Tubulin-binding dibenz[c,e]oxepines as colchinol analogues for targeting tumour vasculature

David J. Edwards,a,b John A. Hadfield,b,c Timothy W. Wallace*a and Sylvie Ducki,c,d

aSchool of Chemistry, The University of Manchester, Oxford Road, Manchester M13 9PL, UK
bDrug Development Group, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester M20 4BX, UK
cKidscan Laboratories, Centre for Molecular Drug Design, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK
dPresent address: Laboratoire de Chimie des Hétérocycles et des Glucides, ENSCCF, Clermont Université, 63174 Aubière, France.

*Corresponding author; e-mail: tim.wallace@manchester.ac.uk; fax +44 (0) 161 275 4939

Supplementary Information

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General Experimental Information
Melting points were determined using Kofler hot-stage, Buchi 512 or Electrothermal 9100 equipment and are uncorrected. Unless otherwise indicated, IR spectra were recorded for neat thin films on NaCl plates, using Perkin-Elmer 1710FT or Nicolet Nexus 670/870 spectrometers. NMR spectra were measured on Bruker AC300 or DPX400 instruments, and assigned with the aid of COSY, HMBC, HSQC and DEPT spectra where appropriate. Coupling constants (J values) are quoted to the nearest 0.1 Hz. Low-resolution mass spectra were measured on a Micromass LCT instrument using a Waters 2790 separations module with electrospray (ES+) ionisation and TOF fragment detection, or a Kratos MS-50 spectrometer with FAB ionisation. High-resolution mass measurements were obtained using ThermoFinnigan MAT95XP or Kratos Concept S1 instruments. Data for most of the peaks of intensity <20% of that of the base peak are omitted. Elemental analyses were carried out by the University of Manchester microanalytical service.

Starting materials and solvents were routinely purified by conventional techniques. Most reactions were carried out under nitrogen or, when appropriate, argon dried by passage through an anhydrous CaCl₂ drying tube and freed from traces of oxygen using an Oxysept cartridge (both Aldrich). Tetrahydrofuran (THF) and N,N,N',N'-tetramethylethylenediamine (TMEDA) were dried using sodium - benzophenone ketyl under argon. Organic solutions were usually dried using anhydrous MgSO₄ and concentrated by rotary evaporation under reduced pressure. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 on aluminium plates containing a 254 nm fluorescent indicator. The chromatograms were visualised by the use of UV light or the following developing agents; ethanolic vanillin or potassium permanganate. Unless otherwise indicated, preparative (column) chromatography was carried out using the flash technique on 60H silica gel (Merck 9385). Compositions of solvent mixtures are quoted as ratios of volume. 'Petroleum' refers to a light petroleum fraction, b.p. 60–80 °C, unless otherwise stated. 'Ether' refers to diethyl ether. The preparative routes to the dibenzoepines 51 and 56 have also been described in a patent application.
Preparative details for compounds 45–51

![Chemical Structures](image)

**Scheme 3**

2-Bromo-3,4,5-trimethoxybenzaldehyde 45
To a stirred refluxing solution of 3,4,5-trimethoxybenzaldehyde (Aldrich T68403; 17.0 g, 86.6 mmol) in water (3 mL) and chloroform (100 mL) was added a solution of bromine (14.7 g, 92 mmol) in chloroform (30 mL) dropwise. The solution was heated under reflux overnight, cooled, washed with water (2 x 100 mL) and saturated aqueous sodium hydrogen carbonate (50 mL), dried and evaporated. The residual orange oil (27 g) crystallised on standing. The solid was washed with petroleum to obtain the title compound 45 (23.4 g, 98%) as white crystals, m.p. 69 °C (lit.38 69.5–71 °C); \( \nu_{\text{max}}/\text{cm}^{-1} \) 2940, 2843, 1685, 1588, 1480, 1460, 1386, 1317, 1196, 1161, 1134, 1103, 1002; \( \delta_{\text{H}} \) (300 MHz, CDCl₃) 10.40 (1 H, s, 1-CHO), 7.29 (1 H, s, 6-H), 4.00 (3 H, s, ArOMe), 3.95 (3 H, s, ArOMe), 3.93 (3 H, s, ArOMe); \( \delta_{\text{C}} \) (75 MHz, CDCl₃) 56.6 (CH₃), 61.6 (2 x CH₃), 106.9 (CH), 116.0 (C), 128.1 (C), 149.2 (C), 150.3 (C), 152.8 (C), 189.2 (CH); \( R_f \) 0.50 (hexane - ethyl acetate, 4:1).

