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Stereochemical variations on the colchicine motif. Peracid oxidation of thiocolchicone. Synthesis, conformation and inhibition of microtubule assembly

Ulf Berg,* Håkan Bladh and Konstantinos Mpampos

Organic Chemistry 1, Dept. of Chemistry, Lund University, P.O. Box 124, S-22100 Lund, Sweden. E-mail: ulf.berg@orgk1.lu.se; Fax: +46 46 2224119; Tel: +46 46 2228123

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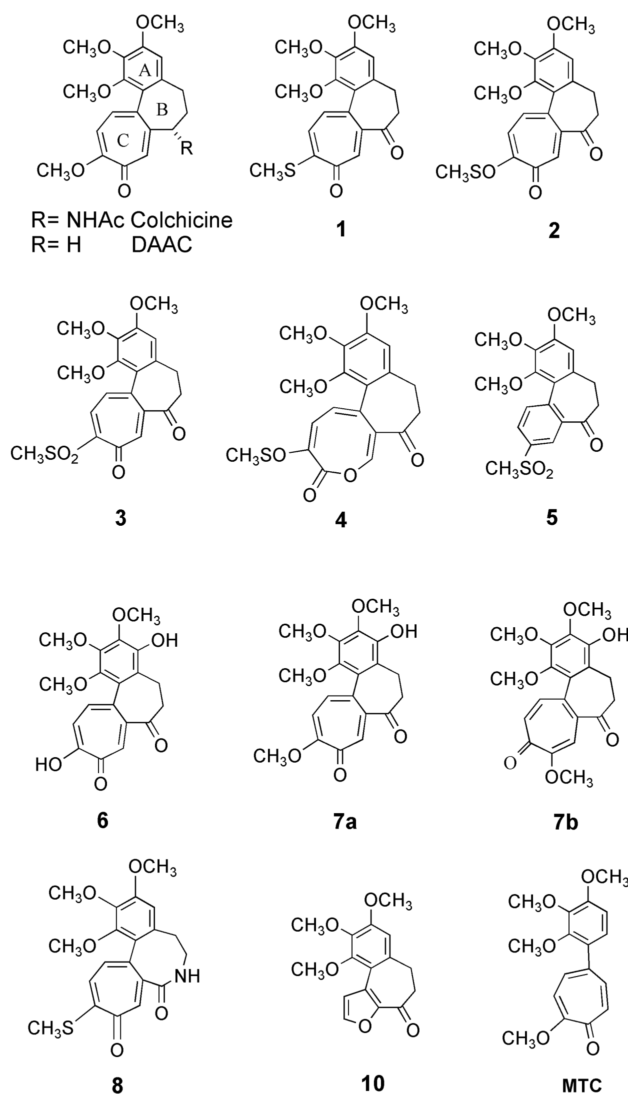
When 7-oxodesacetamidothiocolchicine (**1**) was treated with various peroxides in order to afford a Baeyer–Villiger rearrangement, a complex mixture of products was formed, which included the sulfoxide, (**2**) and sulfone, (**3**). When peracetic acid was used two additional products were formed; a C-ring lactone (**4**) and a ring-contracted allocolchicine derivative (**5**). The sulfoxide (**2**) was semi-preparatively resolved into enantiomers by chromatography on microcrystalline triacetylcellulose. Rotational barriers around the A–C pivot bond of **2**, **4** and **5** were determined by dynamic ^{1}H NMR analysis. The compounds **2**, **3**, **4** and **7a** exhibit moderate inhibition of tubulin polymerization, according to *in vitro* turbidity studies, whereas **5** was inactive.

Introduction

Colchicine, the major alkaloid isolated from *Colchicum autumnale*, is a well-known tubulin toxin. Binding of colchicine to tubulin prevents microtubule assembly and causes cells treated with the drug to arrest in mitosis. The structure of the high-affinity binding site on one of the non-identical subunits of tubulin, probably the β-subunit, is not known, but it is believed that binding induces a conformational change in the protein and thereby inhibits polymerization. Structure–activity relationship studies have indicated that stereochemistry plays an important role in the ability of colchicine and related compounds to bind to tubulin.[1–3] We have been interested in the structural requirements of colchicinoids for binding to tubulin, in particular the conformation around the pivot bond joining the A and C rings.[4–7] The dihedral angle around this bond is close to 54° and the biaryl configuration is *aR* in colchicine and active analogs.[7] A suggestion that colchicine undergoes major conformational changes around this bond upon binding has been put forward[8] and rejected.[4,9,10] One possibility to modify the A–C dihedral angle is to change the size of the B-ring. We have earlier described a Beckmann approach to an eight-membered analog, **8**.[6] An alternative way to expand the B-ring to an eight-membered derivative is *via* a Baeyer–Villiger reaction.

