New *C(4)*-Functionalized Colchicine Derivatives by a Versatile Multicomponent Electrophilic Aromatic Substitution

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Electrophilic alkylation of colchicine at C(4) was accomplished by a multicomponent aromatic electrophilic substitution reaction with electrophilic aldehydes and carboxylic acids or amides in H_2SO_4. A series of new derivatives were obtained and evaluated for their antiproliferative effect towards various tumor cell lines, and their stimulatory effect on the development of polarity in human neutrophils.

Introduction. – Colchicine is a plant alkaloid that shows antimitotic effects on a number of cancer cell lines. Colchicine binds to tubulin, inhibiting the formation of microtubuli and, thus, blocks mitosis and reduces cell motility in certain cell types [1]. In human neutrophils, in contrast, colchicine stimulates the development of polarity and migration [2]. Unfortunately, colchicine is too toxic to be useful as an antitumor agent. Several hundred colchicine derivatives have been synthesized in the search for analogs with lowered toxicity, and their structure/activity relationship (SAR) is, therefore, well characterized [3][4]. Interestingly, substitution at C(4) has not been studied in detail due to serious difficulties in introducing substituents at that position. A formylation reaction [5] and a *Mannich* reaction with Me_2NH and formaldehyde (HCHO) [6] have been described, however, further acylation attempts were unsuccessful [7]. Herein, we report that colchicine can be functionalized at C(4) by a multicomponent electrophilic aromatic substitution reaction with aldehydes, acids, or amides in H_2SO_4 to yield various C(4)-functionalized derivatives (*Scheme 1*). The compounds were evaluated for their antiproliferative effect on various cell lines and for inhibition of tubulin polymerization and stimulation of development of polarity in human neutrophils.

Results and Discussion. – In the *A*-ring of colchicine, C(4) is the only position available for substitution. Considering that this ring is extremely electron-rich due to the presence of three MeO substituents, such a substitution should require only a very mild electrophilic reagent. Indeed, the few substitution reactions described at that position involve very mild electrophiles, as mentioned above. We were interested to attempt functionalization of colchicine at C(4) by means of a procedure recently described for the electrophilic aromatic substitution of vanillin dervatives, involving reaction in concentrated H_2SO_4 in the presence of aldehydes and various amides [8].

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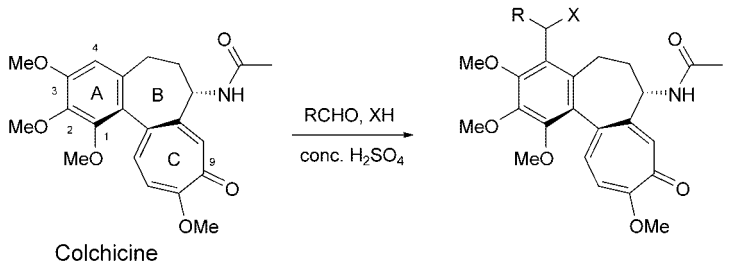
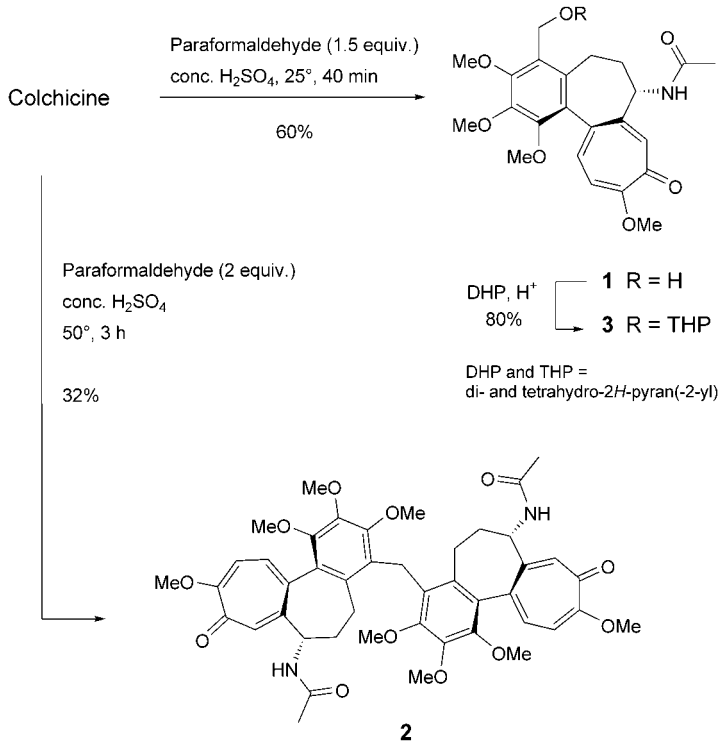
Scheme 1



