b] 1 : 1, %inhibition
1	0.73 ± 0.2	87 ± 11
6	1.0 ± 0.1	83 ± 3
8	1.0 ± 0.03	86 ± 1
9	2.0 ± 0.2	67 ± 2
10	10–40	[c]

[a] Tubulin polymerization was evaluated as described in ref.[22]. A minimum of two independent experiments was performed with each compound. The IC_{50} value is defined as the concentration that inhibits by 50% the extent of assembly after 20 min at 30 °C. [b] The binding of [^{3}H]colchicine (5.0 μM) to tubulin (1.0 μM, 0.1 mg/ml) was measured as described in ref.[23]. Incubation was for 10 min at 37 °C. The values shown in the table represent the averages of two independent experiments, each performed with triplicate samples. [c] Not determined.

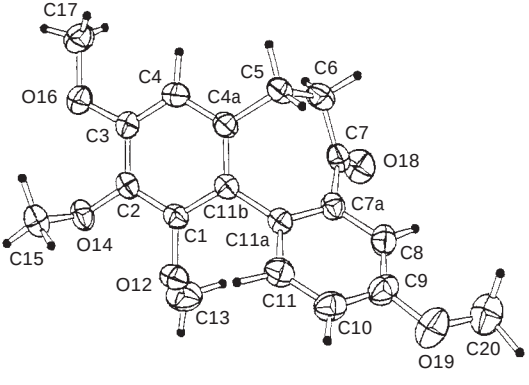


FIG. 1
Structure of compound **6** determined by X-ray crystallography. ORTEP diagrams (40% probability ellipsoids) showing the crystallographic atom-numbering scheme an α*S* solid-state conformation; small filled circles represent hydrogen atoms

tubulin. Data were obtained when the inhibitor and [^{3}H]colchicine were present in the ratios of 1 : 1. The inhibitory activity of **6** and **8** differed little from that of **1**; thus, they showed the strongest tubulin binding ability in this series. Compound **9**, a reduction product of **6**, showed slightly reduced activity. We also examined these compounds for their effect on tubulin polymerization. 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%. A direct correlation was observed between tubulin binding activity and inhibitory effect. Compounds **1**, **6**, and **8**, which displayed the highest potency in the tubulin binding assay were also the strongest antimitotic agents. Compound **9** also showed a significant inhibitory effect with an IC_{50} value of 2.0 ± 0.2 μM. In comparison with **6**, phenol **10**, which is demethylated at C(2), was about 27-fold less active.

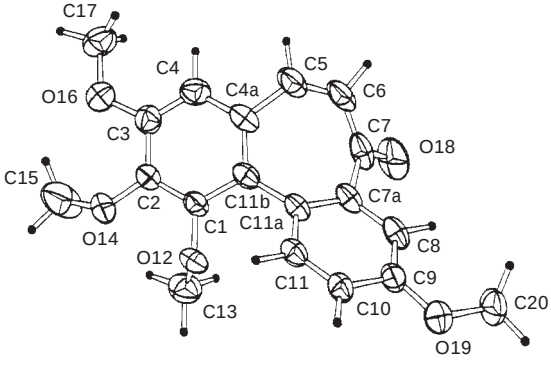


FIG. 2
Structure of compound **8** determined by X-ray crystallography. ORTEP diagrams (40% probability ellipsoids) showing the crystallographic atom-numbering scheme an α*S* solid-state conformation; small filled circles represent hydrogen atoms

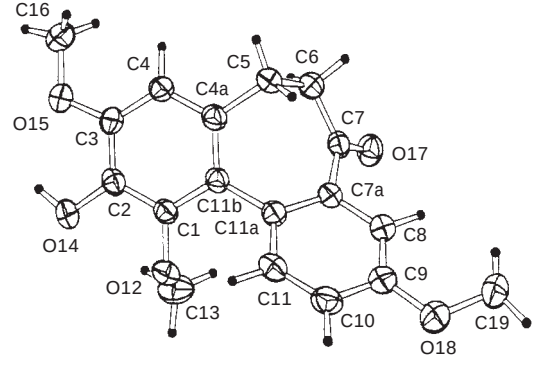


FIG. 3
Structure of compound **10** determined by X-ray crystallography. ORTEP diagrams (40% probability ellipsoids) showing the crystallographic atom-numbering scheme an α*S* solid-state conformation; small filled circles represent hydrogen atoms

