Supporting Information

for

Highly stereocontrolled total synthesis of racemic codonopsinol B through isoxazolidine-4,5-diol vinylation

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Detailed experimental procedures, characterization data and NMR spectra of synthesized compounds, X-ray crystallographic data of 12, and biological evaluation of antiproliferative activities of 1 and 2
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Experimental section

General remarks

Flash column chromatography (FCC) was carried out with a Büchi system (Pump Manager C-615 and Fraction Collector C-660) using Normasil 60 silica gel (0.040–0.063 mm; VWR). Thin layer chromatography (TLC) analysis was carried out using TLC silica gel 60 F254 (aluminium sheets, Merck), and plates were visualized with UV light or by treatment with permanganate solution followed by heating. Melting points were obtained using a Melting Point B-540 (Büchi) instrument. Infrared (IR) spectra were recorded as neat samples with a Nicolet 5700 FTIR spectrometer with an ATR Smart Orbit Diamond adapter (Thermo Electron Corporation). NMR spectra were recorded with a Varian INOVA-300 spectrometer (1H, 299.95 MHz and 13C, 75.42 MHz) and a Varian VNMRS-600 instrument (1H, 599.75 MHz and 13C, 150.81 MHz) in CDCl3 (using tetramethylsilane as the internal standard), CD3OD (residual [D4]methanol, δH = 3.31, 4.87 ppm, δC = 49.00 ppm), and D2O. Chemical shifts are reported in parts per million (ppm). HRMS analysis was carried out with an Orbitrap Velos Pro spectrometer (Thermo Fisher Scientific). All solvents used were dried and distilled according to conventional methods. Oxone®, monopersulfate (2KHSO5·KHSO4·K2SO4, MW: 615.50) and cerium(III) chloride (anhydrous, 99.5%) were purchased from Alfa Aesar. Phosphotungstic acid hydrate (12WO3·H3PO4·H2O, MW: 2880.05 (anhydrous basis)) was purchased from Sigma-Aldrich. 4-Methoxycinnamaldehyde (6) was purchased from Acros Organics. N-Cbz-Hydroxylamine 7 was prepared using a published procedure [1].

Experimental procedures

(±)-Benzyl 5-hydroxy-3-(4-methoxyphenyl)isoxazolidine-2-carboxylate (8)

4-Methoxycinnamaldehyde (6, 120 mg, 0.74 mmol) and N-Cbz-hydroxylamine 7 (100 mg, 0.60 mmol) were added to a stirred solution of Dl-proline (13.8 mg, 0.12 mmol) in anhydrous CHCl3 (1.2 mL) at rt. The mixture was stirred for 48 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc 2:1). The solvent was evaporated under reduced pressure and the product was purified by FCC (hexanes/EtOAc, 85:15) to give isoxazolidine 8 (150 mg, 0.46 mmol, 77%) as a white solid with spectroscopic data in good agreement with those reported in the literature [2]. mp 91–93 °C; Rf = 0.16 (n-hexane/EtOAc, 2:1); 1H NMR (300 MHz, CDCl3): δ = 7.30–7.22 (m, 7 H, Ph-H), 6.88–6.83 (m, 2 H, Ph-H), 5.84 (dd, J = 4.7, 2.7 Hz, 1 H, 5-H), 5.34 (bs, 1 H, OH), 5.33 (t, J = 8.2 Hz, 1 H, 3-H), 5.17 (s, 2 H, OCH2Ph), 3.79 (s, 3 H, OMe), 2.74 (dd, J = 12.6, 8.4 Hz, 1 H, 4-H), 2.33–2.24 (m, 1 H, 4-H); 13C NMR (75 MHz, CDCl3): δ = 159.4, 159.1 (C=O, C-Ph), 135.9 (C-Ph), 133.6 (C-Ph), 128.6 (CH-Ph), 128.3 (CH-Ph), 127.9 (CH-Ph), 127.5 (CH-Ph), 114.2 (CH-Ph), 98.9 (C-5), 68.2 (CH2Ph), 61.0 (C-3), 55.5 (OMe), 45.4 (C-4).
(±)-Benzyl 3-(4-methoxyphenyl)isoxazole-2(3H)-carboxylate (9)

