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Synthesis and tubulin-binding properties of new allocolchicinoids

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ABSTRACT

Allocolchicinoids with B- and C-ring variations were synthesized using sequential enyne-metathesis/Diels–Alder reactions (A → AB → ABC approach) and evaluated for their inhibitory effect on tubulin assembly *in vitro*. (−)-*Allocolchicine* **11** with methyl ester at C10 and (±)-cyclopropyl allocolchicinoid **32** exhibit similar activity than (−)-colchicine (**1**), probably derived from a similar flexibility in the biphenyl system. The presence of methyl ester at C10 led to a little loss in potency in comparison with the series with methyl ester at C9. A complete loss of activity was observed for allocolchicine **9** with methyl ester at C11.

1. Introduction

Colchicine (**1**), present as the major alkaloid in *colchicum autumnale*, is an old drug used in medicine in acute gout attacks and in familial Mediterranean fever. It has long been known for its remarkable antimitotic activity which results from its specific binding to tubulin preventing microtubule assembly, spindle formation, and consequently cell division [1,2]. Colchicine has also been studied as an anticancer agent. However, therapeutic effects are only observed at toxic or nearly toxic doses.

Structure activity studies indicate that the A- and C-rings of colchicine comprise the minimum structural features of the molecule necessary for its high affinity binding to tubulin. Any modification in the A-ring of colchicine causes complete loss of binding, indicating that the requirement of the A-ring is stringent. On the other hand, several changes in the C-ring such as different substitutions at the C-10 position or a replacement of the seven-membered ring with a six-membered ring are not only tolerated, but in some cases increase the activity. For instance, natural allocolchicine **2** with a six-membered aromatic ring in place of ring C binds to tubulin more strongly than the parent alkaloid (−)-(*aR*, 7*S*)-colchicine itself [3]. A significant development in cancer chemotherapy was the discovery that *N*-acetylcolchinol **3a** (Fig. 1), in its water-soluble phosphate (**3b**: ZD6126), selectively induces tumor vascular damage and tumor necrosis at well-tolerated doses [4].

Studies with large members compounds, established clearly that colchicine analogs modified at, or depleted of the B-ring are known to retain potent antimitotic activity, self-assembly inhibitory activity, and to bind to the tubulin colchicine site. The major function of the B-ring is an entropic contribution by suppressing free rotation around the bond between the A- and C-rings. These rings are twisted out of the plane with a torsion angle of about 30° for some derivatives with cyclohexene B-ring and goes to 62° for some of those with cycloheptene moiety (the angle torsion for colchicine itself is 53°).

In the course of our study on the application of the metathesis reaction of enynes to the synthesis of polycyclic compounds containing a seven or eight-membered ring [5], we recently described the synthesis of new allocolchicines [6], analogs of **2**, having the ester group at C10 and/or C11 positions [5c]. Preliminary tubulin assays have shown that some of these compounds inhibited the tubulin assembly. We report here the synthesis of further allocolchicines having a seven- and eight-membered B-ring bearing various substituents and the evaluation for their inhibitory effects on tubulin assembly *in vitro*.

2. Materials and methods

2.1. Chemistry

2.1.1. General procedures

Infrared spectra were recorded as neat or in solutions. ^{1}H and ^{13}C NMR spectra were recorded as solutions in $CDCl_3$, using residual protic solvent $CHCl_3$ (δ_H = 7.24 ppm) or $CDCl_3$ (δ_C = 77.23 ppm) as internal reference. Mass spectra were determined either by elec-

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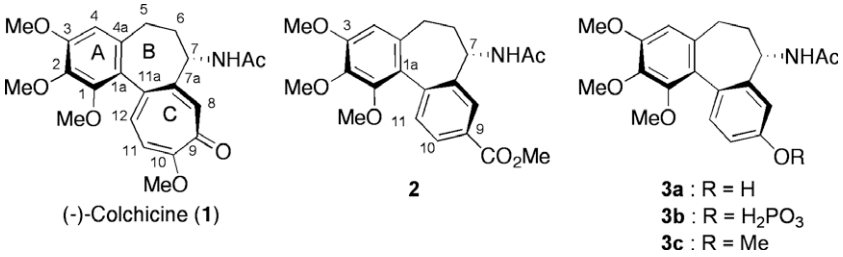


Fig. 1. Structures of colchicine and known allocolchicines.

tronic impact (EI) or chemical ionization with ammonia (CI, NH_3) or electrospray ionization (ESI). All reactions were monitored by TLC carried out on 0.2 mm aluminum silica gel pre-coated plates using UV light and 5% ethanolic solution of phosphomolybdic acid and heat as developing agent. Flash chromatography was performed on 40–63 μm (400–230 mesh) silica gel 60 with ethyl acetate (EtOAc)-petroleum ether (PE) (bp. 40–60 °C) or cyclohexane, heptane as eluents. Commercially available reagents and solvents were purified and dried when necessary by usual methods. THF and Et_2O were purified by distillation, under nitrogen, from sodium/benzophenone. DMF and CH_2Cl_2 were dried by distillation from calcium hydride. Unless otherwise mentioned, all other reagents were purchased from commercial sources and were used without further purification.

2.1.2. (±)-(E)-Methyl 3-(5-(tert-butyldimethylsilyloxy)-2,3,4-trimethoxy-8,9-dihydro-5H-benzocyclohepten-6-yl)acrylate (18)

To a degassed solution of enyne **17** [5f] (20 mg, 0.051 mmol) and methyl acrylate (0.05 mL) in dry CH_2Cl_2 (5 mL) under nitrogen was added Hoveyda–Grubbs II catalyst (**22**) (3.6 mg, 10% mol). The mixture was heated at reflux for 18 h and cooled to room temperature. The solvent was removed and the residue submitted to flash chromatography on silica gel (EtOAc/PE 1/9) to give the diene **18** (22 mg, 0.049 mmol, 96%) as a colorless oil. R_f 0.65 (EtOAc/PE 1/9). ^{1}H NMR: δ = 7.30 (d, J = 15.6 Hz, 1 H), 6.49 (s, 1 H), 6.08 (d, J = 15.6 Hz, 1 H), 6.09–6.07 (m, 1 H), 6.91 (s, 1 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 3.776 (s, 3 H), 3.768 (s, 3 H), 3.64–3.75 (m, 2 H), 2.45–2.38 (m, 2 H), 0.79 (s, 9 H), 0.10 (s, 3 H), −0.2 (s, 3 H). ^{13}C NMR: δ = 168.1 (C), 152.4 (C), 150.5 (C), 149.8 (CH), 144.1 (CH), 139.9 (C), 138.3 (C), 137.7 (C), 127.5 (C), 113.7 (CH), 108.8 (CH), 62.02, 61.98 (CH, CH_3), 60.9 (CH_3), 55.9 (CH_3), 51.6 (CH_3), 30.9 (CH_2), 30.4 (CH_2), 25.7 (3 CH_3), 18.0 (C), −4.8 (CH_3), −5.0 (CH_3). IR ν_{max} (film): 3471, 2938, 2853, 1701, 1615, 1588 cm^{-1}. HRMS (EI) *m/z* calcd for $C_{24}H_{36}O_6Si$, 448.2281, found 448.2277.

2.1.3. (±)-(E)-Methyl 3-(7-hydroxy-1,2,3-trimethoxy-6,7-dihydro-5H-benzocyclohepten-8-yl)acrylate (19)

Eight drops of an aqueous solution of citric acid (5 wt.%) and SiO_2 (200 mg) were added at room temperature to a solution of silyl ether **18** (25 mg, 0.055 mmol) in CH_2Cl_2 (3.4 mL). The reaction mixture was stirred for 18 h at room temperature and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/cyclohexane 3/7) to give the alcohol **19** (15 mg, 0.049 mmol, 81%) as a colorless oil. R_f 0.15 (EtOAc/cyclohexane 3/7). ^{1}H NMR: δ = 7.50 (d, J = 15.6 Hz, 1 H), 7.24 (s, 1 H), 6.51 (s, 1 H), 6.23 (d, J = 15.6 Hz, 1 H), 4.78–4.75 (br s, 1 H), 3.90 (s, 6 H), 3.85 (s, 3 H), 3.77 (s, 3 H), 3.01 (dd, J = 13.9, 10.8 Hz, 1 H), 2.67 (dd, J = 13.9, 8.7 Hz, 1 H), 2.32–2.22 (m, 1 H), 2.18–2.14 (br s, 1 H, OH), 1.94–1.82 (m, 1 H). ^{13}C NMR: δ = 168.0 (C), 154.1 (C), 153.8 (C), 150.0 (CH), 140.6 (C), 140.3 (C), 136.6 (C), 133.5 (CH), 120.5 (C), 116.4 (CH), 108.1 (CH), 68.0 (CH), 61.6 (CH_3), 60.8 (CH_3), 55.9 (CH_3), 51.5 (CH_3), 33.7 (CH_2), 29.7 (CH_2). IR ν_{max} ($CHCl_3$): 2950, 2935, 2893, 2856, 1721, 1622, 1596 cm^{-1}.

