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ANTITUMOR AGENTS 238. ANTI-TUBULIN AND *IN VITRO* CYTOTOXIC EFFECTS OF *N*-SUBSTITUTED ALLOCOLCHICINOIDS

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Abstract – (-)-*N*-Substituted colchinol methyl ethers (**6**-**10**) and *N*-alkyl HCl salts (**8a**–**10a**) were synthesized from (-)-colchicine (**1**). The new compounds were evaluated for *in vitro* cytotoxic activity against five human tumor cell lines and for inhibition of tubulin polymerization. The new carbamate (**6**) and amide (**7**) showed 10-fold stronger activity against human tumor cell line replication than the amines (**8–10**). The corresponding HCl salts (**8a-10a**) generally showed decreased activity. Compounds (**6**) and (**7**) also exerted strong inhibitory effects on tubulin polymerization. All of the colchinol methyl ethers showed essentially equal cytotoxic effects against MDR-resistant (KB-V) and non-resistant (KB) cells, while the potency of colchicine was decreased 100-fold against KB-V cells.

INTRODUCTION

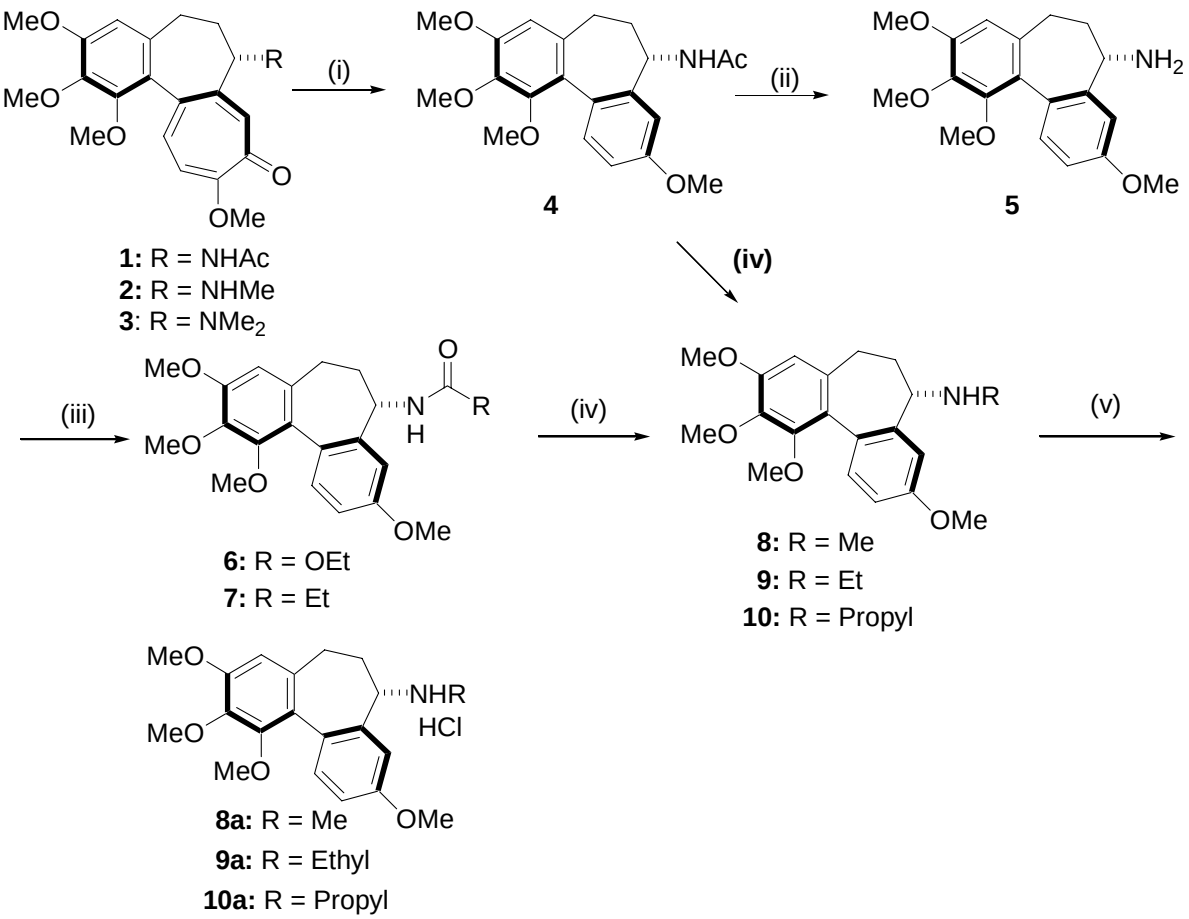
Colchicine (**1**), the major alkaloid from *Colchicium autumnale,* is a well-known compound and is used for

