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Antitumor agents. Part 236: Synthesis of water-soluble colchicine derivatives☆

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Abstract—Water-soluble colchicine derivatives were synthesized from 2-demethylcolchicine and 2-demethylthiocolchicine and evaluated in vitro against human tumor cell replication and for inhibition of tubulin polymerization. The glycinate esters (**4**, **5**) and their tartaric acid salts (**4a**, **5a**) showed potent cytotoxic activity in three different tumor cell lines with IC_{50} values ranging from 0.02 to 0.88 μg/mL. The thiocolchicine analogs (**5**, **5a**) were more potent than the colchicine analogs (**4**, **4a**) in the tubulin polymerization assay. In particular, the water-soluble salt **5a** merits preclinical development as an antitumor agent.

1. Introduction

Colchicine (**1**), isolated from *Colchicum autumnale* and *Gloriosa superba*, is a well-known natural product.[2] It acts as an antimitotic agent and binds to the protein tubulin, thereby suppressing cell division.[2,3] However, the high toxicity of **1** prevents medicinal use as an antitumor agent, although **1** is used for the treatment of gout. Previously, we have reported the syntheses of diverse colchicine analogs in an attempt to reduce toxicity and to study structure–activity relationships.[4] In these studies, compound **2**, which is a phenolic congener of colchicine, was less toxic than the parent compound. Correspondingly, the analogous phenolic thiocolchicine **3** was also less toxic than thiocolchicine and also retained high affinity for tubulin.[5] However, although compounds **2** and **3** have promise as antitumor agents, they show low solubility in water. We therefore continued our synthetic efforts and report herein the syntheses and bioactivities of novel water-soluble derivatives of **2** and **3**.

2-Demethylcolchicine (**2**), obtained from colchicine (**1**) by an improved procedure,[6] was reacted with *N*,*N*-dimethylglycine and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) in the presence of triethylamine (Et_3N) to afford glycinate **4** in 93% yield. [It should be noted that using dicyclohexylcarbodiimide (DCC), one of the most popular condensing agents, failed to produce the target compound.] Employing the same procedure with 2-demethylthiocolchicine (**3**) yielded glycinate **5** (93% yield). Compounds **4** and **5** were treated with L-tartaric acid in water to produce the corresponding salts, **4a** and **5a**, in yields of 84% and 74%, respectively. These salts showed good solubility in water (>20 mg salt/0.1 mL water). Alternatively, 2,3-didemethylthiocolchicine (**6**)[7] was methylated with 1.4 equiv of $TMSCHN_2$ to produce 2-demethylthiocolchicine (**3**) and 3-demethylthiocolchicine (**7**) as a ca. 1:1 mixture in 74% yield. Recycling column chromatography was used to separate the mixture into pure **3** and **7**. An attempt to prepare the glycinate of **7** was unsuccessful. The esterification with *N*,*N*-dimethylglycine proceeded smoothly as followed by TLC analysis. However, due to the extreme instability of the ester, the desired glycinate (**8**) could not be isolated, and substrate **7** was recovered. Glycinates **4** and **5** and their salts **4a** and **5a** were unstable in aqueous solution (the ratio of glycinate and parent compound became ca. 1:1 after 3 days in water solution, as analyzed by TLC). The hydroscopic crystals, however, were stable for several months when kept dry in a desiccator and refrigerated.

☆ For Part 235 of this series, see Ref. 1.