2.1.4. (±)-(E)-Methyl-3-(7-oxo-1,2,3-trimethoxy-6,7-dihydro-benzo cyclohepten-8-yl)acrylate (20)

To a solution of alcohol **19** (72 mg, 0.215 mmol) in dichloromethane (4 mL) was added Dess-Martin reagent (DMP) (1.05 mL, 0.3 M solution in CH_2Cl_2). The reaction mixture was stirred at room temperature for 45 min and saturated aqueous $Na_2S_2O_3$/$NaHCO_3$ (1:1) solution (10 mL) was added. The resultant mixture was stirred for 10 min at room temperature and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL); the combined organic layers were dried ($MgSO_4$) and solvent evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/cyclohexane 1/4) to give the ketone **20** (52 mg, 0.156 mmol, 73%) as a yellow solid. M.p. 98–99 °C (EtOAc/cyclohexane). R_f 0.25 (EtOAc/cyclohexane 1/4). ^{1}H NMR: δ = 7.82 (s, 1 H), 7.72 (dd, J = 16.0, 0.6 Hz, 1 H), 6.58 (s, 1 H), 6.47 (d, J = 16.0, 1 H), 3.94 (s, 3 H), 3.91 (s, 3 H), 3.85 (s, 3 H), 3.77 (s, 3 H), 2.91–2.88 (m, 2 H), 2.83–2.79 (m, 2 H). ^{13}C NMR: δ = 199.4 (C), 167.7 (C), 155.6 (C), 154.5 (C), 143.6 (CH), 140.6 (C), 139.3 (C), 137.6 (CH), 132.3 (C), 120.9 (C), 119.3 (CH), 107.6 (CH), 61.8 (CH_3), 60.9 (CH_3), 56.0 (CH_3), 51.5 (CH_3), 43.9 (CH_2), 26.7 (CH_2). IR ν_{max} (film): 2944, 2897, 1710, 1661, 1590 cm^{-1}. HRMS (EI) *m/z* calcd for $C_{18}H_{20}O_6$, 332.1260, found 332.1271.

2.1.5. 9,10,11-Trimethoxy-5-oxo-6,7-dihydro-5H-dibenzo[a,c]cyclo hepten-2,3-dicarboxylic acid dimethyl ester (12) (±)-5-Hydroxy-9,10, 11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-2,3-dicarbox ylic acid dimethyl ester (23)

A solution of methyl β-nitroacrylate (28 mg, 0.210 mmol) and diene **20** (43 mg, 0.13 mmol) in 0.15 mL of CH_2Cl_2 was stirred at room temperature for 23 h then warmed to 85 °C for 15 h. The reaction mixture was cooled to room temperature and the volatiles were removed by evaporation under reduced pressure to give the crude nitro **21**. DBU (0.4 mL, 2.5 mmol) was added to a solution of crude **31** in THF (1 mL). The reaction mixture was stirred for 2 h at room temperature under argon. The volatiles were removed *in vacuo* and the residue was purified by chromatography on silica gel (EtOAc/PE 1:2) to give 16 mg of the ketone **12** (30%) as a colorless oil and 14 mg of alcohol **23** (26%) as a colorless oil. **22**: ^{1}H NMR: δ = 7.95 (s, 1 H), 7.91 (s, 1 H), 6.61 (s, 1 H), 3.945 (s, 3 H), 3.941 (s, 3 H), 3.92 (s, 3 H), 3.90 (s, 3 H), 3.60 (s, 3 H), 3.10–2.85 (m, 3 H), 2.75–2.70 (m, 1 H). ^{13}C NMR: δ = 205.0 (C), 167.7 (C), 166.9 (C), 154.1 (C), 152.2 (C), 141.7 (C), 141.1 (C), 137.3 (C), 135.8 (C), 134.0 (C), 132.0 (CH), 129.8 (C), 128.8 (CH), 122.5 (C), 107.2 (CH), 61.2 (CH_3), 61.1 (CH_3), 56.1 (CH_3), 52.8 (CH_3), 52.7 (CH_3), 47.7 (CH_2), 29.8 (CH_2). IR ν_{max} ($CHCl_3$): 2934, 2855, 1736, 1694, 1594 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{22}H_{22}O_8$, 414.1315, found 414.1301. **23**: ^{1}H NMR: δ = 8.05 (s, 1 H), 7.86 (s, 1 H), 6.59 (s, 1 H), 4.65 (dd, J = 9.7, 7.3 Hz, 1 H), 3.94 (s, 3 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.63 (s, 3 H), 2.62–2.58 (m, 1 H), 2.45 (dd, J = 12.7, 6.4 Hz, 1 H), 2.32–2.20 (m, 1 H), 2.18–2.10 (m, 1 H, OH), 1.97–1.91 (m, 1 H). ^{13}C NMR: δ = 168.6 (C), 168.1 (C), 153.4 (C), 150.9 (C), 145.2 (C), 141.0 (C), 136.1 (C), 135.6 (C), 130.63 (CH), 130.61 (C), 129.6 (C), 123.6 (CH), 122.6 (C), 107.6 (CH), 66.6 (CH), 61.08 (CH_3), 61.06 (CH_3), 56.0 (CH_3), 52.62 (CH_3), 52.59

(CH_3), 41.3 (CH_3), 30.1 (CH_2). IR ν_{max} ($CHCl_3$): 3616, 3512, 2946, 2858, 1733, 1660, 1595 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{22}H_{24}O_8$, 416.1471, found 416.1504.

2.1.6. (±)-5-Acetamido-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-2,3-dicarboxylic acid dimethyl ester (13)

A solution of ketone **12** (28 mg, 0.067 mmol), NH_4OAc (55 mg, 0.70 mmol) and $NaBH_3CN$ (5 mg, 0.078 mmol) in 400 μL of MeOH was heated in sealed tube at 60 °C for 25 h. After cooling to room temperature 0.50 mL of conc. HCl was added and the mixture was vigorously stirred for 15 min. After that 1 mL of water was added and the mixture was extracted twice with Et_2O. The ethereal layers were combined and set aside, the aqueous layer was treated with 10% NaOH and extracted once with CH_2Cl_2 and once with Et_2O. The organic layers were combined, dried over $MgSO_4$ and evaporated under reduced pressure to give 17 mg of the crude amine that was dissolved in 500 μL of CH_2Cl_2 and 40 μL of Ac_2O. To this solution was added 40 μL of pyridine dropwise, with stirring. After 45 min at room temperature, the mixture was quenched with 1.5 mL of water. The aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were washed with 10% HCl, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by chromatography on silica gel (EtOAc/PE 1:1 then pure EtOAc) to give 10 mg of allocolchicine **13** (0.018 mmol, 26% in 2 steps) as a white solid: M.p. 208–210 °C. 2:1 mixture of atropoisomers in $CDCl_3$ solution. Major rotamer: ^1H NMR δ = 7.87 (s, 1 H), 7.64 (s, 1 H), 6.58 (s, 1 H), 5.93 (d, *J* = 8.0 Hz, 1 H, NH), 4.90–4.84 (m, 1 H), 3.96 (s, 6 H), 3.92 (s, 6 H), 3.40 (s, 3 H), 2.50–2.36 (m, 3 H), 2.06 (s, 3 H), 1.85–1.80 (m, 1 H). ^{13}C NMR δ = 169.3 (C), 168.5 (C), 168.0 (C), 153.6 (C), 151.3 (C), 142.7 (C), 137.8 (C), 134.6 (C), 131.0 (CH), 130.3 (C), 130.1 (C), 126.5 (C), 123.3 (CH), 123.1 (C), 107.8 (CH), 61.37 (CH_3), 61.27 (CH_3), 56.1 (CH_3), 52.7 (CH_3), 52.6 (CH_3), 49.2 (CH), 39.4 (CH_2), 30.2 (CH_2), 23.3 (CH_3). IR ($CHCl_3$): ν_{max} 3447, 2977, 2936, 2863, 1732, 1691, 1596 cm^{-1}. HMRS (EI) *m/z* calcd for $C_{24}H_{27}NO_8$, 457.1736, found 457.1743.

