Journal of Enzyme Inhibition and Medicinal Chemistry, 2010; 25(2): 180–194

informa healthcare

RESEARCH ARTICLE

Lead identification of conformationally restricted benzoxepin type combretastatin analogs: synthesis, antiproliferative activity, and tubulin effects

Irene Barrett[1], Miriam Carr[1], Niamh O'Boyle[1], Lisa M. Greene[2], Andrew J. S. Knox [3], David G. Lloyd[3], Daniela M. Zisterer[2], and Mary J. Meegan[1]

[1]School of Pharmacy and Pharmaceutical Sciences, Centre for Synthesis and Chemical Biology, Trinity College Dublin, Dublin, Ireland, [2]School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland, and [3]Molecular Design Group, School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland

Abstract

We have synthesized a series of polymethoxylated rigid analogs of combretastatin A-4 which contain a benzoxepin ring in place of the usual ethylene bridge present in the natural combretastatin products. The compounds display antiproliferative activity when evaluated against the MCF-7 and MDA human breast carcinoma cell lines. 5-(3-Hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydro-benzoxepine (**11g**) was found to be the most potent product when evaluated against the MCF-7 breast cancer cell line. A brief computational study of the structure–activity relationship for the synthesized compounds is presented. These 4,5-diarylbenzoxepins are identified as potentially useful scaffolds for the further development of antitumor agents which target tubulin.

Keywords: *Benzoxepin; combretastatin analogs; antiproliferative activity*

Introduction

Inhibition of microtubule function by tubulin-targeting compounds is a recognized approach to cancer chemotherapy[1,2] and there have been many known antimitotic agents derived from natural product agents[3,4]. Colchicine[5] and the vinca alkaloids[6], which bind to distinct binding sites of tubulin, result in the destabilization of microtubules and subsequent apoptosis of the cell, while combretastatin A-4 is a powerful antimitotic agent, due to inhibition of tubulin polymerization[7]. Paclitaxel binds to an alternative site on the tubulin and produces a stabilizing effect on microtubules and leads to an accumulation of cells in metaphase arrest[8,9]. The neovasculature present in solid tumors is a target for development of new agents which disrupt the microtubule complex by interacting with β-tubulin. Many synthetic, semisynthetic, and natural compounds have been investigated as tubulin inhibitors and vascular targeting agents[10], and a number are in clinical trials currently, such as combretastatin A-4 (CA-4) as the phosphate ester prodrug[11] and the combretastatin A-4 derivative AVE8062[12]. The *cis* configuration of the CA-4 is known to be essential for activity, together with the 3,4,5-trimethoxy groups on ring A (Figure 1). The *cis*-1,2-diarylethylene scaffold of CA-4 undergoes rapid *cis–trans* isomerization in heat, light, and protic media. Many *cis*-restricted analogs of CA-4 have been synthesized to improve the solubility, stability, and therapeutic index[4] of these drugs. A number of examples where the olefinic group is replaced by a conformationally restricted ring structure have demonstrated significant antiproliferative activity[13], including those based on coumarin (**1**)[14,15], 2(5H)-furanone (**2**)[16], imidazole[17], 1,3-oxazole (**3**)[17], furazan (**4**)[4] and furan (**5**)[18], diarylindole[17,19], and arylthioindole[20], illustrated in Figure 1. We have previously reported the application of the benzoxepin[21] and benzothiepin scaffolds[22] for the design of antiproliferative agents as estrogen receptor (ER) antagonists. We have now investigated the development of a benzoxepin type scaffold as a conformationally restricted analog for combretastatin CA-4. From

Address for Correspondence: Mary J. Meegan, School of Pharmacy and Pharmaceutical Sciences, Centre for Synthesis and Chemical Biology, Trinity College Dublin, Dublin 2, Ireland. Tel: +353-1-8962798. Fax: +353-1-8962793. E-mail: mmeegan@tcd.ie

(Received 24 October 2008; accepted 12 March 2009)

ISSN 1475-6366 print/ISSN 1475-6374 online © 2010 Informa UK Ltd
DOI: 10.3109/14756360903169659

http://www.informahealthcare.com/enz

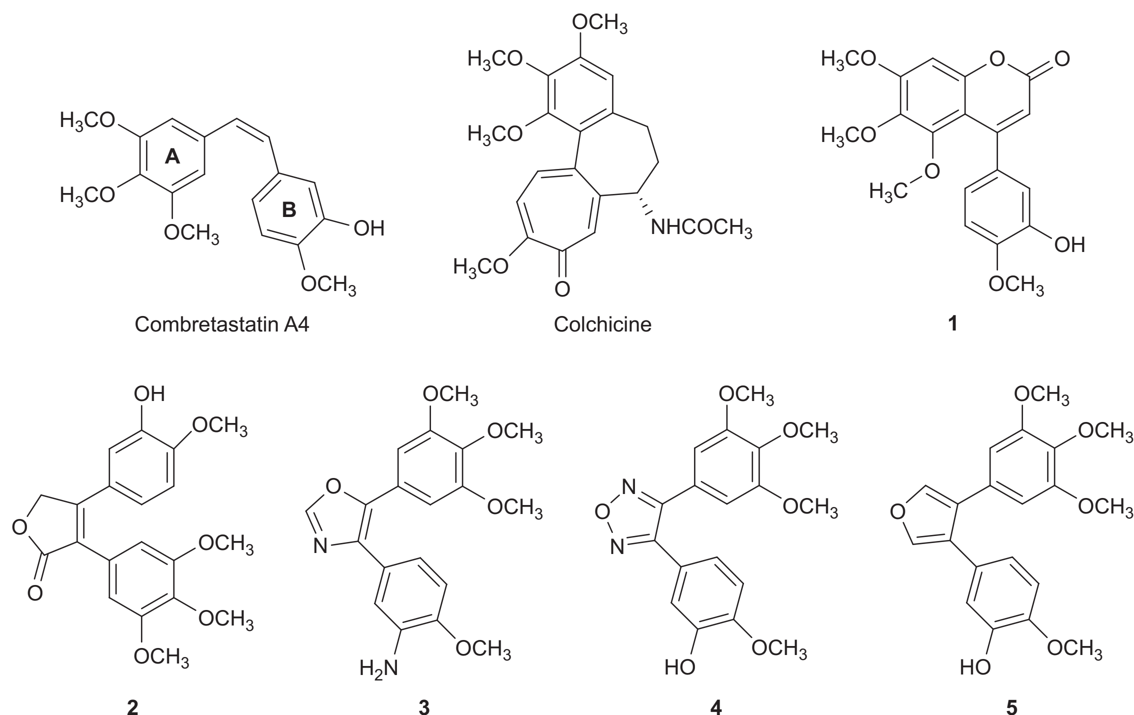


Figure 1. Combretastatin A-4, colchicines, and selected analogs of combretastatin A-4.

preliminary inspection, the aryl rings A and B of the target 5,6-diarylbenzoxepin structures can adopt a configuration in which they are not coplanar and now resemble the 3D orientation of the rings A and B of combretastatin CA-4. We therefore could expect activity at the colchicine binding site of tubulin for suitably substituted benzoxepins. This type of benzoxepin structure will also avoid the inactivation observed for the conventional combretastatin derivatives, which is caused by *cis–trans* isomerism of the olefinic bond observed *in vivo*[23].

We have investigated the development of the benzoxepin type scaffold as a conformationally restricted analog for combretastatin, where the seven-membered oxygen containing ring forms a slightly flexible bridge on the ethylene bond linking the combretastatin rings A and B. Fifteen compounds have been synthesized and evaluated for specific antiproliferative activity in two human breast cancer cell lines, MCF-7 (estrogen receptor positive) and MDA-MB 231(estrogen receptor negative). A study of the cell cycle effects is also presented together with a docking study of the compounds in the colchicine binding site of tubulin, which can be used to rationalize the possible binding mode and activity of these novel compounds.