The strong antimitotic effects of allocolchicinoids led us to examine the effects of these compounds on proliferation of several human tumor cell lines. As shown in Table II, three (**6**, **8**, and **9**) of the five newly synthesized compounds had inhibitory effects essentially comparable to that of **1** in most tested cell lines (less than five-fold difference, except SK-MEL-2). Compound **10** had only insignificant cytotoxicity in CAKI-1 cells (EC_{50} 6.60 μg/ml), and activity close to the cut-off value (EC_{50} 4.0 μg/ml) in other cell lines. In addition, we examined compounds **6**, **8**, and **9** in three drug-resistant KB cell lines (Table III), including KB-7d (with multidrug-resistant protein), KB-VCR (with overexpression of p-glycoprotein), and KB-CPT (with reduced level of topoisomerase II). Interestingly, the cytotoxicities of these three compounds as well as of **1** in drug-resistant cell lines were almost equivalent to those in drug-sensitive KB cells (less than two-fold difference). Gros *et al.*[20] proposed that the intact tropolone ring C and the nitrogen of the acetamido group of colchicine are required for p-glycoprotein recognition. In this study, our findings are consistent with this postulation; allocolchicinoids with C(7) oxygen functionalities (**6**, **8** and **9**) can overcome the drug resistance induced by p-glycoprotein as shown in KB-VCR cells. Moreover, they also showed cytotoxic effect in drug-resistant cell lines containing reduced level of topoisomerase and

TABLE II
Inhibition of *in vitro* tumor cell growth by allocolchicinoids[a]

Compound	Cytotoxicity EC_{50}, μg/ml[b]					
	KB	A549	HCT-8	CAKI-1	MCF-7	SK-MEL-2
1	0.028	0.007	0.007	0.008	0.008	0.009
6	0.057	0.026	0.022	0.036	0.020	0.022
8	0.029	0.019	0.036	0.277	0.039	0.104
9	0.020	0.026	0.026	0.330	0.020	0.099
10	3.14	3.77	3.36	6.60	3.93	3.93

[a] Data obtained from our in-house *in vitro* disease-oriented human tumor cell screen. KB, epidermoid carcinoma of the nasopharynx; A549, lung carcinoma; HCT-8, adenocarcinoma; CAK1-1, kidney carcinoma; MCF-7, breast adenocarcinoma; SK-MEL-2, malignant melanoma. [b] EC_{50} is the concentration of compound that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using SRB assay.

multidrug resistance associated protein as shown in KB-CPT and KB-7d cell lines, respectively.

It was shown that replacing the acetamido group in *N*-acetylcolchinol methyl ether (**1**) with oxygen functionalities afforded highly potent antitubulin agents. The selective cleavage of the methoxy group, as shown with phenol **10**, resulted in a considerable loss of potency. Ketones **6** and **8** have reactive carbonyl groups and may, for this reason, invite further chemical modification.

EXPERIMENTAL

Melting points were measured on a Fisher–Johns melting point apparatus without correction. Optical rotations were determined with a DIP-1000 polarimeter. ^{1}H NMR spectra were measured on a Bruker AC-300 spectrometer with tetramethylsilane as internal reference. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. MS data were determined by NIH. 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.

N-Acetylcolchinol Methyl Ether (**1**)

Colchicine (400 mg, 1.0 mmol) was dissolved in 30% H_2O_2 (20 ml). The reaction mixture was heated to 90 °C and stirred for 12 h. The glassy oil was extracted with CH_2Cl_2 (3 × 20 ml), and the combined organic layers were concentrated to give residue (396 mg). Flash chromatography with 10% CH_3OH_3–CH_2Cl_2 removed impurities and gave a mixture of **1** and **2**. This mixture (213.2 mg, ≈106.6 mg of **2**, 0.26 mmol), was dissolved in dry acetone (13 ml).