This work deals with such an approach and presents the syntheses, NMR spectroscopy, enantiomeric resolution and binding experiments of new derivatives of colchicine: 7-oxodesacetamidothiocolchicine sulfoxide (**2**), 7-oxodesacetamidothiocolchicine sulfone (**3**), 4-hydroxy-7-oxodeacetamidocolchicine (**7a**); a Baeyer–Villiger product was formed, but surprisingly a C-ring lactone (**4**), as well as a C-ring contracted compound (**5**) (Scheme 1).



Scheme 1

Results and discussion

Syntheses

The synthesis of the keto derivative **1** is easily achieved in three steps from colchicine.[5,11] Reaction of **1** with *m*-chloroperbenzoic acid (MCPBA), however, gave no ring-expanded lactone, but instead the sulfone **3** in good yield. Further addition of oxidant or heating did not lead to detectable amounts of lactone. When the reagent was changed to peracetic acid another oxidation product could be isolated in good yield, the sulfoxide **2**, when the reaction was carried out at −10 °C.

Preparative liquid chromatography treatment of **2** on microcrystalline triacetylcellulose, pre-purified by flash chromatography (*vide infra*), afforded very small amounts (≈1 mg) of a byproduct. According to its ^{1}H and ^{13}C NMR, IR and mass

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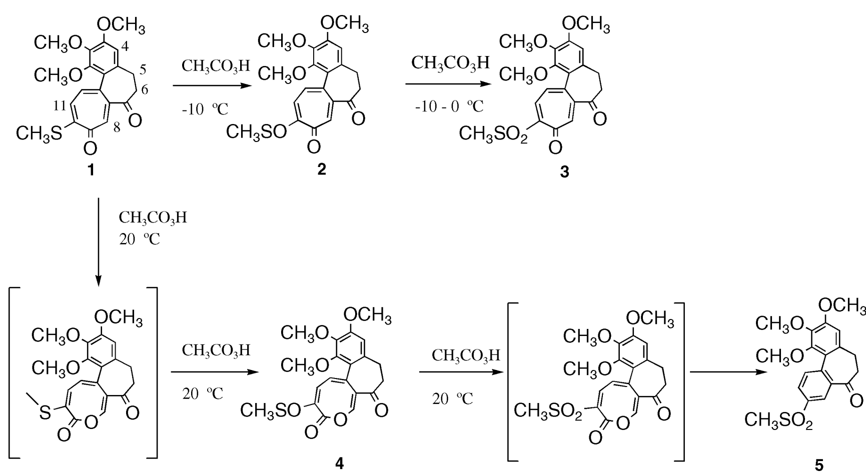
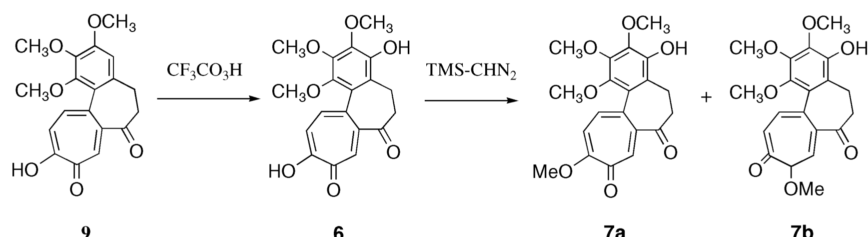


Scheme 2



Scheme 3

spectra the byproduct could be identified as lactone **4** and not the expected B-ring lactone. Prolonged reaction at 20 °C led to further oxidation of **2** to the sulfone **3**, and eventually after 3 days to the ring-contracted benzene analogue **5** in low yield (Scheme 2). The low aptitude of **1** to undergo Baeyer–Villiger reaction and the problems with sulfur oxidation motivated a change of substrate. In 7-oxodeacetamidocolchiceine (**9**) the colchicine and the isocolchicine forms are in a tautomeric equilibrium. If only one of the isomers is able to react, the rearrangement can proceed *via* this tautomer. However, attempts to rearrange **9** using MCPBA, or peroxytrifluoroacetic acid as the oxidizing reagent, did not lead to the desired lactone product. MCPBA gave no reaction, but peroxytrifluoroacetic acid as the reagent resulted in the A-ring hydroxylated product **6**, which could be methylated to **7a/7b** with trimethylsilyldiazomethane. The phenol in C-4 position was not methylated under the reaction conditions, although the reagent was used in excess, probably as a result of steric protection by the 3-methoxy group (Scheme 3).