2.1.7. (±)-5-Fluoro-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c] cyclohepten-2,3-dicarboxylic acid dimethyl ester (24)

To a solution of alcohol **23** (18 mg, 0.043 mmol) in dry CH_2Cl_2 (0.75 mL) cooled at −78 °C and stirred under N_2 was added DAST (20 μL, 0.144 mmol). The mixture was allowed to warm to −5 °C during 1 h. Water was then added and the product extracted with CH_2Cl_2 (3 × 5 mL) to a colorless residue which was purified by chromatography on silica gel (EtOAc/heptane 1:2) affording 16 mg (0.038 mmol, 89%) of **24** as a colorless oil. R_f 0.75 (EtOAc/heptane 1:2). ^1H NMR δ = 7.94 (s, 1 H), 7.89 (s, 1 H), 6.60 (s, 1 H), 5.35 (ddd, *J* = 46.4, 12.6, 11.4 Hz, 1 H), 3.96 (s, 3 H), 3.93 (s, 3 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 3.64 (s, 3 H), 2.82–2.73 (m, 1 H), 2.52–2.47 (m, 1 H), 2.35–2.28 (m, 1 H), 2.21–2.10 (m, 1 H). ^{13}C NMR δ = 168.2 (C), 167.9 (C), 153.7 (C), 151.0 (C), 141.2 (C), 141.1 (d, J_{CF} = 18.9 Hz, C), 134.93 (C), 134.87 (C), 130.7 (CH), 130.6 (C), 130.4 (C), 123.0 (d, J_{CF} = 13.5 Hz, CH), 122.1 (C), 107.7 (CH), 90.2 (d, J_{CF} = 177 Hz, CH), 61.15 (CH_3), 61.1 (CH_3), 56.0 (CH_3), 52.7 (CH_3), 52.6 (CH_3), 38.8 (d, J_{CF} = 23 Hz, CH_2), 29.3 (d, J_{CF} = 12.5 Hz, CH_2). IR ($CHCl_3$): ν_{max} 2998, 2949, 2862, 1735, 1594 cm^{-1}. HMRS (EI) *m/z* calcd for $C_{22}H_{23}O_7F$, 418.1428, found 418.1442.

2.1.8. 9,10,11-Trimethoxy-7H-dibenzo[a,c]cyclohepten-2-carboxylic acid methyl ester (29)

A solution of alcohol **26** [5f] (46 mg, 0.127 mmol) in toluene (1.84 mL) with PTSA (18.4 mg) was warmed to 80 °C and stirred for 1 h. Then the mixture was cooled to room temperature, diluted with Et_2O (10 mL), washed saturated aqueous $NaHCO_3$, dried ($MgSO_4$) and solvent evaporated under reduced pressure. The residue was purified by chromatography on silica gel (EtOAc/heptane 1:1) to give 27 mg (0.079 mmol, 62%) of **29** as a colorless oil. R_f 0.57 (EtOAc/heptane 1:1). ^1H NMR: δ = 8.24 (d, *J* = 1.5 Hz, 1 H), 7.93 (dd, *J* = 8.1, 1.5 Hz, 1 H), 7.35 (d, *J* = 8.1 Hz 1 H), 6.57 (ddd, *J* = 10.0, 8.1, 5.7 Hz 1 H), 6.55 (s, 1 H), 6.30 (ddd, *J* = 10.1 Hz 1 H), 3.92 (s, 3 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 3.52 (s, 3 H), 3.05 (dd, *J* = 13.2, 8.1 Hz, 1 H), 2.73 (ddd, *J* = 13.2, 5.7, 2.1 Hz, 1 H). ^{13}C NMR: δ = 167.4 (C), 153.3 (C), 152.5 (C), 141.0 (C), 140.9 (C), 140.2 (C), 135.1 (C), 134.6 (CH), 133.7 (CH), 129.0 (CH), 128.9 (CH), 127.0 (CH), 126.7 (C), 123.7 (C), 105.9 (CH), 61.3 (CH_3), 61.1 (CH_3), 56.1 (CH_3), 52.0 (CH_3), 33.6 (CH_2). IR ν_{max} (film): 2949, 2847, 1718, 1596 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{20}H_{20}O_5Na$, 363.1208, found 363.1205.

2.1.9. (±)-5-Fluoro-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-2-carboxylic acid methyl ester (28)

Compound **28** was obtained from **26** by the same procedure as for the fluoro compound **24** as a colorless oil (10 mg, 84%). R_f 0.8 (EtOAc/heptane 1:2). ^1H NMR δ = 8.12 (d, *J* = 1.6 Hz 1 H), 8.06 (dd, *J* = 8.4, 1.6 Hz, 1 H), 7.66 (d, *J* = 8.4 Hz 1 H), 6.60 (s, 1 H), 5.36 (ddd, *J* = 46.4, 12.6, 11.4 Hz, 1 H), 3.94 (s, 3 H), 3.92 (s, 6 H), 3.66 (s, 3 H), 2.78–2.71 (m, 1 H), 2.51–2.45 (m, 1 H), 2.38–2.29 (m, 1 H), 2.20–2.13 (m, 1 H). ^{13}C NMR δ = 167.1 (C), 153.2 (C), 151.0 (C), 142.9 (d, J_{CF} = 18.6 Hz, C), 141.3 (C), 134.7 (C), 132.0 (C), 131.2 (CH), 129.0 (C), 128.3 (CH), 123.1 (C), 122.2 (d, J_{CF} = 12.8 Hz, CH), 107.6 (CH), 90.7 (d, J_{CF} = 176 Hz, CH), 61.06 (CH_3), 61.05 (CH_3), 56.0 (CH_3), 52.1 (CH_3), 39.0 (d, J_{CF} = 23 Hz, CH_2), 29.3 (d, J_{CF} = 12.5 Hz, CH_2). IR ($CHCl_3$): ν_{max} 2945, 2861, 1725, 1596 cm^{-1}. HMRS (EI) *m/z* calcd for $C_{20}H_{21}O_5F$, 360.1373, found 360.1364.

2.1.10. 9,10,11-Trimethoxy-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-2-carboxylic acid methyl ester (30)

To a solution of alkene **29** (5 mg, 0.0147 mmol) in MeOH (1 mL) was added the catalyst 10% Pd/C (1 mg). The flask was flushed with H_2, and a positive pressure of H_2 was maintained. The mixture was stirred at room temperature under 1 atm. of H_2 for 3 h. Then the mixture was filtered through a layer of Celite, the solvent removed under reduced pressure. The residue was purified by chromatography on silica gel (EtOAc/heptane 1:1) to give 4.9 mg of tricycle **30** (0.0143 mmol, 97%) as a colorless oil. R_f 0.51 (EtOAc/heptane 1:1). ^1H NMR: δ = 8.12 (d, *J* = 1.8 Hz 1 H), 7.90 (dd, *J* = 7.9, 1.8 Hz, 1 H), 7.28 (d, *J* = 7.9 Hz 1 H), 6.56 (s, 1 H), 3.90 (s, 3 H), 3.89 (s, 6 H), 3.61 (s, 3 H), 2.62–2.55 (m, 1 H), 2.48–2.37 (m, 2 H), 2.27–2.17 (m, 1 H), 2.15–2.05 (m, 2 H). ^{13}C NMR: δ = 167.7 (C), 153.0 (C), 151.1 (C), 145.4 (C), 141.1 (C), 136.7 (C), 135.9 (C), 131.6 (CH), 128.6 (CH), 128.4 (CH), 128.1 (C), 125.3 (C), 107.8 (CH), 61.3 (CH_3), 61.1 (CH_3), 56.2 (CH_3), 52.2 (CH_3), 33.1 (CH_2), 31.62 (CH_2), 31.60 (CH_2). IR ν_{max} (film): 2935, 2856, 1720, 1598 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{20}H_{22}O_5Na$, 365.1365, found 365.1358.