TABLE III
Cytotoxic activities in drug-resistant KB cell lines[a]

Compound	Cytotoxicity EC_{50}, μg/ml[b]			
	KB	KB-7d	KB-VCR	KB-CPT
1	0.028	0.028	0.033	0.011
6	0.057	0.025	>0.063 (42–49)	0.052
8	0.029	0.024	0.016	0.042
9	0.020	0.014	0.013	0.022

[a] See footnote [a] in Table II. KB, epidermoid carcinoma of the nasopharynx; KB-7d, KB cells with multidrug resistant protein; KB-VCR, KB cells overexpression p-glycoprotein; KB-CPT, KB cells with reduced level of topoisomerase. [b] See footnote [b] in Table II.

To the reaction mixture, CH_3I (0.02 ml, 0.3 mmol), K_2CO_3 (50 mg, 0.36 mmol), and Et_4NF (54 mg, 0.36 mmol) were added. The reaction mixture was stirred for 30 h at room temperature. At the completion of the reaction, **2** was converted to its methylated form **3** and was easily separated from **1** by chromatography using 5% CH_3OH–CH_2Cl_2 as eluent. Pure **1** was obtained in a 33% yield (118.9 mg), crystallization from ethyl acetate gave white elongated needles: m.p. 204–205 °C (ref.[4] 202–204 °C). ^{1}H NMR (DMSO-d_6): 1.88 (s, 3 H, $COCH_3$-7); 1.99–2.19 (m, 4 H, H-5,6); 3.46 (s, 3 H, OCH_3-1); 3.77 (s, 3 H, OCH_3-9); 3.79 (s, 3 H, OCH_3-2); 3.83 (s, 3 H, OCH_3-3); 4.53 (m, 1 H, H-7); 6.77 (s, 1 H, H-4); 6.88 (s, 1 H, H-8); 6.89 (d, 1 H, H-10, J = 8.4); 7.25 (d, 1 H, H-11, J = 8.4). CIMS, m/z: 371 M^+.

N-Acetyl-10-methoxycarbonylcolchinol (**2**)

Title compound was prepared according to the procedure reported by Iorio[4]. Crystallization from ethyl acetate gave white plates: m.p. 174–175 °C (ref.[4] 211–212 °C). ^{1}H NMR (DMSO-d_6): 1.89 (s, 3 H, $COCH_3$-7); 2.05–2.20 (m, 4 H, H-5,6); 3.48 (s, 3 H, OCH_3-1); 3.79 (s, 3 H, OCH_3-2); 3.83 (s, 3 H, OCH_3-3); 3.89 (s, 3 H, $COOCH_3$-10); 4.51 (m, 1 H, H-7); 6.79 (s, 1 H, H-4); 6.95 (s, 1 H, H-8); 7.74 (s, 1 H, H-11). CIMS, m/z: 416 $(M + H)^+$. For $C_{22}H_{25}NO_7$ (415.4) calculated: 63.61% C, 6.07% H, 3.37% N; found: 63.51% C, 6.16% H, 3.31% N.

7-Deamino-7-oxocolchinol Methyl Ether (**6**)

