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Antitumor Agents. Part 215:† Antitubulin Effects of Cytotoxic B-Ring Modified Allocolchicinoids

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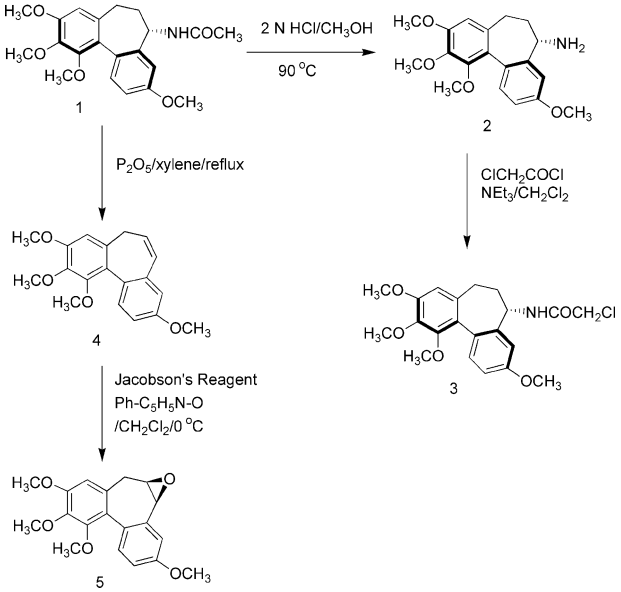
Abstract—*N*-Acetylcolchinol methyl ether **1** served as the starting material to prepare the chloroacetamide (**3**) and epoxide (**5**) analogues. Both **3** and **5** were potent inhibitors of tubulin polymerization in vitro. Compound **3** was also 4-fold more cytotoxic than colchicine against the 1A9 tumor cell line and showed a unique cross-resistance profile.

N-Acetylcolchinol methyl ether **1**, obtained from colchicine by an improved procedure,[2] binds to tubulin with high affinity[3] and has repeatedly been used as a standard to measure the inhibitory effect of compounds related to colchicine on tubulin polymerization and on [^{3}H]-colchicine binding to tubulin.[4,5]

Accordingly, we designed several ligands suitable for a covalent interaction with the colchicine binding site on tubulin. From the known amine **2**,[2] we prepared chloroacetamide **3**, which is related to a chloroacetamide of the colchicine series prepared by Lettré et al.[6] We also prepared the deamino-6,7-dehydrocolchinol methyl ether **4** from **1** via a published procedure,[7] then converted it to the epoxide **5**.[8]

The synthesis[9–12] of **3** and **5** is outlined in Scheme 1 and was based on prior published work.[2] Amine **2** was acylated with chloroacetyl chloride in dichloromethane in the presence of triethylamine to give **3**. After crystallization from methanol, olefin **4** was obtained as the major product on treatment of *N*-acetylcolchinol methyl ether **1** with phosphorous pentoxide in boiling xylene.[7] Epoxide **5** was prepared from **4** with phenylpyridine



Scheme 1. Syntheses of compounds **3** and **5**.

N-oxide and sodium hypochlorite in the presence of Jacobson's reagent.[8]

N-Acetylcolchinol methyl ether **1**, amine **2**, and chloroacetamide **3** have negative rotations and belong to the (*aR*) series of atropisomers.[13] X-ray crystallographic

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Table 1. Inhibition of human tumor cell line replication by **3**–**5** and drug-resistance profiles

Compd	KB[a]	KB-VIN[a]	Fold-resistance	1A9[a]	A9-PTX10[a]	Fold-resistance
	IC_{50} (nM)[b]			IC_{50} (nM)[b]		
3[c]	12.0±0.1[c]	9.1±0.1	0.8	2.7±0.1	13.8±1.2	5.1
4	60.0	68.0	1.1	60.0	78.0	1.3
5	62.0	62.0	1.0	82.0	>100 (17)[d]	>1.2
Colchicine	11.9±0.1	>100 (0–20)	>8.4	11.9±0.1	13.8±1.2	1.2

[a]Human tumor cell lines are epidermoid carcinoma of the nasopharynx (KB) and ovarian cancer (1A9). Drug-resistant cell lines included KB-VIN (expressing P-glycoprotein) and 1A9-PTX10 (a β-tubulin mutant).
[b]Cytotoxicity as IC_{50} values 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.[2]
[c]Values are mean±SEM from two independent determinations.
[d]If inhibition <50% at highest concentration tested, percent inhibition observed is shown in parentheses.