Methyl 6'-formyl-2',3',4,4'-tetramethoxybiphenyl-2-carboxylate 47
To a suspension of copper bronze (7.88 g, 122 mmol) in anhydrous DMF (12 mL) was added a solution of 45 (3.36 g, 12.2 mmol) and 46 (3.00 g, 12.2 mmol) in anhydrous DMF (6 mL) and the suspension was stirred at 165 °C for 3 h. The reaction was cooled, diluted with ethyl acetate (250 mL) and the resulting suspension filtered through Celite® (40 g). The solvent was removed in vacuo, leaving a brown
oil (5.0 g) of which a portion (2.0 g) was purified by chromatography (200 g silica gel, hexane - ethyl acetate, gradient 8:1 to 6:1) followed by crystallisation from petroleum ether 60–80°, which gave the title compound 47 (1.10 g, 63%), m.p. 62–64 °C (Found: C, 63.5; H, 5.7. \( \text{C}_{19}\text{H}_{20}\text{O}_{7} \) requires C, 63.33; H, 5.59%); \( \nu_{\text{max}}/\text{cm}^{-1} \) 2944, 2835, 1724, 1685, 1592, 1532, 1480, 1394, 1332, 1289, 1223, 1192, 1145, 1099, 1072, 1002, 928, 850, 757; \( \delta_{\text{H}} \) (300 MHz, \( \text{CDCl}_3 \)) 9.60 (1 H, s, CHO), 7.60 (1 H, d, \( J \) 2.6 Hz, 3-H), 7.35 (1 H, s, 5'-H), 7.18 (1 H, d, \( J \) 8.4 Hz, 6-H), 7.18 (1 H, dd, \( J \) 2.6, 8.4 Hz, 5-H), 3.97 (3 H, s, ArOMe), 3.95 (3 H, s, ArOMe), 3.91 (3 H, s, ArOMe), 3.55 (3 H, s, CO\(_2\)Me); \( \delta_{\text{C}} \) (75 MHz, \( \text{CDCl}_3 \)) 52.5 (CH\(_3\)), 56.0 (CH\(_3\)), 56.4 (CH\(_3\)), 61.1 (CH\(_3\)), 61.4 (CH\(_3\)), 105.2 (CH), 115.5 (CH), 118.0 (CH), 126.6 (C), 129.8 (C), 132.8 (CH), 134.2 (C), 134.3 (C), 147.6 (C), 151.2 (C), 153.3 (C), 159.6 (C), 167.4 (C), 191.2 (CH); \( m/z \) (ES) 424 \([\text{M}(\text{MeCN})\text{Na}^+]\), 100%; 383 (\( \text{MNa}^+ \), 80); \( R_f \) 0.19 (hexane - ethyl acetate, 5:1). Also isolated was the symmetrical biaryl dimethyl 4,4'-dimethoxy-2,2'-diphenoate 48 (0.25 g, 12%), m.p. 76–77 °C (lit.61 78 °C); \( \delta_{\text{H}} \) (300 MHz, \( \text{CDCl}_3 \)) 7.48 (2 H, d, \( J \) 2.6 Hz, 3 and 3'-H), 7.10 (2 H, d, \( J \) 8.4 Hz, 6,6'-H), 7.04 (2 H, dd, \( J \) 2.6, 8.4 Hz, 5,5'-H), 3.90 (6 H, s, 4,4'-OMe), 3.64 (6 H, s, 2,2'-CO\(_2\)Me); \( R_f \) 0.27 (hexane - ethyl acetate, 5:1).