2.1.11. (±)-6,7-Epoxy-9,10,11-trimethoxy-6,7-dihydro-5H-dibenzo [a,c]cyclohepten-2-carboxylic acid methyl ester (31)

To a solution of alkene **29** (15 mg, 0.044 mmol) in a mixture CH_2Cl_2/sat. aq. $NaHCO_3$ (0.5 mL/0.5 mL) was added *m*-CPBA (17 mg, 70%) at 0 °C. The reaction mixture was warmed to room temperature, stirred at room temperature for 1 h and extracted with CH_2Cl_2 (3 × 5 mL). Then the combined organic layer was washed with a saturated aqueous solution ($NaHCO_3$/$Na_2S_2O_3$ 1:1, 2 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by chromatography on silica gel (EtOAc/heptane 3:7 to 1:1) to give 3.1 mg of epoxide **31** (0.0086 mmol, 20%) as a colorless oil. R_f 0.25 (EtOAc/heptane 1:1). Mixture of 2 diastereoisomers (4:1). Major isomer ^1H NMR: δ = 8.15 (d, *J* = 1.7 Hz 1 H), 7.98 (dd, *J* = 8.1, 1.7 Hz, 1 H), 7.74 (d, *J* = 8.1 Hz 1 H), 6.61 (s, 1 H), 3.99–3.93 (m, 2 H), 3.90 (s, 6 H), 3.89 (s, 3 H), 3.68 (s, 3 H), 3.13–3.05 (m, 1 H), 2.28 (dd, *J* = 13.0, 8.3 Hz, 1 H). ^{13}C NMR: δ = 167.1 (C), 153.0 (C), 152.7 (C), 141.9 (C), 138.8 (C), 135.1 (C), 133.8 (C), 133.5 (CH), 131.2 (CH), 128.3 (CH), 127.9 (C),

124.2 (C), 108.2 (CH), 61.4 (CH_3), 61.2 (CH_3), 58.4 (CH), 56.3 (CH_3), 52.9 (CH), 52.4 (CH_3), 37.1 (CH_2). IR ν_{max} (film): 2951, 2843, 1722, 1597 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{20}H_{20}O_6Na$, 379.1158, found 379.1161.

2.1.12. (±)-1,2,3-Trimethoxy-7-methyl-8-vinyl-6,7-dihydro-5H-benzocyclohepten-7-ol (35)

Methyl lithium (3.5 mL, 5.6 mmol, 1.6 M in Et_2O) was added dropwise to a −70 °C solution of enone **34** (65 mg, 0.227 mmol) in Et_2O (3.5 mL) under argon. The reaction mixture was stirred for 1 h −70 °C and saturated aqueous ammonium chloride (3 mL) added. The mixture was warmed to room temperature and extracted with Et_2O (3 × 5 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by chromatography on silica gel (EtOAc/heptane 1:4) to give 32 mg of alcohol **35** (0.11 mmol, 48%) as a colorless oil. R_f 0.37 (EtOAc/heptane 1:4). ^{1}H NMR: δ = 6.92 (s, 1 H), 6.69 (dd, *J* = 17.4, 11 Hz, 1 H), 6.45 (s, 1 H), 5.59 (d, *J* = 17.2 Hz, 1 H), 5.15 (d, *J* = 11 Hz, 1 H), 3.86 (s, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 2.72 (dd, *J* = 14.6, 10.7 Hz, 1 H), 2.63 (ddd, *J* = 14.6, 8.5 Hz, 1 H), 2.17 (dd, *J* = 14.0, 8.5 Hz, 1 H), 1.90–1.85 (m, 2 H), 1.42 (s, 3 H). ^{13}C NMR: δ = 153.1 (C), 152.5 (C), 142.9 (C), 140.2 (C), 140.0 (C), 139.1 (CH), 121.5 (C), 120.0 (CH), 115.4 (CH_2), 107.3 (CH), 75.1 (C), 61.5 (CH_3), 61.1 (CH_3), 56.0 (CH_3), 44.2 (CH_2), 30.8 (CH_2), 29.0 (CH_3). IR ν_{max} (film): 3456, 2933, 2852, 1593 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{17}H_{22}O_4Na$, 313.1416, found 313.1423.

2.1.13. Nitro (36)

A solution of methyl β-nitroacrylate (28 mg, 0.210 mmol) and diene **35** (30 mg, 0.102 mmol) in 0.5 mL of CH_2Cl_2 was stirred at room temperature for 18 h. The volatiles were removed by evaporation under reduced pressure and the residue was purified by chromatography on silica gel (EtOAc/heptane 3:7, then 1: 1) to give 38.9 mg of **36** (0.092 mmol, 90%) as a colorless oil as a mixture of diastereoisomers. IR ν_{max} (film): 3486, 2938, 2844, 1735, 1596, 1551 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{21}H_{27}NO_8Na$, 444.1634, found 444.1630.

2.1.14. (±)-5-Hydroxy-9,10,11-trimethoxy-5-methyl-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-2-carboxylic acid methyl ester (37)

DBU (30 μL, 0.18 mmol) was added to a solution of **36** (38 mg, 0.09 mmol) in THF (1.5 mL). The reaction mixture was stirred for 18 h at room temperature under N_2. The volatiles were removed *in vacuo* and the residue was filtered through a thin plug of silica gel (EtOAc/heptane 1:4–1:1) to give, after solvent evaporation, 30 mg of elimination product. This was dissolved in 3 mL CH_2Cl_2 and DDQ (60 mg, 0.26 mmol) was added. The solution was stirred for 3 days at room temperature. The solvent was evaporated and the crude mixture was filtered through a layer of Al_2O_3 (EtOAc/heptane 3:7) to give, after solvent removal, 23.6 mg of ester **37** (0.063 mmol, 70%) as a colorless oil (2 isomers 1:1). R_f 0.14 (EtOAc/cyclohexane 3:7). ^{1}H NMR: δ = 8.14–8.12 (br s, 1 H), 7.98 (dd, *J* = 8.2, 1.6 Hz, 0.5 H), 7.94 (dd, *J* = 8.2, 1.6 Hz, 0.5 H), 7.91 (d, *J* = 8.2 Hz, 0.5 H), 7.50 (d, *J* = 8.2 Hz, 0.5 H), 6.62 (s, 0.5 H), 6.55 (s, 0.5 H), 3.90–3.89 (br s, 9 H), 3.62 (s, 1.5 H), 3.61 (s, 1.5 H), 2.45–2.23 (m, 5 H), 1.63 (s, 1.5 H), 1.09 (s, 1.5 H). ^{13}C NMR: δ = 167.5 (C), 167.3 (C), 153.6 (C), 153.3 (C), 151.1 (C), 150.7 (C), 150.1 (C), 148.9 (C), 141.8 (C), 141.7 (C), 135.4 (C), 135.3 (C), 134.6 (C), 133.3 (C), 133.2 (CH), 132.8 (CH), 129.1 (C), 128.7 (C), 128.4 (CH), 128.3 (CH), 126.3 (C), 125.9 (C), 125.0 (CH), 124.3 (CH), 108.0 (CH), 107.6 (CH), 74.7 (C), 61.4 (CH_3), 61.1 (CH_3), 60.6 (CH_3), 56.2 (CH_3), 52.33 (CH_3), 52.26 (CH_3), 49.44 (CH_2), 49.42 (CH_2), 31.59 (CH_2), 31.52 (CH_2), 31.1 (CH_3), 29.0 (CH_3). IR ν_{max} (film): 3470, 2934, 2853, 1714, 1597 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{21}H_{24}O_6Na$, 395.1471, found 395.1472.

2.1.15. 9,10,11-Trimethoxy-5-methyl-7H-dibenzo[a,c]cyclohepten-2-carboxylic acid methyl ester (38)

Compound **38** was obtained from **37** by the same procedure as for alkene **29** as a colorless oil (5.5 mg, 91%). R_f 0.34 (EtOAc/heptane 3:7). ^{1}H NMR: δ = 8.39 (d, *J* = 1.9 Hz 1 H), 7.95 (dd, *J* = 8.3, 1.9 Hz, 1 H), 7.57 (d, *J* = 8.3 Hz 1 H), 6.53 (s, 1 H), 6.09 (ddd, *J* = 7.9, 6.4, 1.5 Hz, 1 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 3.59 (s, 3 H), 2.88 (dd, *J* = 13.2, 8.1 Hz, 1 H), 2.62 (ddd, *J* = 13.2, 5.3, 1.0 Hz, 1 H), 2.09 (t, *J* = 1.0 Hz, 3 H). ^{13}C NMR: δ = 167.4 (C), 153.2 (C), 152.4 (C), 143.9 (C), 141.4 (C), 140.8 (C), 134.9 (C), 133.7 (C), 133.3 (CH), 130.5 (CH), 127.1 (CH), 126.8 (C), 126.6 (CH), 123.7 (C), 105.3 (CH), 61.23 (CH_3), 61.18 (CH_3), 56.1 (CH_3), 52.3 (CH_3), 33.4 (CH_2), 22.2 (CH_3). IR ν_{max} (film): 2950, 2853, 1721, 1620, 1596 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{21}H_{22}O_5Na$, 377.1365, found 377.1367.