Compound **4** (130 mg, 0.39 mmol) was dissolved in a 1 : 1 mixture of CH_2Cl_2–DMF (8 ml). 4-Formyl-1-methylpyridinium benzenesulfonate (FMB) (143 mg, 0.47 mmol) was added to the solution, and the resulting mixture was stirred under N_2 at room temperature for 10 h. To the mixture was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) dropwise (0.15 ml) to afford a deep-purple solution. After stirring for 30 min, oxalic acid-saturated aqueous solution (7 ml) was added, and vigorous stirring was continued overnight. The reaction mixture was extracted with CH_2Cl_2 (3 × 10 ml). The combined organic layers were concentrated to an organic solid, which was purified by preparative TLC and crystallized from CH_3OH to yield **6**: 50 mg (39%); m.p. 134–135 °C (ref.[14] 140–141 °C). ^{1}H NMR ($CDCl_3$): 2.67–2.96 (m, 4 H, H-5,6); 3.50 (s, 3 H, OCH_3-1); 3.88 (s, 3 H, OCH_3-9); 3.90 (s, 3 H, OCH_3-2); 3.91 (s, 3 H, OCH_3-3); 6.60 (s, 1 H, H-4); 7.07 (s, 1 H, H-8); 7.10 (d, 1 H, H-10, J = 8.5); 7.52 (d, 1 H, H-11, J = 8.5). For $C_{19}H_{20}O_5 \cdot 0.25\ H_2O$ (332.9) calculated: 68.56% C, 6.21% H; found: 68.67% C, 6.13% H.

7-Deamino-7-oxo-5,6-dehydrocolchinol Methyl Ether (**8**)

To a 15 ml flask fitted with dry N_2, dry THF (5 ml) was introduced. It was cooled to –78 °C, and 2 M lithium diisopropylamide (in heptane–THF–ethylbenzene, 0.16 ml) was introduced. A solution of **6** (16.8 mg, 0.05 mmol), in dry THF (1 ml) was added from a syringe, and the solution was stirred for 30 min and then brought to 0 °C. Trimethylsilyl chloride (20 mg, 0.13 mmol), in dry THF (0.25 ml) was introduced rapidly to the enolate solution, and the reaction mixture was stirred for 15 min. Saturated sodium hydrogen carbonate solution was added to the reaction mixture, and the silyl enol was extracted with CH_2Cl_2 (3 × 5 ml). The organic layers were washed, dried and evaporated to give an oily residue **7**. The above silyl enol ether was dissolved in dry CH_3CN (4 ml), and palladium acetate (46 mg, 0.1 mmol) was added. The reaction mixture was stirred at room temperature for 2 h, and then filtered through celite. Removal of solvent afforded crude compound (42.3 mg), which was purified

by preparative TLC with 20% acetone–hexane. Crystallization from dibutyl ether and recrystallization from CH_3OH afforded **8**: 8.4 mg (50%); m.p. 95–96 °C (ref.[12] 98–99 °C). ^{1}H NMR ($CDCl_3$): 3.44 (s, 3 H, OCH_3-1); 3.93 (s, 3 H, OCH_3-9); 3.96 (s, 3 H, OCH_3-2); 4.01 (s, 3 H, OCH_3-3); 6.54 (d, 1 H, H-6, *J* = 12.0); 6.79 (s, 1 H, H-4); 7.11 (d, 1 H, H-10, *J* = 9.0); 7.21 (d, 1 H, H-5, *J* = 12.2); 7.31 (s, 1 H, H-8); 7.99 (d, 1 H, H-11, *J* = 8.9). For $C_{19}H_{18}O_5$ (326.4) calculated: 69.93% C, 5.56% H; found: 69.68% C, 5.68% H.

7-Deamino-7-hydroxycolchinol Methyl Ether (**9**)

To a solution of **6** (13 mg, 0.04 mmol) in a mixture of CH_2Cl_2–CH_3OH was added $NaBH_4$ (20 mg, 0.53 mmol), and the solution was stirred at room temperature for 30 min. The reaction mixture was acidified with 50% acetic acid and then extracted with CH_2Cl_2 (3 × 5 ml). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated to give a residue (13.7 mg) which crystallized from CH_3OH to afford **9**: 13 mg (99%); m.p. 156–157 °C. ^{1}H NMR ($CDCl_3$): 2.28–2.61 (m, 4 H, H-5,6); 3.62 (s, 3 H, OCH_3-1); 3.89 (s, 3 H, OCH_3-9); 3.90 (s, 3 H, OCH_3-2); 3.91 (s, 3 H, OCH_3-3); 4.61 (m, 1 H, H-7); 6.59 (s, 1 H, H-4); 6.89 (dd, 1 H, H-10, *J* = 2.8, 8.6); 7.25 (d, 1 H, H-8, *J* = 2.7); 7.40 (d, 1 H, H-11, *J* = 8.5). CIMS, *m/z*: 331 $(M + H)^+$. For $C_{19}H_{22}O_5$ (330.4) calculated: 69.07% C, 6.71% H; found: 69.49% C, 7.07% H.