Solution studies of 2, 3, 4 and 5

Since crystals suitable for X-ray analysis were not available, the identities of the new compounds were deduced from their spectroscopic properties. ^{1}H and ^{13}C NMR data of the compounds are presented in the Experimental section. 2-D NMR spectroscopy, including COSY, NOESY and HETCOR experiments, confirmed the structures. All chemical shifts are close to those of similar colchicine derivatives.[12] The electron-withdrawing effect of the sulfoxide and sulfone groups resulted in downfield shifts of adjacent protons. Thus, for **2** and **3** the 11-H (numbered according to Scheme 2) is shifted 0.86 and 1.21 ppm downfield compared to **1**, and in **4** and **5** the 8-H is shifted further downfield by 0.81 and 1.05 ppm compared to **2** and **3**, respectively. The identity of **4** was deduced from several spectral observations. The sulfoxide function could be identified by the appearance of signals from two diasteromers in the ^{1}H NMR (*vide infra*) spectrum and an IR vibration at 1055 cm^{-1}. The IR spectrum contained two carbonyl stretching vibrations, one at 1696 cm^{-1} in agreement with the corresponding band for the B-ring carbonyl in **1** (1702 cm^{-1}), and one at 1755 cm^{-1}, as expected for an ester or lactone with this type of substitution pattern. The carbonyl vibration in the tropone moiety appears at 1601 cm^{-1} in **1** and possibly below 1600 cm^{-1} in **2**. The ^{13}C NMR carbonyl chemical shift of the B-ring is typical for a ketone, 205.4 ppm, similar to the values in **1** and **2**. The shift for C-11 appeared *ca.* 10 ppm to higher field in **4** compared to the corresponding carbon of **1** and **2** as a result of the change from the β-carbon in an α,β-unsaturated carbonyl compound to a vinyl ester type. The ^{1}H NMR shifts for H-12 (7.79 ppm), H-16 (7.08 ppm) and H-17 (7.44 ppm) in **4** are also in agreement with the proposed structure. The remaining protons did not change appreciably. ^{1}H and ^{13}C NMR data are shown in Fig. 1. Three-bond HETCOR correlations were observed from H-12 to C-1 and C-14, from H-16 to C-1 and C-14, and from H-17 to C-11 in the C-ring.

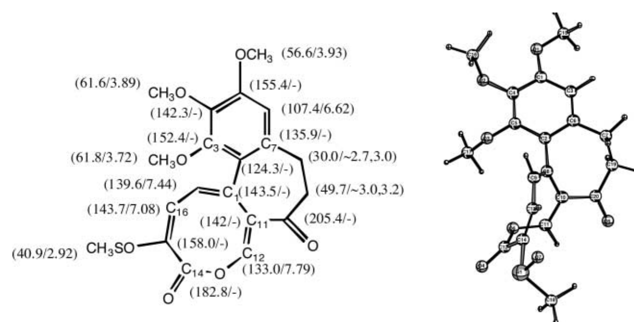


Fig. 1 Assignment of ^{13}C and ^{1}H NMR chemical shifts (left, average of diastereomers) and DFT (pBD/DN**//6-31G**) optimized structure (right) for **4**.

The C5–C6-ethylene bridge showed diastereotopic geminal protons implying that the A- and C-rings are twisted with respect to each other and that rotation around the pivot bond is slow on the NMR time scale at room temperature. The sulfoxides, **2** and **4**, have a stereogenic sulfur atom and exist as diastereomeric atropisomers. Thus, the signals from several protons, *e.g.* the 1-methoxy, the methylthio and A- and C-ring protons, were doubled. By integration, the two diastereomers of **2** were determined to exist in a 55 : 45 ratio in $CDCl_3$. The

Table 1 Experimental barriers to rotation and relative conformer energies for **DAAC** and **1**–**5** and **8**

Compound	$\Delta G^{\ddagger c}$/kJ mol^{-1}	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta G^{\circ c,d}$/kJ mol^{-1}	Solvent
DAAC[a]	92.1 ± 0.4	71.5 ± 1.2	−68.6 ± 10	—	95% EtOH
1[b]	77.4 ± 0.4	—	—	—	Tetrachloroethane-d_2
2	71.8 ± 0.4	61.9 ± 1.7	−33.0 ± 15	1.1	Toluene-d_8
4	76.1 ± 0.4	68.2 ± 1.6	−26.3 ± 15	0.8	Toluene-d_8
5	66.3 ± 0.4	56.6 ± 1.5	−32.3 ± 15	—	Toluene-d_8
8[e]	127 ± 1	—	—	—	Benzene
10[f]	59.0 ± 0.4	—	—	—	Acetonitrile-d_3

[a] Ref. 4. [b] Ref. 5 [c] Value given at 300 K. [d] Referring to the equilibrium between the two diastereomeric atropisomers. [e] Ref. 6. [f] U. Berg, K. Mpampos and F. Hunegnaw, unpublished results.

corresponding values in toluene-d_8 and CD_3OD were 61 : 39 and 50 : 50, respectively, suggesting electrostatic interactions as the major diastereo discriminating factor. In **4** the diastereomeric distribution was 58 : 42 in $CDCl_3$.

Variable-temperature NMR studies[13] in toluene-d_8, using both the 4-H and the 10-CH_3SO signals as probes for **2** and **4** and the ethylene bridge protons for **5**, gave the thermodynamic parameters given in Table 1 for the barrier to rotation around the pivot bond, obtained from the Eyring plots shown in Fig. 2.

