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Novel Allocolchicinoids with an Eight Membered B-Ring: Design, Synthesis and Inhibition of Tubulin Assembly

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Abstract—Several B-ring variations of *O*-methyl androbiphenyline (**8**), newly accessible from (–)-(*M*,7*S*)-colchicine via photo-oxygenation and subsequent endoperoxide-transformation, were synthesized and evaluated for their inhibitory effects on tubulin assembly in vitro. The amino-allocolchicinoid (**9**), a key compound in this study, was transformed to the highly potent ketone **10** and by oxidation with H_2O_2/Na_2WO_4 to a mixture of *syn*/*anti*-oximes, like **11** and **12**. These could easily be transformed to hitherto unknown allocolchicinoids **13** and **14** with an eight membered B-ring lactam obtained via a Beckmann rearrangement. Surprisingly both do not notably affect tubulin assembly, despite obvious structural similarities with active analogues of the thiocolchicine- and azasteganacin-series.

Introduction

Recent findings in our group have revealed that the Diels–Alder reactions of natural (–)-(*M*,7*S*)-colchicine (**1**) with several hetero- and carbodienophiles ($HOMO_{diene}$/$LUMO_{dienophile}$ controlled) lead to a variety of interesting analogues of the antimitotic alkaloid with novel alterations of the C ring.[1–3]

Especially when (–)-colchicine (**1**) was subjected to photo-oxygenation with singlet oxygen in the presence of hematoporphyrin an intriguing endoperoxide **2** was obtained in high yield.[1] This dihydrocolchicine-8,12-endoperoxide (**2**) proved to be an appropriate starting material to gain the well known allocolchicinoids *N*-acetylcolchinol *O*-methyl ether (**3**, NCME) and androbiphenyline (**4**) in sufficient amounts.[2] Both are members of the allo series of **1** with a benzenoid rather than a tropolone C ring, as occurs in **1** and more potent inhibitors than **1** in inhibition of tubulin assembly (ITA); they possess an (*M*,7*S*) absolute configuration consistent with that of the parental alkaloid.

In our program, aimed at the creation of novel semisynthetic structural modifications of allocolchicine,[4–9] these two bioactive allo-congeners were supposed to be promising starting materials. Thus, in our efforts to investigate structure–antitubulin activity relationships on the one side and to further study the effect of allocolchicinoids on tubulin binding and assembly activity on the other side, we were interested in hitherto unknown variations of allocolchicinoids with an extended B-ring, similar to that found in natural (–)-(*M*)-steganacin (**5**) or in the artificial steganacin aza-analogue **6**.[10,11]

In view of their structures the allocolchicinoid (–)-(*M*)-androbiphenyline (**4**) and (–)-(*M*)-steganacin (**5**) bear some conspicuous resemblance. Both are characterized by an atropisomeric biaryl unit comprising a trimethoxyphenyl ring connected with a twice oxygen-substituted ring. Due to the helicity of the pivot bond joining these two phenyl moieties both **4** and **5** are axially chiral species with the absolute configuration (*M*). In addition the biaryl moiety is bridged by a larger ring (thus also referred to as 'o,o′-bridged' biphenyls), however, of different size: (–)-(*M*)-androbiphenyline (**4**) by a seven membered ring, giving rise to a bis(benzocycloheptadiene) skeleton, (–)-(*M*)-steganacin (**5**) by an eight membered ring, furnishing by one-carbon homologation a bis(benzocyclooctadiene) skeleton.[12]

Since on the one hand the steganacin aza-analogue **6** was shown to be endowed with promising in vitro and in vivo activity, higher than that of natural (–)-(*M*)-steganacin (**5**),[13] and the thiocolchicine analogue **7** with an