7-Deamino-O^2-demethyl-7-oxocolchinol Methyl Ether (**10**)

To a solution containing **6** (8.8 mg, 0.027 mmol) in CH_2Cl_2 (1.5 ml) was added 2 equivalents of neat trimethylsilyl iodide *via* a dry syringe. The reaction mixture was stirred at room temperature and monitored by TLC. At the completion of the reaction, the intermediate trimethylsilyl ethers were hydrolyzed to alcohol **10** by pouring the reaction mixture into 4 equivalents of CH_3OH. The volatile components were removed at reduced pressure and the residue was taken up in CH_2Cl_2, washed with brine, and dried. The residue was further purified by preparative TLC to give pure **10** (4.6 mg, 54.8%), crystallization from CH_3OH afforded pale yellow crystals: m.p. 121–123 °C. ^{1}H NMR ($CDCl_3$): 2.64–2.69 (m, 4 H, H-5,6); 3.38 (s, 3 H, OCH_3-1); 3.88 (s, 3 H, OCH_3-9); 3.94 (s, 3 H, OCH_3-2); 6.60 (s, 1 H, H-4); 7.09 (s, 1 H, H-8); 7.10 (d, 1 H, H-10, *J* = 9.3); 7.56 (d, 1 H, H-11, *J* = 9.5). For $C_{18}H_{18}O_5 \cdot 0.5\ H_2O$ (323.3) calculated: 66.86% C, 5.92% H; found: 66.67% C, 6.06% H.

7-Deamino-O^1,O^2,O^3- tridemethyl-7-oxocolchinol (**11**)

To a solution of **10** (115 mg, 0.35 mmol) in anhydrous CH_2Cl_2 (10 ml) was added dropwise a 1 M solution of boron tribromide in CH_2Cl_2 (molar ratio of 1 : 10) under ice cooling. The reaction mixture was stirred at room temperature for 20–24 h. The reaction mixture was cooled in an ice bath, and MeOH (20 ml) was added dropwise. The solution was refluxed for 2 h, and then the solvent was removed *in vacuo*. The residue was purified by MCI Gel CHP-20P column chromatography using water and MeOH as eluents to afford **11** as amorphous solid, yield 7.8 mg (7.4%). ^{1}H NMR (CD_3OD): 2.3–2.7 (m, 4 H, H-5,6); 6.38 (s, 1 H, H-4); 7.28 (dd, 1 H, H-10, *J* = 2.5, 8.6); 7.32 (d, 1 H, H-8, *J* = 2.5); 7.57 (d, 1 H, H-11, *J* = 8.6). For $C_{15}H_{12}O_5 \cdot 1.5\ H_2O$ (299.3) calculated: 60.20% C, 5.05% H; found: 60.65% C, 4.71% H.