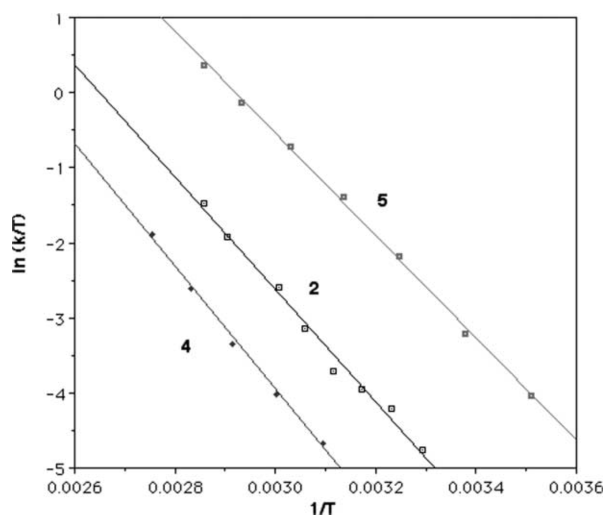


Fig. 2 Eyring plots for the atropisomeric isomerization of compounds **2**, **4** and **5**.

In a previous work[4] the barrier to rotation of desacetamidocolchicine (**DAAC**) was determined as $\Delta G^{\ddagger}_{300K}$ = 92 kJ mol^{-1}. The present experiments show that the barriers are considerably lower for compound **2**, **4** and **5** than in **DAAC**. Thus, these former compounds do not have high enough barriers to allow for resolution with respect to atropisomerism as was performed for **DAAC**.[4] For the C-ring contracted analogue **10**, the barrier is even lower due to an easier accommodation of the less constrained B-ring in the transition state. The surprisingly low barrier for **4** certainly has its origin in the folded and more floppy structure of the eight-membered C-ring. These results also imply that tubulin binding studies have to be performed on atropisomeric mixtures, diastereomers for **2** and **4**, and enantiomers for **3** and **5**.

Structure of 4 and mechanism of the C-ring rearrangement

The structure of **4** can be expected to differ considerably from other colchicine analogs since the eight-membered C-ring is far from planar. Fig. 1 shows the structure calculated by the DFT method using the pBD/DN**//6-31G** basis set in the Spartan program.[14] The C-ring is folded in a tub conformation and the torsional angle around A–C pivot bond is 60°. The surprising Baeyer–Villiger tropone ⟶ lactone exchange reaction in the C-ring of colchicinoids or other tropone derivatives has no precedence in the literature to our knowledge. It was verified that **2** did not lead to Baeyer–Villiger reaction of the C-ring. The low yield of **4** is thus rationalized by the rapid oxidation to sulfoxide. The mechanism is under investigation both experimentally and computationally and will be reported elsewhere.

Enantiomeric resolution and circular dichroism spectrum of 2

The racemate of the sulfoxide **2** was readily resolved on a semi-preparative scale by use of a microcrystalline triacetylcellulose column. The enantiomers were not baseline separated, but by excluding a middle fraction and reinjection of each enantiomer, a >95% ee was obtained. The lactone **4** is eluted prior to the first eluted enantiomer of **2**. Repeated chromatographic resolution afforded *ca.* 5 mg of **4** as a mixture of enantiomers. The chromatogram is shown in Fig. 3.

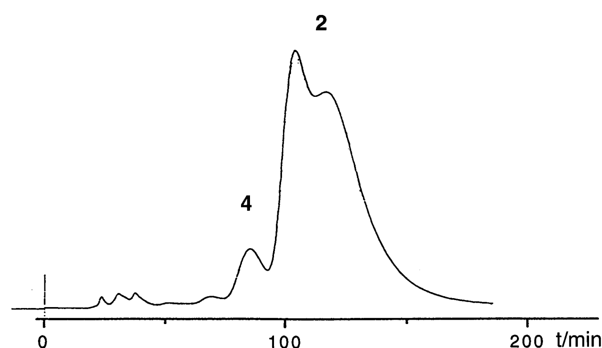


Fig. 3 Enantiomer separation of compound **2** on a microcrystalline triacetylcellulose stationary phase column with 95% ethanol as eluent. Compound **4** is eluted just prior to the first eluted enantiomer of compound **2**.