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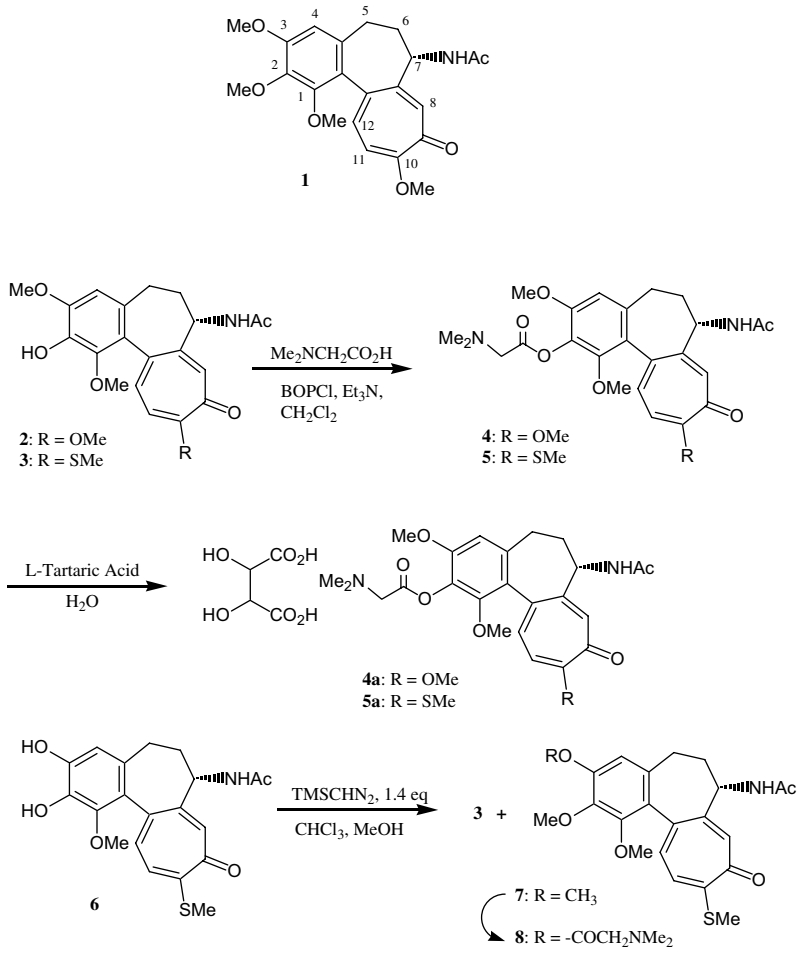

Consequently, compounds **4**, **4a** and **5**, **5a** were evaluated for cytotoxic and antitubulin[8] activities immediately after being dissolved. The IC_{50} values against four human tumor cell lines [A549 lung carcinoma, 1A9 ovarian carcinoma, KB nasopharyngeal carcinoma, and KB-V, a multidrug resistant (p170 PgP) variant] are shown in Table 1. The thiocolchicine glycinate **5** and its salt **5a** both had significant activity against the tumor cell lines, except for the multidrug resistant subline (KB-V). In particular, the activities of the salt **5a** and the parent phenolic compound **3** against 1A9 and KB cells were almost identical. The colchicine glycinate **4** and its salt **4a** showed decreased activity compared to **5** and **5a**. This observation was in correspondence with results for the original phenolic compounds, **2** and **3**. All derivatives were good substrates for P-glycoprotein based on cross-resistance in the KB-V cell line.

Table 1. In vitro anticancer activities of colchicine analogs

Compound	IC_{50}[a] (μg/mL)/cell line			
	A549[b]	1A9[b]	KB[b]	KB-V[b]
1	0.002	0.003	0.003	0.3
2	0.1	0.1	0.1	[24][c]
3	0.01	0.01	0.01	[56][c]
4	0.44	0.04	0.09	[5][c]
4a (L-Tartarate of **4**)	0.88	0.11	0.16	[4][c]
5	0.05	0.05	0.02	[45][c]
5a (L-Tartarate of **4**)	0.06	0.01	0.02	[28][c]

[a] Cytotoxicity as IC_{50} value for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay.
[b] Human lung carcinoma (A549), human ovarian carcinoma (lA9), human epidermoid carcinoma of the nasopharynx (KB), multi-drug resistant KB subclone expressing P-glycoprotein (KB-V).
[c] % Inhibition of cell growth at 4 μg/mL is given in brackets.

Table 2 shows the data for inhibition of tubulin polymerization by compounds **1**–**5**. The antitubulin activities

Table 2. Inhibition of tubulin assembly by colchicine analogs[a]

Compound	IC_{50} (μM) ± SD	Compound	IC_{50} (μM) ± SD
1	2.9 ± 0.7	**4**	15 ± 5
2	4.8 ± 0.9	**4a**	13 ± 2
3	1.5 ± 0.3	**5**	8.7 ± 2
		5a	3.8 ± 0.6

[a] Tubulin (10 μM) and varying compound concentrations were preincubated in 0.8 M monosodium glutamate at 30 °C for 15 min. Samples were chilled on ice, and GTP (final concentration, 0.4 mM) was added. Samples were transferred to cuvettes held at 0 °C in a recording spectrophotometer, and polymerization was followed for 20 min at 30 °C. The IC_{50} is defined as the concentration that inhibits the extent of the reaction by 50%. See Ref. 8 for further details.

generally paralleled the antiproliferative activities, suggesting that tubulin was the critical biochemical target of the novel compounds. We expected that the glycinated derivatives **4** and **5** and their salts **4a** and **5a** would be readily metabolized by cells to generate the parent compounds **2** and **3** and, thus, have comparable activity. However, all of the glycinates showed lower activity than their corresponding phenolic congeners. Two possible reasons are poor absorption into the cell or variable intracellular cleavage of the ester bonds.