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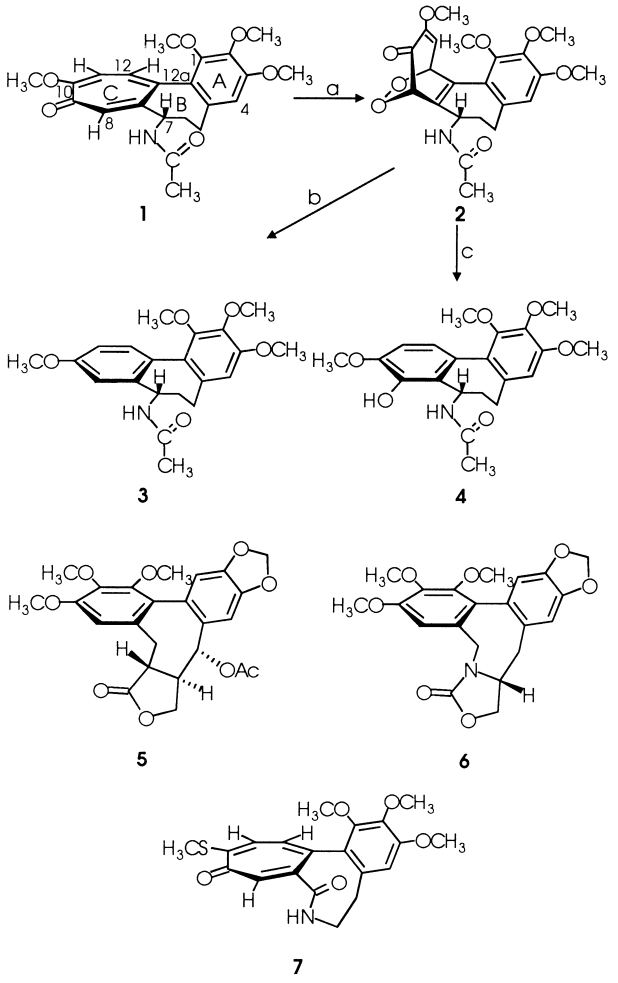


Scheme 1. Reagents and conditions: (a) $^1O_2$, hv, hematoporphyrin, $CHCl_3$, 80%; (b) Ph_3P, CH_2Cl_2, rt, 40%; (c) CH_2Cl_2/CH_3OH, SiO_2, 60%.

eight membered B-ring on the other hand was recently shown to inhibit tubulin assembly,[14–16] we chose the allocolchicinoid **8** as starting material for the syntheses of intriguing variants of **6** and **7** each with an eight membered B-ring and a necessarily modified dihedral angle of the pivot bond. The *O*-methyl androbiphenyline **8** was easily available by treatment of the phenolic **4** with an excess of diazomethane. This could successfully be transformed in a three step procedure to two structurally different azacyclooctanoids **13** and **14**. Synthesis, spectroscopic data and tubulin binding experiments of these novel allocolchicinoids with an eight membered B-ring lactam and some other variations of the natural androbiphenyline (**4**) are described in this paper.

Results and Discussion

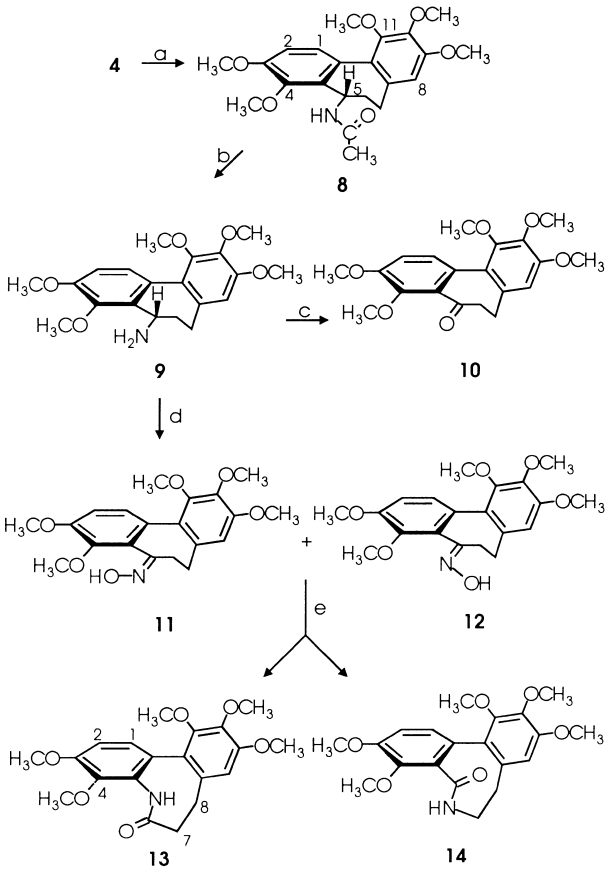
According to a reported procedure for thiocolchicine[17–19] the acetamido group of **8** was easily hydrolysed by acidolysis with methanolic hydrochloric acid to yield the amino-substituted allocolchicinoid **9**. Utilizing Rapoports biomimetic conversion of amines to carbonyl compounds taking place under mild conditions and allowing the transamination in high yields and in a one pot reaction,[20] the primary amine **9** could easily be transformed to the ketone **10** with 67% yield. This is a racemic mixture, suggesting that its enantiomers with a chiral biphenyl backbone equilibrate quickly. On the other hand compound **9** could be directly transformed to a mixture of the corresponding *syn*- and *anti*-oximes **11** and **12**, utilizing an approved methodology with H_2O_2/Na_2WO_4 as oxidizing agent.[21,22] The *syn*/*anti*-ratio of the 50% yield isolated mixture of oximes could be determined to be 1:3 by ^1H NMR spectroscopic analysis in $CDCl_3$ as solvent.