Materials and methods

Chemistry

All reagents were commercially available and were used without further purification unless otherwise indicated. Anhydrous tetrahydrofuran (THF) was obtained by distillation from benzophenone-sodium under nitrogen immediately before use. All reactions were performed under a nitrogen atmosphere unless specifically noted. Infrared (IR) spectra were recorded as thin films on NaCl plates or as KBr disks on a PerkinElmer Paragon 100 Fourier transform (FT)-IR spectrometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance DPX 400 instrument at 20°C, 400.13 MHz for ^{1}H spectra, 100.61 MHz for ^{13}C spectra, in $CDCl_3$, CD_3COCD_3, or CD_3OD (internal standard tetramethylsilane). Low resolution mass spectra were run on a Hewlett-Packard 5973 MSD gas chromatography-mass spectrometry (GC-MS) system in an electron impact mode, while high resolution accurate mass determinations for all final target compounds were obtained on a Micromass time-of-flight (TOF) mass spectrometer equipped with electrospray ionization (ES) interface operated in the positive ion mode at the High Resolution Mass Spectrometry Laboratory by Dr. Martin Feeney in the Department of Chemistry, Trinity College Dublin. Thin layer chromatography (TLC) was performed using Merck silica gel 60 TLC aluminum sheets with fluorescent indicator, visualizing with ultraviolet (UV) light at 254 nm. Flash chromatography was carried out using standard silica gel 60 (230–400 mesh) obtained from Merck. All products isolated were homogeneous on TLC. All samples were analyzed using reversed phase high performance liquid chromatography (Waters Alliance system). The analysis was performed at 254 nm using a Phenomonex column (4 µm, 250 × 4.60 mm) using a mobile phase of acetonitrile:water (0.1% trifluoroacetic acid (TFA)) 70:30 delivered at a flow rate of 1.0 mL/min.

4-(3,4-Dimethoxy-phenoxy)-butyric acid ***6b*** A mixture of 3,4-dimethoxyphenol (50 mmol) and potassium carbonate (6.9 g, 55 mmol) in acetone (100 mL) was stirred for 30 min. Ethyl 4-bromobutyrate (7.9 mL, 55 mmol) was

added dropwise via syringe and catalytic KI was added. The reaction mixture was refluxed for 24 h. The reaction mixture was cooled to ambient temperature and the solids removed by filtration. The solid was washed with acetone (50 mL) and the combined filtrate and washings were concentrated under reduced pressure. The residue was taken up in diethyl ether (150 mL), washed with 5% sodium hydroxide (50 mL), and the solvent removed under reduced pressure. The residue was dissolved in ethanol (20 mL) and treated with 10% sodium hydroxide in water (100 mL) and heated at 110°C for 3 h or until the solution went clear. The solution was cooled and acidified with concentrated hydrochloric acid and the product which precipitated was filtered and dried, (Yield 5g, 41%). M.P. 89–91°C[24]. IR ν_{max} (film): 1682 (C=O), 1601, 1450 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 6.80 (1 H, d,*J* = 8.52 Hz, Ar-H), 6.54 (1 H, d, *J* = 2.52 Hz, Ar-H), 6.41 (1 H, dd, *J* = 2.76 Hz, 6.04 Hz, Ar-H), 3.99 (2 H, t, *J* = 6.02 Hz, CH_2), 3.88 (3 H, s, OCH_3), 3.85 (3 H, s, OCH_3), 2.62 (2 H, t, *J* = 7.28 Hz, CH_2), 2.13 (2 H, t, *J* = 6.78 Hz, CH_2) ppm; ^{13}C (101 MHz, $CDCl_3$): δ 178.4 (C), 152.9 (C), 149.4 (C), 143.1 (C), 111.3 (CH), 103.2 (CH), 100.3 (CH), 66.5 (CH_2), 55.9 (OCH_3), 55.4 (OCH_3), 30.0 (CH_2), 23.9 (CH_2) ppm.

4-(3,5-Dimethoxy-phenoxy)-butyric acid ***6c*** Preparation as above from 3,5-dimethoxyphenol to afford a brown solid, (3.8 g, 31%). M.P. 62°C. IR ν_{max} (film) cm^{-1}: 1682 (C=O), 1601, 1450; ^{1}H (400 MHz, $CDCl_3$): δ 6.08 (3 H, t, *J* = 2.00 Hz, Ar-H), 3.98 (2 H, t, *J* = 6.02 Hz, OCH_2CH_2), 3.76 (6 H, s, OCH_3), 2.58 (2 H, t, *J* = 7.28 Hz, OCH_2CH_2), 2.09 (2 H, qn, *J* = 6.54 Hz, 7.00 Hz, $OCH_2CH_2CH_2$) ppm; ^{13}C (101 MHz, $CDCl_3$): δ 178.5 (C), 161.0 (C), 160.2 (C), 92.9 (CH), 92.6 (CH), 66.1 (CH_2), 54.9 (OCH_3), 30.0 (CH_2), 23.8 (CH_2) ppm; Found 263.0888; $C_{12}H_{16}O_5Na$ requires 263.0895.

3,4-Dihydro-2H-1-benzoxepin-5-one ***7a*** 4-Phenoxybutyric acid (**6a**) (5 g) and polyphosphoric acid (51 g) were heated together at 80°C for 2 h. The brown syrup was poured into ice-water and the aqueous solution extracted with dichloromethane. The organic layers were washed with water (100 mL), brine (100 mL), and then dried over sodium sulfate and the solvent removed under reduced pressure. The crude product was purified by chromatography (silica, 5% diethyl ether in hexane) to give the product as a yellow oil (1.37 g, 37%)[25]. IR ν_{max} (film) cm^{-1}: 1682 (C=O), 1601, 1450; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (1 H, dd, *J* = 2.0 Hz, 8.1 Hz, Ar-H), 7.44-7.39 (1 H, td, *J* = 1.3 Hz, 7.0 Hz, Ar-H), 7.11–7.06 (2 H, m, Ar-H), 4.23 (2 H, t, *J* = 7.0 Hz, OCH_2), 2.89 (2 H, t, *J* = 7.0 Hz, (O=C-CH_2), 2.21 (2 H, m, C-CH_2-C); ^{13}C NMR (101 MHz, $CDCl_3$): δ 200.6 (C), 161.9 (C), 133.6 (CH), 129.4 (CH), 128.9 (C), 122.6 (CH), 120.7 (CH), 72.6 (CH_2), 40.5 (CH_2), 26.3 (CH_2) ppm; HRMS: Found 162.1850; $C_{10}H_{10}O_2$ requires 162.1900.

6,7-Dimethoxy-3,4-dihydro-2H-benzo[b]oxepin-5-one ***7b*** Preparation as above from 4-(3,4-dimethoxy-phenoxy)-butyric acid **6b** (5 g) and polyphosphoric acid (51 g) or Eaton's reagent (20 g). The crude product was purified by chromatography (silica, 5% diethyl ether in hexane) to afford the product as a brown solid, (1.36g, 29%). M.P. 77°C[24]. IR ν_{max} (KBr): 2966.2, 1659.1 (C=O), 1604.3, 1502.4 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.27 (1 H, s, Ar-H), 6.56 (1 H, s, Ar-H), 4.19 (2 H, t, *J* = 7.02 Hz, CH_2), 3.89 (3 H, s, OCH_3), 3.87 (3 H, s, OCH_3), 2.88 (2 H, t, *J* = 6.78 Hz, CH_2), 2.15 (2 H, qn, *J* = 6.76 Hz, 7.00 Hz, $OCH_2CH_2CH_2$) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.8 (C), 157.6 (C), 153.3 (C), 144.6 (C), 120.4 (C), 109.5 (C), 103.5 (CH), 72.6 (CH_2), 55.7 (OCH_3), 40.0 (CH_2), 25.5 (CH_2) ppm; HRMS: Found 245.0798; $C_{12}H_{14}O_4Na$ requires 245.0790.

6,8-Dimethoxy-3,4-dihydro-2H-benzo[b]oxepin-5-one ***7c*** Preparation as above from 4-(3,5-dimethoxy-phenoxy)-butyric acid 6c (2 g, 8.32 mmol) and polyphosphoric acid (20 g) or Eaton's reagent (20 g). The product was purified by flash column chromatography (SiO_2, 5% diethyl ether/hexane) to afford the product as a yellow gel, (1.25 g, 67.6%). IR ν_{max} (film):1573.58, 1604, 1682 (C=O), 2941 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 6.19 (2 H, s, Ar-H), 4.16 (2 H, t, *J* = 6.26 Hz, CH_2), 3.82 (3 H, s, OMe), 3.81 (3H, s, OMe), 2.78 (2H, t, *J* = 6.76 Hz, CH_2), 2.14 (2H, qn, *J* = 6.65 Hz, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.9 (C), 163.1 (C), 163.0 (C), 115.8 (C), 113.6 (C), 97.0 (CH), 94.1 (CH), 71.9 (CH_2), 56.0 (OCH_3), 53.0 (OCH_3), 41.5 (CH_2), 26.2 (CH_2) ppm; HRMS: Found 245.0793, $C_{12}H_{14}O_4Na$ requires 245.0790.