the treatment of gout,[1,2] familial Mediterranean fever,[3] and liver cirrhosis.[4] Colchicine has also been studied as an antitumor agent; however, its high toxicity prevents clinical use. The biological effects of colchicine, including pharmacological activities and toxicity, are caused by its interference with microtubule-dependent cell functions *via* binding of **1** to the protein tubulin, the subunit of microtubules.[5] Because of the interesting biological actions of **1**, various colchicinoid derivatives have been synthesized and structure-activity relationships (SAR) investigated in order to develop more effective and less toxic antitumor agents.[1,6] Recent studies have shown that active colchicinoid structures require *R* chirality of the biaryl and *S* configuration at C-7 in order to bind to tubulin.[6,7] Active allocolchicinoids, with a benzenoid rather than a troponoid ring C, also bind to tubulin and retain the (*aR*) atropisomer and *7S* absolute configuration.[8]

In earlier studies, colchicine was used as the starting point for modification of the nitrogen functionality in colchicinoids.[9] However, the same studies have not been done in the allocolchicine series.[8a,10] For example, demecolcine (**2**) and *N*-methyldemecolcine (**3**) are *Colchicum* alkaloids that give water soluble salts.[11] Similar compounds are not available in the allo-series. In order to fill this gap and toward a more thorough SAR study of allocolchicinoids, we now have prepared several C-7-substituted amines of the allo-series from colchicine (**1**) via *N*-acetylcolchicinoid methyl ether (**4**). Hydrochlorides of the obtained amine derivatives were also prepared in order to increase water solubility. The effects of these novel compounds on in vitro tumor cell replication and tubulin binding were investigated, and the results are reported here.

RESULTS AND DISCUSSION

(-)-*N*-Acetylcolchinol methyl ether (**4**) was obtained from (-)-colchicine (**1**) without loss of chirality by rearrangement of the troponoid C-ring to benzenoid using H_2O_2.[12] Hydrolysis of acetamide (**4**) under acidic conditions gave amine (**5**) in 61% yield, based on recovered starting material. The amine (**5**) was treated with ethyl chloroformate or propionyl chloride in the presence of Et_3N to produce the corresponding carbamate and amide (**6**) or (**7**) in 79% and 98% yield, respectively. Methylamine (**8**), ethylamine (**9**), and propylamine (**10**) were obtained by reduction of **6**, **4**, and **7**, respectively with excess $LiAlH_4$. The resulting amines (**8**–**10**) were converted to their HCl salts (**8a**–**10a**). The salts (**8a**) and (**9a**) showed good solubility in water (10 mg/1.5 mL water) compared to the corresponding amines with no water solubility. Amazingly, the salt (**10a**) had no solubility in water. All novel compounds showed the (-)-rotation and a*R*, 7*S* absolute configuration of natural (-)-colchicine.[13,14] The new *N*-modified colchicinol methyl ethers were evaluated for cytotoxic activity, and most were also examined for inhibitory effects on tubulin assembly in comparison with **4**.



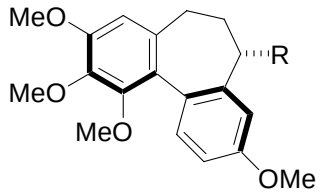
Scheme 1. Conditions: (i) H_2O_2; (ii) HCl; (iii) RCOCl, Et_3N; (iv) $LiAlH_4$, THF, reflux; (v) HCl.