The subsequent Beckmann rearrangement of the not separated *syn*/*anti*-mixture **11/12** was successfully realized employing the conditions of Berg et al.[14] Thus, use of polyphosphoric acid (PPA) at ca. 70 °C for 20 h provided a mixture of the two expected lactams in 68% total yield, which could be easily separated by careful column chromatography. Both compounds **13** and **14** are atropisomers and exist as racemic mixtures with their chiral biphenyl backbone in a (*M*)/(*P*) equilibrium.

The structural assignment of the two crystalline lactams **13** and **14** obtained with 25 and 41% yield, respectively, was secured on the basis of the spectroscopic data (MS, ^1H and ^{13}C NMR). The identical parent ion peaks of **13** and **14** in the MS (*m*/*z* 373 [100%, M^+]) indicated that these products must be structural isomers. The ^1H and ^{13}C NMR data allowed an unequivocal decision between the two constitutions. Firstly they could be distinguished on the basis of the multiplicity of the N–H-protons in the ^1H NMR spectra. Whilst lactam **13** exhibits a triplet at $\delta = 5.81$ ($^3J = 6.3$ Hz) arising from a coupling of the N–H-proton with the two vicinal protons of C-7, the ^1H NMR spectrum of lactam **14** is characterized by a singlet for the 'isolated' N–H-proton, as expected. Furthermore the chemical shifts of C-7 and C-8 at $\delta = 35.46$ and 29.54 in the ^{13}C NMR spectrum of lactam **14** are consistent with those reported for ϵ-caprolactam. In contrast the corresponding signals of C-7 and C-8 of lactam **13** are shifted to lower field ($\delta = 40.38$ for C-7 and 33.70 for C-8) indicating the vicinity to the amide nitrogen (concerning the atomic numbering used, see Experimental).

The hitherto unknown allocolchicinoids **8**, **9**, **10**, **13** and **14** and androbiphenyline **4** were evaluated for inhibition of tubulin assembly in vitro. The data are presented in terms of IC_{50} values (μM), which are the drug concentrations required to inhibit the extent of the assembly by 50% (see Experimental). The IC_{50} values of the newly synthesized compounds were compared to that of (–)-colchicine, measured the same day under the same conditions (Table 1).

Compared with (–)-(*M*,7*S*)-colchicine (**1**) (–)-(*M*,7*S*)-androbiphenyline (**4**) was ca. 2.5 times and (–)-(*M*,7*S*)-*N*-acetylcolchinol *O*-methyl ether (**3**) ca. 3.8 times as active as inhibitors of tubulin assembly. Surprisingly the *O*-methyl derivative of **4**, the novel allocolchicinoid **8**, turned out to be a significant weaker inhibitor, ca. 41-fold less active than the natural substance **4**. This obviously seemed to be due to a steric interaction of the



Scheme 2. Reagents and conditions: (a) CH_2N_2, excess, rt, 96%; (b) 2 M aqueous hydrochloric acid, CH_3OH, reflux, 70%; (c) FMT/DMF, DBN, $(CO_2H)_2/H_2O$, 67%; (d) H_2O_2/Na_2WO_4, CH_3OH, rt, 50%; (e) PPA, 70°C, 20 h; 41% and 25%, resp. (Concerning the atomic numbering used see Experimental.)