5-(4-Methoxyphenyl)-2,3-dihydro-1-benzoxepin ***9a*** To a mixture of 3,4-dihydro-2*H*-1-benzoxepin-5-one **7a** (0.40 g, 2.50 mmol) and sodium carbonate (0.79 g, 7.2 mmol) in dichloromethane (30 mL) at 0°C under nitrogen was added trifluoromethanesulfonic anhydride (1.20 mL, 7.2 mmol). The mixture was stirred overnight at room temperature and water (30 mL) was added. The aqueous layer was extracted with dichloromethane (2 × 50 mL), washed with brine (30 mL), dried over sodium sulfate, and the solvent was removed under reduced pressure. The triflate (**8a**) was dissolved in THF (30 mL), and 4-methoxyphenylboronic acid (493 mg, 2.86 mmol) and 2 M Na_2CO_3 (4.8 mL) were added and the mixture stirred under nitrogen for 10 min. Tetrakis(triphenylphosphine)palladium(0) $Pd(PPh_3)_4$ (149 mg) was added and the reaction refluxed for 6 h at 85°C. The solution was cooled to room temperature and acidified with 2 M hydrochloric acid. The aqueous layer was extracted with dichloromethane (3 × 30 mL) and the combined organic layers were washed with water (30 mL), brine (30 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a yellow solid, (198 mg, 31%). M.P. 71°C[21]. IR ν_{max} (KBr):1657.7, 1602.3, 1510.3, 1510.3, 1484.6, 1247.8, 1177.5 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.25 (3H, m, Ar-H), 7.13 (1H, d, *J* = 2.0 Hz, Ar-H), 7.03 (2H, m, Ar-H), 6.91 (2H, d, *J* = 8.80 Hz, Ar-H), 6.29 (1H, t, *J* = 6.36 Hz, CH), 4.53 (2 H, t, *J* = 6.36 Hz, CH_2), 3.85 (3 H, s, OCH_3), 2.51 (2 H, t, *J* = 6.20 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.4 (C), 157.4 (C), 140.5 (C), 134.8 (C), 132.60 (C), 130.7 (CH), 129.2 (CH), 129.1 (CH), 128.9 (CH), 125.4 (CH), 122.7 (CH), 114.1 (CH), 114.0 (CH), 113.1 (CH), 77.5 (CH_2), 54.8 (CH_3), 29.4 (CH_2) ppm; HRMS: Found 253.1241; $C_{17}H_{17}O_2$ requires 253.1229.

5-(4-Hydroxy-phenyl)-2,3-dihydro-1-benzoxepin ***9b*** A mixture of 3,4-dihydro-2*H*-benzoxepin-5-one **7a** (0.33 g, 2.04 mmol) and phosphorus tribromide (1.00 mL, 10.4

mmol) was heated at 90°C for 24 h. The reaction was cooled to room temperature, added slowly dropwise to ice-water (20 mL), and the aqueous layer extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water (20 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The product was immediately purified by chromatography (silica, 5% diethyl ether in hexane) to give a yellow oil, 5-bromo-2,3-dihydro-benzo[*b*]oxepin (0.26 g, 56%), which was used immediately in the following reaction. $Pd(PPh_3)_4$ (40 mg) was added to a solution, 4-hydroxyphenylboronic acid (0.76 g, 5.5 mmol) and 2 M Na_2CO_3 (12.8 mL, 25 mmol) were added to this product, and the solution heated to 80°C for 24 h. The solution was cooled, water was added (20 mL), and the aqueous layer extracted with dichloromethane (4 × 50 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (silica, 5% diethyl ether in hexane) to give 4-(2,3-dihydro-benzo[*b*]oxepin-5-yl)-phenol as a white solid, (0.62 g, 52%), which was used in subsequent reactions without further purification. M.P. 110°C. IR ν_{max} (KBr): 3384 (OH), 1606, 1513, 1485, 1263, 1201, 758 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.23 (1 H, m, Ar-H), 7.14 (3 H, m, Ar-H), 7.00 (2 H, m, Ar-H), 6.77 (2 H, d, *J* = 6.0Hz, Ar-H), 6.27 (1 H, t, *J* = 6.0 Hz, C*H*), 2.51 (2 H, t, *J* = 6.0 Hz, OCH_2), 2.46 (2 H, m, CH_2);^{13}C NMR (101 MHz, $CDCl_3$): δ 157.6 (C), 154.8 (C), 140.9 (C), 135.3 (C), 133.0 (CH), 131.0 (CH), 129.8 (CH), 128.4 (CH), 126.9 (CH), 123.3 (CH), 121.9 (CH), 114.9 (CH), 78.0 (CH_2), 29.7 (CH_2) ppm; HRMS: Found: 261.0902; $C_{16}H_{14}O_2Na$ requires 261.0891.

5-(3,4-Dimethoxyphenyl)-2,3-dihydro-1-benzoxepin ***9c*** Preparation as described above for **9a** from 3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one **7a** (1.36 g, 8.88 mmol) and 3,4-dimethoxyphenylboronic acid (1.29 g, 8.88 mmol) via the triflate **8c**. The crude product was purified by column chromatography (silica, 5% diethyl ether/hexane) to afford the product as a yellow oil which was used in subsequent reactions without further purification, (1.12 g, 45%). IR ν_{max} (KBr): 2935, 1606, 1508, 1246 (C=C) cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.23–7.21 (1 H, m, Ar-H), 7.13 (1 H, d, *J* = 7.52 Hz, Ar-H), 7.04–7.02 (2 H, m, Ar-H), 6.86 (1 H, d, 1.52 Hz, Ar-H), 6.85 (1 H, s, Ar-H), 6.82 (1 H, d, *J* = 2.04 Hz, Ar-H), 6.29 (1H, t, *J* = 6.28 Hz, CH), 4.50 (2 H, t, *J* = 6.02 Hz, OCH_2), 3.90 (3 H, s, OCH_3), 3.83 (3 H, s, OCH_3), 2.47 (2 H, q, *J* = 6.03 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.4 (C), 148.1 (C), 147.9 (C), 140.7 (C), 135.2 (C), 132.4 (C), 130.7 (CH), 128.0 (CH), 126.8 (CH), 122.7 (CH), 121.4 (CH), 120.5 (CH), 111.4 (CH), 110.3 (CH), 77.3 (CH_2), 55.5 (OCH_3), 55.4 (OCH_3), 29.4 (CH_2) ppm.

5-(3-Fluoro-4-methoxyphenyl)-2,3-dihydro-1-benzoxepin ***9d*** Preparation as described above for **9a** from 3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one **7a** (1.36 g, 8.88 mmol) and 3-fluoro-4-methoxyphenylboronic acid (0.76 g, 3.82 mmol) via the triflate **8a**. The crude product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a white solid, (0.60 g, 58%). M.P. 88°C. IR ν_{max} (KBr): 2932.9, 1596.7, 1574.9, 1483.3, 1209.4 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.27–7.24 (1 H, m, Ar-H), 7.14 (1 H, dd, *J* = 1.00 Hz, 7.04 Hz, Ar-H), 7.09 (1 H, d, *J* = 3.48 Hz, Ar-H), 7.04–7.01 (3 H, m, Ar-H), 6.94 (1 H, t, *J* = 8.54 Hz, Ar-H), 6.31 (1H, t, *J* = 6.02 Hz, CH), 4.52 (2 H, t, *J* = 6.02 Hz, OCH_2), 3.93 (3 H, s, OCH_3), 2.50 (2 H, q, *J* = 6.19 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.4 (C), 152.8 (C), 150.4 (C), 146.5 (C), 146.4 (C), 139.7 (C), 135.4 (C), 132.0 (CH), 130.5 (C), 129.1 (CH), 128.9 (CH), 128.2 (CH), 127.4 (CH), 123.8 (CH), 122.9 (CH), 121.1 (CH), 115.9 (CH), 115.7 (CH), 114.1 (CH), 112.5 (CH), 112.4 (CH), 77.4 (CH_2), 58.8 (OCH_3), 29.3 (CH_2) ppm; HRMS: Found 271.1169; $C_{17}H_{16}O_2F$ requires 271.1134.