2.1.16. (±)-9,10,11-Trimethoxy-5-methyl-6,7-dihydro-5H-dibenzo[a,c]cyclohepten-2-carboxylic acid methyl ester (39)

Compound **39** was obtained from **38** by the same procedure as for the tricycle **30** as a colorless oil (9.4 mg, 77%). R_f 0.40 (EtOAc/heptane 3:7). ^{1}H NMR: δ = 8.03 (d, *J* = 1.3 Hz 1 H), 7.97 (dd, *J* = 8.2, 1.3 Hz, 1 H), 7.38 (d, *J* = 8.2 Hz, 1 H), 6.55 (s, 1 H), 3.90 (s, 6 H), 3.89 (s, 3 H), 3.65 (s, 3 H), 2.71–2.63 (m, 1 H), 2.42–2.33 (m, 1 H), 2.24–2.15 (m, 2 H), 1.68–1.62 (m, 1 H), 1.31 (d, *J* = 6.6 Hz, 3 H). ^{13}C NMR: δ = 167.7 (C), 153.0 (C), 151.1 (C), 148.5 (C), 141.1 (C), 136.6 (C), 136.2 (C), 131.3 (CH), 128.5 (CH), 127.7 (C), 125.1 (C), 124.3 (CH), 107.5 (CH), 61.3 (CH_3), 61.2 (CH_3), 56.3 (CH_3), 52.2 (CH_3), 42.3 (CH), 33.8 (CH_2), 32.1 (CH_2), 18.2 (CH_3). IR ν_{max} (film): 2933, 2848, 1718, 1597 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{21}H_{24}O_5Na$, 379.1521, found 379.1524.

2.1.17. (±)-5-Azido-9,10,11-trimethoxy-5,6,7,8-tetrahydro-dibenzo[a,c]cyclooctadien-2-carboxylic acid methyl ester (41)

Methanesulfonyl chloride (0.12 mL, 1.55 mmol) was added dropwise to a solution of alcohol **40** [5f] (45 mg, 0.12 mmol) and DMAP (5 mg) in dry pyridine (0.785 mL) at 0 °C under argon. The reaction mixture was stirred for 1 h at 0 °C, for 3 h at room temperature and diluted with CH_2Cl_2 (20 mL). The organic phase was washed with aqueous saturated $CuSO_4$ solution (2 × 10 mL), brine (10 mL), dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was diluted with dry DMF (1 mL) and NaN_3 (40 mg, 0.6 mmol). The reaction mixture was warmed for 7 h at 90 °C and diluted with CH_2Cl_2 (20 mL). The organic phase was washed with water (2 × 10 mL), brine (10 mL), dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by chromatography on silica gel (EtOAc/cyclohexane 1:4) to give 17 mg of azide **41** (0.043 mmol, 35%) as a colorless oil. R_f 0.55 (EtOAc/cyclohexane 3:7). Mixture of atropoisomers (4:1). Major atropoisomer: ^{1}H NMR: δ = 8.09 (dd, *J* = 8.4, 1.5 Hz, 1 H), 7.95 (d, *J* = 8.4 Hz, 1 H), 7.93 (d, *J* = 1.5 Hz, 1 H), 6.57 (s, 1 H), 4.81 (d, *J* = 10.2 Hz, 1 H), 3.93 (s, 6 H), 3.92 (s, 3 H), 3.60 (s, 3 H), 2.58–2.46 (m, 2 H), 2.21–1.99 (m, 2 H), 1.86–1.63 (m, 2 H). ^{13}C NMR: δ = 166.8 (C), 153.9 (C), 150.8 (C), 147.1 (C), 140.8 (C), 138.6 (C), 133.9 (C), 131.5 (CH), 129.1 (CH), 127.9 (C), 128.6 (C), 127.2 (CH), 124.3 (CH), 108.2 (C), 61.1 (CH_3), 61.0 (CH_3), 59.1 (CH_3), 56.1 (CH_3), 52.1 (CH_3), 41.2 (CH_2), 31.4 (CH_2), 28.8 (CH_2), 26.9 (CH_2). IR ν_{max} (film): 2933, 2853, 2093, 1722, 1597 cm^{-1}.

2.1.18. 9,10,11-Trimethoxy-5,6,7,8-tetrahydro-dibenzo[a,c]cyclooctadien-2-carboxylic acid methyl ester (43)

To a solution of azide **41** (17 mg, 0.042 mmol) in absolute EtOH (1 mL) was added the catalyst 10% Pd/C (5 mg). The flask was flushed with H_2, and a positive pressure of H_2 was maintained. The mixture was stirred at room temperature under 1 atm. of H_2 for 72 h. Then the mixture was filtered through a layer of Celite, the Celite was washed with acetone and the solvents removed un-

der reduced pressure. The residue was purified by chromatography on silica gel (EtOAc/PE 1:4) to give 8 mg of the tricycle **43** (0.022 mmol, 53%) as a colorless oil. R_f 0.5 (EtOAc/cyclohexane 3:7). ^{1}H NMR: δ = 7.99–7.95 (m, 2 H), 7.36 (d, J = 7.8 Hz, 1 H), 6.60 (s, 1 H), 3.92 (s, 6 H), 3.91 (s, 3 H), 3.57 (s, 3 H), 2.78 (dd, J = 12.9, 8.1 Hz, 1 H), 2.59 (dd, J = 13.5, 8.4 Hz, 1 H), 2.26 (t, J = 11.7 Hz, 1 H), 2.11–1.94 (m, 3 H), 1.50–1.40 (m, 2 H). ^{13}C NMR: δ = 167.2 (C), 153.1 (C), 150.7 (C), 148.4 (C), 140.3 (C), 138.8 (C), 135.7 (C), 131.9 (CH), 129.4 (CH), 128.7 (CH), 127.1 (C), 125.9 (C), 108.1 (CH), 61.1 (CH_3), 60.9 (CH_3), 56.1 (CH_3), 52.0 (CH_3), 32.7 (CH_2), 32.5 (CH_2), 29.6 (CH_2), 29.5 (CH_2). IR ν_{max} (film): 2930, 2852, 1720, 1596 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{21}H_{24}O_5Na$, 379.1521, found 379.1528.

2.1.19. (±)-5-Fluoro-9,10,11-trimethoxy-5,6,7,8-tetrahydro-dibenzo[a,c]cyclooctadien-2-carboxylic acid methyl ester (44)

Compound **44** was obtained from **40** by the same procedure as for the fluoro compound **24** as a colorless oil (2.5 mg, 83%). R_f 0.75 (EtOAc/cyclohexane 1:2). ^{1}H NMR δ = 7.99 (dd, J = 8.0, 1.6 Hz, 1 H), 7.94 (d, J = 1.6 Hz, 1 H), 7.29 (d, J = 8 Hz, 1 H), 6.65 (s, 1 H), 5.79 (ddd, J = 47.2, 8.4, 1.6 Hz, 1 H), 3.93 (s, 6 H), 3.91 (s, 3 H), 3.58 (s, 3 H), 2.61 (ddd, J = 8.0, 6.8, 1.6 Hz, 1 H), 2.30–2.23 (m, 1 H), 2.10–1.95 (m, 2 H), 1.90–1.65 (m, 2 H). ^{13}C NMR δ = 166.7 (C), 153.1 (C), 150.4 (C), 143.6 (d, J_{CF} = 16.8 Hz, C), 140.3 (C), 136.5 (C), 134.5 (C), 134.2 (CH), 129.3 (C), 128.4 (d, J_{CF} = 6.1 Hz, CH), 128.1 (C), 126.7 (C), 107.4 (CH), 94.6 (J_{CF} = 176 Hz, CH), 61.1 (CH_3), 60.7 (CH_3), 55.9 (CH_3), 52.1 (CH_3), 32.0 (d, J_{CF} = 23 Hz, CH_2), 31.7 (CH), 24.2 (d, J_{CF} = 4.4 Hz, CH_2). HMRS (EI) *m/z* calcd for $C_{21}H_{23}O_5F$, 374.1529, found 374.1531.

2.2. Biological activities

2.2.1. Tubulin binding assay

Sheep brain tubulin was purified according to the method of Shelanski et al. [7], 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. We determined for all newly synthesized drugs the IC_{50} values of their concentrations that decreased by 50% the maximum assembly rate of tubulin without drug. The IC_{50} for all compounds were compared to the IC_{50} of deoxypodophyllotoxine or colchicine, measured the same day under the same conditions (see Supporting Information).