Table 1. Inhibition of tubulin assembly

Compound	IC_{50} (μM)
1	8.8
3	2.3
4	3.4
8	140.0
9	Inactive
10	3.1
13	Inactive
14	Inactive

ortho-standing OCH_3-moiety at C-4, with the C-5 *N*-acetyl group, indicated by an unusual reversal of molecular chirality of compound **8** with a (*M*):(*P*) = 3:7 ratio. *N*-Deacetylation of compound **8** exhibited an unexpected negative impact on the inhibitor power, thus the more hydrophilic amino-substituted allocolchicinoid **9** proved to be inactive. In contrast the ketone **10** reveals potent activity in the tubulin-based assay probably deriving from an increased flexibility in the biaryl system with the already mentioned (*M*)/(*P*) equilibration, induced by reduction of the conformational rigidity of the molecule after introduction of a C-5 carbonyl group. To our surprise both novel racemic allocolchicinoids with an eight membered B-ring lactam **13** and **14** do not notably affect tubulin assembly, despite the structural similarities especially with the colchicinoid (–)-(*M*)-**7**, the concentration of which was shown to be 10 times higher than that for (–)-(*M*,7*S*)-colchicine (**1**) needed to inhibit tubulin assembly.[14] The drastically reduced tubulin binding activity among other things may be a result of a significant increase of the torsional angle between the least-squares planes A and C from 54° in most active compounds like **1** and **10** to more than 75° in inactive compounds like the lactames **13** and **14**. As molecular models reveal, both lactams are comparatively rigid molecules without the possibility of induced fit for drug/tubulin interaction, possibly an additional explanation for the loss of activity.

Conclusion

B-ring variations of the newly accessible natural alkaloid androbiphenyline (**4**), a highly potent allocolchicinoid, were synthesized and evaluated for their inhibitory effects on tubulin assembly in vitro. A key compound in this study was the amino-allocolchicinoid (**9**) which could be transformed to the ketone **10** on the one hand and to the novel allocolchicinoids **13** and **14** on the other hand, each with an eight membered B-ring lactam. Whilst the racemic ketone **10** exhibits greater activity than (–)-colchicine, probably derived from an increased flexibility in the biphenyl system, the ring expanded racemic lactams **13** and **14** do not arrest tubulin assembly despite their narrow structural similarities with active analogues like the azasteganacin **6** or the thiocolchicinoid **7**.

Experimental

General procedures

Standard vacuum techniques were used in handling of air sensitive materials. Melting points are uncorrected: 'Leitz–Heiztischmikroskop' HM-Lux. Solvents were dried and freshly distilled before use according to literature procedures. IR: Perkin–Elmer 257, 398 and FT–IR spectrometer 510-P (Nicolet). Liquids were run as films, solids as KBr pellets. ^1H NMR and ^{13}C NMR: Jeol JNM-GX 400 and LA 500; δ/ppm = 0 for tetramethylsilane, 7.24 for chloroform. MS: Vacuum Generators 7070 (70 eV; ^{11}B). Column chromatography: purifications were carried out on Merck silica gel 40 (40–60 mesh), flash chromatography. Reactions were monitored by thin-layer chromatography (TLC) by using plates of silica gel (0.063–0.200 mm, Merck) or silicagel-60F_{254} microcards (Riedel de Haen). Optical rotations: Mod. Dip-370 polarimeter (Jasco). UV: UV–vis scanning spectrophotometer UV-2101 PC (Shimadzu).

(*S*)-*N*-(6,7-Dihydro-3,4,9,10,11-pentamethoxy-5*H*-dibenzo[*a*,*c*]cyclohepten-5-yl)acetamide (8). To a solution of 387 mg (1 mmol) of androbiphenyline (**4**) in 10 mL of a mixture of CH_2Cl_2:CH_3OH (4:1) was added a solution of excess diazomethane in diethylether, generated from 336 mg of Diazald. The mixture was kept at room