5-(3-Formyl-4-methoxyphenyl)-2,3-dihydro-1-benzoxepin ***9e*** Preparation as described above for **9a** from 3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one **7a** (1.36 g, 8.88 mmol) and 3-formyl-4-methoxyphenylboronic acid (0.61 g, 3.37 mmol) via the triflate **8a**. The product was purified by column chromatography (silica, 2.5% methanol/dichloromethane) to give the product as a yellow oil, (0.57 g, 26%). IR ν_{max} (film): 1759 (C=O), 3384 (OH), 1603, 1494, 1269, 1180 cm^{-1}. ^{1}H (400 MHz, $CDCl_3$): δ 10.5 (1 H, s, CHO), 7.81 (1 H, d, *J* = 2.52 Hz, Ar-H), 7.42 (1 H, dd, *J* = 2.52 Hz, 6.00 Hz, Ar-H), 7.25 (1 H, d, *J* = 7.56 Hz, Ar-H), 7.09 (1 H, d, *J* = 3.00 Hz, Ar-H), 6.89–7.01 (3H, m, Ar-H), 6.29 (1H, t, *J* = 6.04 Hz, CH), 4.47 (2 H, t, *J* = 6.02 Hz, OCH_2), 3.93 (3 H, s, OCH_3), 2.47 (2 H, q, *J* = 6.01 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 189.7 (CHO), 161.1 (C), 157.9 (C), 139.8 (CH), 136.1 (C), 135.3 (C), 132.2 (C), 130.7 (CH), 129.4 (C), 129.3 (C), 128.8 (CH), 128.6 (CH), 128.3 (CH), 126.6 (C), 124.4 (CH), 123.3 (CH), 121.9 (CH), 121.5 (CH), 114.4 (CH), 111.4 (CH), 76.7 (CH_2), 55.7 (CH_3), 29.9 (CH_2) ppm; HRMS: Found 281.1185; $C_{18}H_{17}O_3$ requires 281.1178.

5-(3,4,5-Trimethoxyphenyl)-2,3-dihydro-1-benzoxepin ***9f*** Preparation as described above for **9a** from 3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one **7a** (1.36 g, 8.88 mmol) and 3,4,5-trimethoxyphenylboronic acid (0.93 g, 4.38 mmol) via the triflate **8a**. The crude product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a yellow oil, (0.311 g, 27%). IR ν_{max} (film): 2936.9, 1579.9, 1124.3, 1007.4 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.25–7.21 (1 H, m, Ar-H), 7.12 (1 H, d, *J* = 8.00 Hz, Ar-H), 7.04 (2 H, d, *J* = 4.00 Hz, Ar-H), 6.52 (2 H, s, Ar-H), 4.50 (2 H, t, *J* = 6.04 Hz, OCH_2), 3.89 (3 H, s, OCH_3), 3.83 (3 H, s, OCH_3), 2.53 (2 H, q, *J* = 6.19 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.5 (C), 152.4 (C), 140.9 (C), 138.2 (C), 136.8 (C), 131.9 (CH), 130.8 (C), 128.1 (CH), 127.5 (CH), 122.7 (CH), 121.4 (CH), 105.4 (CH), 76.9 (CH_2), 60.5 (OCH_3), 55.6 (OCH_3), 29.6 (CH_2) ppm; HRMS: Found 335.1272; $C_{19}H_{20}O_4Na$ requires 335.1259.

6,8-Dimethoxy-5-(4-methoxyphenyl)-2,3-dihydro-benzoxepin ***9g*** Preparation as described above for **9a** from 6,7-dimethoxy-3,4-dihydro-2*H*-benzo[*b*]oxepin-5-one **7b** (1.36 g, 8.88 mmol) and 4-methoxyphenylboronic acid (0.532 g, 3.5mmol) via the triflate **8b**. The crude product was purified by column chromatography using silica and 5% diethylether in hexane to afford the product as an oil which was used in subsequent reactions without further purification, (0.232 g, 27.5%). IR ν_{max} (film): 2967, 1604, 1573, 1251 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.16 (2 H, d, *J* = 9.0 Hz,

Ar-H), 6.79 (2 H, d, J = 8.5 Hz, Ar-H), 6.39 (2 H, m, Ar-H), 6.29 (1 H, d, J = 2.5 Hz, Ar-H), 4.48 (2 H, t, J = 6.0 Hz, CH_2), 3.82 (3 H, s, OCH_3), 3.79 (3 H, s, OCH_3), 3.46 (3 H, s, OCH_3), 2.31 (2 H, t, J = 6.5 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.7 (C), 158.4 (C), 158.0 (C), 139.3 (C), 134.4 (C), 126.9 (CH), 124.9 (CH), 115.0 (C), 113.1 (CH), 99.4 (CH), 95.8 (CH), 80.4 (CH_2), 55.6 (OCH_3), 55.3 (OCH_3), 55.2 (OCH_3), 27.3 (CH_2) ppm.

7,8-Dimethoxy-5-(4-methoxy-phenyl)-2,3-dihydro-benzo[b]oxepin ***9h*** Preparation as described above for **9a** from 6,8-dimethoxy-3,4-dihydro-2H-benzo[*b*]oxepin-5-one **7c** (0.5 g, 2.25 mmol) and 4-methoxyphenylboronic acid (0.445 g, 2.93 mmol) via the triflate **8b**. The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a white solid, (0.393g, 55.9%). M.P. 81–90°C. IR ν_{max} (KBr): 2967, 1604, 1573, 1251 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.23 (2 H, m, Ar-H), 6.86 (2 H, m, Ar-H), 6.69 (1 H, s, Ar-H), 6.48 (1 H, s, Ar-H), 6.21 (1 H, t, OCH_2CH_2CH), 4.48 (2 H, t, CH_2), 3.88 (3 H, s, OCH_3), 3.81 (3 H, s, OCH_3), 3.65 (3 H, s, 3 × OCH_3), 2.44 (2H, t, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.8 (C), 151.8 (C), 148.7 (C), 144.5 (C), 140.8 (C), 134.8 (C), 129.4 (CH), 125.7 (CH), 124.0 (CH), 113.4 (CH), 105.4 (CH), 78.5(CH_2), 56.0 (OCH_3), 55.8 (OCH_3), 55.1 (OCH_3), 29.6 (CH_2) ppm; HRMS: Found 335.1260; $C_{19}H_{20}O_4Na$ requires 335.1259.

4-Bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin ***10a*** To a solution of 5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin **9a** (1.62 g, 5.74 mmol) in dichloromethane (30 mL) at 0°C was added pyridinium tribromide (1.08 g, 5.74 mmol, technical grade 90%) and stirred for 1 h with careful monitoring by TLC. Water (30 mL) was added and the solution extracted with dichloromethane (2 × 30 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate (30 mL), brine (30 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a brown solid which was used in the following reaction without further purification, (1.64 g, 86%). M.P. 69°C[21]. IR ν_{max} (KBr): 1604.7, 1509.3, 1248.5, 1172.9, 1036.9, 753.9 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.21 (2H, m, Ar-H), 7.13 (1H, dd J = 0.98 Hz, 6.84 Hz, Ar-H), 6.95 (1H, m, Ar-H), 6.91 (2H, d, J = 8.80 Hz, Ar-H), 6.82 (1H, dd, J = 1.46 Hz, 6.36 Hz, Ar-H), 4.62 (2 H, t, J = 6.86 Hz, CH_2), 3.86 (3 H, s, OCH_3), 3.04 (2 H, t, J = 5.86 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.4 (C), 156.0 (C), 139.0 (C), 134.3 (C), 133.2 (C), 130.9 (CH), 130.8 (CH), 129.1 (CH), 129.0 (CH), 128.5 (CH), 123.0 (CH), 121.6 (C), 121.4 (CH), 115.3 (CH), 114.1 (CH), 113.0 (CH), 77.1 (CH_2), 54.7 (CH_3), 40.7 (CH_2) ppm.