Hydroxyisoxazolidine 8 (1.0 g, 3.04 mmol) was placed in a reaction flask, which was subsequently sealed with a rubber septum, evacuated, and filled with argon. Anhydrous NMP (30 mL) was added, and the resulting solution was cooled in ice/NaCl bath (−20 °C). 2-Fluoropyridine (1.83 mL, 21.3 mmol) and Tf₂O (0.76 mL, 4.52 mmol, 1.5 equiv) were added. The mixture was stirred at −20 °C for 20 min, and then allowed to warm to rt within 16 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc 2:1). The mixture was poured into H₂O (100 mL), and sat. solution of NaHCO₃ (10 mL) was added. The mixture was subsequently extracted with Et₂O (3 × 100 mL). The combined organic layers were washed with H₂O (2 × 100 mL), dried (MgSO₄), and concentrated in vacuo. The product was purified by FCC (hexanes/EtOAc 9:1) to give 2,3-dihydroisoxazole 9 (640 mg, 2.06 mmol, 68%) as a white solid which gradually became yellow over time. mp 77–79 °C; Rf = 0.29 (n-hexane/EtOAc, 7:3); ¹H NMR (300 MHz, CDCl₃): δ = 7.35–7.30 (m, 5 H, H-Ph), 7.30–7.25 (m, 2 H, H-Ph), 6.89–6.84 (m, 2 H, H-Ph), 6.68 (dd, J = 3.1, 2.1 Hz, 1 H, 5-H), 5.90 (pseudo t, J = 2.3, 2.2 Hz, 1 H, 3-H), 5.25 (d, J = 12.2 Hz, 1 H, OCH₂Ph), 5.19 (d, J = 12.2 Hz, 1 H, OCH₂Ph), 5.15 (dd, J = 3.1, 2.4 Hz, 1 H, 4-H), 3.79 (s, 3 H, OCH₃); ¹³C NMR (75 MHz, CDCl₃): δ = 159.6, 156.8 (C=O, C-Ph), 141.6 (C-5), 135.5 (C-Ph), 132.2 (C-Ph), 128.5 (CH-Ph), 128.3 (× 2, CH-Ph), 128.2 (CH-Ph), 114.0 (CH-Ph), 101.9 (C-4), 68.2 (CH₂Ph), 66.9 (C-3), 55.3 (OCH₃); IR (ATR): νmax = 3105, 2837, 1703, 1342, 1238, 1154, 1073, 1031, 823, 776, 730, 694, 561 cm⁻¹. HRMS (ESI): m/z calcld for C₁₈H₁₈NO₄: 312.1231 [M+H⁺]; found: 312.1232.

(±)-Benzyl 4-(4-methoxyphenyl)-2,6-dioxa-3-azabicyclo[3.1.0]hexane-3-carboxylate (10)

Solid NaHCO₃ (2.0 g; 23.8 mmol) was placed in a reaction flask, and H₂O (13 mL) followed by acetone (21 mL) were added. The resulting mixture was cooled to 0 °C in an ice bath and stirred for 20 min. Oxone (2.09 g, 3.40 mmol) was added in one portion, and stirring was continued at 0 °C for further 15 min. Then, 2,3-dihydroisoxazole 9 (530 mg, 1.70 mmol) was added in one portion. The ice bath was removed, and the reaction mixture was stirred at rt for an additional 80 min. After this time, TLC showed that the reaction was complete (hexanes/EtOAc 4:1). The reaction mixture was diluted with H₂O (40 mL) and extracted with CH₂Cl₂ (3 × 30 mL). The organic layers were dried (MgSO₄), and the solvent was evaporated in vacuo to give isoxazolidinyl epoxide 10 (550 mg, 1.68 mol, 99%) as a white solid with satisfactory purity. mp 83–85 °C; Rf = 0.24 (n-hexane/EtOAc, 7:3); ¹H NMR (600 MHz, CDCl₃, 25 °C): δ = 7.40–7.30 (m, 5 H, H-Ph), 7.28–7.23 (m, 2 H, H-Ph), 6.90–6.88 (m, 2 H, H-Ph), 5.59 (bs, 1 H, 3-H), 5.56 (bs, 1 H, 5-H), 5.26 (d, J = 12.3 Hz, 1 H, OCH₂Ph), 5.22 (d, J = 12.5 Hz, 1 H, OCH₂Ph), 3.86 (d, J = 1.7 Hz, 1 H, 4-H), 3.79 (s, 3 H; OCH₃); ¹³C NMR (150 MHz, CDCl₃, 25 °C): δ = 159.8 (C-Ph), 135.5 (C-Ph), 128.5 (CH-Ph), 128.3 (CH-Ph), 128.1 (CH-Ph), 128.0 (CH-Ph), 126.7 (C-Ph), 114.4 (CH-Ph), 80.4 (C-5), 68.6 (CH₂Ph), 63.7 (C-3), 60.0
(±)-Benzy1 4,5-dihydroxy-3-(4-methoxyphenyl)isoaxazolidine-2-carboxylate (3)