Colchicine

First, the reaction with HCHO alone was investigated (*Scheme 2*). Reaction of colchicine with paraformaldehyde in H_2SO_4 at 25° led cleanly to **1**, the hydroxymethyl derivative at C(4). This colchicine derivative has been previously prepared by reduction of the corresponding formyl derivative [5b]. The same reaction gave the methylene-bridged dimer of colchicine **2** upon heating at 50°. The primary OH group in **1** was protected as the corresponding tetrahydropyranyl (THP) ether **3** for biological-activity studies (see below).

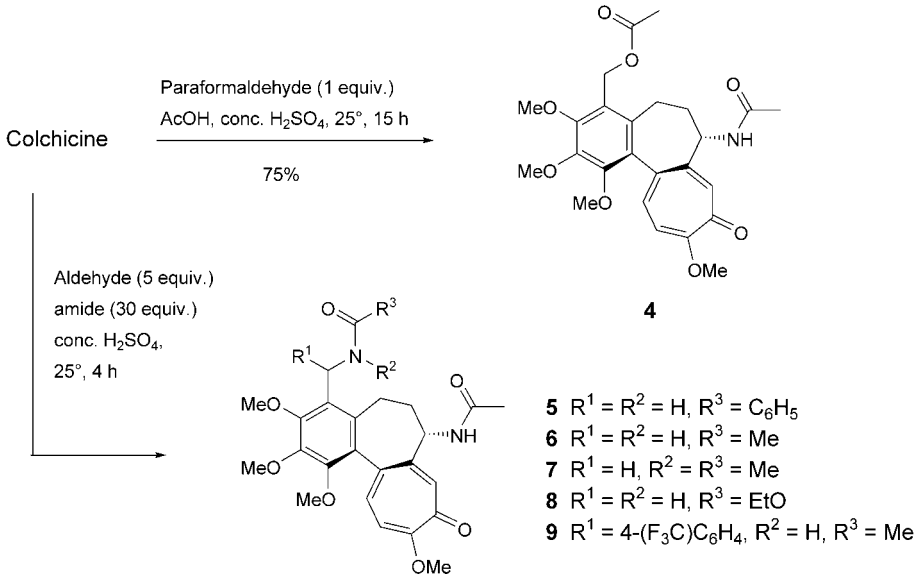
Scheme 2



Aromatic substitution in the presence of AcOH gave the acetoxymethyl derivative **4**, which has been prepared previously in three steps *via* the formyl derivative [5b]. Only AcOH gave an acyloxymethyl derivative, but there was no product formed in the presence of other carboxylic acids such as benzoic or pivalic acid.

The reaction with amides according to [8] lead to the new (acylamino)methyl derivatives **5**–**8** (*Scheme 3*). When HCHO was replaced by 4-(trifluoromethyl)benzaldehyde, compound **9** was isolated as a mixture of stereoisomers. However, reaction with less-electrophilic aldehydes such as benzaldehyde gave no product.

Scheme 3



The mechanism of the above reactions probably involves an acetyl(methylene)oxonium ($AcO^+=CH_2$) electrophile formed by condensation of HCHO with AcOH, or an *N*-acyl imminium cation ($RCON^+=CHR$) from the reaction between aldehyde and amide. Although colchicine possesses a potentially acid-labile enolether function in the *C*-ring, the compound is stable in conc. H_2SO_4, probably due to the formation of a stable tropylium cation by protonation of the C(9)=O group in ring *C*.