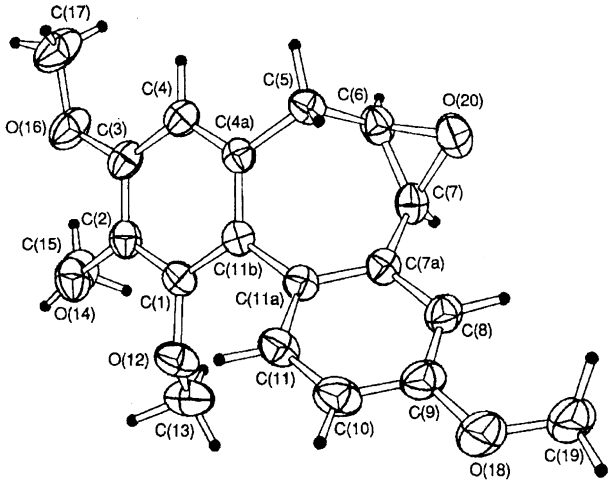

Figure 1. ORTEP diagram (40% probability ellipsoids) showing the crystallographic atom numbering scheme and solid-state conformation of the *aS*,5*R*,6*S* enantiomer of **5**; small filled circles represent hydrogen atoms.

analysis of **5**[14] showed it to be a racemic mixture of a*S*,5*R*,6*S* and a*R*,5*S*,6*R* enantiomers; the solid-state conformation of the former is illustrated in Figure 1.

Compounds **3**–**5** were tested for inhibition of tubulin polymerization as described elsewhere.[15] Colchicine and **1**, as controls, yielded IC_{50} values of 3.0±0.1 and 0.90±0.01 μM, respectively. Compounds **3**–**5** all showed significant inhibitory effects. The IC_{50} value for *N*-chloroacetylcolchinol methyl ether (**3**) was 1.3±0.06 μM, and the olefin **4** yielded a similar value, 1.3±0.2 μM. With an IC_{50} value of 1.9±0.08 μM, the epoxide **5** was somewhat less active.

In addition, **3**–**5** were evaluated for cytotoxic activity against the following four tumor cell lines: nasopharyngeal carcinoma KB and KB-VIN, a multi-drug resistant (MDR) variant, and ovarian carcinoma 1A9 and 1A9-PTX10, which has a mutation in the M40 β-tubulin gene. The IC_{50} values are shown in Table 1. Compound **3** was ca. 4-fold more potent than colchicine against 1A9 cell replication. Unlike colchicine, **3** did not appear to be a p-glycoprotein substrate, since the KB-VIN cell line was even more susceptible to **3** than was the parent cell line. In addition, β-tubulin mutation (1A9-PTX10 cell line) decreased sensitivity of **3** by 5-fold. Compounds **4** and **5** were ca. 5-fold less active than colchicine in the KB, 1A9, and 1A9-PAX cell lines. However, **4** and **5** were not p-glycoprotein substrates, as sensitivities of the KB and KB-VIN cell lines were similar, and did not display cross-resistance.

Acknowledgements

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Supplementary Information

Crystallographic data and parameter tables are available from the author.

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9. All melting points were taken on a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1320 spectrophotometer. ^{1}H NMR spectra were obtained using a Varian 300 spectrometer with TMS as the internal standard. All chemical shifts are reported in ppm. FABMS and ESIMS spectral analyses were determined on a JEOL HX-110 instrument. Elemental ana-

lyses were performed by Atlantic MicroLab, Inc., Norcross, GA. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). EM Kieselgel 60 (230–400 mesh ASTM) was used for column chromatography. Optical rotations were measured with a JASCO DIP-1000 polarimeter.

10. ***N*-Chloroacetylcolchinol methyl ether (3).** Compound **2** (70 mg, 0.21 mmol) was dissolved in 5 mL of dry CH_2Cl_2. Et_3N (0.5 mL) and chloroacetyl chloride (48 mg, 0.42 mmol) were added to the solution at 0 °C, and the resulting mixture was stirred under N_2 at room temperature overnight. The solvent was evaporated, and the residue was purified by flash liquid chromatography to give 76 mg (88%) of compound **3**. White solid, mp 158–159 °C.$[\alpha]_D^{25}$ −15.0 (*c* 0.12, $CHCl_3$); IR 3407, 3291, 2910, 2844, 1733, 1695; EI$^+$MS *m*/*e* 405[M]$^+$; ^{1}H NMR (DMSO-d_6) 8.79 (d, 1H, J=8.7, NH), 7.26 (d, 1H, J=9.0, 11-H), 6.89 (s, 1H, 8-H), 6.88 (d, 1H, J=8.5, 10-H), 6.77 (s, 1H, 4-H), 4.50 (m, 1H, 7-H), 4.10 (AB, 2H, J=12.6, 22.8, 5-H), 3.82 (s, 3H, 3-OCH_3), 3.78 (s, 3H, 2-OCH_3), 3.76 (s, 3H, 9-OCH_3), 3.46 (s, 3H, 1-OCH_3), 3.33 (s, 2H, CH_2Cl), 2.26–2.00 (m, 2H, 6-H). Anal. $C_{21}H_{24}ClNO_5$, C, 62.14, H, 5.96, N, 3.45; Cl, 8.73. Found: C, 61.87; H, 5.97; N, 3.50; Cl, 8.84.