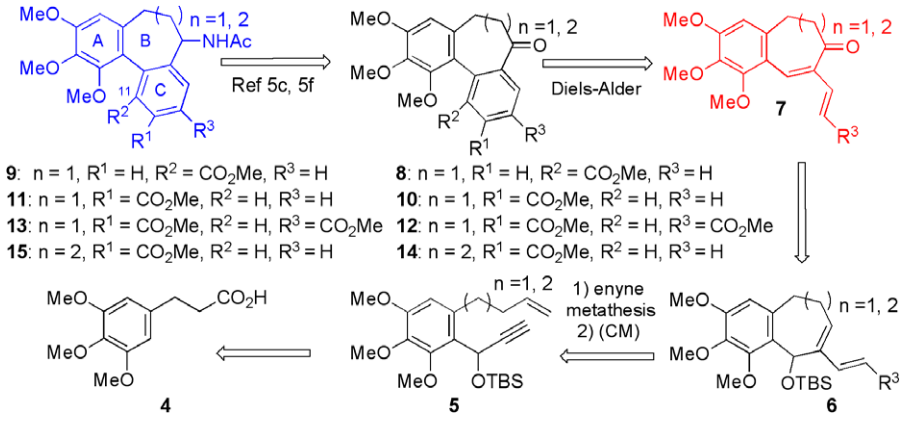
3. Results and discussion

3.1. Chemistry: synthesis of allocolchicinoids

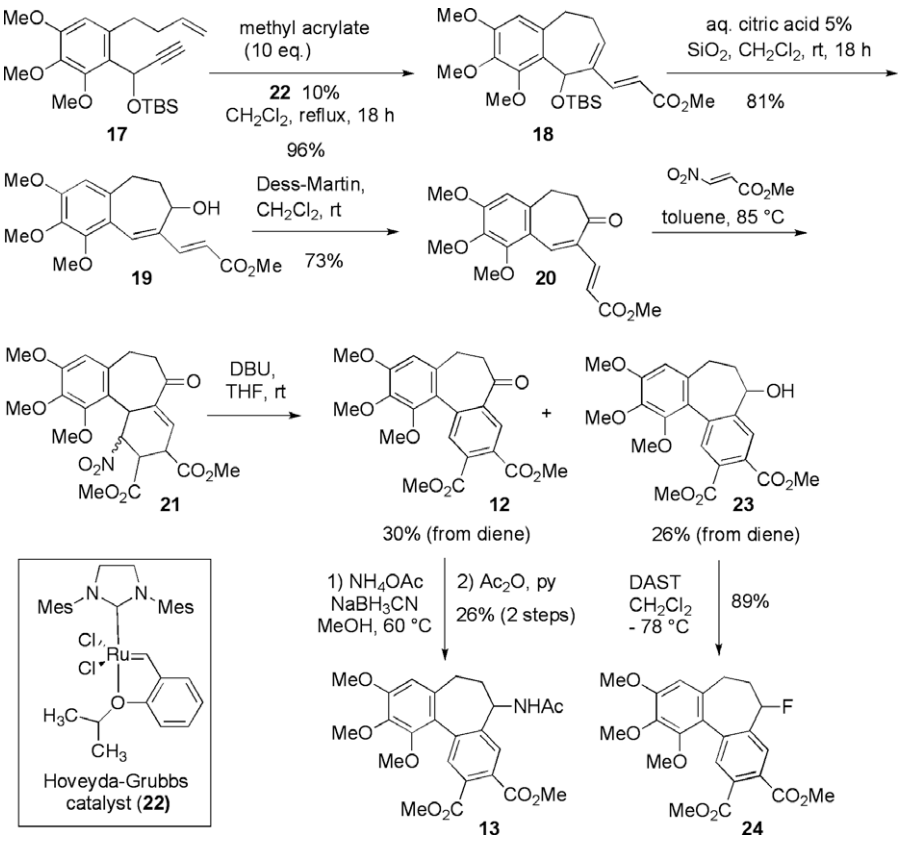
For a long time allocolchicinoids were obtained by transformation of colchicine itself, thus limiting the type of structural variations. Recently, total syntheses of **2** and **3** have been reported, and a number of syntheses of various allocolchicinoids have also been described [8]. While most approaches involve a strategy based on the construction of the seven-membered ring by an intramolecular biaryl-coupling reaction (AC → ABC approach), a different route was developed by Wulff and co-workers and distinguished by constructing the aromatic ring C by a Diels–Alder reaction of a suitably substituted diene containing the seven-membered ring (AB → ABC approach) [8a]. In all these approaches, the described allocolchicinoids bear functionalities at the C9 position in the C ring [9].

Our route was based on a conceptually similar approach to Wulff's one. The construction of the tricyclic core of allocolchicines was planed by a Diels–Alder reaction on a suitably substituted diene containing either seven- or eight-membered ring fused to the aromatic A-ring (AB → ABC approach). As shown in the retrosynthetic analysis (Scheme 1), target compounds **9**, **11, 13** and **15** were to be obtained by direct reductive amination of ketones **8**, **10, 12** and **14**, respectively. These ketones could be prepared by a Diels–Alder – aromatization sequence from intermediate dienones **7** (n = 1 or 2) which can be traced back to **6** (R = H, CO_2Me). Our initial plan involved the preparation of **6** by the ring-closing metathesis (RCM) reaction of enyne **5** [10]. This precursor was to be obtained from the cheap and commercially available acid **4**.

According to this strategy, we previously described total syntheses of allocolchicines **9**, **11,** and **15** [5c,5f]. The synthesis of allocolchicine **13**, outlined in Scheme 2, began by converting the enyne **17** to bicyclic diene **18** by tandem ring-closing metathesis/cross-metathesis (RCM–CM) [11]. Treatment of **17** with excess methyl acrylate in the presence of Hoveyda–Grubbs catalyst (**22**) in refluxing CH_2Cl_2 gave **18** in almost quantitative yield (96%). Complete deprotection of the secondary alcohol followed by acidic rearrangement in the presence of citric acid furnished the allylic alcool **19**. Oxidation of alcohol **19** with Dess-Martin reagent at room temperature for 1 h, afforded dienone **20** in 73% yield. As for dienones **7** [5f] (R^1 = H, n = 1 or 2), **20** reacted regioselectively with β-nitroacrylate [12] giving cycloadduct **21** in a near quantitative yield. Unexpectedly, treatment of **21** with DBU at room temperature for 4 h achieved both elimination of nitrous acid and aromatization leading to a mixture of ketone **12** and alcohol **23** (1:1 ratio) in 55% combined yield. Reductive amination of ketone **12**, followed by *N*-



Scheme 1.



Scheme 2.

acylation produced allocolchicinoid **13** as a 2:1 mixture of atropoisomers as shown by ^{1}H NMR (Scheme 2).

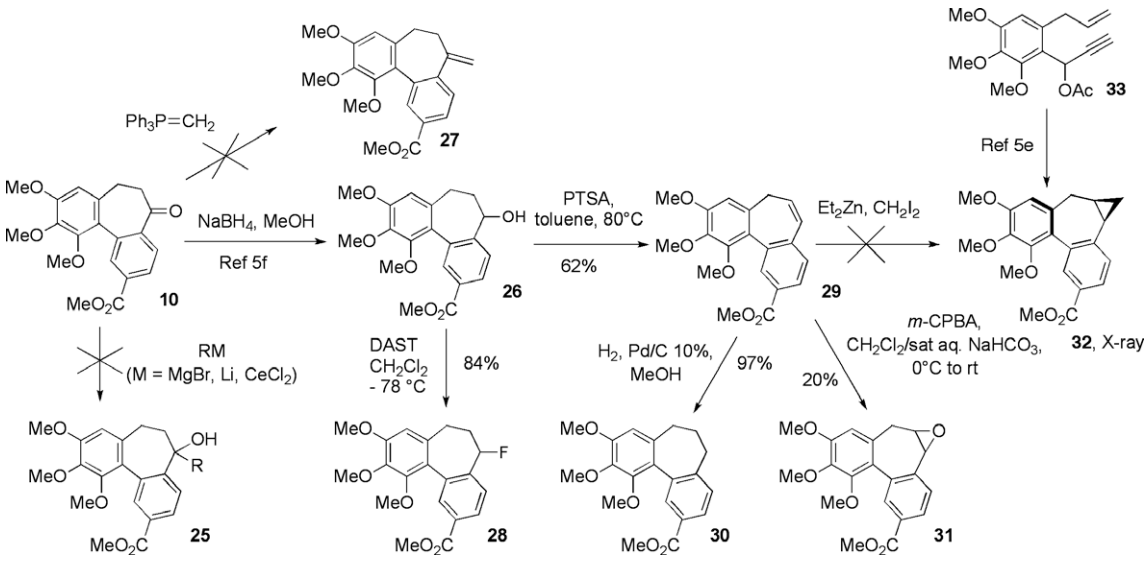
Fluorinated compounds have attracted attention in various areas, particularly in pharmaceutical research, due to their interesting biological and physical properties. Nowadays, fluoro-organic compounds are routinely synthesized in the pharmaceutical industry [13]. In order to assess the effect of fluorine on the biological activity, allocolchicinoid **24** bearing fluorine atom at C7 was prepared in high yield by treatment of alcohol **23** with diethylaminosulfur trifluoride (DAST) at −78 °C (Scheme 2). In the same way, alcohol **26** prepared by reduction of **10** with $NaBH_4$, was transformed into fluoro compound **28** in 84% yield (Scheme 3). On the other hand, dehydration of alcohol **26** furnished alkene **29** easily transformed to tricyclic compound **30** by catalytic hydrogenation in 60% overall yield. Treatment of alkene **29** with *m*-CPBA led to the epoxide **31** in poor yield (20%).