The biological activities of the new colchicine derivatives were investigated (*Table*). Antiproliferative activities were evaluated for HL-60 cells, as well as for drug-sensitive (KB-31) and multi-drug resistant (KB-8511) human epidermoid cancer cells. The latter overexpress P-glycoprotein (P-gp), rendering it resistant to various xenobiotics, including paclitaxel and colchicine [9]. Only compound **4** showed antimitotic activity against HL-60 cells, comparable to that of colchicine. Unfortunately, none of the C(4)-derivatives showed antiproliferative activity comparable to that of colchicine, towards the epidermoid cancer-cell lines. Only the alcohol **1** displayed weak activity against colchicine-sensitive KB-31 cells.

Table. *Bioactivities of C(4)-Functionalized Colchicine Derivatives Against Various Cell Lines.* Standard-deviation values refer to three to four experiments.

Compound	HL-60 Inhibition of mitosis [%] at 10 μM	Inhibition of tubulin polymerization [%][a]) at 10 μM	Neutrophils [b]) Polarized cells [%] at 10 μM	KB-31 [c]) IC_{50} [nM]	KB-8511 [c]) IC_{50} [nm]
Colchicine	88 ± 14	100	78 ± 6	8.8	540
1	33 ± 20	100	8 ± 3	260	> 1000
2	29 ± 25	0	3/0 ($n = 2$)	> 1000	> 1000
3	24 ± 25	100	11 ± 14	> 1000	> 1000
4	90 ± 11	100	58 ± 10	> 1000	> 1000
5	n.d. [d])	0	n.d.	> 1000	> 1000
6	n.d.	17	n.d.	> 1000	> 1000
7	n.d.	n.d.	n.d.	n.d.	n.d.
8	n.d.	57	n.d.	> 1000	> 1000
9	n.d.	0	n.d.	> 1000	> 1000

[a]) *In vitro* assay of drug-induced inhibition of GTP-stimulated tubulin polymerization. [b]) 8 ± 10% of the neutrophils were polarized in the control without compound. [c]) Antiproliferative activity on drug-sensitive (KB-31) and multi-drug-resistant (KB-8511) cancer cell lines. The drug concentration that reduced the net cell-mass increase by 50%, after a 96-h incubation, is shown. [d]) Not determined.

Interestingly, the acetoxymethyl derivative **4** was not hydrolyzed to the corresponding alcohol **1** in the presence of lysed HL-60 cells, implying that **4** does not function as a prodrug of **1**. *In vitro* testing of tubulin-polymerization inhibition, indeed, showed that both the alcohol **1** and its acetate **4** were as active as colchicine itself. The much lower activity of **1** compared to **4** as an antimitotic agent with respect to HL-60 might be due to impaired cellular uptake.

The THP-derivative **3** showed a weak, variable activity against HL-60 cells, but inhibited tubulin polymerization completely, again suggesting impaired entry into cells. The hydrophobic THP-ether **3** might act as a prodrug of **1** by diffusing into the cells, after which a small fraction might be hydrolyzed to **1** by acid catalysis. The dimeric colchicine derivative **2** did not show any activity in the assays, except for a small variable effect on HL-60 cells.

The acetate derivative **4** was only slightly less active than colchicine itself in inducing neutrophil polarity, which reflects interaction with the tubulin cytoskeleton in neutrophils. In contrast, derivates **1**, **2**, and **3** were inactive on neutrophils. Also, none of the amide derivatives **5**–**9** showed significant activity in either the tubulin polymerization or antiproliferative assays.

Conclusions. – C(4)-Functionalization of colchicine was realized by electrophilic alkylation with paraformaldehyde and a carboxylic acid or amide in H_2SO_4. This multicomponent reaction provides a simple one-step protocol to introduce a variety of functionalities into the 4-position of colchicine. The procedure can probably be extended to other natural products bearing electron-rich aromatic rings. Of the various C(4)-derivatives studied, only the 4-acetoxymethyl compound **4** showed biological activity comparable to that of colchicine.

