Supplementary Material (ESI) for Organic & Biomolecular Chemistry

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

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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.[52] Most reactions were carried out under nitrogen or, when appropriate, argon dried by passage through an anhydrous $CaCl_2$ 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 $MgSO_4$ 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[53] 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 dibenzoxepines **51** and **56** have also been described in a patent application.[32]

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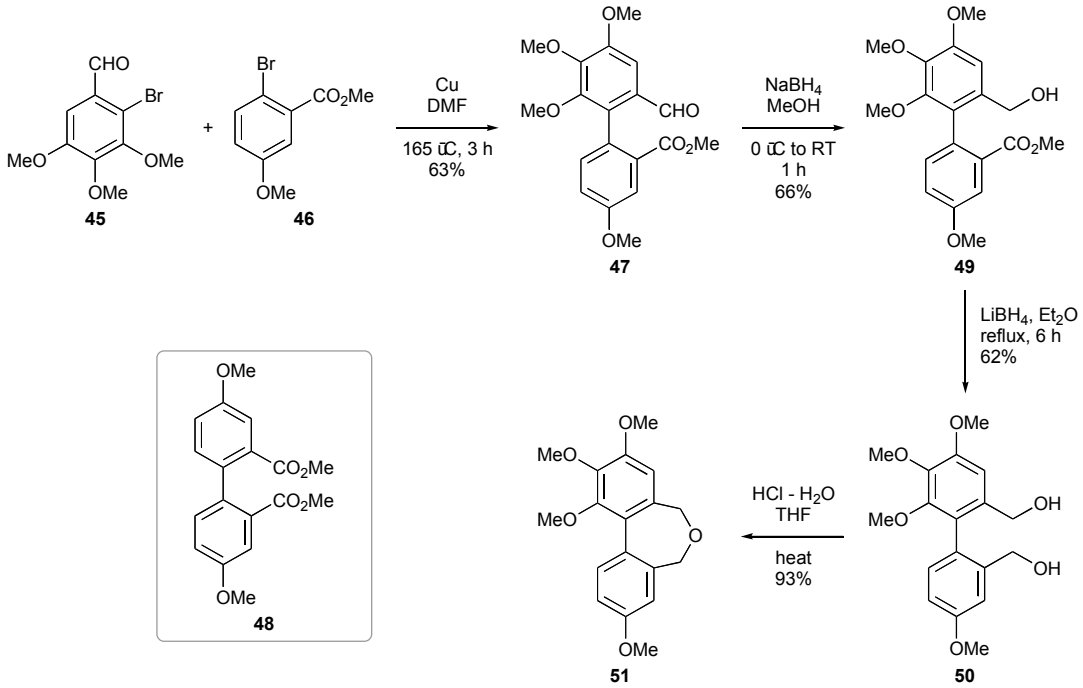
Preparative details for compounds 45–51



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); ν_{max}/cm^{-1} 2940, 2843, 1685, 1588, 1480, 1460, 1386, 1317, 1196, 1161, 1134, 1103, 1002; δ_H (300 MHz, $CDCl_3$) 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); δ_C (75 MHz, $CDCl_3$) 56.6 (CH_3), 61.6 (2 x CH_3), 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**[60] (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. $C_{19}H_{20}O_7$ requires C, 63.33; H, 5.59%); ν_{max}/cm^{-1} 2944, 2835, 1724, 1685, 1592, 1480, 1394, 1332, 1289, 1223, 1192, 1145, 1099, 1072, 1002, 928, 850, 757; δ_H (300 MHz, $CDCl_3$) 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_2Me); δ_C (75 MHz, $CDCl_3$) 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 [*M*(MeCN)Na^+, 100%], 383 (*M*Na^+, 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); δ_H (300 MHz, $CDCl_3$) 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_2Me); R_f 0.27 (hexane - ethyl acetate, 5:1).