We turned next to the synthesis of allocolchicinoids bearing an alkyl group at C7 by addition of an organometallic reagent to ketone **10**. Unfortunately, all attempts of addition of alkyl magnesium bromide, alkyllithium, or other nucleophiles prepared from these reagents and cerium(III) chloride to **10** gave only traces of the desired tertiary alcohol **25** (Scheme 3). In the same manner, the Wittig reaction on the ketone **10** with methylene triphenylphosphorane failed to furnish the exocyclic alkene **27**. Preparation of cyclopropane derivative **32** from alkene **29** was then considered. However, treatment of **29** under Simmons–Smith conditions failed to give the desired compound. Finally allocolchicinoid **32** has been successfully synthesized via gold(I)-catalyzed cycloisomerization of 1,7-enyne propargylic acetate **33** [5e]. As for other members of this family, **32** displays molecular asymmetry resulting from a noncoplanar arrangement of rings A and C. X-ray crystallographic data showed that these rings are twisted with a torsion angle of 45°.

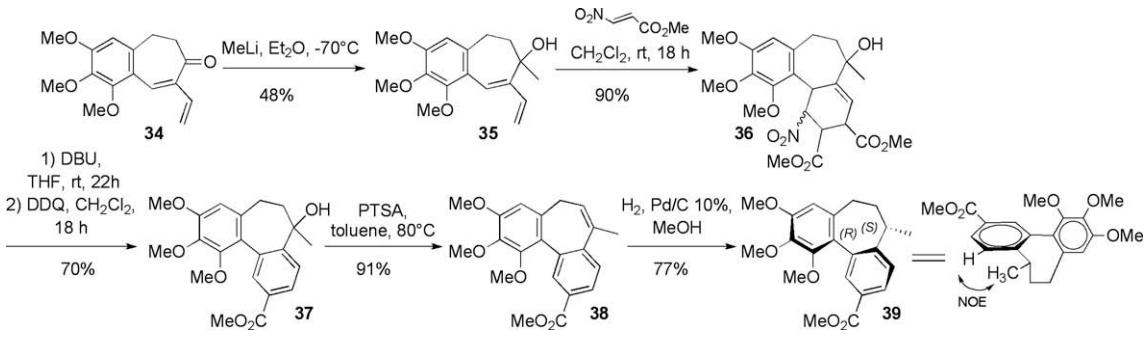
On the basis of the preceding results we deduced that the introduction of alkyl group at C7 position must be done prior to the formation of C-ring by alkylation of bicyclic dienone **34** (Scheme 4). Addition of methyllithium to **34** led to tertiary alcohol **35**, which was submitted to Diels–Alder reaction with β-nitroacrylate at room temperature to afford cycloadduct **36** in 90% yield. Elimination of nitrous acid using DBU and subsequent aromatization by DDQ furnished the tricyclic compound **37** as a mixture of 2 isomers (1:1) in 70% overall yield. Dehydration of **37** readily afforded alkene **38** that led, by catalytic hydrogenation, to the tricyclic compound **39** as a single atropoisomer as shown by ^{1}H and ^{13}C NMR spectra. In addition, NOESY correlations observed for **39** confirmed its (±)-(*aR*,7*S*) relative configuration.

The antimitotic activity of the allocolchicine **11** (see Table 1) prompted us to be interested in hitherto unknown allocolchicinoids having an eight membered B-ring. Such compounds belong to lignan derivatives possessing the dibenzocyclooctadiene skeleton and, like allocolchicinoids, they are characterized by an atropoisomeric biaryl unit [14]. A number of these products display wide variety of biological activities. For example, natural occurring steganacin binds to the colchicine site in tubulin and shows *in vivo* anti-tumor activity (Fig. 2) [15].

The syntheses of all dibenzocyclooctadiene lignan derivatives described to date, including aza and oxa analogues of steganacin, involved inter- or intramolecular biaryl-coupling reaction (AC → ABC approach) [8k,15,16]. As for the allocolchicines **9, 11** and **13**, we planed the construction of the tricyclic core of the lignan derivatives according to the AB → ABC approach. The AB part containing the eight-membered ring fused to the aromatic A-ring has to be built using a ring-closing metathesis reaction of a suitably substituted enyne (Scheme 1) [17]. Following this approach, we previously reported the preparation of the allocolchicinoid **15**



Scheme 3.



Scheme 4.

Table 1
Inhibition of tubulin assembly.[a]

Entry	Compound	IC_{50}(cpd)/ IC_{50}(**1**)	Entry	Compound	IC_{50}(cpd)/ IC_{50}(**1**)
1	(−)-Colchicine (**1**)	1	14	**24**	>7
2	(−)-Allocolchicine [8b] (**2**)	0.2	15	**26**	1.4
3	(−)-NCME [8b] (**3c**)	0.25	16	**28**	3.9
4	DPPT [8b] (**46**)	0.2	17	**29**	Inactive
5	**45** [8b]	0.2	18	**30**	1.8
6	**9**	Inactive[c]	19	**31**	>7
7	**10**	2.5	20	**32**	0.86
8	**(−)-11**	1	21	**37**	>7
9	**(±)-11**	2.6	22	**38**	Inactive
10	**13**	>7	23	**39**	>7
11	**14**	>7	24	**40**	4.3
12	**15**	>7	25	**43**	>7
13	**(−)-16**	1.5	26	**44**	6.5

[a] IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly, average of three experiments; IC_{50}(**1**) varies from 2 to 6 μM for the different experiments according to the tubulin preparation and concentration.
[b] Unless specified, the tested compounds are racemic.
[c] Inactive: IC_{50} > 100 μM.

[5f]. It is worthy of note that initial attempt to prepare **15** from azide **41** via alcohol **40** failed. Catalytic hydrogenation of **41** led to the hydrogenolysis product **43** (53% yield) instead of the awaited amine **42** (Scheme 5). Assignment of the relative configuration (a*S*,8*R*) of alcohol **40** was based on the X-ray crystal analysis [5f]. For this diastereoisomer the two aryl rings are twisted out of plane with a torsion angle of about 61°. Finally, fluoride **44** was synthesized from **40** as the same manner as for **24** and **28**.

3.2. Biological activities: inhibition of tubulin assembly

All the newly synthesized allocolchicinoids **11–16**, **24**, **26**, **28–32**, **37–40**, **43–44** were subjected to our standard assay conditions [8b,18] for evaluation of tubulin assembly inhibition *in vitro* using sheep brain tubulin. In order to allow a precise comparison between the novel ligands and both parent leads, allocolchicine **(2)** and *N*-acetyl colchinol-*O*-methylether (NCME) **(3c)** were included in the evaluation. The IC_{50} value of all the allocolchicinoids under consideration were compared to that of colchicine (**1**) as the standard, measured within the same day with the same tubulin preparation. The data are compiled in Table 1, presented in terms of the relative IC_{50}(cpd)/IC_{50}(**1**) value determined from the IC_{50} values of the test compounds. As can be seen from the data in Table 1 (entries 1–5), the parent allocolchicine **2** and allocolchicinoid **3c** possess a 4–5 times higher inhibitory effect than the standard colchicine **1**.