Experimental Part

General. All reagents were purchased from *Aldrich* or *Fluka*, or synthesized according to literature procedures. Thin-layer chromatography (TLC): with fluorescent F_{254} glass plates. Flash or regular column chromatography (CC): with *Silica gel 60* (0.040–0.063 mm; *Merck*). Prep. reverse-phase high-performance liquid chromatography (prep. RP-HPLC): HPLC-grade MeCN and *MilliQ* de-ionized H_2O using *Waters prepak cartridge 500g* (*RP-C_{18}* 20 μm, 300-Å pore size) installed on a *Waters Prep LC-4000* system (*Millipore*); flow rate 100 ml/min; gradient: +0.5% MeCN/min. Anal. RP-HPLC: *Chromolith SpeedROD RP-18e* (50–4.6 mm); MeCN/H_2O mixture (H_2O with 1% CF_3COOH; TFA) at a flow rate of 3 ml/min, gradient of 0–50% MeCN in 10 min; UV detection at 254 nm; retention time t_R in min. EI-MS (70 eV): in *m*/*z*.

N-*[(7*S*)-5,6,7,9-Tetrahydro-4-(hydroxymethyl)-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-7-yl]acetamide* (**1**). Paraformaldehyde (12 mg, 1.5 equiv.) was dissolved in 95% H_2SO_4 (4 ml). Then, colchicine (100 mg, 0.25 mmol) was added at r.t., and the mixture was stirred for 40 min. The mixture was neutralized at 0° by slow addition of 2N aq. NaOH soln., and extracted with CH_2Cl_2 (2 × 75 ml). The org. phase was dried (Na_2SO_4) and concentrated, and the residue was purified by RP-HPLC to afford, after lyophilization, 65 mg (60 %) of **1**. Yellow solid. M.p. 149°. Anal. HPLC: t_R 5.1 min. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.58 (*s*, 1 H); 7.43 (*d*, *J* = 5.6, 1 H); 7.31 (*d*, *J* = 10.7, 1 H); 6.90 (*d*, *J* = 10.7, 1 H); 4.71 (*s*, 2 H); 4.61 (*m*, 1 H); 4.02 (*s*, 3 H); 3.98 (*s*, 3 H); 3.96 (*s*, 3 H); 3.61 (*s*, 3 H); 3.02 (*m*, 1 H); 2.22–2.11 (*m*, 2 H); 1.99 (*s*, 3 H); 1.85 (*m*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 180.1; 171.1; 164.9; 153.3; 152.7; 151.8; 146.0; 137.4; 136.6; 134.4; 130.8; 130.3; 127.3; 113.7; 62.4; 62.1; 61.9; 57.4; 57.2; 53.3; 37.0; 25.7; 23.5. EI-MS: 429 (M^+). HR-ESI-MS: 430.1865 ($[M+H]^+$, $C_{23}H_{28}NO_7^+$; calc. 430.1874).

N-*{(7*S*)-5,6,7,9-Tetrahydro-1,2,3,10-tetramethoxy-9-oxo-4-[(tetrahydro-2*H*-pyran-2-yloxy)methyl]benzo[*a*]heptalen-7-yl}acetamide* (**3**). Compound **1** (40 mg, 0.090 mmol), dihydro-2*H*-pyran (13 μl, 1.5 equiv), and a cat. amount (2 mg) of camphor sulfonic acid were dissolved in anh. CH_2Cl_2 (1.5 ml) at 0°. The mixture was stirred at r.t. for 23 h, cooled to 0°, and neutralized by addition of 5 drops of a 25% aq. ammonia soln. The residue was purified by RP-HPLC (gradient 25–35% MeCN in 20 min, without TFA). The main fraction was lyophilized to give **3** (38 mg, 80%). Yellow solid. M.p. 133°. Anal. HPLC: t_R 5.2 min. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.67 (*m*, 1 H); 7.55 (*d*, *J* = 3.21, 1 H); 7.28 (*d*, *J* = 10.0, 1 H); 6.86 (*d*, *J* = 10.0, 1 H); 4.81 (*m*, 2 H); 4.67 (*m*, 1 H); 4.51 (*m*, 1 H); 4.01 (*s*, 3 H); 3.96 (*s*, 3 H); 3.96–3.88 (*m*, 2 H); 3.93 (*s*, 3 H); 3.62 (*s*, 3 H); 3.03–2.95 (*m*, 1 H); 2.37–2.33 (*m*, 2 H); 2.00 (*s*, 3 H); 1.85–1.72 (*m*, 7 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 179.5; 169.9; 164.2; 153.1; 151.7; 151.6; 151.2; 145.5; 136.4; 135.5; 134.6; 130.3; 124.4; 112.5; 98.8; 94.7; 62.9; 61.2; 60.9; 60.7; 56.4; 52.6; 36.3; 30.7; 25.4; 25.2; 22.9; 19.6. EI-MS: 513 (M^+). HR-EI-MS: 513.236268 (M^+, $C_{28}H_{35}NO_8^+$; calc. 513.236270).