temperature for 3 h, then the solvent was evaporated in vacuo and the residue again treated with an excess of diazomethane as described before. Evaporation of the solvent afforded a crystalline raw material which was recrystallized from CH_2Cl_2:*n*-hexane 1:1 to yield 387 mg (96%) colorless crystals, mp 125 °C; (*M*):(*P*)-ratio = 3:7. UV (MeOH): λ_{max} (lgε) 288 nm (3.99), 262 (4.34), 213 (4.71). $[\alpha]_D^{20}$ −2° (*c* 0.35, $CHCl_3$). IR (KBr): ν (cm^{-1}) 3302 (NH), 1648 (CO). ^{1}H NMR ($CDCl_3$), (*P*) conformer: δ 1.54 (s, 3H, COC*H*$_3$), 2.11 (m, 1H, 7-H), 2.40 (m, 2H, 6-H, 7-H), 2.46 (m, 1H, 6-H), 3.60 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 3.89 (s, 3H, OC*H*$_3$), 3.90 (s, 3H, OC*H*$_3$), 3.93 (s, 3H, OC*H*$_3$), 5.17 (d, ^3J = 8.95 Hz, 1H, N*H*), 5.83 (m, 1H, 5-H), 6.61 (s, 1H, 8-H), 6.87 (d, ^3J = 8.7 Hz, 1H, 2-H), 7.17 (d, ^3J = 8.5 Hz, 1H, 1-H), (*M*) conformer: δ 1.85 (m, 1H, 6-H), 1.94 (s, 3H, COC*H*$_3$), 2.16 (m, 1H, 7-H), 2.40 (m, 1H, 7-H), 2.46 (m, 1H, 6-H), 3.61 (s, 3H, OC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 3.90 (s, 3H, OC*H*$_3$), 3.91 (s, 3H, OC*H*$_3$), 4.90 (m, 1H, 5-H), 6.53(s, 1H, 8-H), 6.87 (d, ^3J = 8.7 Hz, 1H, 2-H), 7.18 (d, ^3J = 8.7 Hz, 1H, 1-H), 7.83 (d, ^3J = 8.25 Hz, 1H, NH); ^{13}C NMR ($CDCl_3$), (*P*) conformer: δ 23.29 (CO*C*H$_3$), 31.81 (CH_2, C-7), 39.95 (CH_2, C-6), 43.71 (CH, C-5), 55.64 (O*C*H$_3$), 56.01 (O*C*H$_3$), 60.51 (O*C*H$_3$), 61.16 (2×O*C*H$_3$), 107.88 (CH, C-8), 110.52 (CH, C-2), 125.91 (C-11a), 126.81 (CH, C-1), 127.22 (C-4a), 133.27 (C-11b), 135.89 (C-7a), 141.39 (C-10), 145.56 (C-3*), 150.91 (C-4*), 151.64 (C-11*), 152.65 (C-9*), 167.72 (*C*OCH$_3$); (*M*) conformer: δ 23.76 (CO*C*H$_3$), 31.12 (CH_2, C-7), 39.23 (CH_2, C-6), 50.02 (CH, C-5), 55.64 (O*C*H$_3$), 55.93 (O*C*H$_3$), 61.02 (O*C*H$_3$), 61.08 (O*C*H$_3$), 61.27 (O*C*H$_3$), 107.96 (CH, C-8), 110.28 (CH, C-2), 124.60 (C-11a), 126.39 (CH, C-1), 128.74 (C-4a), 130.68 (C-11b), 134.83 (C-7a), 141.09 (C-10), 146.13 (C-3*), 150.73 (C-4*), 151.52 (C-11*), 152.61 (C-9*), 168.73 (*C*OCH$_3$). *Assignments not confirmed. MS (70 eV), *m*/*z* (%): 401 (100, M^+), 369 (11), 342 (44). $C_{22}H_{27}NO_6$: calcd: 401.1838, found: 401.1837 (MS). $C_{22}H_{27}NO_6$ (401.46): calcd: C 65.82, H 6.78; found: C 65.85, H 6.73.