In the first part of our SAR studies, we focused our attention on the influence of the methyl ester function at different positions on C-ring in comparison with the well-known natural allocolchicine **2**

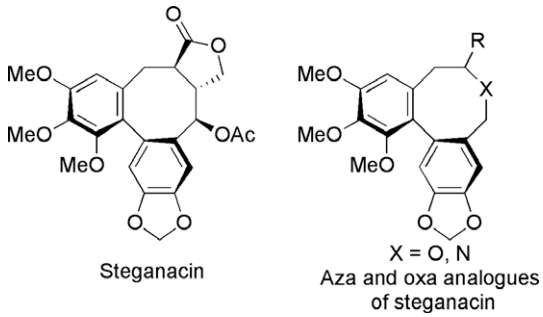


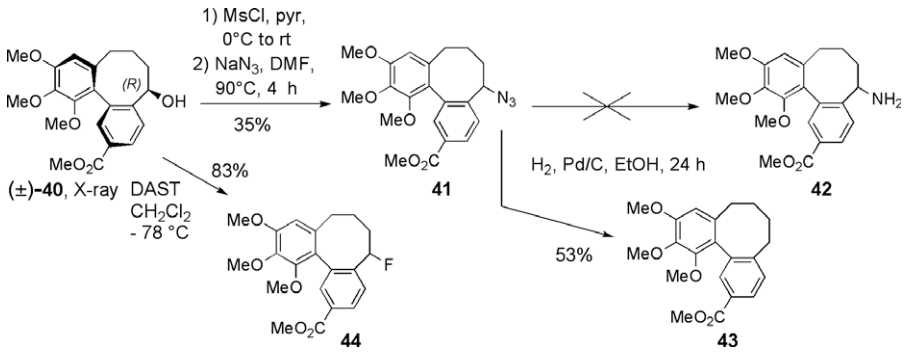
Fig. 2. Structures of steganacin and analogues.

having the methyl ester group at C9 position on C-ring (Table 1, entries 6–10).

Compound **9** with an ester at C11 position was completely inactive. On the contrary, allocolchicine (−)-**11**, bearing a methyl ester at C10 position, was found to be as potent as colchicine (**1**) to inhibit tubulin assembly, but still less active than natural allocolchicine **2**. These results prompted us to test the activity of compound **13** having two methyl ester functions at C9 and C10 positions that proved to be inactive. Ketone **10** was also found to be moderately active, contrary to ketone **45** [8b] (Fig. 3) that displayed IC_{50} value in same range as natural allocolchicine (**2**). The geometry of allocolchicines **9**, **11**, **13** in comparison with the active structures **45** and **2** already described could explain their antimicrotubule activity. It is worth mentioning that most of our studied compounds exist as a diastereoisomeric mixture of two atropoisomers due to the hindered rotation around the pivot bond connecting the A- and C-rings. It is well-known that the key structural factor for the cytotoxic activity is the presence of the B-ring, forcing the two aromatic rings to be noncoplanar and that only the a*R* isomer can bind to tubulin [8b,19]. The comparison of the structures **9, 11, 13** and **24** with **2** in one hand and **10** with **45** in the other hand indicates that the relative position of the oxygen atom 3-O(Me) born by the phenyl A-ring and the oxygen atom of the CO_2Me born by the phenyl C-ring is also important for the activity. Moreover, in **9**, **13** and **24**, steric effects inhibit the coplanarity of the carbonyl group with the phenyl C-ring [20]: this coplanarity seems essential for antitubulin activity.

In the second part of our SAR studies (Table 1, entries 8–9, 13, 15–23), we were interested in the influence of the substitution at C7 position on allocolchicines bearing a methyl ester at C10 position. B-ring modifications affect the torsion angle between the least squares planes of the A- and C-rings and do not seem to take part productively in the binding process to tubulin. The acetamido group can be replaced without loss in potency by the hydroxyl function (compound **26**), the azido group (compound **16** [5f]), fluorine (compound **28**) or hydrogen (compound **30**) suggesting that the substitution at C7 position influences slightly the activity. It is worthy of note that compound **30** has been already prepared and evaluated by Banwell et al. for its ability to prevent polymerization [21]. Surprisingly, we found with our test conditions a weaker activity ((IC_{50}(**30**)/IC_{50}(**1**) = 1.8) in comparison with the results of Banwell (IC_{50}(**30**)/IC_{50}(**1**) = 0.8). However our result is in good agreement with that obtained with similar structures synthesized in this present work. The difference of activity of **26** compared to **10** suggests that the hybridization of the C7 can affect slightly the activity. The experimental observation that atropoisomer (−)-**11** (a*RS*) is about 2- to 3-fold more active than the corresponding racemic mixture confirms the well known effect of the C7 chirality on tubulin binding.

The presence of a double bond at C7 led to inactive compounds **29**, **38**. In contrast, the presence of an epoxide, a quaternary center or a methyl at C7 position gave compounds (**31**, **37**, **39**) that displayed moderate inhibitory effects toward tubulin assembly. The cyclopropane derivative (±)-**32** was an exception. Although it does not surpass the activity of NCME (**3**) or allocolchicine (**2**), it proved to be a potent inhibitor of tubulin assembly with IC_{50} value in the same range as that of colchicine (**1**). The X-ray structure of **32** revealed a biaryl dihedral angle of 45° to be compared with a 49° value in natural allocolchicine (**2**) [22], 53° in colchicine (**1**) [1] and 54° in NCME (**3**) [23] which could explain the good antimicrotubule properties of racemic **32**. The loss of activity of epoxide **31**



Scheme 5.

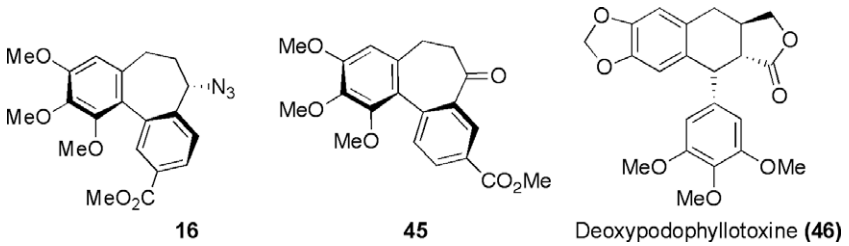


Fig. 3. Structures of compounds **16**, **45** and deoxypodophyllotoxine.

indicates that the oxygen atom in this position is not favourable compared to the carbon atom.

The last part of our SAR studies allowed a comparison between allocolchicines previously described and their hitherto unknown analogues possessing an eight-membered B-ring (Table 1, entries 11–12 and 24–26). It was shown that ring expansion of (allo)colchicinoids to derivatives with aza [24,16a] or oxa [8b] eight-membered B-ring leads to less potent inhibitors. Surprisingly, the homologous allocolchicine **15** and ketone **14** showed no antimicrotubule activity. In contrast, alcohol **40** displayed inhibition of tubulin assembly but turned out to be significantly weaker inhibitor, ca 4-fold less active than the corresponding alcohol **26** possessing a seven-membered B-ring. The X-ray structure of **40** revealed that the torsion angle between the least-squares of the two aromatic A- and C-rings is about 61°. This angle is in accordance with limit value for which activity is possible which spans from an angle of about 30° [25] to 62° [26]. As shown by molecular models for eight membered B-ring analogues, the change from a three- to a four-atom bridge linking the A–C biaryl backbone results in comparatively more rigid structures with stable conformation. It generates a significant increase of the torsion angle between the planes of the A- and C-ring and a decrease of the antimicrotubule activity. The fluoride compound **44** and its analogue **28** exhibited still similar moderate activity. The comparison of the structures **10**, **11**, **26** and **30** with **14**, **15**, **40** and **43** respectively, confirms that B-ring expansion from seven- to eight-membered ring leads to less potent inhibitors [8b]. The torsion angle between the planes of the A- and C-rings increases from about 48–49° to 61–66° [27].

4. Conclusion

Allocolchicinoids with B-and C-ring variations were prepared by total synthesis using sequential enyne-metathesis/Diels–Alder reactions (A → AB → ABC approach) and evaluated for their inhibitory effect on tubulin assembly *in vitro*. These results show that the key structural factor for the cytotoxic activity is the size of the B-ring, giving rise to a certain torsion angle between the planes of the A- and C-rings. (−)-Allocolchicine **11** with methyl ester at C10 and (±)-cyclopropyl allocolchicinoid **32** exhibit an activity close to that of (-)-colchicine (**1**), probably because of a similar flexibility in the biphenyl system. Another important position effect concerns the oxygen atom of the CO_2Me of the phenyl C-ring. Its alignment with the oxygen atom 3-O(Me) born by the phenyl A-ring and the axis connecting the A- and C-rings favors the activity. The presence of a methyl ester at C10 led to a little loss in potency in comparison with the series with a methyl ester at C9. A complete loss of activity was observed for the allocolchicine **9** with a methyl ester at C11. Nevertheless, the C7 coordination must not be negligible.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2010.03.003.

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