N,N′-*[Methylenebis[(7*S*)-5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalene-4,7-diyl]]diacetamide* (**2**). Paraformaldehyde (7.5 mg, 2.0 equiv.) was dissolved in 95% H_2SO_4 (4 ml) at r.t. After 10 min of stirring, colchicine (50 mg, 0.12 mmol) was added, the mixture was heated for 3 h at 50°, and neutralized at 0° by slow addition of an aq. 2N NaOH soln. Then, the mixture was extracted with CH_2Cl_2 (2 × 75 ml), and the org. phase was dried (Na_2SO_4) and concentrated. The residue was purified by RP-HPLC to afford, after lyophilization, **2** (32 mg, 32%). Yellow solid. M.p. 213°. Anal. HPLC: t_R 8.7 min. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.54 (*s*, 2 H); 7.34 (*d*, *J* = 11.0, 2 H); 7.19 (*m*, 2 H); 6.93 (*d*, *J* = 11.0, 2 H); 4.39 (*m*, 2 H); 4.10 (*s*, 2 H); 4.00 (*s*, 6 H); 4.93 (*s*, 6 H); 3.71 (*s*, 6 H); 3.62 (*s*, 6 H); 2.99 (*m*, 2 H); 2.05–1.92 (*m*, 2 H); 1.92 (*s*, 6 H); 1.70–1.64 (*m*, 2 H); 1.44–1.40 (*m*, 2 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 179.2; 171.1; 164.8; 153.5; 153.1; 150.3; 146.1; 138.4; 137.1; 133.7; 130.6; 130.1; 128.7; 114.3; 62.4; 61.8; 61.5; 57.2; 53.4; 36.1; 25.9; 24.1; 23.5. EI-MS: 811 (M^+). HR-ESI-MS: 811.3442 ($[M+H]^+$, $C_{45}H_{51}N_2O_{12}^+$; calc. 811.3446).

*[(7*S*)-7-(Acetylamino)-5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-4-yl]methyl Acetate* (**4**). Paraformaldehyde (22.5 mg, 1.0 equiv.) was dissolved in 95% H_2SO_4/AcOH 1:9 (4 ml). Then, colchicine (300 mg, 0.75 mmol) was added at r.t., the mixture was stirred overnight, and then neutralized at 0° by slow addition of 2N aq. NaOH soln. The mixture was extracted with CH_2Cl_2 (2 × 150 ml), and the org. phase was dried (Na_2SO_4) and concentrated. The residue was purified by RP-HPLC to afford, after lyophilization, **4** (273 mg, 75 %). Yellow solid. M.p. 94°. Anal. HPLC: t_R 6.4 min. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.61 (*s*, 1 H); 7.33 (*d*, *J* = 10.8, 1 H); 6.91 (*d*, *J* = 10.8, 1 H); 5.25 (*d*, *J* = 11.7, 1 H); 5.13 (*d*, *J* = 11.7, 1 H); 4.65 (*m*, 1 H); 4.03 (*s*, 3 H); 3.97 (*s*, 3 H); 3.95 (*s*, 3 H); 3.63 (*s*, 3 H); 2.84 (*m*, 1 H); 2.25–2.17 (*m*, 2 H); 2.09 (*s*, 3 H); 2.02 (*s*, 3 H); 1.89–1.83 (*m*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 180.1; 171.6; 171.5; 165.0; 154.2; 153.3; 152.5; 146.3; 137.5; 137.1; 135.2; 130.7; 130.1; 122.6; 114.1; 62.4; 62.1; 61.9; 58.8; 57.2; 53.4; 36.6; 25.9; 23.2; 21.8. EI-MS: 471 (M^+). HR-EI-MS: 471.189317 (M^+, $C_{25}H_{29}NO_8^+$; calc. 471.189670).