4-Bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin ***10b*** Preparation as described above for **10a** from 4-(2,3-dihydro-benzoxepin-5-yl)phenol **9b** (0.67 g, 2.8 mmol). The impure product was purified by column chromatography (silica, dichloromethane:hexane 1:1) to give the product as a white solid, (0.59 g, 67%)[21]. M.P. 58°C. IR ν_{max} (KBr): 3384 (OH), 1606, 1513, 1485, 1263, 1201, 758cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.20 (1 H, t, J = 6.0 Hz, m, Ar-H), 7.14 (2 H, d, J = 8.5 Hz, Ar-H), 7.07 (1 H, dd, J = 8.0 Hz, 1.0 Hz, Ar-H), 6.95 (1 H, t, J = 6.5Hz, Ar-H), 6.85–6.77 (3 H, m, Ar-H), 4.59 (2 H, t, J = 6.0 Hz, OCH_2), 3.00 (2 H, t, J = 6.0 Hz, CH_2);^{13}C NMR (101 MHz, $CDCl_3$): δ 156.4 (C), 154.8 (C), 131.4 (CH), 128.9 (CH), 123.4 (CH), 122.0 (CH), 114.9 (CH), 77.4 (CH_2), 41.1 (CH_2) ppm; HRMS: Found 317.1842; $C_{16}H_{14}BrO_2$ requires 317.1848.

4-Bromo-5-(3,4-dimethoxyphenyl)-2,3-dihydro-1-benzoxepin ***10c*** Preparation as described above for **10a** from 5-(3,4-dimethoxyphenyl)-2,3-dihydro-1-benzoxepin **9c** (1.62 g, 5.74 mmol). The crude residue was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product, (1.64 g, 79%) as a white solid. M.P. 88°C. IR ν_{max} (KBr): 2934.1, 1582.4, 1512.4, 1440.1, 1247.5, 1223.7 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.18 (1 H, m, Ar-H), 7.06 (1 H, m, Ar-H), 6.92 (1 H, m, Ar-H), 6.80 (1 H, dd, J = 8.00 Hz, 1.50 Hz, Ar-H), 4.55 (2 H, t, J = 5.80 Hz, CH_2), 3.87 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 2.99 (2 H, t, J = 6.00 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.0 (C), 148.0 (C), 147.8 (C), 139.1 (C), 134.5 (C), 132.9 (C), 130.9 (CH), 128.5 (CH), 123.0 (CH), 122.0 (CH), 121.6 (CH), 121.5 (CH), 112.7 (CH), 110.1 (CH), 76.8 (CH_2), 55.5 (OCH_3), 55.4 (OCH_3), 40.8 (CH_2) ppm; HRMS: Found 359.0300; $C_{18}H_{17}BrO_3$ requires 359.0283.

4-Bromo-5-(3-fluoro-4-methoxyphenyl)-2,3-dihydro-1-benzoxepin ***10d*** Preparation as described above for **10a** from 5-(3-fluoro-4-methoxyphenyl)-2,3-dihydro-1-benzoxepin 9d (0.60 g, 2.22 mmol). The crude residue was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a brown solid which was used in subsequent reactions without further purification, (0.72 g, 93%). M.P. 47°C. IR ν_{max} (KBr): 2924.9, 1511.2, 1483.9, 1267.4, 1126.8 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.28–7.22 (1 H, m, Ar-H), 7.09 (1 H, d, J = 7.36 Hz, Ar-H), 7.02 (1 H, d, J = 11.24 Hz, Ar-H), 6.99–6.96 (3 H, m, Ar-H), 6.81 (1 H, dd, J = 1.48 Hz, 6.32 Hz, Ar-H), 4.60 (2 H, t, J = 5.86 Hz, CH_2), 3.94 (3 H, s, OCH_3), 3.03 (2 H, t, J = 5.86 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.2 (C), 152.8 (C), 150.3 (C), 147.8 (C), 146.7 (C), 138.2 (C), 134.9 (C), 134.8 (C), 132.7 (C), 131.0 (CH), 128.8 (CH), 125.8 (CH), 123.3 (CH), 122.3 (C), 121.9 (CH), 117.7 (CH), 117.5 (CH), 112.5 (CH), 76.5 (CH_2), 55.9 (CH_3), 40.9 (CH_2) ppm; ^{19}F NMR (376 MHz, $CDCl_3$, Me_4Si): δ–135.94 ppm; HRMS: Found: 371.0060; $C_{17}H_{14}O_2FBrNa$ requires 371.0059.

4-Bromo-5-(3-formyl-4-methoxyphenyl)-2,3-dihydro-1-benzoxepin ***10e*** Preparation as described above for **10a** from 5-(3-formyl-4-methoxyphenyl)-2,3-dihydro-1-benzoxepin **9e** (217 mg, 0.77 mmol). The crude residue was purified by chromatography (silica, 5% diethyl ether/hexane) to give the product as a white solid which was used in subsequent reactions without further purification, (184 mg, 60%). M.P. 145°C. IR ν_{max} (KBr): 2917.5, 1677.2, 1599.8, 1484.9, 1257.0 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 10.50 (1 H, s, CHO), 7.76 (1 H, d, J = 2.48 Hz, Ar-H), 7.43 (1 H, dd, J = 2.52 Hz, 6.00 Hz, Ar-H), 7.23 (1 H, dt, J = 1.52 Hz, 6.76 Hz, Ar-H), 7.10 (1 H, d, J = 8.04 Hz, Ar-H), 7.03 (1 H, d, J = 8.56 Hz, Ar-H), 6.95 (1 H, dt, J = 1.00 Hz, 7.52 Hz, Ar-H), 6.74 (1H, dd, J = 1.52 Hz, 6.52 Hz, Ar-H), 4.58 (2 H, t, J = 6.20 Hz, OCH_2), 3.96 (3 H, s, OCH_3), 3.01 (2 H, t, J = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 189.5 (CHO), 160.9 (C), 156.5 (C), 138.3

(C), 137.4 (CH), 134.8 (CH), 132.8 (CH), 131.1 (CH), 130.2 (C), 129.4 (CH), 129.3 (CH), 129.0 (CH), 126.6 (C), 124.3 (CH), 123.5 (CH), 123.3 (CH), 122.8 (CH), 122.1 (CH), 121.5 (CH), 116.2 (CH), 111.4 (CH), 77.2 (CH_2), 55.7 (CH_3), 53.1 (CH_3), 41.1 (CH_2) ppm; HRMS: Found: 359.0292; $C_{18}H_{16}BrO_3$ requires 359.0283.

*4-Bromo-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin **10f*** Preparation as described above for **10a** from 5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin **9f** (0.95 g, 3.02mmol). The crude residue was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a white solid, (0.80 g, 67%). M.P. 111°C. IR ν_{max} (KBr): 2929.6, 1581.9, 1482.6, 1459.3, 1410.0, 1235.3, 1130.2 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.23 (1 H, dt, J = 1.32 Hz, 6.54 Hz, Ar-H), 7.09 (1 H, dd, J = 1.00 Hz, 7.00 Hz, Ar-H), 6.98 (1 H, dt, J = 1.35 Hz, 6.26 Hz, Ar-H), 6.87 (1 H, dd, J = 1.50 Hz, 6.52 Hz, Ar-H), 6.47 (2 H, s, Ar-H), 4.61 (2 H, t, J = 5.78 Hz, OCH_2), 3.92 (3 H, s, OCH_3), 3.83 (6 H, s, OCH_3), 3.05 (2 H, t, J = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.0 (C), 152.4 (C), 139.3 (C), 137.4 (C), 136.8 (C), 132.4 (CH), 130.9 (C), 128.5 (CH), 123.1 (CH), 121.9 (CH), 121.5 (C), 106.6 (CH), 77.1 (CH_2), 60.5 (OCH_3), 55.7 (OCH_3), 40.9 (CH_2) ppm; HRMS: Found 413.0381; $C_{19}H_{19}O_4NaBr$ requires 413.0364.