Table 1 lists the data for inhibition of tubulin polymerization by compounds (**1**, **4**–**9**, **8a**, and **9a**). The newly synthesized carbamate (**6**) and propionamide (**7**) showed strong inhibition of tubulin polymerization, with IC_{50} values of 1.7 and 1.2 μM, respectively, but they were less potent than acetamide (**4**)(0.7 μM). Compared with **6** and **7**, compounds (**8**) and (**9**), which bear a basic secondary amine group at C-7, were about 10-fold less active. Moreover, the corresponding HCl salts (**8a** and **9a**) showed almost twofold lower potency, with IC_{50} values of 27 and 31 μM, than the free amines (**8**) and (**9**).

It is not clear why the IC_{50} values differ between the free amine and salt. One conceivable reason is that rotation of the atropisomer might have occurred when the amine was treated with HCl due to the comparatively low rotation energy around the two aromatic rings. However, this hypothesis appears unlikely based on the following observations. 1) The free amines and the corresponding salts showed almost the same optical rotation values in MeOH solution. 2) After converting the HCl salts (**8a**) and (**9a**) to the parent amines (**8**) and (**9**) by treatment with 2 N NaOH, the reformed free amines showed only slightly lower optical rotation values than the original free amines (Table 2). Finally, the primary amine (**5**) had only feeble activity (IC_{50} = 27 μM) as an inhibitor of tubulin assembly. Within the limits of this

small series of compounds, a carbonyl group in the side chain seems to be required for antitubulin activity. This finding is in contrast to the situation with the colchicinoids, where both the amine and demecolcine (**2**) are potent inhibitors of tubulin assembly.[9e]

Table 1. Inhibition of Tubulin Assembly[a] by Allocholchinoids (**4–9, 8a** and **9a**)



Compound	R	IC_{50} (μM)±SD
1 (Colchicine)	-	2.9±0.7
4	NHCOMe	0.7±0.1
5	NH_2	27±2
6	NHCOOEt	1.7±0.2
7	NHCOEt	1.2±0.2
8	NHMe	13±4
9	NHEt	17±2
8a	NHMe·HCl	27±10
9a	NHEt·HCl	31±6

[a] For experimental details, see E. Hamel, *Cell Biochem. Biophys.*, **2003**, *38*, 1.

Table 2. The Optical Rotations of Free Amines (**8**) and (**9**) Before and After Making Salts

Compound	Optical Rotation Original Free Amine	Optical Rotation HCl Salt	Optical Rotation Reformed Free Amine
8	$[\alpha]_D^{30}$ = –121.5°	--	$[\alpha]_D^{26}$ = –114.0°
	(c 0.13, $CHCl_3$)	--	(c 0.17, $CHCl_3$)
	$[\alpha]_D^{26}$ = –87.7°	$[\alpha]_D^{31}$ = –89.6°	--
	(c 0.47, CH_3OH)	(c 0.24, CH_3OH)	
9	$[\alpha]_D^{30}$ = –169.9°	--	$[\alpha]_D^{26}$ = –152.5°
	(c 0.13, $CHCl_3$)	--	(c 0.13, $CHCl_3$)
	$[\alpha]_D^{26}$ = –77.8°	$[\alpha]_D^{31}$ = –81.3°	--
	(c 0.23, CH_3OH)	(c 0.25, CH_3OH)	

The IC_{50} values against the growth of five human tumor cell lines [A549 lung carcinoma, 1A9 ovarian carcinoma, MCF-7 breast cancer, KB nasopharyngeal carcinoma, and KB-V, a multidrug resistant (p170 PgP) variant] are shown in Table 3.

Table 3. Activities of Colchicine Analogs against Human Tumor Cell Line and Drug Resistant Cell Line Replication

	IC_{50} (μg/mL) [a]				
Compound	A549[b]	1A9 [b]	KB [b]	KB-V [b]	MCF-7 [b]
1	0.017±0.002	0.003±0.001	0.0036±0.0002	0.192±0.013	0.006±0.0007
4	0.007±0.001	0.004±0.003	0.007±0.001	0.007±0.000	0.004±0.005
5	0.291±0.030	0.179±0.010	0.272±0.024	0.207±0.017	0.177±0.012
6	0.019±0.002	0.010±0.001	0.024±0.005	0.016±0.002	0.017±0.003
7	0.021±0.003	0.013±0.004	0.029±0.005	0.025±0.005	0.013±0.002
8	0.292±0.040	0.184±0.037	0.272±0.031	0.277±0.030	0.214±0.020
9	0.327±0.039	0.215±0.027	0.336±0.046	0.297±0.041	0.197±0.012
10	0.332±0.044	0.210±0.040	0.328±0.040	0.267±0.033	0.217±0.004
8a	0.463±0.041	0.261±0.038	0.371±0.043	0.362±0.069	0.330±0.097
9a	0.667±0.066	0.428±0.079	0.663±0.075	0.561±0.101	0.441±0.025
10a	0.738±0.094	0.517±0.071	0.695±0.097	0.543±0.093	0.412±0.028