General Procedure for the Synthesis of the Colchicine Derivatives **5**–**9**. The aldehyde (2.5 mmol, 5 equiv.) and the amide (or carbamate; 7.5 mmol, 30 equiv.) were dissolved in 95% H_2SO_4 (1 ml) and stirred at r.t. for

10 min. Then, colchicine (100 mg, 0.25 mmol, 1 equiv.) was added, whereupon the mixture turned yellow. All reactions were complete after 4 h of stirring. The products were isolated by RP-HPLC after dilution of the crude mixture with cold H_2O (30 ml). For the synthesis of **7**, see below.

N-*{[(7*S*)-7-(Acetylamino)-5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-4-yl]methyl}-benzamide* (**5**). Yield: 90 mg (68%). Yellow solid. M.p. 152°. Anal. HPLC: t_R 7.0 min. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.75 (*d*, *J* = 6.6, 2 H); 7.64 (*s*, 1 H); 7.40 (*m*, 3 H); 7.30 (*m*, 1 H); 7.01 (*m*, 1 H); 6.93 (*d*, *J* = 11, 1 H); 6.59 (*m*, 1 H); 4.76 (*m*, 1 H); 4.65 (*m*, 2 H); 4.04 (*s*, 3 H); 4.02 (*s*, 3 H); 3.99 (*s*, 3 H); 3.61 (*s*, 3 H); 3.21 (*m*, 1 H); 2.17 (*m*, 2 H); 2.01 (*s*, 3 H); 1.85 (*m*, 1 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 179.1; 170.8; 167.1; 164.3; 152.9; 152.4; 151.1; 145.3; 140.1; 137.1; 136.5; 134.0; 133.2; 131.7; 130.2; 129.5; 128.6; 126.9; 124.1; 113.6; 61.4; 61.3; 61.2; 56.5; 52.6; 36.4; 35.8; 25.1; 22.7. EI-MS: 532 (M^+). HR-ESI-MS: 533.2287 ($[M+H]^+$, $C_{30}H_{33}N_2O_7^+$; calc. 533.2271).

N-*{(7*S*)-4-[(Acetylamino)methyl]-5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-7-yl}acetamide* (**6**). Yield: 80 mg (68%). Yellow solid. M.p. 161°. Anal. HPLC: t_R 5.3 min. ^{1}H-NMR ($CDCl_3$/CD_3OD, 300 MHz): 8.09 (*m*, 1 H); 7.28 (*s*, 1 H); 7.20 (*m*, 2 H); 6.83 (*d*, *J* = 11,0, 1 H); 4.48 (*m*, 2 H); 4.27 (*d*, *J* = 14,0, 1 H); 3.93 (*s*, 3 H); 3.90 (*s*, 3 H); 3.89 (*s*, 3 H); 3.51 (*s*, 3 H); 2.93 (*m*, 1 H); 2.01 (*m*, 2 H); 1.97 (*s*, 3 H); 1.93 (*s*, 3 H); 1.75 (*m*, 1 H). ^{13}C-NMR ($CDCl_3$/CD_3OD, 75 MHz): 179.5; 170.6; 169.9; 163.9; 152.4; 152.0; 150.6; 144.9; 136.4; 135.5; 133.1; 129.9; 129.5; 123.8; 112.7; 61.1; 61.0; 60.9; 56.1; 51.7; 35.7; 34.9; 24.8; 22.6; 22.2. EI-MS: 470 (M^+). HR-EI-MS: 470.205302 (M^+, $C_{25}H_{30}N_2O_7^+$; calc. 470.205350).