To a stirred solution of epoxide 10 (650 mg, 1.99 mol) in acetone/water 4:1 (v/v, 40 mL) at 0 °C was added concentrated HCl (37 wt % in H2O, 3 drops). The reaction mixture was stirred for 30 min. After this time, TLC showed that the reaction was complete (hexanes/EtOAc 1:1). The mixture was diluted with water (40 mL) and extracted with CH2Cl2 (3 × 30 mL). The combined organic layers were diluted with MgSO4, and the solvent was evaporated under reduced pressure. The residue was crystallized from hexanes/CH2Cl2 to give single 4,5-cis-anomer 3 (640 mg, 1.85 mmol, 93%) as a white solid. mp 143–144 °C; Rf = 0.14 (n-hexane/EtOAc, 1:1); 1H NMR (300 MHz, CDCl3): δ = 7.32‒7.19 (m, 7 H, H-Ph), 6.92–6.87 (m, 2 H, H-Ph), 6.39 (bs, 1 H, 5-OH), 5.63 (pseudo t, J = 4.1 Hz, 1 H, 5-H), 5.19 (s, 2 H, OCH2Ph), 4.84 (d, J = 5.7 Hz, 1 H, 3-H), 4.40–4.30 (m, 1 H, 4-H), 3.81 (s, 3 H, OCH3), 2.95 (d, J = 10.1 Hz, 1 H, 4-OH); 1H NMR (300 MHz, CDCl3 + D2O): δ = 7.32‒7.20 (m, 7 H, H-Ph), 6.92–6.87 (m, 2 H, H-Ph), 5.60 (d, J = 4.3 Hz, 1 H, 5-H), 5.18 (s, 2 H, OCH2Ph), 4.84 (d, J = 5.7 Hz, 1 H, 3-H), 4.33 (dd, J = 5.7, 4.3 Hz, 1 H, 4-H), 3.81 (s, 3 H, OCH3); 13C NMR (150 MHz, CDCl3): δ = 159.3 (C-Ph), 135.3 (C-Ph), 131.1 (C-Ph), 128.5 (CH-Ph), 128.2 (CH-Ph), 127.6 (CH-Ph), 127.2 (CH-Ph), 114.1 (CH-Ph), 96.0 (C-5), 82.2 (C-4), 68.4 (OCH2Ph), 67.6 (C-3), 55.3 (OCH3), (the signal corresponding to the carbonyl carbon is missing); IR (ATR): νmax = 3350, 2954, 1704, 1513, 1302, 1244, 1141, 1065, 1028, 801, 737, 696, 554 cm⁻¹; HRMS (ESI): m/z calcd for C18H19NNaO6: 368.1105 [M + Na]+; found: 368.1105.

(±)-Benzy1 [2,3-dihydroxy-1-(4-methoxyphenyl)pent-4-en-1-yl](hydroxy)carbamate (4)

In a manner similar to [3], a reaction flask containing diol 3 (760 mg, 2.20 mmol) and anhydrous CeCl3 (815 mg, 3.31 mmol) was sealed with a rubber septum, evacuated, and filled with argon. Anhydrous THF (22 mL) was added, and the stirred mixture was cooled to 0 °C. Vinyl-MgBr (8.83 mL, 8.83 mmol, 1 M solution in THF) was added dropwise over 5 min. The reaction mixture was allowed to slowly warm to rt over 16 h. When TLC showed that the reaction was complete [CH2Cl2/acetone/NH3 4:1:0.3 (aq., 26%)], the reaction was quenched with 1 M HCl (100 mL), and the product was extracted into Et2O (3 × 100 mL). The combined organic layers were dried over MgSO4, and the solvent was evaporated under reduced pressure. The residue was purified by FCC (CH2Cl2/acetone 9:1) to give single diastereoisomer anti,syn-4 (600 mg, 1.61 mmol, 73%) as a pale yellow sticky oil which turned to a solid in the refrigerator. mp 100–101 °C (recrystallized from hexanes/CH2Cl2); Rf = 0.28 [CH2Cl2/acetone/NH3 (aq., 26%), 4:1:0.3]; 1H NMR (600 MHz, CD3OD): δ = 7.39–7.28 (m, 7 H, H-Ph), 6.87–6.82 (m, 2 H, H-Ph), 6.05 (ddd, J = 17.3, 10.5, 5.8 Hz, 1 H, 4-
H), 5.34 (dt, J = 17.3, 1.7 Hz, 1 H, 5-Ha), 5.21 (d, J = 9.9 Hz, 1 H, 1-H), 5.18 (d, J = 12.4 Hz, 1 H, OCH2Ph), 5.17 (td, J = 10.5, 1.7 Hz, 1 H, 5-Hb), 5.09 (d, J = 12.4 Hz, 1 H, OCH2Ph), 4.34 (dq, J = 5.8, 1.6 Hz, 1 H, 3-H), 4.22 (dd, J = 9.9, 1.8 Hz, 1 H, 2-H), 3.77 (s, 3 H, OMe); 13C NMR (75 MHz, CD3OD): δ = 160.6, 158.8 (C=O, C-Ph), 140.3 (C-4), 137.8 (C-Ph), 131.5 (C-Ph), 131.4 (CH-Ph), 129.4 (CH-Ph), 129.1 (CH-Ph), 129.0 (CH-Ph), 115.9 (C-5), 114.4 (CH-Ph), 73.5 (C-2), 73.1 (C-3), 68.7 (OCH2Ph), 63.8 (C-1), 55.7 (OCH3); IR (ATR): νmax = 3325, 2935, 1693, 1513, 1247, 1178, 1111, 1086, 1028, 744, 697, 563 cm−1; HRMS (ESI): m/z calcd for C20H24NO6: 374.1599 [M + H]+; found: 374.1610; m/z calcd for C20H23NNaO6: 396.1418 [M + Na]+; found: 396.1429.