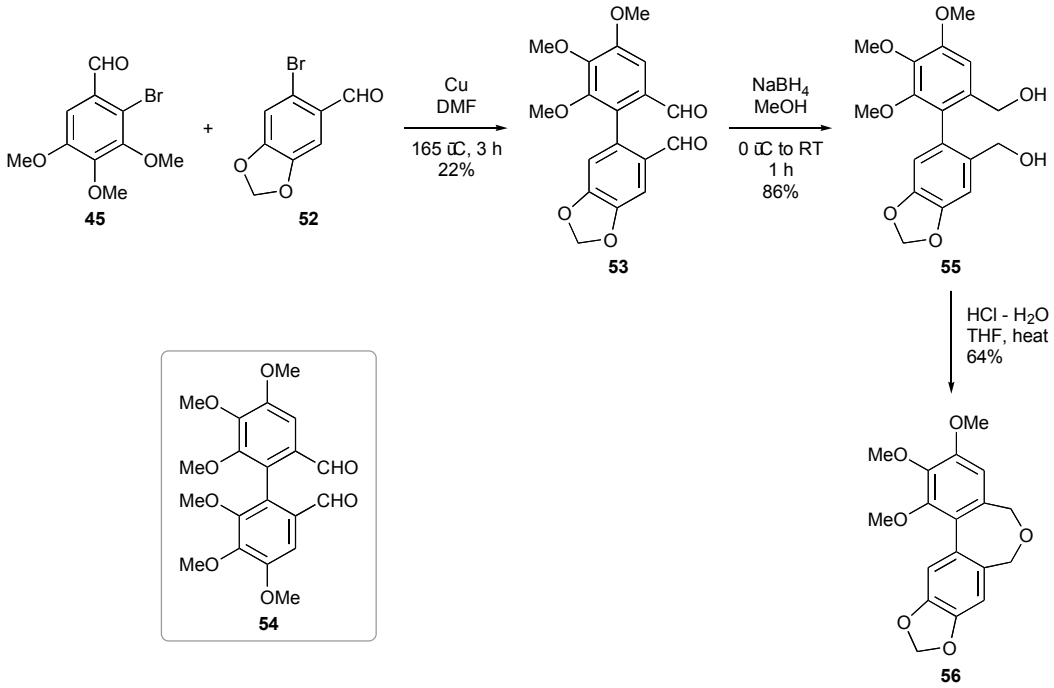
Methyl 6'-(hydroxymethyl)-2',3',4,4'-tetramethoxybiphenyl-2-carboxylate **49**
(4,4',5,6-Tetramethoxybiphenyl-2,2'-diyl)dimethanol **50**
To a solution of crude **47** (55% pure by ^1H NMR spectroscopy; 3.0 g, 4.6 mmol) in methanol (100 mL) was added sodium borohydride (0.61 g, 16.0 mmol) and the solution 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_2SO_4 and filtered. The solvent was removed *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 δ_H (300 MHz, $CDCl_3$) 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'-C*H*$_A$OH), 4.25 (1 H, dd, *J* 5.2, 11.7 Hz, 6'-C*H*$_B$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_2Me), 2.44 (1 H, br s, 6'-CH_2OH); 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 *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, 1402, 1386, 1332, 1289, 1250, 1200, 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, C*H*$_2$OH), 4.28 (2 H, s, C*H*$_2$OH), 3.93 (3 H, s, ArOMe), 3.91 (3 H, s, ArOMe) 3.88 (3 H, s, ArOMe), 3.55 (3 H, s, ArOMe), 3.05 (1 H, br s, CH_2O*H*), 2.59 (1 H, br s, CH_2O*H*); 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 (*M*Na^+, 75); R_f 0.28 (hexane - ethyl acetate, 1:2).

5,7-Dihydro-1,2,3,9-tetramethoxydibenz[*c*,*e*]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_2SO_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*H^+–CH_2O, 100); R_f 0.39 (hexane - ethyl acetate, 3:1).

Preparative details for compounds 53–56



Scheme 4

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**[62] (ratio 10:1 by ^{1}H 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%); ν_{max}/cm^{-1} 2940, 2850, 1685, 1612, 1588, 1476, 1386, 1332, 1250, 1137; δ_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 (3H, s, ArOMe); δ_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 (*M*Na$^+$, 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)]; ν_{max}/cm^{-1} 2939, 2843, 1685, 1588, 1480, 1460, 1386, 1317,

1196, 1161, 1134, 1103, 1002; δ_H (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); δ_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_2SO_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; $C_{18}H_{20}O_7Na$ requires 371.1102); ν_{max}/cm^{-1} 3254, 2944, 2889, 1603, 1480, 1410, 1328, 1235, 1146, 1111, 1033, 928; δ_H (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_2OH), 4.21 (2 H, m, CH_2OH), 3.93 (3 H, s, OMe), 3.91 (3 H, s, OMe), 3.62 (3 H, s, OMe); δ_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/z* (ES) 412 [*M*(MeCN)Na^+, 100%], 371 (*M*Na^+, 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_2SO_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%); ν_{max}/cm^{-1} 2967, 2932, 2866, 1600, 1484, 1460, 1414, 1324, 1239, 1146, 1107, 1045; δ_H (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 Hz, 10-H_2), 4.40 (2 H, d, *J* 11.2 Hz, 5-H_2), 4.04 (1 H, d, *J* 10.8 Hz, 7-H_A), 4.01 (1 H, d, *J* 10.8 Hz, 7-H_B), 3.96 (3 H, s, OMe), 3.91 (3 H, s, OMe), 3.71 (3 H, s, OMe); δ_C (75 MHz,

$CDCl_3$) 56.3 (CH_3), 61.2 (CH_3), 61.5 (CH_3), 67.6 (CH_2), 67.8 (CH_2), 101.6 (CH_2), 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$^+$–CH_2O, 100%); R_f 0.28 (hexane - ethyl acetate, 4:1).

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