N-*{[(7*S*)-7-(Acetylamino)-5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-4-yl]methyl}*-N-*methylacetamide* (**7**). Prepared according to the *General Procedure*, with a slight modification: parafomaldehyde and *N*-methylacetamide were heated at 60° for 3 h before addition of colchicine. Yield: 10 mg (8%). Yellow oil. Anal. HPLC: t_R 5.8 min. ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.2 (*m*, 1 H); 7.56 (*s*, 1 H); 7.32 (*d*, *J* = 10.9, 1 H); 6.90 (*d*, *J* = 10.9, 1 H); 4.88 (*d*, *J* = 13.7, 1 H); 4.54 (*d*, *J* = 13.7, 2 H); 4.01 (*s*, 3 H); 3.99 (*s*, 3 H); 3.94 (*s*, 3 H); 3.63 (*s*, 3 H); 2.97 (*m*, 1 H); 2.85 (*s*, 3 H); 2.14 (*m*, 5 H); 2.04 (*s*, 3 H); 1.8 (*m*, 1 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 179.4; 170.8; 170.7; 164.2; 153.3; 152.2; 150.9; 145.1; 136.6; 135.9; 134.5; 129.9; 129.7; 122.6; 113.0; 61.4; 61.3; 61.2; 56.4; 52.7; 40.3; 35.6; 34.2; 24.9; 22.6; 21.9. EI-MS: 484 (M^+). HR-ESI-MS: 485.2287 ($[M+H]^+$, $C_{26}H_{33}N_2O_7^+$; calc. 485.2293).

*Ethyl {[(7*S*)-7-(Acetylamino)-5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-4-yl]methyl}carbamate* (**8**). Yield: 124 mg (99%). Yellow solid. M.p. 102°. Anal. HPLC: t_R 6.6 min. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.67 (*s*, 1 H); 7.36 (*d*, *J* = 10.7, 1 H); 7.34 (*m*, 1 H); 6.95 (*d*, *J* = 10.7, 1 H); 5.03 (*m*, 1 H); 4.62 (*m*, 1 H); 4.37 (*m*, 2 H); 4.10 (*q*, *J* = 7.0, 2 H); 4.03 (*s*, 3 H); 3.98 (*s*, 3 H); 3.96 (*s*, 3 H); 3.59 (*s*, 3 H); 3.12 (*m*, 1 H); 2.27–2.06 (*m*, 2 H); 2.03 (*s*, 3 H); 1.88–1.79 (*m*, 1 H); 1.23 (*t*, *J* = 7.0, 3 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 179.0; 170.9; 164.3; 158.9; 156.3; 152.9; 152.8; 150.9; 145.2; 137.4; 136.7; 133.0; 130.0; 129.3; 124.7; 113.8; 61.4; 61.2; 61.0; 56.5; 52.7; 36.7; 36.2; 25.0; 22.6; 14.6. EI-MS: 500 (M^+). HR-ESI-MS: 501.2236 ($[M+H]^+$, $C_{26}H_{33}N_2O_8^+$; calc.: 501.2230).

N-*{[(7*S*)-7-(Acetylamino)-5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-4-yl][4-(trifluoromethyl)phenyl]methyl}acetamide* (**9**). Yield: 55 mg (36%). Yellow solid. M.p. 169°. Anal. HPLC: t_R 8.3 min. ^{1}H-NMR ($CDCl_3$, 300 MHz): 8.17 (*m*, 1 H); 7.71 (*s*, 1 H); 7.64 (*d*, *J* = 8.1, 2 H); 7.43 (*d*, *J* = 10.6, 1 H); 7.25 (*d*, *J* = 8.1, 2 H); 6.98 (*m*, 2 H); 6.72 (*d*, *J* = 9.9, 1 H); 4.74 (*m*, 1 H); 4.04 (*s*, 3 H); 3.91 (*s*, 3 H); 3.64 (*s*, 3 H); 3.29 (*s*, 3 H); 3.17 (*m*, 1 H); 2.25 (*m*, 2 H); 2.16 (*s*, 3 H); 2.10 (*s*, 3 H); 2.05 (*m*, 1 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 179.3; 171.6; 170.5; 164.5; 158.8; 152.5; 152.2; 151.5; 146.3 146.0; 136.9; 136.8; 132.7; 130.3; 129.7; 129.2; 127.1; 125.7; 125.7; 125.6; 122.2; 113.6; 61.4; 61.0; 60.4; 56.6; 53.0; 50.0; 36.0; 25.4; 23.3; 22.5. EI-MS: 614 (M^+). HR-ESI-MS: 615.2318 ($[M+H]^+$, $C_{32}H_{34}F_3N_2O_7^+$; calc.: 615.2336).