Data for γ-(hydroxyamino)-α,β-diol anti,anti-4 isolated in a small amount (≈ 10%) when the reaction was performed in the absence of anhydrous CeCl3: Pale yellow sticky oil. Rf = 0.05 (n-hexane/EtOAc, 55:45); Rf = 0.11 [CH2Cl2/acetone/NH3 (aq., 26%), 4:1:0.3]; 1H NMR (300 MHz, CD3OD): δ = 7.39–7.27 (m, 7 H, H-Ph), 6.87–6.82 (m, 2 H, H-Ph), 6.02 (ddd, J = 17.4, 10.5, 7.0 Hz, 1 H, 4-H), 5.26–5.16 (m, 2 H, 5-H), 5.14 (d, J = 12.3 Hz, 1 H, OCH2Ph), 5.08 (d, J = 12.3 Hz, 1 H, OCH2Ph), 4.92 (d, J = 9.5 Hz, 1 H, 1-H), 4.37 (dd, J = 9.5, 3.5 Hz, 1 H, 2-H), 4.28 (tdd, J = 7.0, 3.5, 1.1 Hz, 1 H, 3-H), 3.77 (s, 3 H, OCH3); 13C NMR (75 MHz, CD3OD): δ = 160.7, 158.5 (C=O, C-Ph), 137.7 (C-Ph), 137.4 (C-4), 131.4 (CH-Ph), 131.1 (C-Ph), 129.5 (CH-Ph), 129.2 (CH-Ph), 129.1 (CH-Ph), 118.0 (C-5), 114.4 (CH-Ph), 74.8, 74.3 (C-2, C-3), 68.6 (OCH2Ph), 64.4 (C-1), 55.7 (OCH3); IR (ATR): νmax = 3369, 2935, 1693, 1513, 1247, 1178, 1111, 1086, 1028, 744, 697, 563 cm−1; HRMS (ESI): m/z calcd for C20H24NO6: 374.1599 [M + H]+; found: 374.1604.

(±)-Benzyl [2,3-dihydroxy-1-(4-methoxyphenyl)pent-4-en-1-yl]carbamate (11)

In a manner similar to [3], to a well-stirred solution of (hydroxyamino)dil anti,syn-4 (700 mg, 1.87 mmol) in acetic acid (13 mL) was added zinc dust (4.90 g, 75.0 mmol), and the reaction mixture was stirred at 40 °C. The reaction progress was monitored by TLC [CH2Cl2/acetone/NH3 5:1:0.3 (aq., 26%)]. After stirring for 24 h, the reaction mixture was cooled to rt, diluted with 0.1 M NaOH solution (60 mL) and CH2Cl2 (40 mL), and the resulting slurry was vigorously stirred for 10 min. Insoluble solids were removed by filtration through a pad of Celite and sequentially washed with 0.1 M NaOH solution (15 mL) and CH2Cl2 (15 mL). After phase separation, the aqueous layer was extracted with CH2Cl2 (2 × 50 mL). The combined organic layers were dried over MgSO4 and concentrated under reduced pressure. The product was isolated by FCC (CH2Cl2/acetone 9:1) to give N-Cbz-protected amino diol 11 (570 mg, 1.59 mmol, 85%) as a white solid. mp 93–94 °C; Rf = 0.18 (CH2Cl2/acetone, 9:1); 1H NMR (300 MHz, CD3OD): δ = 7.37–7.19 (m, 7 H, H-Ph), 6.90–6.85 (m, 2 H, H-Ph), 5.94 (ddd, J = 17.2, 10.5, 6.3 Hz, 1 H, 4-H), 5.21 (ddd, J = 17.2, 1.8, 1.4 Hz, 1 H, 5-Ha), 5.14 (ddd, J = 10.5, 1.8, 1.3 Hz, 1 H, 5-Hb), 5.08 (d, J = 12.5 Hz, 1 H, OCH2Ph), 5.01 (d, J = 12.5 H, 1 H, OCH2Ph), 4.76 (d, J = 6.7 Hz, 1 H, 1-H), 4.06–3.96 (m, 1 H, 3-H), 3.77 (s, 3 H, OCH3), 3.66 (dd, J = 6.6, 3.4 Hz, 1 H, 2-H); 13C NMR (75 MHz, CD3OD): δ = 160.4, 158.2 (C=O, C-Ph), 139.8 (C-4), 138.3 (C-
Ph), 133.6 (C-Ph), 129.9 (CH-Ph), 129.4 (CH-Ph), 129.0 (CH-Ph), 128.9 (CH-Ph), 116.4 (C-5), 114.6 (CH-Ph), 76.7, 73.4 (C-2, C-3), 67.6 (OCH2Ph), 58.5 (C-1), 55.7 (OCH3); IR (ATR): \( \nu_{\text{max}} = 3356, 2957, 1683, 1517, 1249, 1138, 1013, 836, 756, 700, 587 \text{ cm}^{-1} \); HRMS (ESI): \( m/z \) calcd for \( \text{C}_{20}\text{H}_{23}\text{NNaO}_5: 380.1469 \text{ [M + Na]}^+ \); found: 380.1469.