11. **7-Deamino-6,7-dehydrocolchinol methyl ether (4).** Phosphorous pentoxide (1.0 g) was added to a solution of **1** (513 mg, 1.38 mmol) in pure dry xylene (25 mL), and the mixture was boiled for 15 min. The hot xylene solution was decanted from insoluble material, which was well washed with more boiling xylene. The gum obtained by evaporating the combined solutions under reduced pressure was passed through a short silica gel column and crystallized from MeOH to yield 228 mg (53%) of **4**. Mp 98–100 °C. MS *m*/*e* 312 [M]$^+$; ^{1}H NMR ($CDCl_3$) 7.75 (d, 1H, J=8.7, 11-H), 6.88 (dd, 1H, J=2.7, 8.7, 10-H), 6.82 (d, 1H, J=2.7, 8-H), 6.57 (s, 1H, 4-H), 6.53 (dd, 1H, J=1.8, 9.9, 7-H), 6.22 (ddd, 1H, J=6.0, 8.1, 9.9, 6-H), 3.90 (s, 3H, 3-OCH_3), 3.89 (s, 3H, 2-OCH_3), 3.87 (s, 3H, 9-OCH_3), 3.45 (s, 3H, 1-OCH_3), 3.04 (dd, 1H, J=8.1, 12.9, 5-H), 2.79 (ddd, 1H, J=1.8, 6.0, 12.9, 5β-H).

12. **7-Deamino-6,7-epoxy-6,7-dehydrocolchinol methyl ether (5)**. Compound **4** (60 mg, 0.2 mmol) was dissolved in 1 mL of CH_2Cl_2 under N_2 atmosphere, then 1.27 mg (0.002 mmol) of (*S*,*S*)-(*N*,*N*')-bis-(3,5-di-*tert*-butylsalicylidiene)-1,2-cyclohexanediamino manganese(III) chloride and 1.03 mg (0.006 mmol) of 4-phenylpyridine *N*-oxide were added. The resulting brown mixture was cooled to −5 °C, and a cold sodium hypochlorite solution (1.5 equiv, 1.7 M, 0.3 mmol, 0.18 mL) was added slowly with vigorous stirring while maintaining the reaction temperature at 0–2 °C. Upon complete addition of the bleach, stirring was continued for 1 h at 0 °C, then 20 mL of CH_2Cl_2 was added and the mixture washed with satd NaCl solution. The organic layer was dried over Na_2SO_4, evaporated, and purified by flash liquid chromatography (EtOAc/hexane, 1/3) to give 38 mg (63%) of recovered substrate and 15 mg (24%) of **5**, after recrystallization from diisopropyl ether. Mp 116–117 °C. FB$^+$-MS *m*/*e* 328[M]$^+$; ^{1}H NMR ($CDCl_3$) 7.43 (d, 1H, J=9.0, 11-H), 7.20 (d, 1H, J=3.0, 8-H), 6.92 (dd, 1H, J=3.0, 9.0, 10-H), 6.63 (s, 1H, 4-H), 3.92 (s, 3H, 3-OCH_3), 3.91 (s, 3H, 2-OCH_3), 3.88 (s, 3H, 9-OCH_3), 3.62 (s, 3H, 1-OCH_3), 3.56 (ddd, 1H, J=5.4, 5.4, 8.1, 6-H), 3.50 (d, 1H, J=5.4, 7-H), 3.10 (dd, 1H, J=5.4, 12.9, 5-H), 2.36 (dd, 1H, J=8.1, 12.9, 5β-H). Anal. $C_{19}H_{20}O_5$, C 69.50, H 6.14. Found: C 69.36, H 6.21.

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14. Compound **5**, mp 116–117 °C, was crystallized from diisopropyl ether. Crystal data: $C_{19}H_{20}O_5$, M=328.37, triclinic, space group $P\bar{1}(C_i^1)$, a=9.028(1) Å, b=12.003(1) Å, c=7.793(1) Å, α=99.84(1)°, β=94.14(1)°, γ=102.46(1)°, V=807.2(2) Å^3, Z=2, D_{calcd}=1.351 g cm^{-3}, μ(Cu K_α radiation, γ=1.5418 Å)=7.6 cm^{-1}, crystal size: 0.26×0.26×0.30 mm. Intensity data (+h, ±k, ±l, 3324 non-equivalent reflections, θ_{max}=75°) were recorded on an Enraf-Nonius CAD4 diffractometer. The crystal structure was solved by direct methods. Full-matrix least-squares refinement of atomic positional and thermal parameters (anisotropic C, O; isotropic H) converged (max. shift: esd=0.03) at R=0.047 (R_w=0.071) over 2746 reflections with I>2.0σ(I). Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre, deposition number CCDC 179553. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44–1223–336033 or e-mail: deposit@ccdc.cam.ac.uk).

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