Assay for Proliferation Inhibition of HL-60 Cell Mitosis. HL-60 Promyelocytic leukemia cells were obtained from the *American Type Culture Collection* (*ATCC*, Manassas, Virginia, U.S.A.; No CCL-240) and cultured in *Iscove*'s medium (*Fakola AG*, Basel, Switzerland) supplemented with 10% fetal calf serum, penicillin (2 U/ml), and streptomycin (2 U/ml) [10]. For mitosis assays, cells (0.5×10^6/ml) were incubated in the above medium at 37° in a humidified atmosphere (CO_2/O_2 5:95) for 24 h in the absence (control) or presence of colchicine derivatives. Subsequently, the cell number was determined. In untreated samples, this number increased to $0.9 \pm 0.1 \times 10^6$ cells/ml ($n = 4$) after 24 h. The increase in cell number obtained in the absence of added compounds was taken as 100% before calculating inhibitory effects of the compounds.

Assay of Neutrophil Morphology. Neutrophils were isolated as described from heparinized human blood obtained from healthy donors [2]. For analysis of the development of polarity, neutrophils (3×10^6 cells/ml) were incubated for 10 minutes in *Gey*'s medium (supplemented with 0.1% human serum albumin, 1 mM $CaCl_2$, and 1 mM $MgSO_4$) in a shaking water bath at 37°. Subsequently, the buffer or colchicine derivate was added, and incubation was continued for another 30 min. Then, the cells were fixed in 1% glutaraldehyde for 30 min, and

the percentage of polarized cells was determined as described in [2]. Per sample, the morphologies of a total of 100 cells were assessed.

Assay for Tubulin-Polymerization Inhibition. Inhibition of GTP-induced tubulin polymerization by test compounds (10 μM) was assessed by centrifugal separation of polymerized tubulin (pellet) from non-polymerized tubulin (supernatant), basically as described in [11]. Briefly, 50 μl of bovine brain tubulin (*Cytoskeleton*, Denver, CO, U.S.A.) reconstituted to 2 mg/ml in MSG-buffer (=1.4M L-glutamic acid monosodium monohydrate, 1 mM $MgCl_2$, pH 6.6) was mixed with 5 μl of a 2 mM DMSO soln. of test compound, and pre-incubated for 15 min at 30° in a final volume of 95 μl. After chilling the sample for 5 min on ice, 5 μl of a 20 mM GTP (Guanosine-5′-triphosphate disodium dihydrate) soln. was added, and the tubulin polymerization reaction was started by transferring the incubation mixture to a water bath held at 37°, and continued for 30 min. Following centrifugation in a microcentrifuge for 20 min at 14000 r.p.m., the level of nonpolymerized tubulin in the sample supernatant was assessed by the *Lowry* protein-quantification method (*DC Assay Kit*, *Bio-Rad Laboratories*), using a spectrophotometer. The degree of tubulin-polymerization inhibition by test compound (expressed in percent) is reflected by the extent to which the decrease in optical density is diminished compared to that induced by a 1 mM soln of GTP/5% DMSO (maximal polymerization, 0% inhibition).

Assay for Proliferation Inhibition of Drug-Sensitive and Multi-Drug-Resistant Cancer-Cell Lines. Human KB-31 (drug-sensitive) and KB-8511 (multidrug-resistant due to P-gp overexpression) epidermoid carcinoma-cell lines were obtained from Dr. *R. M. Baker* (Roswell Park Memorial Institute, Buffalo, NY, U.S.A.), and have been previously described [9]. Antiproliferative assays were performed as described in [12]. Briefly, cells were seeded at 1.5×10^3 cells/well into 96-well microtiter plates, and incubated overnight at 37° in a CO_2/O_2 5:95 atmosphere at 80% rel. humidity. Twofold serial compound dilutions were performed after 24 h, with the highest drug concentration being 1 μM. Following incubation of the cell plates for an additional 96 h, cells were fixed with 3.3 vol-% glutaraldehyde, washed with H_2O, and stained with 0.05 weight-% Methylene Blue. After washing, the dye was eluted with 3% aq. HCl soln., and the optical density was measured at 665 nm with a spectrophotometer. IC_{50} values, defined as the drug concentration leading to 50% inhibition of net cell-mass increase compared to untreated control cultures, were determined mathematicallly by means of curve-fitting.

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