(±)-Benzyl [2,3-dihydroxy-1-(4-methoxyphenyl)-3-(oxiran-2-yl)propyl]carbamate (5)

To a stirred solution of amino diol 11 (120 mg, 0.34 mmol) in EtOAc (0.8 mL) at rt was added pyridine (1.6 µL, 0.02 mmol), phosphotungstic acid hydrate (10 mg, 3.4 µmol) and hydrogen peroxide solution (86 µL, 1.0 mmol, 35 wt % in H2O). The reaction mixture was stirred for 48 h. After this time, TLC showed that the reaction was complete (CH2Cl2/acetone 85:15). The reaction mixture was cooled to 0 °C, and sat. aq. Na2S2O3 (20 mL) was added dropwise over 10 min. The mixture was diluted with EtOAc (15 mL), warmed to rt, and stirred for additional 5 min. The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were dried over MgSO4 and the solvent was evaporated under reduced pressure. The residue was purified by FCC (CH2Cl2/acetone 9:1) to give a single anti, syn, syn-isomer of epoxide 5 (90 mg, 0.24 mmol, 70%) as a white solid. mp 112–113 °C; \( R_f = 0.10 \) (CH2Cl2/acetone, 9:1); \(^1\)H NMR (300 MHz, CD3OD): \( \delta = 7.37–7.19 \) (m, 7 H, H-Ph), 6.90–6.85 (m, 2 H, H-Ph), 5.07 (d, \( J = 12.4 \text{ Hz} \), 1 H, OCH2Ph), 5.01 (d, \( J = 12.4 \text{ Hz} \), 1 H, OCH2Ph), 4.80 (d, \( J = 7.7 \text{ Hz} \), 1 H, 1-H), 3.80 (dd, \( J = 7.8, 2.1 \text{ Hz} \), 1 H, 2-H), 3.77 (s, 3 H, OCH3), 3.27 (dd, \( J = 6.4, 2.1 \text{ Hz} \), 1 H, 3-H), 3.13 (ddd, \( J = 6.4, 4.2, 2.7 \text{ Hz} \), 1 H, 4-H), 2.72 (dd, \( J = 4.9, 4.2 \text{ Hz} \), 1 H, 5-Ha), 2.49 (dd, \( J = 4.9, 2.7 \text{ Hz} \), 1 H, 5-Hb); \(^{13}\)C NMR (75 MHz, CD3OD): \( \delta = 160.4, 158.3 \text{ (C=O, C-Ph)}, 138.2 \text{ (C-Ph)}, 133.7 \text{ (C-Ph)}, 129.7 \text{ (CH-Ph)}, 129.4 \text{ (CH-Ph)}, 129.0 \text{ (CH-Ph)}, 128.9 \text{ (CH-Ph)}, 114.7 \text{ (CH-Ph)}, 75.1, 73.4 \text{ (C-2, C-3)}, 67.6 \text{ (OCH2Ph)}, 58.5 \text{ (C-1)}, 55.7, 55.2 \text{ (OCH3, C-4)}, 45.0 \text{ (C-5)}; IR (ATR): \( \nu_{\text{max}} = 3361, 2935, 1690, 1514, 1247, 1030, 833, 738, 698, 585 \text{ cm}^{-1} \).

(±)-Benzyl 3,4-dihydroxy-2-(hydroxymethyl)-5-(4-methoxyphenyl)pyrrolidine-1-carboxylate (12)

To a stirred solution of epoxide 5 (340 mg, 0.91 mmol) in CH2Cl2 (9 mL) at 0 °C was added BF3·OEt2 (0.14 mL, 1.13 mmol) and the reaction mixture was stirred for 15 min. When TLC showed that the reaction was complete [CH2Cl2/MeOH/NH3 19:1:0.1 (aq., 26%)], sat. aq. NH4Cl (50 mL) and CH2Cl2 (50 mL) were added, and the mixture was vigorously stirred at rt for additional 5 min. Afterwards, the organic layer was separated, and the aqueous layer was extracted with CH2Cl2 (2 × 50 mL). The combined organic layers were dried over MgSO4 and the solvent was evaporated under reduced pressure. The residue was purified by FCC (CH2Cl2/MeOH 95:5) to give N-Cbz-protected pyrrolidine 12 (235 mg, 0.63 mmol, 69%) as a white solid. mp 139–140 °C; \( R_f = 0.08 \) (CH2Cl2/MeOH, 95:5); \(^1\)H
and $^{13}$C NMR spectra obtained at 25 °C show the presence of two N-Cbz rotamers in a ~2:1 ratio:

$^1$H NMR (600 MHz, DMSO-d$_6$, 25 °C, major rotamer): $\delta = 7.19–7.11$ (m, 5 H, H-Ph), 6.85–6.79 (m, 2 H, H-Ph), 6.72–6.69 (m, 2 H, H-Ph), 5.55 (d, $J = 4.9$ Hz, 1 H, OH), 5.09 (d, $J = 3.2$ Hz, 1 H, OH), 5.05–5.00 (m, 1 H, OH), 4.90 (d, $J = 13.1$ Hz, 1 H, OCH$_2$Ph), 4.77 (d, $J = 13.1$ Hz, 1 H, OCH$_2$Ph), 4.61–4.59 (m, 1 H, 5-H), 4.06–4.03 (m, 1 H, 3-H), 3.91–3.87 (m, 1 H, 2-H), 3.82–3.79 (m, 1 H, 4-H), 3.76–3.72 (m, 5 H, C$_2$H$_2$OH, OCH$_3$)$_3$; $^{13}$C NMR (150 MHz, DMSO-d$_6$, 25 °C, major rotamer): $\delta = 157.9$ (C-Ph), 154.0 (C=O), 136.6 (C-Ph), 134.4 (C-Ph), 127.9 (CH-Ph), 127.5 (CH-Ph), 127.2 (CH-Ph), 126.7 (CH-Ph), 113.2 (CH-Ph), 83.2 (C-4), 77.0 (C-3), 70.4 (C-5), 68.7 (C-2), 65.5 (OCH$_2$Ph), 58.9 (CH$_2$OH), 55.1 (OCH$_3$); $^1$H NMR (600 MHz, DMSO-d$_6$, 25 °C, minor rotamer): $\delta = 7.39–7.27$ (m, 5 H, H-Ph), 7.19–7.11 (m, 2 H, H-Ph), 6.85–6.79 (m, 2 H, H-Ph), 5.57 (d, $J = 4.6$ Hz, 1 H, OH), 5.06–5.00 (m, 4 H, OCH$_2$Ph, 2 × OH), 4.59–4.58 (m, 1 H, 5-H), 4.10–4.07 (m, 1 H, 3-H), 3.91–3.87 (m, 1 H, 2-H), 3.79–3.77 (m, 1 H, 4-H), 3.71 (s, 3 H, OCH$_3$), 3.69–3.65 (m, 2 H, C$_2$H$_2$OH); $^{13}$C NMR (150 MHz, DMSO-d$_6$, 25 °C, minor rotamer): $\delta = 157.7$ (C-Ph), 153.6 (C=O), 137.0 (C-Ph), 133.5 (C-Ph), 128.4 (CH-Ph), 127.8 (CH-Ph), 127.6 (CH-Ph), 127.5 (CH-Ph), 127.3 (C-Ph), 82.6 (C-4), 77.9 (C-3), 71.0 (C-5), 68.3 (C-2), 65.8 (OCH$_2$Ph), 60.0 (CH$_2$OH), 55.0 (OCH$_3$); IR (ATR): $\nu_{\text{max}}$ = 3236, 2903, 1673, 1427, 1351, 1200, 1035, 861, 744, 695, 586 cm$^{-1}$; HRMS (ESI): $m/z$ calcd for C$_{20}$H$_{24}$NO$_5$: 374.1599 [M + H]$^+$/found: 374.1600; $m/z$ calcd for C$_{20}$H$_{24}$NaNO$_6$: 396.1418 [M + Na]$^+$/found: 396.1418.

(±)-2-(Hydroxymethyl)-5-(4-methoxyphenyl)pyrrolidine-3,4-diol (2)

To a solution of pyrrolidine 12 (180 mg, 0.48 mmol) in anhydrous MeOH (28 mL) was added Pd(OH)$_2$/C (90 mg, 5 wt %), and the reaction mixture was vigorously stirred under H$_2$ atmosphere (1 atm) at rt for 2 h. After this time, TLC showed that the reaction was complete [CH$_2$Cl$_2$/MeOH/NH$_3$ 9:1:0.1 (aq., 26%)]. The catalyst was removed by filtration through a pad of Celite and washed with MeOH (10 mL). The filtrate was concentrated under reduced pressure. The residue was crystallized from EtOAc to give unprotected pyrrolidine 2 (81 mg, 0.34 mmol, 71%) as a white needle-like crystals with spectroscopic data in good agreement with those reported in the literature [4]. mp 143–144 °C; $R_f = 0.06$ (CH$_2$Cl$_2$/MeOH, 9:1); $^1$H NMR (300 MHz, D$_2$O): $\delta = 7.44–7.39$ (m, 2 H, H-Ph), 7.07–7.02 (m, 2 H, H-Ph), 4.12 (dd, $J = 9.1,7.5$ Hz, 1 H, 4-H), 3.97 (t, $J = 7.4$ Hz, 1 H, 3-H), 3.93 (d, $J = 9.1$ Hz, 1 H, 5-H), 3.86 (s, 3 H, OCH$_3$), 3.77 (dd, $J = 11.6, 4.7$ Hz, 1 H, C$_2$H$_2$OH), 3.71 (dd, $J = 11.6, 6.4$ Hz, 1 H, C$_2$H$_2$OH), 3.26 (td, $J = 6.7, 4.7$ Hz, 1 H, 2-H); $^1$H NMR (300 MHz, CD$_3$OD): $\delta = 7.37–7.32$ (m, 2 H, H-Ph), 6.93–6.88 (m, 2 H, H-Ph), 3.97–3.85 (m, 3 H, 3-H, 4-H, 5-H), 3.78 (s, 3 H, OCH$_3$), 3.72 (dd, $J = 11.1, 4.3$ Hz, 1 H, C$_2$H$_2$OH), 3.64 (dd, $J = 11.1, 6.2$ Hz, 1 H, C$_2$H$_2$OH), 3.19 (bd, $J = 6.2, 4.3$ Hz, 1 H, 2-H); $^{13}$C NMR (75 MHz, CD$_3$OD): $\delta = 160.7$ (C-Ph), 134.8 (C-Ph), 129.5 (CH-Ph), 114.9 (CH-Ph), 85.0, 79.5 (C-3, C-4), 66.1 (C-5), 64.5 (C-2), 63.8 (CH$_2$OH), 55.7 (OCH$_3$); IR (ATR): $\nu_{\text{max}}$ = 3210, 2912, 2398, 1513, 1250, 1183, 1028, 967, 825, 753, 669, 560 cm$^{-1}$.
(±)-2-(Hydroxymethyl)-5-(4-methoxyphenyl)-1-methylpyrrolidine-3,4-diol, (±)-codonopsinol B (1)