[a]Cytotoxicity as ED_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to unreacted cells using the sulforhodamine B assay.
[b]Human lung carcinoma (A549), human ovarian carcinoma (1A9), human epidermoid carcinoma of the nasopharynx (KB), multi-drug resistant KB expressing P-glycoprotein (KB-V), breast cancer (MCF-7).

As in the above tubulin results, compounds (**6**) and (**7**) showed 10-fold stronger activities (0.01 – 0.02 μg/mL) against tumor cell replication than the amines (**8-10**) (0.2 – 0.3 μg/mL). Although there is a close correlation between antitubulin activity and cytotoxicity in this compound series, there could be other reasons for the relatively weak cytotoxicity of the amines (**8-10**). For example, these compounds might have reduced ability to enter the cells, or they might bind to a component in the growth medium. The corresponding HCl salts (**8a-10a**) generally showed still lower activity. These results demonstrated that, amongst C-7 substituted allocolchicinoids, a basic amine was deleterious for both antitubulin and

cytotoxic activity**.** All of the colchinol methyl ethers showed essentially equal cytotoxic effects against MDR-resistant (KB-V) and non-resistant (KB) cells, similar to other compounds in the allocolchicinoid series,[11a, 12] while the potency of colchicine was decreased 100-fold in resistant KB-V cells. The two most active novel compounds (**6**) and (**7**) were 4 to 10-fold less cytotoxic than colchicine in all cell lines.

CONCLUSIONS

The acetamide (**4**), carbamate (**6**), and the propionamide (**7**) are good candidates for further development.

EXPERIMENTAL

All melting points were taken on a Fisher-Johns or Mel-Temp II melting point instrument and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1320 spectrophotometer. ^{1}H NMR spectra were obtained using a Varian Gemini 2000 (300 MHz) NMR spectrometer with TMS as the internal standard. All chemical shifts are reported in ppm. MS spectral data were obtained on a TRIO 1000 mass spectrometer. FABMS and HRFABMS spectral analyses were determined on a JEOL HX-110 instrument. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). Optical rotations were measured with a JASCO DIP-1000 polarimeter. All target compounds were characterized by ^{1}H-NMR and IR spectroscopic and MS analyses.

N-Ethoxycarbonylcolchinol methyl ether (6)

To a solution of *N*-deacetylcolchinyl methyl ether (**5**, 109 mg, 0.33 mmol) in anhydrous CH_2Cl_2 (1 mL), anhydrous Et_3N (0.2 mL, excess) and ethyl chloroformate (0.05 mL, 0.53 mmol) were added. The reaction mixture was stirred at rt for 3 h, then quenched with saturated aq. $NaHCO_3$ and extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with MeOH–CH_2Cl_2 (5:95, v/v) as eluent to afford **6** (105 mg, 79%) as a colorless oil. $[\alpha]_D^{30}$ = –56.7° (*c* 0.15, $CHCl_3$). IR (KBr): 3334, 2935, 1707, 1608, 1483, 1459, 1293, 1238, 1093 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 7.80 (d, 1H, *J* = 8.5 Hz, NH), 7.25 (d, 1H, *J* = 8.2 Hz, 11-H), 6.91 (d, 1H, *J* = 2.6 Hz, 8-H), 6.87 (dd, 1H, *J* = 8.2 and 2.6 Hz, 10-H), 6.77 (s, 1H, 4-H), 4.32-4.19 (m, 1H, 7-H), 3.94 (q, 2H, *J* = 7.0 Hz, OC*H*$_2$CH$_3$), 3.82 (s, 3H, 3-OC*H*$_3$), 3.79 (s, 3H, 2-OCH$_3$), 3.78 (s, 3H, 9-OCH$_3$), 3.46 (s, 3H, 1-OCH$_3$), 2.52–2.42 (m, 1H, 5-H), 2.22–2.10 (m, 1H, 5 or 6-H), 2.08–1.97 (m, 1H, 5 or 6-H), 1.94–1.81 (m, 1H, 6-H), 1.15 (t, 3H, *J* = 7.0 Hz, OCH$_2$C*H*$_3$). HRMS calcd for $C_{22}H_{27}NO_6$: 401.1838, found: 401.1836.