The CD spectrum of the first eluted enantiomer of **2** in ethanol is shown in Fig. 4. It contains four bands above 220 nm, centered at 230, 310, 370 and 420 nm. The CD-spectrum of **2** is perturbed by the stereogenic sulfoxide substituent, and the two

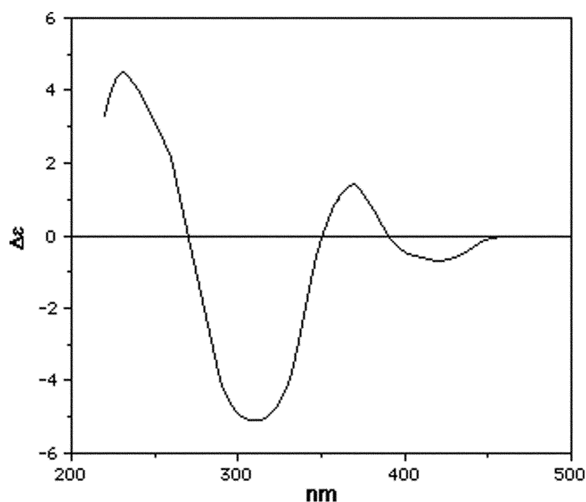


Fig. 4 CD Spectrum of the first eluted enantiomer of **2** in 95% ethanol.

diastereomeric atropisomers are essentially equally populated in ethanol. Thus, the CD spectrum of **2** does not contain the features typical of colchicinoids and could not be used to assign the absolute configuration or the helical stereochemistry by analogy with other colchicine derivatives.[4–6,10]

Binding studies to tubulin of 1, 2, 3, 5 and 7a

Thiocolchicone **1** has been investigated earlier and it was shown that the compound binds rapidly to tubulin and depolymerizes microtubules in human prostate cancer cells.[5] In the present study the effect on assembly of microtubules *in vitro* was determined in a temperature-controlled spectrophotometer by measuring the change in absorbance at 450 nm.[15] The increase of absorbance is a result of light scattering induced by the assembling microtubules. The results are shown in Fig. 5. It turns out that, judging from these experiments, the thioether **1** is the most powerful microtubule inhibitor of the compounds studied. The enantiomers of the sulfoxide **2** were found to be of equal activity. The sulfone **3** was shown to be the least potent microtubule inhibitor. Thus, the experiments indicate that oxidation of the thioether results in decreasing tubulin binding ability. Competition experiments were performed with the polymerization inhibiter **MTC**, which binds rapidly to tubulin but with moderate affinity.[16,17] The results suggest that **2** and **3** bind slightly more strongly to tubulin than **MTC**. No binding results are available for **4**. Compound **5** is not affecting microtubule assembly, in contrast to its analog allocolchicine, in which the methylsulfonyl group is replaced by a methyl ester. 4-Hydroxy-7-oxodeacetamidocolchicine (**7a**; not shown in Fig. 5) was found to inhibit microtubule polymerization at low concentrations, indicating that colchicine analogs hydroxylated at C-4 retain tubulin binding ability.

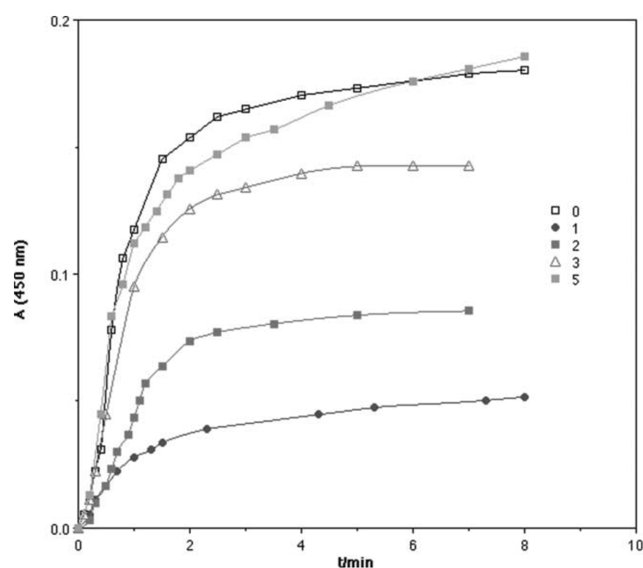


Fig. 5 Effect of the compounds **1**, **2**, **3** and **5** on the turbidity time course of the assembly of microtubule protein at 37 °C. All samples contained 2.5 mg ml^{-1} bovine tubulin (including 20% MAPs). The compound concentrations are 25 mM. The curve marked 0 is without any inhibitor.

Conclusions

In summary, we report the synthesis and tubulin binding properties of the sulfoxide and sulfone analogs of thiocolchicone. We present the isolation of the lactone analog **4** and the ring-contracted analog **5**, although in very low yield in the former case. In addition a DNMR investigation of the dynamic stereochemistry of the new compounds was undertaken. It was shown that the rotational barriers for **2**, **4** and **5** are lower than in **1**, which make them too low to allow for biological studies to be made on individual atropisomers. The results of the tubulin binding experiments indicate that the activity decreases upon oxidation of the 10-methylthio substituent. The differences in potency were relatively minor, and according to these results, the steric and electronic effects of the C-10 substituent on tubulin binding are rather small. Finally, we report the synthesis and tubulin binding properties of a 4-hydroxycolchicone and demonstrate that colchicine analogs hydroxylated in the C-4 position retain tubulin binding activity.