*6,8-Dimethoxy-4-bromo-5-(4-methoxyphenyl)-2,3-dihydrobenzoxepin **10g*** Preparation as described above for **10a** from 6,8-dimethoxy-5-(4-methoxyphenyl)-2,3-dihydrobenzoxepin **9g** (0.232 g, 0.74 mmol). The crude product was then purified by column chromatography over silica gel (eluent: hexane 95%, diethylether 5%). The product was isolated as a brown solid which was used in subsequent reactions without further purification, (0.083 g, 28.5%). M.P. 96–102°C. IR ν_{max} (KBr): 2927.1, 1610.3, 1573.7, 1241.8 (C=C), 736.58 (C-Br) cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.20 (2 H, d, J = 8.52 Hz, Ar-H), 6.84 (2 H, d, J = 8.52 Hz, Ar-H), 6.33 (1 H, d, J = 2.52 Hz, Ar-H), 6.22 (1 H, d, J = 2.52 Hz, Ar-H), 4.59 (2 H, t, J = 6.16 Hz, CH_2), 3.80 (3 H, s, OCH_3), 3.79 (3 H, s, OCH_3), 3.35 (3 H, s, OCH_3), 2.86 (2 H, t, J = 5.78 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.1 (C), 158.3 (C), 158.1 (C), 156.7 (C), 136.9 (C), 134.0 (C), 129.9 (CH), 119.1 (C), 116.6 (C), 112.5 (C), 99.3 (CH), 96.2 (CH), 78.8 (CH_2), 77.3 (C), 77.0 (C), 76.7 (C), 55.7 (OCH_3), 55.3 (OCH_3), 55.1 (OCH_3), 39.2 (CH_2) ppm; HRMS: Found 413.0376; $C_{19}H_{19}O_4NaBr$ requires 413.0364.

*4-Bromo-7,8-dimethoxy-5-(4-methoxy-phenyl)-2,3-dihydro-benzo[b]oxepin **10h*** Preparation as described for **10a** from 7,8-dimethoxy-5-(4-methoxy-phenyl)-2,3-dihydro-benzo[*b*]oxepin **9h** (262 mg, 0.96 mmol). The residue was purified by flash column chromatography (silica, 5% diethyl ether/hexane) to give the product as off-white crystals, (284 mg, 75.6 %). M.P. 79°C. IR ν_{max} (KBr): 2916.7, 1607,9, 1510.7, 1465.5, 1208.9, 1026.6, 854.0 cm^{-1}; ^1H (400 MHz, $CDCl_3$, Me_4Si): δ 7.22 (2 H, d, J = 9.04 Hz, Ar-H), 6.93 (2 H, d, J = 8.52 Hz, Ar-H), 6.65 (1 H, s, Ar-H), 6.24 (1 H, s, Ar-H), 4.59 (2 H, t, J = 5.76 Hz, CH_2), 3.88 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 3.59 (3 H, s, OCH_3), 3.03 (2H, t, J = 5.78 Hz, CH_2) ppm; ^{13}C (101 MHz, $CDCl_3$, Me_4Si): δ 158.3 (C), 150.3 (C), 148.8 (C), 144.3 (C), 138.7 (C), 133.9 (C), 130.8 (CH), 124.2 (C), 119.9 (C), 113.1 (CH), 104.9 (CH), 78.1 (CH_2), 55.7 (OCH_3), 55.5 (OCH_3), 54.8 (OCH_3), 40.7 (CH_2) ppm; HRMS: Found 413.0344; $C_{19}H_{19}O_4NaBr$ requires 413.0364.

*5-(4-Methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin **11a*** To a solution of 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin **10a** (208 mg, 0.63 mmol) in THF (20 mL) under nitrogen was added tetrakis(triphenylphosphine)palladium(0), $Pd(PPh_3)_4$ (96 mg) and the reaction stirred for 10 min. 3,4,5-Trimethoxyphenylboronic acid (200 mg, 0.94 mmol) and 2 M sodium carbonate (1.55 mL, 3.14 mmol) were added and the solution heated to 80°C and refluxed overnight. The solution was cooled and 2 M hydrochloric acid was added. The solution was extracted with dichloromethane (3 × 50 mL) and the combined organic layers were washed with water (50 mL), brine (50 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by column chromatography (silica, 10% diethyl ether/hexane) to give the product as a white solid, (193mg, 73%). IR ν_{max} (KBr): 1685 (C=O) cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.27 (1H, m, Ar-H), 7.16 (1H, dd J = 1.02 Hz, 7.00 Hz, Ar-H), 7.05 (1H, dt, J = 1.49 Hz, 6.04 Hz, Ar-H), 6.91 (3H, d, J = 5.00 Hz, Ar-H), 6.70 (2H, d, J = 9.04 Hz, Ar-H), 6.39 (2H, s, Ar-H), 4.66 (2 H, t, J = 6.26 Hz, CH_2), 3.84 (3 H, s, OCH_3), 3.76 (3 H, s, OCH_3), 3.65 (6 H, s, 2 × OCH_3), 2.73 (2 H, t, J = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.1 (C), 154.9 (C), 152.5 (C), 137.7 (C), 137.2 (C), 137.1 (C), 136.5 (C), 133.9 (CH), 132.2 (CH), 130.9 (CH), 128.4 (CH), 123.6 (CH), 122.0 (CH), 113.1 (CH), 106.8 (CH), 80.7 (CH_2), 60.8 (CH_3), 55.9 (CH_3), 55.1 (CH_3), 35.3 (CH_2) ppm.

*4-[4-(3,4,5-Trimethoxyphenyl)-2,3-dihydro-1-benzoxepin-5-yl-phenol **11b*** Preparation as described above for **11a** from 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin **10b** (158 mg, 0.5 mmol) and 3,4,5-trimethoxyphenylboronic acid (138 mg, 0.65 mmol). The crude product residue was purified by column chromatography (silica, 10% diethyl ether/hexane) to give the product as a beige solid, (133 mg, 67%). M.P. 151°C. IR ν_{max} (KBr): 3365.9 (OH), 1610.9, 1578.2, 1127.3 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.22 (1 H, t, J = 7.00 Hz, Ar-H), 7.13 (1 H, dd, J = 1.00 Hz, 8.00 Hz, Ar-H), 7.03 (1 H, dt, J = 1.50 Hz, 7.50 Hz, Ar-H), 6.90 (1 H, dt, J = 1.50 Hz, 7.50 Hz, Ar-H), 6.82 (2 H, d, J = 5.00 Hz, Ar-H), 6.57 (2 H, d, J = 8.50 Hz, Ar-H), 6.37 (2 H, s, Ar-H), 4.64 (2 H, t, J = 6.00 Hz, CH_2), 3.82 (3 H, s, OCH_3), 3.62 (6 H, s, OCH_3), 2.70 (2 H, t, J = 6.00 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.7 (C), 154.4 (C), 152.5 (C), 137.8 (C), 137.6 (C), 137.2 (C), 137.1 (C), 133.7 (C), 132.4 (CH), 130.9 (CH), 128.4 (CH), 123.7 (CH), 122.0 (CH), 114.6 (CH), 106.8 (CH), 80.7 (CH_2), 60.9 (OCH_3), 55.9 (OCH_3), 35.2 (CH_2) ppm; HRMS: Found 427.1519; $C_{25}H_{24}O_5Na$ requires 427.1521.

*5-(3,4-Dimethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin **11c*** Preparation as described above for **11a** from 4-bromo-5-(3,4-dimethoxyphenyl)-2,3-dihydro-1-benzoxepin **10c** (240 mg, 851 μmol) and 3,4,5-trimethoxyphenylboronic acid (225 mg, 1.06 mmol). The crude residue was purified by column chromatography (silica, 10% diethyl ether/hexane) to give the product as a white solid, (200 mg, 52%). M.P. 152°C. IR ν_{max} (KBr): 1577.9, 1515.9,

1407.5, 1232.9, 1125.2, 1024.4 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.22 (1 H, dt, *J* = 1.51 Hz, 6.28 Hz, Ar-H), 7.09 (1 H, dt, *J* = 1.50 Hz, 6.02 Hz, Ar-H), 7.02 (1 H, d, *J* = 8.56 Hz, Ar-H), 6.94 (1 H, dd, *J* = 1.48 Hz, 7.04 Hz, Ar-H), 6.84–6.81 (1 H, m, Ar-H), 6.78 (1 H, d, *J* = 2.00 Hz, Ar-H), 6.60 (2 H, s, Ar-H), 4.60 (2 H, t, *J* = 5.76 Hz, CH_2), 3.92 (3 H, s, OCH_3), 3.87 (9 H, s, OCH_3), 3.84 (3 H, s, OCH_3), 3.02 (2 H, t, *J* = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.0 (C), 153.1 (C), 148.0 (C), 147.8 (C), 139.1 (C), 137.6 (C), 134.5 (C), 132.8 (C), 130.1 (CH), 128.5 (CH), 123.2 (CH), 123.0 (CH), 122.0 (CH), 121.6 (CH), 121.5 (CH), 112.7 (CH), 110.1 (CH), 104.7 (CH), 76.8 (CH_2), 60.4 (OCH_3), 55.7 (OCH_3), 55.6 (OCH_3), 54.5 (OCH_3), 55.3 (OCH_3), 40.8 (CH_2) ppm; HRMS: Found 471.1786; $C_{27}H_{28}O_6Na$ requires 471.1784.