Methyl 6'-(hydroxymethyl)-2',3',4,4'-tetramethoxybiphenyl-2-carboxylate 49 (4,4',5,6-Tetramethoxybiphenyl-2,2'-diyl)dimethanol 50

To a solution of crude 47 (55% pure by 1H NMR spectroscopy; 3.0 g, 4.6 mmol) in methanol (100 mL) was added sodium borohydride (0.61 g, 16.0 mmol) and the solution was stirred at room temperature for 1 h. Water (120 mL) and ethyl acetate (120 mL) were then added to the reaction, the layers were separated and the aqueous layer was extracted with ethyl acetate (2 x 80 mL). The combined organic extract was dried over Na\(_2\)SO\(_4\) and filtered. The solvent was removed \textit{in vacuo} and the residue was purified by chromatography (150 g silica gel, hexane - ethyl acetate, 1:1), which yielded a clear oil (1.30 g). This was shown by 1H NMR spectroscopy to be a 3:1 mixture of 49 and 3,4,5-trimethoxybenzyl alcohol 17 (identified by TLC and 1H NMR comparison with an authentic sample). The crude 49 (1.10 g, 66%), which was used without further purification in the next step, had \( \delta_{\text{H}} \) (300 MHz, \( \text{CDCl}_3 \)) 7.46 (1 H, d, \( J \) 2.1 Hz, 3-H), 7.16–7.07 (2 H, m, 5,6-H), 6.87 (1 H, s, 5'-H), 4.34 (1 H, d, \( J \) 11.7 Hz, 6'-CH\(_3\)OH), 4.25 (1 H, dd, \( J \) 5.2, 11.7 Hz, 6'-CH\(_3\)OH), 3.92 (3 H, s, ArOMe) 3.89 (3 H, s, ArOMe), 3.88 (3 H, s, ArOMe), 3.71 (3 H, s, ArOMe), 3.56 (3 H, s, 2-CO\(_2\)Me), 2.44 (1 H, br s, 6'-CH\(_2\)OH); \( R_f \) 0.30 (hexane - ethyl acetate, 1:1). To a solution of lithium borohydride (78 mg, 3.6 mmol) in dry ether (10 mL) under argon was added a solution of 49 (1.3 g, purity \textit{ca.} 85% w/w, 2.9 mmol) in dry ether (10 mL) and the mixture was then heated under reflux for 18 h. After being cooled to room
temperature, the mixture was acidified with conc. hydrochloric acid (1 mL), diluted with water (50 mL) and extracted with ether (3 x 30 mL). The combined organic extract was dried and evaporated, giving a crude solid (1.2 g) which was purified by chromatography (60 g silica gel, hexane - ethyl acetate, 2:1), followed by crystallisation (twice) from ethyl acetate - hexane. This gave the title compound 50 (600 mg, 62%), m.p. 137–139 °C (Found: C, 64.9; H, 6.7. C_{18}H_{22}O_{6} requires C, 64.66; H, 6.63%); ν_{max}/cm^{-1} (nujol mull) 3266, 2924, 2846, 1612, 1596, 1484, 1460, 1360, 1332, 1289, 1250, 1157, 1095, 1049, 998; δ_{H} (300 MHz, CDCl_{3}) 7.05 (2 H, m, 3,6-H), 6.90 (1 H, dd, J 2.5, 9.5 Hz, 5-H), 6.87 (1 H, s, 5'-H), 4.30 (2 H, s, CH_{2}OH), 4.28 (2 H, s, CH_{2}OH), 3.93 (3 H, s, ArOMe), 3.91 (3 H, s, ArOMe) 3.88 (3 H, s, ArOMe), 3.65 (3 H, s, ArOMe), 3.55 (3 H, s, ArOMe), 3.05 (1 H, br s, CH_{2}OH), 2.59 (1 H, br s, CH_{2}OH); δ_{C} (75 MHz, CDCl_{3}) 55.7 (CH_{3}), 56.4 (CH_{3}), 61.4 (2 x CH_{3}), 63.3 (CH_{2}), 64.2 (CH_{2}), 108.6 (CH), 114.0 (CH), 115.1 (CH), 126.6 (C), 127.6 (C), 131.8 (CH), 135.5 (C), 141.3 (C), 142.0 (C), 151.5 (C), 153.4 (C), 159.7 (C); m/z (ES) 398 [M(MeCN)Na^{+}, 100%], 357 (MNa^{+}, 75); R_{f} 0.28 (hexane - ethyl acetate, 1:2).