Experimental

Spectra

^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 400 spectrometers, using the solvent peak, usually $CDCl_3$, as internal shift standard. The dynamic NMR experiments were performed as described by Sandström.[13] Temperature calibration of the NMR spectrometer was performed with methanol according to the method described by van Geet.[18] The populations and rate constants were evaluated by visual fitting of the experimental spectra to spectra calculated by the McConnell classical formalism[19] or by DNMR5.[20] The evaluation of T_2 and $\delta\nu$ values for bandshape calculations were performed as previously described.[21] The enthalpies and entropies of activation are obtained by a linear regression analysis of $\ln(k/T)$ *vs.* $1/T$ according to the Eyring equation.[22] Errors in activation parameters have been given with the assumption that the temperature could be determined with an accuracy of ±0.5 K.[13]

CD spectra were recorded with a JASCO Model J-500A spectrometer, UV spectra with a Cary Model 2290 spectrophotometer, IR spectra with a Nicolet spectrometer, and high-resolution mass spectra with a JEOL SX-102 mass spectrometer (electron impact, direct inlet or FAB$^+$).

Chromatographic enantiomer resolution

Chromatographic enantiomer resolution of **2** was performed as described earlier using swollen, microcrystalline triacetylcellulose as stationary phase and 95% ethanol as eluent.[23]

Tubulin binding assay

The effect on assembly of microtubules was determined as described earlier using a temperature-controlled spectrophotometer by measuring the change in light scattering at 450 nm. This experiment has been described in detail earlier.[15]

The DFT calculations were performed on a Silicon Graphics IRIS workstation using the SPARTAN 3.0 program.[14]

Syntheses. *7-Oxodeacetamidothiocolchicine sulfoxide (2).* To 7-oxodeacetamidothiocolchicine (**1**) (50 mg, 0.13 mmol) in 5 ml 90% acetic acid at 0 °C was added 2.5 ml 30% hydrogen peroxide in 3 ml AcOH. After 24 h at 0 °C the solution was poured on ice and extracted with dichloromethane. The organic extracts were washed with $NaHSO_3$ and water, dried and concentrated to a yellow oil. Purification with flash chromatography (ethyl acetate–heptane) yielded a yellow oil, 46 mg as a mixture of enantiomers. Yield 91%; IR (neat) 1710, 1570, 1055 cm^{-1} (S=O); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.95 (1H, 11-H d, 9.7 Hz, minor), 7.93 (1H, 11-H, d, 9.6 Hz, major), 7.44 (1H, 12-H, d, 9.7 Hz, minor), 7.41 (1H, 12-H, d, 9.6 Hz, major), 6.98 (1H, 8-H, s, minor), 6.97 (1H, 8-H, s, major), 6.55 (2H, 4-H, s, minor + major), 3.90 (6H, 3-CH_3O, s, minor + major), 3.88 (3H, 2-CH_3O, s, minor), 3.88 (3H, 2-CH_3O, s, major), 3.68 (3H, 1-CH_3O, s, major), 3.64 (3H, 1-CH_3O, s, minor), 3.20–3.10 (2H, CH_2, m), 3.03–2.81 (4H, CH_2, m), 2.92 (3H, CH_3S, s, major), 2.91 (3H, CH_3S, s, minor), 2.79–2.72 (2H, CH_2, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.9, 204.6 (C_7=O), 183.0,182.9 (C_9=O), 158.5, 158.5 (C_{10}), 155.3 (C_3), 152.5, 152.4 (C_1), 152.3, 152.2 (C_{7a}), 142.2, 142.1 (C_2), 141.7, 141.6, 137.4, 137.3 (C_{12}),

136.8, 136.8 (C_{11}), 135.9, 135.8 (C_{4a}), 134.2, 134.0 (C_{12a}), 124.2 (C_{1a}), 107.4 (C_4), 61.9, 61.8 (CH_3O–), 61.6, 61.5 (CH_3O–), 56.3 (CH_3O–), 47.4 (CH_3SO–), 40.8, 40.6 (C_6), 30.1, 29.6 (C_5); HRMS, *m*/*z*: $C_{20}H_{20}O_6S$ calc. 388.0981, found 388.0980.