*5-(3,4-Dimethoxyphenyl)-4-(2,3,4-trimethoxyphenyl)-2,3-dihydro-benzoxepin **11d*** Preparation as described above for **11a** from 4-bromo-5-(3,4-dimethoxyphenyl)-2,3-dihydro-1-benzoxepin **10c** (1.26 g, 3.49 mmol) and 2,3,4-trimethoxyphenylboronic acid (0.92 g, 4.36 mmol). The crude residue was purified by column chromatography (silica, 10% diethyl ether/hexane) to give the product as a white solid, (1.42 g, 90%). M.P. 130°C. IR ν_{max} (KBr): 2932.1, 1587.4, 1514.2, 1488.9, 1289.3, 1091.5, 1025.7 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.26–7.23 (1 H, m, Ar-H), 7.15 (1 H, dd, *J* = 1.00 Hz, 7.04 Hz, Ar-H), 7.05 (1 H, dt, *J* = 1.17 Hz, 6.28 Hz, Ar-H), 6.97 (1 H, dd, *J* = 1.52 Hz, 6.00 Hz, Ar-H), 6.66 (2 H, t, *J* = 8.28 Hz, Ar-H), 6.57 (2H, d, *J* = 11.52 Hz, Ar-H), 6.45 (1H, d, *J* = 8.52 Hz, Ar-H), 4.64 (2 H, t, *J* = 6.26 Hz, OCH_2), 3.96 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 3.83 (3 H, s, OCH_3), 3.82 (3 H, s, OCH_3), 3.58 (3 H, s, OCH_3), 2.69 (2 H, t, *J* = 6.26 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.7 (C), 152.3 (C), 150.9 (C), 147.2 (C), 146.9 (C), 141.6 (C), 137.4 (C), 136.3 (C), 135.4 (C), 133.7 (C), 130.7 (CH), 128.8 (C), 127.9 (CH), 125.6 (CH), 122.9 (CH), 121.6 (CH), 113.8 (CH), 109.7 (CH), 106.3 (CH), 79.9 (CH_2), 60.4 (CH_3), 60.2 (CH_3), 55.4 (CH_3), 55.2 (CH_3), 55.1 (CH_3), 34.6 (CH_2) ppm; HRMS: Found 471.1784; $C_{27}H_{28}O_6Na$ requires 471.1784.

*5-(3-Fluoro-4-methoxyphenyl)-4-(2,3,4-trimethoxyphenyl)-2,3-dihydro-benzoxepin **11e*** Preparation as described above for **11a** from 4-bromo-5-(3-fluoro-4-methoxyphenyl)-2,3-dihydro-1-benzoxepin **10d** (146 mg, 420 µmol) and 3,4,5-trimethoxyphenylboronic acid (111 mg, 515 µmol). The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give an orange solid, (170 mg, 94%). M.P. 69°C. IR ν_{max} (KBr): 2919.1, 1578.3, 1507.8, 1407.2, 1125.7 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.33–7.30 (1 H, m, Ar-H), 7.18 (1 H, dd, *J* = 1.00 Hz, 7.00 Hz, Ar-H), 7.08 (1 H, dt, *J* = 1.02 Hz, 6.52 Hz, Ar-H), 6.91 (1 H, dd, *J* = 1.52 Hz, 6.52 Hz, Ar-H), 6.79–6.74 (3 H, m, Ar-H), 6.40 (1H, s, Ar-H), 4.68 (2 H, t, *J* = 6.02 Hz, OCH_2), 3.87 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 3.69 (6 H, s, OCH_3), 2.74 (2 H, t, *J* = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.6 (C), 152.3 (C), 150.1 (C), 145.8 (C), 145.7 (C), 138.3 (C), 136.9 (C), 136.4 (CH), 136.1 (C), 135.7 (C), 134.2 (C), 134.1 (C), 130.4 (CH), 130.2 (CH), 130.1 (CH), 128.3 (CH), 126.7 (CH), 126.6 (CH), 125.9 (CH), 123.3 (CH), 121.8 (CH), 118.3 (CH), 118.1 (CH), 112.0 (CH), 106.3 (CH), 80.1 (CH_2), 60.5 (OCH_3), 55.7 (OCH_3), 55.5 (OCH_3), 35.0 (CH_2) ppm; HRMS: Found 459.1584; $C_{26}H_{25}O_5FNa$ requires 459.1584.

*5-(3-Formyl-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydro-benzoxepin **11f*** Preparation as described above for **11a** from 4-bromo-5-(3-formyl-4-methoxyphenyl)-2,3-dihydro-1-benzoxepin **10e** (146 mg, 407 µmol) and 3,4,5-trimethoxyphenylboronic acid (147 mg, 682 µmol). The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give a yellow solid, (175 mg, 96%). M.P. 98°C. IR ν_{max} (KBr): 2839, 1682 (C=O), 1603, 1579, 1505cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 10.3 (1 H, s, CHO), 7.50 (1 H, d, *J* = 2.04 Hz, Ar-H), 7.26 (1 H, dt, *J* = 1.68 Hz, 6.26 Hz, Ar-H), 7.17 (2 H, dt, *J* = 2.01 Hz, 6.01 Hz, Ar-H), 7.01 (1 H, t, *J* = 6.52 Hz, Ar-H), 6.83 (1 H, dd *J* = 1.50 Hz, 6.04 Hz, Ar-H), 6.77 (1 H, d, *J* = 8.52 Hz, Ar-H), 6.37 (2H, s, Ar-H), 4.67 (2 H, t, *J* = 6.02 Hz, OCH_2), 3.94 (6 H, s, OCH_3), 3.90 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3), 2.71 (2 H, t, *J* = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 189.5 (CHO), 160.3 (C), 156.0 (C), 153.3 (C), 152.7 (C), 139.1 (C), 138.6 (CH), 137.5 (C), 137.4 (C), 136.2 (C), 136.0 (C), 134.1 (C), 131.1 (CH), 130.6 (CH), 128.7 (CH), 124.1 (C), 123.8 (C), 122.2 (CH), 110.9 (CH), 106.7 (CH), 104.5 (CH), 92.7 (CH), 80.4 (CH_2), 60.9 (OCH_3), 60.8 (OCH_3), 56.2 (OCH_3), 55.8 (OCH_3), 35.5 (CH_2) ppm; HRMS: Found 469.1615; $C_{27}H_{26}O_6Na$ requires 469.1627.

*5-(3-Hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydro-benzoxepin **11g*** Hydrogen peroxide (30%, 10 drops) and sulfuric acid (2.0 mL) were added to a solution of 5-(3-formyl-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzoxepin **11f** (132 mg, 300 µmol) in methanol (10 mL) and the solution stirred at room temperature for 6 h. Water (20 mL) was added, the solution neutralized to pH 7, the aqueous layer extracted with ethyl acetate (2 × 25 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a yellow solid, (75 mg, 58%). IR ν_{max} (film): 3436.9 (OH), 1580.5, 1509.4, 1242.4, 1125.6 cm^{-1}. ^{1}H (400 MHz, $CDCl_3$): δ 7.22 (1 H, dt, *J* = 1.76 Hz, 6.78 Hz, Ar-H), 7.12 (1 H, dd, *J* = 1.00 Hz, 7.04 Hz, Ar-H), 7.03 (1 H, dt, *J* = 1.00 Hz, 7.02 Hz, Ar-H), 6.91 (1 H, dd *J* = 1.76 Hz, 6.00 Hz, Ar-H), 6.60 (1H, d, *J* = 8.04 Hz, Ar-H), 6.58 (1 H, d, *J* = 2.00 Hz, Ar-H), 6.48 (1 H, dd *J* = 2.00 Hz, 6.04 Hz, Ar-H), 6.21 (2 H, s, Ar-H), 4.63 (2 H, t, *J* = 6.02 Hz, OCH_2), 3.81 (6 H, s, 2 × OCH_3), 3.65 (6 H, s, 2 × OCH_3), 2.71 (2 H, t, *J* = 6.26 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.4 (C), 152.1 (C), 144.8 (C), 144.4 (C), 137.5 (C), 137.3 (C), 136.8 (C), 136.5 (C), 136.1 (C), 134.5 (C), 130.6 (CH), 127.9 (CH), 123.2 (CH), 122.7 (CH), 121.6 (CH), 116.9 (CH), 109.4 (CH), 106.3 (CH), 80.2 (CH_2), 60.4 (OCH_3), 55.8 (OCH_3), 55.5 (OCH_3), 55.4 (OCH_3), 34.9 (CH_2) ppm; HRMS: Found 457.1640; $C_{26}H_{26}O_6Na$ requires 457.1627.