To a solution of pyrrolidine 12 (180 mg, 0.48 mmol) in anhydrous MeOH (28 mL) was added Pd(OH)$_2$/C (90 mg, 5 wt %) and the reaction mixture was vigorously stirred under H$_2$ atmosphere (1 atm) at rt for 2 h. When TLC showed that the protecting group was removed [CH$_2$Cl$_2$/MeOH/NH$_3$ 9:1:0.1 (aq., 26%)], formaldehyde solution (0.54 mL, 7.2 mmol, 37 wt % in H$_2$O) was added, and the reaction mixture was stirred at rt under H$_2$ atmosphere (1 atm) for additional 16 h. After this time, the catalyst was removed by filtration through a pad of Celite and washed with MeOH (10 mL). The filtrate was concentrated under reduced pressure. The residue was purified by FCC (CH$_2$Cl$_2$/MeOH 9:1) to give (±)-codonopsinol B (1, 70 mg, 0.28 mmol, 58% over two steps) as a colourless sticky oil with spectroscopic data in good agreement with those reported in the literature [4,5].

R$_f$ = 0.10 (CH$_2$Cl$_2$/MeOH, 9:1); $^1$H NMR (300 MHz, CD$_3$OD): δ = 7.33–7.28 (m, 2 H, H-Ph), 6.94–6.89 (m, 2 H, H-Ph), 4.04 (pseudo t, J = 4.7, 4.6 Hz, 1 H, 3-H), 3.99 (dd, J = 6.6, 4.8 Hz, 1 H, 4-H), 3.88 (dd, J = 11.6, 4.2 Hz, 1 H, CH$_2$OH), 3.83 (dd, J = 11.6, 4.2 Hz, 1 H, CH$_2$OH), 3.79 (s, 3 H, OCH$_3$), 3.74 (d, J = 6.6 Hz, 1 H, 5-H), 3.12 (pseudo q, J = 4.2, 4.3 Hz, 1 H, 2-H), 2.22 (s, 3 H, NCH$_3$); $^{13}$C NMR (75 MHz, CD$_3$OD): δ = 161.2 (C-Ph), 131.1 (CH-Ph), 131.0 (C-Ph), 115.0 (CH-Ph), 84.1 (C-4), 79.0 (C-3), 75.2 (C-5), 71.5 (C-2), 60.1 (CH$_2$OH), 55.7 (OCH$_3$), 35.4 (NCH$_3$).

Crystallography

Data collection and cell refinement for 12 were carried out with a Stoe StadiVari diffractometer equipped with a Pilatus3R 300K HPD detector, and using CuKα radiation (λ = 1.54186 Å, microfocussed source Xenocs Genix3D Cu HF) at 100 K. The structure was solved using Sir14 [6] and refined by a full-matrix least-squares procedure of independent atom model (AIM) with the SHELXL (ver. 2018/3) [7]. The 4-methoxyphenyl group is disordered in two positions with occupancy factors 0.64 and 0.36 for main and minor parts (Figures S1 and S2). The disordered 4-methoxyphenyl group has been modelled using SAME, SADI, RIGU, and EADP instructions. The Hirshfeld atom refinement (HAR) was carried out using IAM model as a starting point. The wave function was calculated using ORCA software [8,9] with basis set def2-TZVP and method PBE0. The least-squares refinements of HAR model were then carried out with olex2.refine [10], while keeping the same constrains and restrains as for the SHELXL refinement. The NoSpherA2 implementation [11] of HAR makes used for tailor-made aspherical atomic factors calculated on-the-fly from a Hirshfeld-partitioned electron density. For the HAR approach, all H atoms were refined isotropically and independently. All calculations and structure drawings were done in the OLEX2 package [12].
Crystal data for 12

C_{20}H_{23}NO_{6} (M = 373.39 g/mol): monoclinic, space group C2/c (no. 15), a = 35.2705(13) Å, b = 5.8905(2) Å, c = 17.6025(7) Å, β = 94.974(3)°, V = 3643.3(2) Å^3, Z = 8, T = 100.0 K, μ(CuKα) = 0.837 mm\(^{-1}\), D_{calc} = 1.361 g/cm\(^3\), 51482 reflections measured (5.03° ≤ 2θ ≤ 144.05°), 3538 unique (R_{int} = 0.0785, R_{sigma} = 0.0296) which were used in all calculations. IAM: The final R_1 was 0.0391 (I > 2σ(I)) and wR_2 was 0.1017 (all data). CCDC no 2058692. HAR: The final R_1 was 0.0294 (I > 2σ(I)) and wR_2 was 0.0693 (all data). CCDC no 2099851.

Cell culture and cultivation conditions

The antiproliferative effect of the tested compounds was evaluated on different cell lines. Cells were cultivated in humidified atmosphere with 5% CO_2 concentration at 37 °C.