(*S*)-6,7-Dihydro-3,4,9,10,11-pentamethoxy-5*H*-dibenzo-[*a*,*c*]cyclohepten-5-amine (9). To a solution of 400 mg (1 mmol) of the allocolchicinoid **8** in 7 mL of CH_3OH was added 7 mL of 2 M aqueous hydrochloric acid. The mixture was heated at reflux for 20 h, then the CH_3OH evaporated and a solution of 560 mg of NaOH in 7 mL of water added. The resulting solution was extracted with 5×7 mL of $CHCl_3$, the combined organic layers evaporated in vacuo and the residue purified and separated by column chromatography to yield two fractions. Fraction 1 contained 100 mg (25%) of the educt **8**; fraction 2 contained 251 mg (70%) of **9**, which could be recrystallized from CH_2Cl_2:*n*-hexane (1:1), mp 95–97 °C. UV (MeOH): λ_{max} (lgε) 289 nm (3.99), 263 (4.30), 214 (4.66). $[\alpha]_D^{20}$ +7.4° (*c* 0.37, $CHCl_3$). IR (KBr): ν (cm^{-1}) 3364 (NH). ^{1}H NMR ($CDCl_3$), (*P*) conformer: δ 2.09 (m, 1H, 7-H), 2.40 (m, 2H, 6-H, 7-H), 2.43 (m, 1H, 6-H), 3.61 (s, 3H, OC*H*$_3$), 3.82 (s, 3H, OC*H*$_3$), 3.86 (s, 6H, 2×OC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 4.37 (broad signal, 2H, N*H*$_2$), 4.87 (d, ^3J = 7.1 Hz, 1H, 5-H), 6.59 (s, 1H, 8-H), 6.88 (d, ^3J = 8.75 Hz, 1H, 2-H), 7.21 (d, ^3J = 8.5 Hz, 1H, 1-H), (*M*) conformer: δ 2.09 (m,1H, 6-H), 2.29 (m, 1H, 6-H), 2.46 (m, 1H, 7-H), 2.56 (m, 1H, 7-H), 3.59 (s, 3H, OC*H*$_3$), 3.86 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 3.89 (s, 3H, OC*H*$_3$), 3.93 (s, 3H, OC*H*$_3$), 4.01 (m, 1H, 5-H), 4.37 (broad signal, 2H, N*H*$_2$), 6.51 (s, 1H, 8-H), 6.88 (d, ^3J = 8.75 Hz, 1H, 2-H), 7.15 (d, ^3J = 8.5 Hz, 1H, 1-H); ^{13}C NMR ($CDCl_3$), (*P*) conformer: δ 30.99 (CH_2, C-7), 40.20 (CH_2, C-6), 45.91 (CH, C-5), 55.63 (O*C*H$_3$), 55.93 (O*C*H$_3$), 60.73 (O*C*H$_3$), 61.09 (O*C*H$_3$), 61.40 (O*C*H$_3$), 108.03 (CH, C-8), 110.69 (CH, C-2), 124.76 (C-11a), 127.26 (C-4a), 127.48 (CH, C-1), 134.14 (C-11b), 135.56 (C-7a), 141.51 (C-10), 145.41 (C-3*), 150.97 (C-4*), 151.40 (C-11*), 152.83 (C-9*); (*M*) conformer: δ 30.65 (CH_2, C-7), 38.64 (CH_2, C-6), 51.99 (CH, C-5), 55.65 (O*C*H$_3$), 55.96 (O*C*H$_3$), 61.09 (O*C*H$_3$), 61.40 (O*C*H$_3$), 61.57 (O*C*H$_3$), 107.94 (CH, C-8), 111.25 (CH, C-2), 124.12 (C-11a), 126.15 (CH, C-1), 127.70 (C-11b), 128.13 (C-4a), 134.22 (C-7a), 141.25 (C-10), 146.06 (C-3*), 150.80 (C-4*), 151.47 (C-11*), 152.78 (C-9). *Assignments not confirmed. MS (70 eV), *m*/*z* (%): 359 (100, M^+), 342 (57), 328 (44). $C_{20}H_{25}NO_5$: calcd: 359.1759, found: 359.1766 (MS).

6,7-Dihydro-3,4,9,10,11-pentamethoxy-5*H*-dibenzo[*a*,*c*]-cyclohepten-5-one (10). To a solution of 530 mg (1.8 mmol) of 4-formyl-1-methylpyridinium tosylate (FMT) in 9 mL of dry dimethyl formamide was added a solution of 433 mg (1.2 mmol) of the allocolchicinoid **9** in 27 mL of CH_2Cl_2. The mixture was heated at reflux for 4 h, cooled to 0 °C and after addition of 0.43 mL of 1.5-diazabicyclo[4.3.0]non-5-ene (DBN) stirred for a further 30 min. Then an oxalic acid-saturated aqueous solution (6.3 g in 45 mL of water) was added, and vigorous stirring was continued at room temperature for 24 h. The organic layer was separated and to the aqueous layer was added 36 mL of a 3:1 mixture of CH_2Cl_2:dimethyl formamide. After vigorous stirring for 24 h the organic layer was again separated and the same procedure repeated three times. The combined organic layers were concentrated in vacuo and the residue purified by column chromatography on silica gel (10×3 cm, CH_2Cl_2:CH_3OH, 9.5:0.5) to yield 291 mg (67%) of colorless crystals, mp 156–157 °C (CH_2Cl_2:*n*-hexane, 1:1). UV (CH_3OH): λ_{max} (lgε) 258 nm (3.32), 213 (4.63). IR (KBr): ν (cm^{-1}) 1694 (C=O). ^{1}H NMR ($CDCl_3$): δ 2.58 (m, 1H, 7-H), 2.83 (m, 1H, 6-H), 2.99 (m, 2H, 6-H, 7-H), 3.54 (s, 3H, OC*H*$_3$), 3.84 (s, 3H, OC*H*$_3$), 3.85 (s, 3H, OC*H*$_3$), 3.85 (s, 3H, OC*H*$_3$), 3.90 (s, 3H, OC*H*$_3$), 6.54 (s, 1H, 8-H), 6.99 (d, ^3J = 8.45 Hz, 1H, 2-H), 7.22 (d, ^3J = 8.45 Hz, 1H, 1-H); ^{13}C NMR ($CDCl_3$): δ 30.16 (CH_2, C-7), 49.70 (CH_2, C-6), 55.82 (OCH_3), 56.00 (O*C*H$_3$), 60.90 (O*C*H$_3$), 61.05 (O*C*H$_3$), 62.33 (O*C*H$_3$), 107.57 (CH, C-8), 113.01 (CH, C-2), 123.86 (C-11a), 126.22 (C-4a), 126.30 (CH, C-1), 135.30 (2C, C-7a, C-11b), 141.48 (C-10), 144.39 (C-3*), 151.74 (C-4*), 151.87 (C-11*), 152.66 (C-9*), 204.92 (CO). *Assignments not confirmed. MS (70 eV) *m*/*z* (%): 358 (100, M^+). $C_{20}H_{22}O_6$ (358.39): calcd: 358.1416, found: 358.1380 (MS). $C_{20}H_{22}O_6$: calcd: C 67.03, H 6.19; found: C 67.49, H 6.32.