5,7-Dihydro-1,2,3,9-tetramethoxydibenz[cd,ef]oxepine 51

A solution of 50 (170 mg, 0.51 mmol) in THF (2 mL), 2 M hydrochloric acid (2 mL) and conc. hydrochloric acid (1 mL) was stirred under reflux for 3 h. Water (15 mL) and ethyl acetate (15 mL) were added to the reaction, the layers were separated and the aqueous layer was extracted with ethyl acetate (2 x 10 mL). The combined organic extract was dried over Na_{2}SO_{4} and concentrated in vacuo. Chromatography of the residue (20 g silica gel, hexane - ethyl acetate, 4:1) gave the title compound 51 (150 mg, 93%) as a colourless solid, m.p. 151–153 °C (Found: C, 68.5; H, 6.5. C_{18}H_{20}O_{5} requires C, 68.34; H, 6.37%); ν_{max}/cm^{-1} 2963, 2936, 2858, 2839, 1612, 1491, 1456, 1332, 1243, 1150, 1104, 1052, 1006; δ_{H} (300 MHz, CDCl_{3}) 7.63 (1 H, d, J 8.4 Hz, 11-H), 6.98 (1 H, dd, J 2.6, 8.4 Hz, 10-H), 6.96 (1 H, d, J 2.6 Hz, 8-H), 6.75 (1 H, s, 4-H), 4.42 (2 H, m), 4.08 (2 H, m), 3.94 (3 H, s, ArOMe), 3.91 (3 H, s, ArOMe), 3.86 (3 H, s, ArOMe), 3.65 (3 H, s, ArOMe); δ_{C} (75 MHz, CDCl_{3}) 55.7 (CH_{3}), 56.4 (CH_{3}), 61.2 (CH_{3}), 61.5 (CH_{3}), 68.1 (2 x CH_{2}), 109.1 (CH), 114.2 (CH), 114.8 (CH), 126.7 (C), 129.7 (C), 131.1 (CH), 131.4 (C), 136.8 (C), 143.1 (C), 150.9 (C), 153.1 (C), 159.4 (C); m/z (ES) 380 [M(MeCN)Na^{+}, 100%], 287 (M Na^{+}–CH_{2}O, 100); R_{f} 0.39 (hexane - ethyl acetate, 3:1).
Preparative details for compounds 53–56