N-Propionylcolchinol methyl ether (7)

To a solution of *N*-deacetylcolchinyl methyl ether (**5**, 87 mg, 0.26 mmol) in anhydrous CH_2Cl_2 (1 mL), anhydrous Et_3N (0.2 mL, excess) and propionyl bromide (0.03 mL, 0.33 mmol) were added. The

reaction mixture was stirred at rt for 1 h, then treated as described above. Chromatography on silica gel with MeOH–CH_2Cl_2 (3:97, v/v) as eluent afforded **7** (98 mg, 98%). Colorless prisms, mp 194-195 °C (EtOAc–MeOH). $[\alpha]_D^{29}$ = –85.7° (*c* 0.28, $CHCl_3$). IR (KBr): 3299, 2936, 1648, 1608, 1539, 1484, 1459, 1402, 1291, 1237, 1103 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 8.31 (d, 1H, *J* = 8.2 Hz, NH), 7.25 (d, 1H, *J* = 8.5 Hz, 11-H), 6.91–6.84 (m, 2H, 8- and 10-H), 6.77 (s, 1H, 4-H), 4.56-4.45 (m, 1H, 7-H), 3.83 (s, 3H, 3-OCH_3), 3.78 (s, 3H, 2-OCH_3), 3.77 (s, 3H, 9-OCH_3), 3.46 (s, 3H, 1-OCH_3), 2.51–2.44 (m, 1H, 5-H), 2.24–2.00 (m, 4H, 5-, 6-H and C(O)C$H_2$$CH_3$), 1.94–1.80 (m, 1H, 6-H), 0.99 (t, 3H, *J* = 7.5 Hz, OCH_2CH_3). HRMS calcd for $C_{22}H_{27}NO_5$: 385.1889, found: 385.1890.

***N*-Methylcolchinol methyl ether (8)**

To a solution of *N*-ethoxycarbonylcolchinyl methyl ether (**6**, 91 mg, 0.23 mmol) in anhydrous THF (2 mL), $LiAlH_4$ (150 mg, 3.95 mmol) was added. The reaction mixture was refluxed for 3 h. After quenching with MeOH at 0 °C, 10% Rochelle salt (aqueous) was added, and the solution was extracted with CH_2Cl_2 three times. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with MeOH–CH_2Cl_2–28% NH_4OH (5:95:0.5, v/v) as eluent to afford **8** (59 mg, 76%) as a colorless oil. $[\alpha]_D^{30}$ = –121.5° (*c* 0.13, $CHCl_3$). IR (KBr): 2934, 2849, 1604, 1483, 1460, 1402, 1292, 1240, 1147, 1102, 1091 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.40 (d, 1H, *J* = 8.5 Hz, 11-H), 7.17 (d, 1H, *J* = 2.8 Hz, 8-H), 6.88 (dd, 1H, *J* = 8.5 and 2.8 Hz, 10-H), 6.58 (s, 1H, 4-H), 3.92 (s, 3H, 3-OCH_3), 3.91 (s, 3H, 2-OCH_3), 3.89 (s, 3H, 9-OCH_3), 3.62 (s, 3H, 1-OCH_3), 3.45 (dd, 1H, *J* = 11.5 and 5.2 Hz, 7-H), 2.48–2.38 (m, 2H, 5- and/or 6-H), 2.42 (s, 3H, NHCH_3), 2.34–2.25 (m, 1H, 5 or 6-H), 1.88–1.781 (m, 1H, 6-H). HRMS calcd for $C_{20}H_{25}NO_4$: 343.1784, found: 343.1784.