The human glioblastoma astrocytoma cancer cell line U87-MG (purchased from ATCC, USA) was cultivated in Dulbecco’s Modified Eagle’s Medium (DMEM) High Glucose containing L-glutamine (2 mM), sodium pyruvate (1 mM), 1% non-essential amino acids (NAA) and 10% fetal bovine serum (FBS).

The HK-2 (purchased from ATCC, USA) is an immortalized proximal tubular cell line. The cells were maintained in DMEM High Glucose supplemented with 2 mM L-glutamine and 10% FBS.

The hepatocellular carcinoma cell line HepG2 (purchased from ECACC, UK) was cultivated in DMEM High Glucose containing L-glutamine (2 mM), 10% FBS, and 1% NAA.

The human placental choriocarcinoma cells JEG-3 (purchased from ECACC, UK) were maintained in Minimum Essential Medium Eagle (MEM) supplemented with Earle's Salts, sodium bicarbonate, 1% NAA, sodium pyruvate (1 mM), and 10% FBS.

The acute myeloid leukemia MOLM-13 cell line (DSMZ, GE) was cultivated in RPMI-1640 medium supplemented with 12% fetal bovine serum (both purchased from Gibco, Miami, OK, USA), 100,000 units/mL penicillin and 50 mg/L streptomycin.

All used supplements and mediums were purchased from Sigma-Aldrich, USA except for FBS purchased from Biosera, South America.

Cell viability testing

For the U87-MG, HepG2, JEG-3 cancer cell lines, as well as immortalized proximal tubular cells HK2 the potential antiproliferative effect of tested substances was determined using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, USA). The whole evaluation was proceeded according to the manufacturer’s instructions. The tested cells were seeded in a 96-well plate (TPP, Switzerland) at low density 4 × 10^3 cells per well and subsequently incubated for 24 h. The cells were exposed to the tested compounds at a concentration range (1–1000 µM), 1% DMSO
(non-toxic control, 100% viability) or 10% DMSO (toxic control, 100% mortality). The compounds were dissolved in DMSO (Sigma-Aldrich, USA), the volume of solvent was 1% (v/v) in the cultivation medium. The cells were incubated for 24, 48, or 72 h under standard culture conditions. The IC$_{50}$ values were calculated using the GraphPad Prism 8.3.1 software.

In the case of AML cell line MOLM-13, the cells were treated with compounds at a concentration range (0.5–500 µM). The DMSO concentration to dissolve the tested substances was 1% (non-toxic concentration). After the treatment, the cells were incubated for 48 h under standard culture conditions and the antiproliferative effect of the tested substances was also determined using CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, USA). Numerical data are expressed as the mean ± SD of three independent measurements.
Spectrum A
an epoxidation with
H₂O₂/12WO₃·H₃PO₄·xH₂O

Spectrum B
an epoxidation with
m-CPBA
**Figure S1:** The ORTEP-like style drawn of IAM model of 12 with the thermal ellipsoids shown at a 50% probability level. The green and purple lines represent the main and minor part of the disorder.
**Figure S2:** The ORTEP-like style drawn of HAR model of 12 with the thermal ellipsoids shown at a 50% probability level. The green and purple lines represent the main and minor part of the disorder.
Figure S3: The O–H···O hydrogen bonds in the crystal structure of 12. The minor part of disorder has been removed for clarity.
Table S1: Summarization of IC$_{50}$ values for compounds (±)-1 and (±)-2.

| Tested compound | Cell line  | The concentration range (µM) | IC$_{50}$ (µM) treatment 24 h | IC$_{50}$ (µM) treatment 48 h | IC$_{50}$ (µM) treatment 72 h |
|-----------------|------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 1               | U87-MG     | 1–1000                        | > 1000                        | > 1000                        | > 1000                        |
| 2               | U87-MG     | 1–1000                        | > 1000                        | > 1000                        | > 1000                        |
| 1               | HK-2       | 1–1000                        | > 1000                        | > 1000                        | > 1000                        |
| 2               | HK-2       | 1–1000                        | > 1000                        | > 1000                        | > 1000                        |
| 1               | HepG2      | 1–1000                        | > 1000                        | > 1000                        | > 1000                        |
| 2               | HepG2      | 1–1000                        | > 1000                        | > 1000                        | > 1000                        |
| 1               | JEG-3      | 1–1000                        | > 1000                        | > 1000                        | —*                            |
| 2               | JEG-3      | 1–1000                        | > 1000                        | > 1000                        | —*                            |

Note: The viability of JEG-3 cell line was not tested after 72 hours treatment of compounds.
**Figure S4:** Antiproliferative effect of the tested compounds (±)-1 and (±)-2 on U87-MG cells after 24, 48, and 72 h treatment.
Figure S5: Antiproliferative effect of the tested (±)-1 and (±)-2 compounds on HK-2 cells after 24, 48, and 72 h treatment.
**Figure S6**: Antiproliferative effect of the tested compounds (±)-1 and (±)-2 on HepG2 cells after 24, 48, and 72 h treatment.
**Figure S7:** Antiproliferative effect of the tested compounds (±)-1 and (±)-2 on JEG-3 cells after 24 or 48 h treatment.
Figure S8: Antiproliferative effect of the tested compounds (±)-1 and (±)-2 on MOLM-13 cells after 48 h treatment.
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