6-((6-Formyl-2,3,4-trimethoxyphenyl)benzo[d][1,3]dioxole-5-carbaldehyde 53
To a suspension of copper bronze (2.81 g, 43.6 mmol) in anhydrous DMF (5 mL) was added a solution of 45 (1.2 g, 4.36 mmol) and 52 (1.00 g, 4.36 mmol) in anhydrous DMF (3 mL) and the suspension was stirred at 165 °C for 3 h. The reaction was cooled, diluted with ethyl acetate (100 mL) and the resulting suspension was filtered through Celite® (15 g). The solvent was removed in vacuo and the residue chromatographed (50 g silica gel, hexane - ethyl acetate, 6:1), which gave a mixture of the desired dialdehyde 53 and the homocoupled product 54 (ratio 10:1 by 1H NMR spectroscopy). Two crystallisations from ethyl acetate gave the title compound 53 (330 mg, 22%), m.p. 142–143 °C [lit.38 138–142 °C (benzene)] (Found: C, 63.0; H, 4.8. C_{18}H_{16}O_{7} requires C, 62.79; H, 4.68%); $\nu_{\text{max}}$/cm$^{-1}$ 2940, 2850, 1685, 1612, 1588, 1476, 1386, 1332, 1250, 1137; $\delta_{\text{H}}$ (300 MHz, CDCl$_3$) 9.64 (1 H, s, CHO), 9.60 (1 H, s, CHO), 7.51 (1 H, s, 4-H), 7.40 (1 H, s, 7-H), 6.74 (1 H, s, 5'-H), 6.16 (2 H, s, 2-H), 4.00 (3 H, s, ArOMe), 3.98 (3 H, s, ArOMe), 3.65 (3 H, s, ArOMe); $\delta_{\text{C}}$ (100 MHz, CDCl$_3$) 56.6 (CH$_3$), 61.4 (CH$_3$), 61.5 (CH$_3$), 102.8 (CH$_2$), 106.2 (CH), 107.0 (CH), 112.2 (CH), 129.5 (C), 130.7 (C), 130.8 (C), 133.8 (C), 147.7 (C), 148.9 (C), 151.6 (C), 152.5 (C), 154.3 (C), 189.8 (CH), 190.2 (CH); $m/z$ (ES) 389 [M(MeCN)Na$^+$, 100%], 367 (MNa$^+$, 40); R$_f$ 0.21 (hexane - ethyl acetate, 5:1). A sample of the dialdehyde 54 prepared by the published procedure$^{62}$ had m.p. 128 °C (ether) [lit.$^{38}$ 128–129 °C (ether)]; $\nu_{\text{max}}$/cm$^{-1}$ 2939, 2843, 1685, 1588, 1480, 1460, 1386, 1317,
1196, 1161, 1134, 1103, 1002; \( \delta_T \) (400 MHz, CDCl\(_3\)) 9.58 (2 H, s, CHO), 7.39 (2 H, s, 3,3'-H), 3.974 (6 H, s, OMe), 3.971 (6 H, s, OMe), 3.61 (6 H, s, OMe); \( \delta_C \) (100 MHz, CDCl\(_3\)) 56.3 (CH\(_3\)), 60.8 (CH\(_3\)), 61.2 (CH\(_3\)), 105.7 (CH), 124.5 (C), 130.5 (C), 147.3 (C), 151.7 (C), 154.0 (C), 190.3 (CH); \( R_f \) 0.32 (EtOAc - hexane, 1:4), 0.70 (ether).

(6-(6-(Hydroxymethyl)-2,3,4-trimethoxyphenyl)benzo[d][1,3]dioxol-5-yl)methanol 55