6,7-Dihydro-3,4,9,10,11-pentamethoxy-5*H*-dibenzo[*a*,*c*]-cyclohepten-5-one oximes, *syn*/*anti*-mixture 11/12. To a solution of 108 mg (0.3 mmol) of the 5-amino-allo-

colchicinoid **9** and 10 mg of Na_2WO_4 in a mixture of 4 mL of water and 3 mL of CH_3OH was added 60 μL of H_2O_2 (30%) and the solution kept at ambient temperature for 2 h. The resulting oxime/amine complex, which was precipitated after that time was extracted with 2×10 mL of $CHCl_3$. The combined organic layers were evaporated in vacuo and the residue purified and separated by column chromatography to yield two fractions. Fraction 1 contained the *syn*/*anti*-mixture of the oximes **11** and **12**, 56 mg (50%) which could be recrystallized from acetone, mp 209–220 °C, Fraction 2 contained 54 mg of **9**. UV (MeOH): λ_{max} (lgε) 291 nm (3.81), 264 (4.28), 215 (4.65). IR (KBr): ν (cm^{-1}) 3463 (OH), 1596 (CN). **12**: ^{1}H NMR ($CDCl_3$): δ 2.49–2.66 (m, 3H, 6-H, 7-H), 3.47 (m, 1H, 6-H), 3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.52 (s, 1H, 8-H), 6.95 (d, ^3J = 8.7 Hz, 1H, 2-H), 7.21 (d, ^3J = 8.7 Hz, 1H, 1-H), 7.77 (bs, 1H, OH). **11**: ^{1}H NMR ($CDCl_3$): δ 2.49–2.66 (m, 2H, 6-H, 7-H), 2.84–2.90 (m, 2H, 6-H, 7-H), 3.48 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.53 (s, 1H, 8-H), 6.96 (d, ^3J = 8.7 Hz, 1H, 2-H), 7.07 (bs, 1H, OH), 7.25 (d, ^3J = 8.7 Hz, 1H, 1-H). **12**: ^{13}C NMR (DMSO-d_6): δ 30.04 (CH_2, C-7), 34.34 (CH_2, C-6), 56.14 (OCH_3), 56.28 (OCH_3), 60.98 (2×OCH_3), 61.80 (OCH_3), 108.31 (CH, C-8), 112.77 (CH, C-2), 124.61 (C-11a), 126.12 (CH, C-1), 128.88 (CH, C-4a), 130.21 (C-11b), 136.32 (C-7a), 140.98 (C-10), 146.45 (C-3*), 151.15 (C-4*), 151.86 (C-11*), 152.53 (C-9*), 154.59 (CN). *Assignments not confirmed. The ^{13}C NMR signals of **11** could not be fully identified. MS (70 eV), *m*/*z* (%): 373 (100, M^+). $C_{20}H_{23}NO_6$: calcd: 373.1525, found: 373.1533 (MS). $C_{20}H_{23}NO_6$ (373.41): calcd: C 64.33, H 6.21; found: C 64.26, H 6.11.