***N*-Ethylcolchinol methyl ether (9)**

Compound (**9**) (100 mg, 74%, colorless oil) was obtained from *N*-acetylcolchinol methyl ether (**4**, 140 mg, 0.38 mmol) as described for compound (**8**). $[\alpha]_D^{30}$ = –169.9° (*c* 0.13, $CHCl_3$). IR (KBr): 2933, 2833, 1603, 1482, 1460, 1401, 1290, 1237, 1144, 1101 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.38 (d, 1H, *J* = 8.4 Hz, 11-H), 7.16 (d, 1H, *J* = 2.8 Hz, 8-H), 6.86 (dd, 1H, *J* = 8.4 and 2.8 Hz, 10-H), 6.58 (s, 1H, 4-H), 3.92 (s, 3H, 3-OCH_3), 3.91 (s, 3H, 2-OCH_3), 3.89 (s, 3H, 9-OCH_3), 3.59 (s, 3H, 1-OCH_3), 3.49 (dd, 1H, *J* = 11.0 and 5.8 Hz, 7-H), 2.63 (dd, 1H, *J* = 11.0 and 7.1 Hz, 5-H), 2.50 (q, 2H, *J* = 7.2 Hz, C$H_2$$CH_3$) 2.50–2.24 (m, 2H, 5- and 6-H), 1.80–1.70 (m, 1H, 6-H), 1.35 (br s, 1H, NH), 1.09 (t, 3H, *J* = 7.2 Hz, CH_2CH_3). HRMS calcd for $C_{21}H_{27}NO_4$: 357.1940, found: 357.1939.

***N*-Propylcolchinol methyl ether (10)**

Compound (**10**) (36 mg, 69%, colorless oil) was obtained from *N*-propionylcolchinol methyl ether (**7**, 54 mg, 0.14 mmol) as described for compound (**8**). $[\alpha]_D^{29}$ = –98.8° (*c* 0.16, $CHCl_3$). IR (KBr): 2930, 1602,

1482, 1458, 1401, 1291, 1236, 1144, 1101, 1008 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.38 (d, 1H, *J* = 8.5 Hz, 11-H), 7.18 (d, 1H, *J* = 2.8 Hz, 8-H), 7.07 (br s, 1H, NH), 6.86 (dd, 1H, *J* = 8.5 and 2.8 Hz, 10-H), 6.58 (s, 1H, 4-H), 3.92 (s, 3H, 3-OCH_3), 3.90 (s, 3H, 2-OCH_3), 3.89 (s, 3H, 9-OCH_3), 3.59 (s, 3H, 1-OCH_3), 3.47 (dd, 1H, *J* = 11.0 and 5.9 Hz, 7-H), 2.60–2.49 (m, 1H, 5-H), 2.46–2.24 (m, 2H, 5- and 6-H), 1.82–1.71 (m, 1H, 6-H), 1.56–1.41 (m, 4H, NH(CH_2)$_2$$CH_3$), 0.90 (t, 3H, *J* = 7.4 Hz, NH(CH_2)$_2$$CH_3$). HRMS calcd for $C_{22}H_{29}NO_4$: 371.2097, found: 371.2097.

***N*-Methylcolchinol methyl ether HCl salt (8a)**

A solution of *N*-methylcolchinol methyl ether (**8**, 27 mg, 0.08 mmol) in benzene (1 mL) and 0.5 N HCl (0.3 mL) was stirred at rt for 30 min. The volatile solvents were removed under reduced pressure to afford **8a** (25 mg, 83%) as a colorless amorphous solid. mp 127–130 °C; $[\alpha]_D^{32}$ = –89.6° (*c* 0.24, MeOH). IR (KBr): 3403 (br) 2938, 2836, 2703, 2449, 1611, 1599, 1484, 1460, 1404, 1327, 1246, 1147, 1108, 1091, 1005, 752 cm^{-1}. ^{1}H-NMR (CD_3OD): δ 7.46 (d, 1H, *J* = 8.5 Hz, 11-H), 7.04 (dd, 1H, *J* = 8.5 and 2.2 Hz, 10-H), 6.94 (d, 1H, *J* = 2.2 Hz, 8-H), 6.78 (s, 1H, 4-H), 3.93 (dd, 1H, *J* = 11.5 and 5.5 Hz, 7-H), 3.89 (s, 3H, 3-OCH_3), 3.88 (s, 3H, 2-OCH_3), 3.85 (s, 3H, 9-OCH_3), 3.61 (s, 3H, 1-OCH_3), 2.76 (s, 3H, NHCH_3), 2.70–2.57 (m, 2H, 5- and 6-H), 2.24 (dt, 1H, *J* = 14.8 and 7.2 Hz, 5-H), 2.01 (ddd, 1H, *J* = 18.6, 11.5 and 7.2 Hz, 6-H).