In summary, the salt **5a**, in particular, merits further study as an antitumor agent. It is nearly as active as the parent compound **3**, highly soluble in water, and probably, like **3**, much less toxic than colchicine. In further studies, the preparation of additional water-soluble colchicinoid derivatives will be explored to increase the drug development potential of this lead compound.

2. Experimental

2.1. General

All melting points were taken on a Fisher–Johns or MelTemp 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. 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). Recycling preparative HPLC (JAI Company) used the following conditions: column, Shodex Asahipak GS 310 21G; eluent, MeOH; flow rate, 2.5 mL/min; recycled 6–7 times. Optical rotations were measured with a JASCO DIP-1000 polarimeter. All target compounds were characterized by ^{1}H and IR spectral analyses and MS analyses.

2.1.1. 2-*O*-(*N*,*N*-Dimethylglycinyl)-2-demethylcolchicine (4). A solution of BOPCl (1.309 g, 4.08 mmol) and *N*,*N*-dimethylglycine (537 mg, 5.21 mmol) in anhydrous CH_2Cl_2 (10 mL) was cooled to 0 °C, and Et_3N (0.75 mL, 5.4 mmol) was added. The mixture was stirred and warmed to room temperature for 0.5 h. After cooling to 0 °C, 2-demethylcolchicine (282 mg, 0.73 mmol) in anhydrous CH_2Cl_2 (6 mL) was added. After stirring for 18 h at room temperature, the reaction mixture was partitioned between satd $NaHCO_3$ aq and CH_2Cl_2. The aqueous layer was extracted with 10% MeOH/CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (5% MeOH/CH_2Cl_2) to provide glycinate **4** (319 mg, 93% yield): colorless prisms; mp 160–162 °C (hexane/acetone); $[\alpha]_D^{32}$ −240 (*c* 0.22, MeOH); IR (KBr) 3271 (br), 2941, 2840, 2779, 1770, 1663, 1558, 1486, 1460, 1254, 1134, 752 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (br d, 1H, *J* = 5.8 Hz, N*H*), 7.57 (s, 1H, H-8), 7.34 (d, 1H, *J* = 10.8 Hz, H-12), 6.84 (d, 1H, *J* = 10.8 Hz, H-11), 6.58 (s, 1H, H-4), 4.68–4.58 (m, 1H, H-7), 3.99 (s, 3H, OCH_3-3), 3.84 (s, 3H, OCH_3-1), 3.55 (d, 1H, *J* = 16.6 Hz, C*H*HNMe$_2$), 3.54 (s, 3H, OCH_3-10), 3.48 (d, 1H, *J* = 16.6 Hz, CH*H*NMe$_2$), 2.60–2.50 (m, 1H, H-5), 2.46 [s, 6H, N(CH_3)$_2$], 2.45–2.28 (m, 2H, H-5 and H-6), 1.96–1.86 (m, 1H, H-6), 1.94 (s, 3H, NHCOCH_3); MS *m*/*z* 469 (M^+−1), 384 (M^+−$COCH_2NMe_2$).

2.1.2. 2-*O*-(*N*,*N*-Dimethylglycinyl)-2-demethylthiocolchicine (5). Compound **5** (71 mg, 93% yield) was prepared from 2-demethylthiocolchicine (63 mg, 0.16 mmol) in a similar manner as for the synthesis of **4**. Yellow powder; mp 250–251 °C (dec) (hexane/acetone); $[\alpha]_D^{31}$ −230 (*c* 0.21, MeOH); IR (KBr) 3288 (br), 2941, 2836, 2779, 1771, 1660, 1604, 1544, 1485, 1460, 1425, 1322, 1132, 753 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (br d, 1H, *J* = 4.7 Hz, N*H*), 7.41 (s, 1H, H-8), 7.33 (d, 1H, *J* = 10.4 Hz, H-12), 7.06 (d, 1H, *J* = 10.4 Hz, H-11), 6.60 (s, 1H, H-4), 4.72–4.61 (m, 1H, H-7), 3.86 (s, 3H, OCH_3-3), 3.58 (d, 1H, *J* = 15.3 Hz, C*H*HNMe$_2$), 3.57 (s, 3H, OCH_3-1), 3.51 (d, 1H, *J* = 15.3 Hz, CH*H*NMe$_2$), 2.62–2.54 (m, 1H, H-5), 2.49 [s, 6H, N(CH_3)$_2$], 2.43 (s, 3H, SCH_3), 2.44–2.26 (m, 2H, H-5 and H-6), 1.99 (s, 3H, NHCOCH_3), 1.98–1.86 (m, 1H, H-6); MS *m*/*z* 487 (M^+), 402 (M^+−$COCH_2NMe_2$).