To a solution of the crude dialdehyde 53 (265 mg, 0.77 mmol) in methanol (7 mL) was added sodium borohydride (80 mg, 2.1 mmol) and the solution was stirred at room temperature for 1 h. Water (30 mL) and ethyl acetate (30 mL) were then added, the layers were separated and the aqueous layer was extracted with ethyl acetate (2 x 30 mL). The combined organic extract was dried over Na\(_2\)SO\(_4\) and concentrated in vacuo. The residue was purified by chromatography (40 g silica gel, hexane - ethyl acetate, 1:2) followed by crystallisation (ethyl acetate), which gave the title compound 55 (230 mg, 86%) as a colourless solid, m.p. 123–124 °C (Found: \( M + Na^+ \), 371.1105; C\(_{18}\)H\(_{20}\)O\(_7\)Na requires 371.1102); \( \nu_{\text{max}}/\text{cm}^{-1} \) 3254, 2944, 2889, 1603, 1480, 1410, 1328, 1235, 1146, 1111, 1033, 928; \( \delta_T \) (300 MHz, CDCl\(_3\)) 7.00 (1 H, s, 7-H), 6.88 (1 H, s, 4-H), 6.63 (1 H, s, 5'-H), 6.04 (2 H, s, 2-H), 4.30 (2 H, m, CH\(_2\)OH), 4.21 (2 H, m, CH\(_2\)OH), 3.93 (3 H, s, OMe), 3.91 (3 H, s, OMe), 3.62 (3 H, s, OMe); \( \delta_C \) (75 MHz, CDCl\(_3\)) 56.4 (CH\(_3\)), 61.4 (CH\(_3\)), 61.5 (CH\(_3\)), 63.1 (CH\(_2\)), 63.7 (CH\(_2\)), 101.7 (CH\(_2\)), 108.7 (CH), 110.4 (CH), 110.6 (CH), 126.6 (C), 129.1 (C), 133.8 (C), 135.5 (C), 142.0 (C), 147.5 (C), 147.8 (C), 151.4 (C), 153.5 (C); \( m/\text{z} \) (ES) 412 [M(MeCN)Na\(^+\), 100%], 371 (MNa\(^+\), 60); \( R_f \) 0.14 (hexane - ethyl acetate, 1:2).

5,7-Dihydro-1,2,3-trimethoxybenzo[d][1,3]dioxolo[4,5-h][2]benzoxepine 56

A solution of 55 (170 mg, 0.48 mmol) in THF (2 mL), 2 M hydrochloric acid (2 mL) and conc. hydrochloric acid (1 mL) was stirred under reflux for 3 h. Water (15 mL) and ethyl acetate (15 mL) were added, the layers were separated and the aqueous layer was extracted with ethyl acetate (2 x 10 mL). The combined organic extract was dried over Na\(_2\)SO\(_4\) and concentrated in vacuo. Chromatography of the residue (20 g silica gel, hexane - ethyl acetate, 4:1) followed by crystallisation (ethyl acetate) gave the title compound 56 (103 mg, 64%) as large clear crystals, m.p. 154–156 °C (Found: C, 65.3; H, 5.5. C\(_{18}\)H\(_{18}\)O\(_6\) requires C, 65.45; H, 5.49%); \( \nu_{\text{max}}/\text{cm}^{-1} \) 2967, 2932, 2866, 1600, 1484, 1460, 1414, 1324, 1239, 1146, 1107, 1045; \( \delta_T \) (300 MHz, CDCl\(_3\)) 7.21 (1 H, s, 12-H), 6.98 (1 H, s, 8-H), 6.75 (1 H, s, 4-H), 6.04 (2 H, d, \( J = 4.8 \text{ Hz}, 10-H_2 \)), 4.40 (2 H, d, \( J = 11.2 \text{ Hz}, 5-H_2 \)), 4.04 (1 H, d, \( J = 10.8 \text{ Hz}, 7-H_A \)), 4.01 (1 H, d, \( J = 10.8 \text{ Hz}, 7-H_B \)), 3.96 (3 H, s, OMe), 3.91 (3 H, s, OMe), 3.71 (3 H, s, OMe); \( \delta_C \) (75 MHz,
CDCl₃) 56.3 (CH₃), 61.2 (CH₃), 61.5 (CH₃), 67.6 (CH₂), 67.8 (CH₂), 101.6 (CH₂), 109.0 (CH), 109.9 (CH), 110.2 (CH), 126.8 (C), 129.5 (C), 131.3 (C), 131.7 (C), 143.0 (C), 147.3 (C), 147.7 (C), 150.8 (C), 153.3 (C); m/z (ES) 301 (MH⁺–CH₂O, 100%); Rf 0.28 (hexane - ethyl acetate, 4:1).

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