***N*-Ethylcolchinol methyl ether HCl salt (9a)**

Compound (**9a**) (37 mg, 99%) was obtained from *N*-ethylcolchinol methyl ether (**9**, 34 mg, 0.10 mmol) as described for compound (**8a**). mp 156–158 °C; $[\alpha]_D^{31}$ = –81.3° (*c* 0.25, MeOH). IR (KBr): 3383 (br), 2938, 2834, 2695, 1611, 1486, 1459, 1243, 1089 cm^{-1}. ^{1}H-NMR (CD_3OD): δ 7.47 (d, 1H, *J* = 8.4 Hz, 11-H), 7.05 (d, 1H, *J* = 8.5 and 2.5 Hz, 10-H), 6.97 (d, 1H, *J* = 2.5 Hz, 8-H), 6.78 (s, 1H, 4-H), 4.02–3.92 (m, 1H, 7-H), 3.90 (s, 3H, 3-OCH_3), 3.89 (s, 3H, 2-OCH_3), 3.86 (s, 3H, 9-OCH_3), 3.61 (s, 3H, 1-OCH_3), 3.19–2.98 (m, 2H, NC$H_2$$CH_3$), 2.69–2.55 (m, 2H, 5- and 6-H), 2.30–2.20 (m, 1H, 5-H), 2.09–1.97 (m, 1H, 6-H), 1.33 (t, 3H, *J* = 6.9 Hz, CH_2CH_3).

***N*-Propylcolchinol methyl ether HCl salt (10a)**

Compound (**10a**)(11 mg, 100%) was obtained from *N*-ethylcolchinol methyl ether (**10**, 10 mg, 0.03 mmol) as described for compound (**8a**). mp 227–229 °C; $[\alpha]_D^{31}$ = –36.8° (*c* 0.25, $CHCl_3$). IR (KBr): 2935,2712, 1610, 1597, 1485, 1458, 1404, 1245, 1092 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 10.19 (br s, 1H, NH), 7.52 (br s, 1H, 8-H), 7.41 (d, 1H, *J* = 8.4 Hz, 11-H), 6.97 (d, 1H, *J* = 8.4 Hz, 10-H), 6.55 (s, 1H, 4-H), 4.02–3.92 (m, 1H, 7-H), 3.95 (s, 3H, 3-OCH_3), 3.91 (s, 3H, 2-OCH_3), 3.89 (s, 3H, 9-OCH_3), 3.71 (s, 3H, 1-OCH_3), 3.20–2.92 (m, 2H, NCH_2Et), 2.82 (br s, 1H, 5- or 6-H), 2.55–2.39 (m, 2H, 5- and/or 6-H), 2.27 (br s, 1H, 6-H), 2.17–1.89 (m, 2H, $NCH_2C$$H_2$$CH_3$), 0.94 (br s, 3H, $NCH_2CH_2C$$H_3$).

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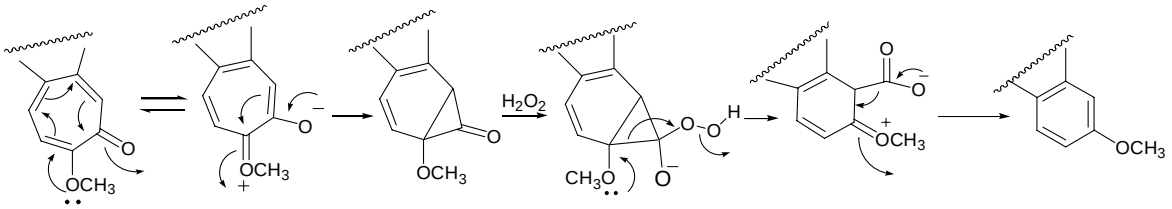

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