7-Oxodeacetamidothiocolchicine sulfone (3). 7-Oxodeacetamidothiocolchicine (**1**) (50 mg, 0.13 mmol) and *m*-chloroperbenzoic acid (95 mg, 85%, 0.47 mmol) were dissolved in 5 ml dry dichloromethane at 0 °C under an argon atmosphere. Concentrated sulfuric acid (one drop) was added to the stirred solution. After four days at 0 °C the solution was diluted with dichloromethane and then washed with water, 5% sodium hydrogen carbonate, 5% sodium thiosulfate, water and brine, dried and concentrated to a yellow oil. Purification with flash chromatography (ethyl acetate–methanol) yielded 35 mg as a yellow oil. Yield 67%; IR (neat) 1710, 1590, 1305, 1160 cm^{-1} (SO_2); ^1H NMR (400 MHz; $CDCl_3$) δ 8.29 (1H, 11-H, d, 9.6 Hz), 7.25 (1H, 12-H, d, 9.6 Hz), 7.11 (1H, 8-H, s), 6.55 (1H, 4-H, s), 3.90 (3H, 3-CH_3O, s), 3.87 (3H, 2-CH_3O, s), 3.67 (3H, 1-CH_3O, s), 3.38 (3H, CH_3SO_2, s), 3.20–2.75 (4H, 5-CH_2 + 6-CH_2, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.0 (C_7=O), 181.8 (C_9=O), 155.6 (C_3), 152.5 (C_1), 150.9 (C_{7a}), 147.3, 145.4, 142.2 (C_2), 141.0, 139.0, 135.9 (C_{4a}), 135.1, 123.8 (C_{1a}), 107.6 (C_4), 62.0 (CH_3O–), 61.6 (CH_3O–), 56.6 (CH_3O–), 47.1 (CH_3SO_2–), 43.5 (C_6), 29.5 (C_5); HRMS, *m*/*z*: $C_{20}H_{20}O_7S$ calc. 404.0930, found 404.0932.

15-Methanesulfinyl-3,4,5-trimethoxy-13-oxa-tricyclo-[9.6.0.0^{2,7}]heptadeca-1(17),2,4,6,11,15-hexaene-10,14-dione (4). The title compound was formed as a byproduct in the reaction of 7-oxodeacetamidothiocolchicine with peroxyacetic acid and was eluted as a mixture of enantiomers, prior to **2** from the TAC-column.

Yield ≈1%; IR (neat) 1755, 1696, 1613, 1595, 1490, 1312, 1055 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ 7.80, (1H, 12-H, s, minor), 7.78 (1H, 12-H s, major), 7.46 (1H, 17-H, d, 12.7 Hz, minor), 7.42 (1H, 17-H, d, 12.7 Hz, major), 7.09 (1H, 16-H, d, 12.7 Hz, major), 7.08 (1H, 16-H d, 12.7 Hz, minor), 6.62 (1H, 6-H, s, major + minor), 3.93 (3H, 5-CH_3O, s, major + minor), 3.89 (3H, 4-CH_3O, s, major + minor), 3.74 (3H, 3-CH_3O, s, major), 3.71 (3H, 3-CH_3O, s, minor), 3.25–3.15 (1H, 8-CH_2, m), 3.10–2.95 (2H, 9-CH_2, m), 2.93 (3H, CH_3SO, s, major), 2.91 (3H, CH_3SO, s, minor), 2.77–2.71 (H, 8-CH_2, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.87, 205.32 (C_7=O), 182.85 (C_9=O), 158.01, 157.89 (C_{10}), 155.38, 155.33 (C_3), 153.44, 152.40 (C_1), 143.72, 143.67 (C_{11}), 143.56, 143.43 (C_{12a}), 142.32, 142.22 (C_2), 142.07, 142.00 (C_{7a}), 139.57, 139.52 (C_{12}), 135.92, 135.89 (C_{4a}), 133.00, 132.91 (C_8), 124.39, 124.32 (C_{1a}), 107.49 (C_4), 61.86, 61.83 (CH_3O–), 61.60, 61.56 (CH_3O–), 56.56 (CH_3O–), 49.78, 49.61 (C_6), 41.06, 40.78 (CH_3SO–), 30.12, 29.95 (C_5); HRMS, *m*/*z*: $C_{20}H_{20}O_7S$ calc. 404.0930, found 404.0930.

3-Methanesulfonyl-9,10,11-trimethoxy-6,7-dihydrodibenzo-[a,c]cyclohepten-5-one (5). To 7-oxodeacetamidothiocolchicine (**1**) (80 mg, 0.21 mmol) in 11 ml 90% acetic acid at 11 °C was added 4 ml of 35% hydrogen peroxide in 4 ml acetic acid. After 24 h at this temperature TLC analysis showed that the starting material had oxidized mainly to the sulfoxide (**2**) and partially further, to the sulfone (**3**). The temperature was raised to 20 °C and after another 24 h the main product was the sulfone (**3**) and a new byproduct (**5**). The solution was poured on ice and extracted with dichloromethane. The organic extracts were washed with saturated aqueous sodium thiosulfate solution and dried over Na_2SO_4. The allocolchcine derivative (**5**) was semi-preparatively (10 mg) isolated by HPLC on a normal phase (YMC-packed, 300 × 20 mm i.d.) column, with EtOAc–heptane 1 : 1 as eluent.