2.1.3. 2-*O*-(*N*,*N*-Dimethylglycinyl)-2-demethylcolchicine tartaric acid salt (4a). Glycinate **4** (65 mg, 0.14 mmol) was dissolved in water (1 mL). L-Tartaric acid (20 mg, 0.13 mmol) was added. After stirring for 1 h at room temperature, the solvent was removed under reduced pressure. The residue was triturated with CH_2Cl_2, and the solid (72 mg, 84%) was collected: colorless amorphous solid; mp 143–144 °C (CH_2Cl_2); $[\alpha]_D^{33}$ −130 (*c* 0.22, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.40 (d, 1H, *J* = 10.8 Hz, H-12), 7.38 (s, 1H, H-8), 7.18 (d, 1H, *J* = 10.8 Hz, H-11), 6.86 (s, 1H, H-4), 4.56–4.44 (m, 1H, H-7), 4.47 (s, 2H, C*H*OH × 2), 4.14 (s, 2H, CH_2NMe$_2$), 3.99 (s, 3H, OCH_3-3), 3.88 (s, 3H, OCH_3-1), 3.48 (s, 3H, OCH_3-10), 2.80 [s, 6H, N(CH_3)$_2$], 2.71 (dd, 1H, *J* = 12.5 and 6.1 Hz, H-5), 2.48–2.32 (m, 1H, H-5 or H-6), 2.31–2.16 (m, 1H, H-5 or H-6), 2.04–1.88 (m, 1H, H-6), 1.99 (s, 3H, COCH_3).

2.1.4. 2-*O*-(*N*,*N*-Dimethylglycinyl)-2-demethylthiocolchicine tartaric acid salt (5a). Glycinate **5** (97 mg, 0.20 mmol) was treated in the same manner as **4** to give **5a** (94 mg, 74% yield): yellow amorphous solid; mp 199–200 °C (acetone); $[\alpha]_D^{33}$ −190 (*c* 0.20, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.34 (s, 2H, H-11 and H-12), 7.18 (s, 1H, H-8), 6.86 (s, 1H, H-4), 4.46 (s, 2H, C*H*OH × 2), 4.16 (s, 2H, CH_2NMe$_2$), 3.88 (s, 3H, OCH_3-3), 3.50 (s, 3H, OCH_3-1), 2.82 [s, 6H, N(CH_3)$_2$], 2.71 (dd, 1H, *J* = 13.8 and 7.7 Hz, H-5), 2.47–2.34 (m, 1H, H-5 or H-6), 2.46 (s, 3H, SCH_3), 2.30–2.17 (m, 1H, H-5 or H-6), 2.02–1.88 (m, 1H, H-6), 1.99 (s, 3H, COCH_3).

2.1.5. Methylation of 2,3-didemethylthiocolchicine (6). To a solution of **6** (105 mg, 0.27 mmol) in $CHCl_3$ (1 mL)

and MeOH (0.5 mL), $TMSCHN_2$ (0.14 mL, 2 M solution in hexane) was added at 0 °C. After stirring for 16.5 h, the mixture was allowed to warm to room temperature, and stirring was continued for 7.5 h. After addition of HOAc, the volatiles were removed in vacuo. The residue was purified by column chromatography on SiO_2 using 5% $MeOH/CH_2Cl_2$ as eluent to provide a mixture of **3** and **7** (79 mg, ca. 1:1 by ^1H NMR analysis, 74% yield) along with recovered starting material (25 mg, 22%). Pure compounds **3** and **7** were isolated using recycling preparative HPLC; 3-demethylthicolchicine (**7**) eluted first and 2-demethylthiocolchicine (**3**) eluted last. The compounds obtained were identical to those reported previously,[6] and the methylated positions of both compounds were confirmed by NOESY analysis.

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