7,8-Dihydro-3,4,10,11,12-pentamethoxydibenz[*c*,*e*]azocin-5(6*H*)-one (13) and 7,8-dihydro-3,4,10,11,12-pentamethoxydibenz[*b*,*d*]azocin-6(5*H*)-one (14). A solution of 60 mg (0.16 mmol) of the **11/12**-mixture in 3.0 g of polyphosphoric acid (PPA) was heated at 65–70 °C for 20 h. The mixture was diluted with 10 mL of water and extracted with 3×10 mL of $CHCl_3$. The combined organic layers were evaporated in vacuo and the residue purified and separated by column chromatography on silica gel (8×3 cm, CH_2Cl_2:CH_3OH, 9.8:0.2). Fraction 1 contained **14** (25 mg, 41%), which could be recrystallized from ethyl acetate to yield colorless crystals, mp 242–246 °C. Fraction 2 contained **13** (16 mg, 25%), mp 270–272 °C. Compound **13**: UV (MeOH): λ_{max} (lgε) 285 nm (3.85), 247 (4.25), 211 (4.74). IR (KBr): ν (cm^{-1}) 3204 (NH), 1672; 1642. ^{1}H NMR ($CDCl_3$): δ 2.68 (m, 2H, 8-H), 3.23 (m, 1H, 7-H), 3.36 (m, 1H, 7-H), 3.50 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.81 (t, ^3J = 6.3 Hz, 1H, NH), 6.43 (s, 1H, 9-H), 6.96 (d, ^3J = 8.5 Hz, 1H, 1-H), 6.99 (d, ^3J = 8.5 Hz, 1H, 2-H); ^{13}C NMR ($CDCl_3$): δ 33.70 (CH_2, C-8), 40.38 (CH_2, C-7), 55.83 (OCH_3), 56.01 (OCH_3), 61.06 (OCH_3), 61.10 (OCH_3), 61.84 (OCH_3), 109.33 (CH, C-9), 112.92 (CH, C-2), 126.22 (CH, C-1), 126.36 (C-12a*), 126.98 (C-4a*), 131.79 (C-12b*), 132.25 (C-8a*), 141.13 (C-11), 145.16 (C-3*), 151.92 (C-4*), 152.01 (C-12*), 152.85 (C-10*), 170.96 (CO). *Assignments not confirmed. MS (70 eV), *m*/*z* (%) 373 (100, M^+), 358 (3), 344 (97), 329 (22). $C_{20}H_{23}NO_6$: calcd: 373.1525; found: 373.1552 (MS). $C_{20}H_{23}NO_6$ (373.41): calcd: C 64.33, H 6.21; found: C 64.02, H 6.16. Compound **14**: UV (MeOH): λ_{max} (lgε) 285 nm (3.76), 215 (4.74). IR (KBr): ν (cm^{-1}) 3255 (NH), 1678; 1642. ^{1}H NMR ($CDCl_3$): δ 2.59–2.74 (m, 4H, 7-H, 8-H), 3.52 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.54 (s, 1H, 9-H), 6.92 (s, 1H, NH), 6.93 (d, ^3J = 8.7 Hz, 1H, 2-H), 6.99 (d, ^3J = 8.45 Hz, 1H, 1-H); ^{13}C NMR ($CDCl_3$): δ 29.54 (CH_2, C-8), 35.46 (CH_2, C-7), 55.89 (OCH_3), 55.95 (OCH_3), 60.74 (OCH_3), 60.87 (OCH_3), 60.90 (OCH_3), 108.06 (CH, C-9), 110.90 (CH, C-2), 124.07 (C-12a), 126.19 (CH, C-1), 128.24 (C-4a), 130.32 (C-12b), 134.79 (C-8a), 140.94 (C-11), 143.63 (C-3*), 151.10 (C-4*), 151.20 (C-12*), 153.07 (C-10*), 174.14 (CO). *Assignments not confirmed. MS (70 eV), *m*/*z* (%): 373 (100, M^+), 358 (8), 344 (10), 330 (26), 314 (25). $C_{20}H_{23}NO_6$: calcd: 373.1525; found: 373.1579 (MS).

Tubulin binding assay. Calf brain tubulin was purified according to the method of Shelanski,[23] by three cycles of assembly–disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 2 mM EGTA, and 1 mM GTP pH 6.6 (the concentration of tubulin was about 2–3 mg/mL). Tubulin assembly was monitored and recorded continuously by turbidimetry at 400 nM in a UV spectrophotometer, equipped with a thermostated cell at 37 °C.[12] We determined for all newly synthesized drugs the IC_{50} values of their concentrations which decreased by 50% the maximum assembly rate of tubulin without drug. The IC_{50} for all compounds were compared to the IC_{50} of colchicine, measured the same day under the same conditions.

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