IR (KBr) 1689, 1314, 1154 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ 8.11 (1H, 4-H, d, 1.9 Hz), 8.06 (1H, 2-H, dd, 8.3/2.0 Hz), 7.78 (1H, 1-H, d, 8.3 Hz), 6.64 (1H, 8-H, s), 3.94 (3H, 9-CH_3O, s), 3.91 (3H, 10-CH_3O, s), 3.62 (3H, 11-CH_3O, s), 3.16 (3H, CH_3SO_2, s), 3.20–2.95 (3H, CH_2, m), 2.72 (1H, CH_2, d); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.6 (C_7=O), 154.7 (C_3), 152.6 (C_1), 142.0 (C_2), 140.8, 140.0, 139.7, 136.3 (C_{4a}), 133.1, 129.1, 127.5, 122.9 (C1a), 107.7 (C_4), 61.7 (CH_3O–), 61.5 (CH_3O–), 56.51 (CH_3O–), 48.1 (CH_3SO_2–), 44.8 (C6), 30.3, 30.1 (C5); HRMS, *m*/*z*: $C_{19}H_{20}O_6S$ calc. 376.0981, found 376.0982.

4-Hydroxy-7-oxodeacetamidocolchiceine (6). To an ice-cooled solution of 7-oxodeacetamidocolchiceine (100 mg, 0.29 mmol) in dichloromethane (3 ml) was added a solution of peroxytrifluoroacetic acid in trifluoroacetic acid–dichloromethane (3.0 ml, 0.13 M, 0.38 mmol). After 2 h aqueous sodium thiosulfate (15 ml, 5%) was added and the product was extracted twice with chloroform, washed with brine, dried and concentrated to a yellow solid. The crude product was directly used in the following step. Yield 85–90%; ^1H NMR (400 MHz; $CDCl_3$) δ 7.62 (1H, 11-H, d, 12.2 Hz), 7.48 (1H, 12-H, d, 12.1 Hz), 6.59 (1H, 8-H, s), 6.50–6.20 (1H, –OH, s, br), 3.90 (3H, 3-CH_3O, s), 3.89 (3H, 2-CH_3O, s), 3.58 (3H, 1-CH_3O, s), 3.2–2.8 (3H, CH_2, m), 2.78–2.62 (1H, CH_2, m).

*4-Hydroxy-7-oxodeacetamidocolchicine (7**a**) and 4-hydroxy-7-oxodeacetamidoisocolchicine (7**b**).* The crude 4-hydroxy-7-oxodeacetamidocolchiceine (**6**) (*ca.* 0.29 mmol) was dissolved in diethyl ether–dichloromethane–methanol (2 : 2 : 1, 10 ml). To the stirred solution was added Me_3SiCHN_2 (2 ml, 2 M) under an argon atmosphere. After 50 min the solvents were evaporated, water was added, and the products were extracted with chloroform. The organic phase was dried and concentrated to a brownish oil (120 mg). The isomers were separated by flash chromatography (ethyl acetate) yielding **7b** as the first eluted isomer and **7a** as the second eluted isomer. **7a**: Yield 37%, ^1H NMR (400 MHz; $CDCl_3$) δ 7.10 (1H, 11-H, d, 11.0 Hz), 7.15 (1H, 8-H, s), 6.86 (1H, 12-H, d, 11.0 Hz), 5.70 (1H, –OH, s), 4.02 (3H, 3-CH_3O, s), 4.00 (3H, 2-CH_3O, s), 3.93 (3H, 1-CH_3O, s), 3.45 (3H, 10-CH_3O, s), 3.36–3.28 (1H, m), 2.98–2.60 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.7 (C_7=O), 179.9 (C_9=O), 165.6, 150.8, 145.4, 145.0, 142.4, 140.5, 137.0, 133.3, 132.1, 128.4, 120.6 (C_{1a}), 112.7 (C_4), 61.8 (CH_3O–), 61.7 (CH_3O–), 61.6 (CH_3O–), 57.0 (CH_3O–), 46.8 (C_6), 21.0 (C_5); HRMS, *m*/*z*: $C_{20}H_{20}O_7$ calc. 372.1209, found 372.1207.

7b: Yield 46%, ^1H NMR (400 MHz; $CDCl_3$) δ 7.40 (1H, 11-H, d, 12.8 Hz), 7.37 (1H, 12-H, d, 12.8 Hz), 6.64 (1H, 8-H, d, 0.5 Hz), 5.76 (1H, –OH, s), 4.02 (3H, 3-CH_3O, s), 3.99 (3H, 9-CH_3O, d, 0.5 Hz), 3.94 (3H, 2-CH_3O, s), 3.50 (3H, 1-CH_3O, s), 3.36–3.28 (1H, CH_2, m), 3.02–2.87 (2H, CH_2, m), 2.78–2.68 (1H, CH_2, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.5 (C_7=O), 179.9 (C_9=O), 164.6, 145.3, 145.0, 142.6, 142.3, 141.8, 140.6, 136.5, 134.3, 128.2, 120.2 (C_{1a}), 110.9 (C_4), 61.8 (CH_3O–), 61.62 (CH_3O–), 61.6 (CH_3O–), 56.9 (CH_3O–), 48.8 (C_6), 20.9 (C_5); HRMS, *m*/*z*: $C_{20}H_{20}O_7$ calc. 372.1209, found 372.1208.

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