X-Ray Crystal Structure Analyses of **6**, **8**, and **10**

Crystal data for **6**: $C_{19}H_{20}O_5$, M = 328.36, triclinic, space group $P1(C_i^1)$, a = 8.441(2) Å, b = 14.328(2) Å, c = 7.932(1) Å, α = 104.14(1)°, β = 117.58(1)°, γ = 82.84(1)°, V = 824.5(5) Å^3, Z = 2, D_{calc} = 1.323 g cm^{-1}, μ(CuKα radiation, λ = 1.5418 Å) = 7.5 cm^{-1}; crystal dimensions: 0.24 × 0.30 × 0.40 mm. Crystal data for **8**: $C_{19}H_{18}O_5$, M = 326.35, monoclinic, space group $P2_1/c(C_{2h}^5)$, a = 7.732(1) Å, b = 9.951(2) Å, c = 22.124(1) Å, β = 107.21(1)°, V = 1 624.1(6) Å^3, Z = 4, D_{calc} = 1.339 g cm^{-1}, μ(CuKα radiation, λ = 1.5418 Å) = 7.6 cm^{-1}; crystal dimensions: 0.06 × 0.14 × 0.26 mm. Crystal data for **10**: $C_{18}H_{18}O_5$, M = 314.34, monoclinic, space group $P2_1/c(C_{2h}^5)$, a = 9.575(1) Å, b = 12.011(1) Å, c = 14.753(1) Å, β = 112.41(1)°, V = 1 568.5(6) Å^3, Z = 4, D_{calc} = 1.331 g cm^{-1}, μ(CuKα radiation, λ = 1.5418 Å) = 7.6 cm^{-1}; crystal dimensions: 0.07 × 0.16 × 0.44 mm.

All crystal structures were solved by direct methods (MULTAN11/82). Initial coordinates for all non-hydrogen atoms were derived from an *E*-map. Positional and thermal parameters of these atoms (first isotropic and then anisotropic) were adjusted by means of several rounds of full-matrix least-squares calculations during which $\Sigma w\Delta^2[w = 1/\sigma^2(|F_o|), \Delta = (|F_o| - |F|_c)]$ was minimized. Hydrogen atoms were located in difference Fourier synthesis and incorporated at their calculated positions during the subsequent least-squares cycles; an extinction correction was included as a variable during the later iterations. A final difference Fourier synthesis contained no unusual features.

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf–Nonius Structure Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from literature[21]. Detailed crystallographic data have been deposited in the Cambridge Crystallographic Data Centre, deposition codes: **6**: CCDC 113323, **8**: CCDC 113324, and **10**: CCDC 113325.

Biological Assays

The tubulin polymerization and cytotoxicity assays were all performed as described previously[22,23].

a) *Tubulin polymerization assay*. In brief, tubulin at 1.2 mg/ml (12 μM) was preincubated for 15 min at 26 °C in a 0.24-ml volume of 0.8 M monosodium glutamate (pH 6.6 with NaOH in a 2 M stock solution) with varying drug concentrations. The drug stock solutions was in DMSO, and the final solvent concentration was 4% (v/v). All concentrations were in terms of the final reaction volume (0.25 ml). The reaction mixtures were chilled on ice, and 10 μl of 10 mM GTP was added to each reaction mixture. Samples were transferred to cuvettes held at 0 °C by an electronic temperature controller in Gilford spectrophotometers. Baselines were estalished at 350 nm, and polymerization was initiated by a temperature jump to 26 °C. After 20 min, turbidity readings were recorded, and the temperature controller was set to 0 °C. When depolymerization was complete, a turbidity reading was again recorded. Generally, turbidity readings were about 90% cold-reversible, and cold-reversible turbidity was taken to represent the extent of assembly for each reaction mixture. IC_{50} values were obtained graphically from inhibition of polymerization by different drug concentrations.

b) *Colchicine binding assay*. In brief, each 0.1 ml reaction mixture contained 0.1 mg (1.0 μM) of tubulin, 1.0 M commercial monosodium glutamate (pH 6.6 with HCl), 1 mM $MgCl_2$, 0.1 mM GTP, 5.0 μM [^{3}H] colchicine, 5% (v/v) DMSO, and 5.0 μM inhibitor. Incubation was for 20 min at

37 °C. Each reaction mixture was filtered under vacuum through a stack of two DEAE-cellulose paper filters, washed with water, and radioactivity quantitated in a liquid scintillation counter.

c) *Cytotoxicity assay*. For testing, samples diluted in culture medium immediately prior to use were prescreened at 4, 2, and 1 μM *vs* HTCL. HTCL panel cultured in RPMI-1640, FBS 10% (v/v), and kanamycin (100 μg/ml). Sulforhodamine B assays, triplicate dose treatment and three-day exposure were standard procedures. Based on prescreening results, compounds were further tested *vs* HTCL to establish ED_{50} values.

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