*4-(3,4-Dimethoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin **11h*** Preparation as described above for **11a** from 4-bromo-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin **10f** (196 mg, 0.5 mmol) and 3,4-dimethoxyphenylboronic acid (114 mg, 0.625 mmol).

The crude residue was purified by column chromatography (silica, gradient ethyl acetate/hexane) to give the product as a white solid, (185 mg, 83%). M.P. 131°C. IR ν_{max} (KBr): 1579.6, 1465.6, 1127.3 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.28–7.24 (1 H, m, Ar-H), 7.14 (1 H, dd, *J* = 1.00 Hz, 7.00 Hz, Ar-H), 7.04 (1 H, dt, *J* = 1.50 Hz, 6.78 Hz, Ar-H), 6.96 (1 H, dd, *J* = 1.74 Hz, 6.04 Hz, Ar-H), 6.66 (1 H, d, *J* = 8.00 Hz, Ar-H), 6.55 (1 H, dd, *J* = 2.02 Hz, 6.00 Hz, Ar-H), 6.52 (1 H, d, *J* = 2.00 Hz, Ar-H), 6.41 (2 H, s, Ar-H), 4.67 (2 H, t, *J* = 6.26 Hz, OCH_2), 3.84 (3 H, s, OCH_3), 3.83 (3 H, s, OCH_3), 3.66 (6 H, s, OCH_3), 3.57 (3 H, s, OCH_3), 2.78 (2 H, t, *J* = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.9 (C), 152.7 (C), 147.9 (C), 147.6 (C), 137.9 (C), 137.8 (C), 137.3 (C), 136.8 (C), 136.6 (C), 133.9 (C), 130.9 (CH), 128.5 (CH), 123.7 (CH), 123.6 (CH), 122.1 (CH), 114.5 (CH), 110.3 (CH), 106.8 (CH), 104.5 (CH), 80.7 (CH_2), 60.9 (OCH_3), 60.8 (OCH_3), 56.2 (OCH_3), 55.9 (OCH_3), 55.6 (OCH_3), 35.2 (CH_2) ppm; HRMS: Found 471.1791; $C_{27}H_{28}O_6Na$ requires 471.1784.

2-Methoxy-5-[5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin-4-yl]-benzaldehyde ***11i*** Preparation as described above for **11a** from 4-bromo-5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin **10f** (389 mg, 1 mmol) and 3-formyl-4-methoxyphenylboronic acid (243 mg, 1.3 mmol). The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a yellow solid, (307 mg, 69%). M.P. 141°C. IR ν_{max} (KBr): 1681.0 (C=O), 1496.3, 1237.9, 1121.6 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 10.41 (1 H, s), 7.76 (1 H, d, *J* = 2.00 Hz, Ar-H), 7.30 (1 H, t, *J* = 1.50 Hz, Ar-H), 7.25 (1 H, d, *J* = 2.00 Hz, Ar-H), 7.13 (1 H, dd, *J* = 1.00 Hz, 8.00 Hz, Ar-H), 7.04 (1 H, dt, *J* = 1.00 Hz, 7.50 Hz, Ar-H), 6.95 (1 H, dd, *J* = 1.50 Hz, 8.00 Hz, Ar-H), 6.76 (1 H, d, *J* = 8.50 Hz, Ar-H), 6.19 (2 H, s, Ar-H), 4.61 (1 H, t, *J* = 6.00 Hz, O-CH_2), 3.87 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 3.56 (6 H, s, OCH_3), 2.72 (2 H, t, *J* = 6.00 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 189.2 (CH), 160.3 (C), 155.8 (C), 152.3 (C), 137.9 (CH), 137.6 (C), 136.7 (C), 136.5 (C), 136.3 (C), 136.1 (C), 134.7 (C), 131.9 (CH), 130.8 (CH), 128.5 (CH), 127.9 (CH), 124.0 (C), 123.5 (CH), 121.9 (CH), 111.1 (CH), 108.5 (CH), 80.2 (CH_2), 60.6 (CH_3), 55.7 (CH_3), 55.5 (CH_3), 35.1 (CH_2) ppm; HRMS: Found 469.1626; $C_{27}H_{26}O_6Na$ requires 469.1627.

2-Methoxy-5-[5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin-4-yl]-phenol ***11j*** Hydrogen peroxide (30%, 233fL) and sulfuric acid (50 μL) were added to a solution of 2-methoxy-5-[5-(3,4,5-trimethoxyphenyl)-2,3-dihydro-1-benzoxepin-4-yl]-benzaldehyde **11i** (45 mg, 0.1 mmol) in methanol (10 mL) and the solution stirred at room temperature for 6 h. Water (20 mL) was added, the solution neutralized to pH 7, the aqueous layer extracted with ethyl acetate (2 × 25 mL), dried over sodium sulfate, and the solvent removed under reduced pressure and the product obtained as an orange solid, (21 mg, 50%). IR ν_{max} (KBr): 3410.4 (OH), 1579.9, 1115.3 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.25–7.24 (1 H, m, Ar-H), 7.13 (1 H, d, *J* = 8.04 Hz, Ar-H), 7.06 (1 H, t, *J* = 6.78 Hz, Ar-H), 6.96 (1 H, dd, *J* = 1.50 Hz, 6.04 Hz, Ar-H), 6.82 (1 H, d, *J* = 6.04 Hz, Ar-H), 6.68–6.62 (2 H, m, Ar-H), 6.22 (2 H, s, Ar-H), 4.65 (3 H, t, *J* = 6.28 Hz, O-CH_2), 3.86 (3 H, s, OCH_3), 3.84 (3 H, s, OCH_3), 3.58 (6 H, s, 2 × OCH_3), 2.69 (2 H, t, *J* = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.0 (C), 152.3 (C), 145.2 (C), 138.2 (C), 137.1 (C), 136.9 (C), 136.7 (C), 136.5 (C), 135.9 (C), 131.0 (CH), 128.4 (CH), 123.5 (CH), 122.1 (CH), 121.6 (CH), 114.9 (CH), 110.2 (CH), 108.7 (CH), 80.6 (CH_2), 60.9 (CH_3), 55.9 (CH_3), 35.7 (CH_2) ppm; HRMS: Found 457.1645; $C_{26}H_{26}O_6Na$ requires 457.1627.

6,8-Dimethoxy-5-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzoxepin ***11k*** Preparation as described above for **11a** from 4-bromo-6,8-dimethoxy-5-(4-methoxyphenyl)-2,3-dihydrobenzoxepin **10j** (0.069 g, 0.14 mmol) and. 3,4,5-trimethoxyphenylboronic acid (1.3 eq., 0.19 mmol, 0.039 g). The crude product was purified using column chromatography (silica and 5% diethylether in hexane) to afford the product as a white solid, (13 mg, 18.9%). ^{1}H (400 MHz, $CDCl_3$): δ 6.91 (2 H, d, *J* = 9.05 Hz, Ar-H), 6.71 (1 H, s, Ar-H), 6.67 (2 H, d, *J* = 8.56 Hz, Ar-H), 6.37 (3 H, d, *J* = 3.00 Hz, Ar-H), 4.64 (2 H, t, *J* = 6.02 Hz, CH_2), 3.84 (6 H, s, 2 × OCH_3), 3.76 (3 H, s, OCH_3), 3.62 (9 H, s, 3 × OCH_3), 2.72 (2 H, t, *J* = 6.02 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.8 (C), 158.8 (C), 157.4 (C), 157.2 (C), 152.5 (C), 137.4 (C), 136.9 (C), 134.4 (C), 130.0 (C), 118.0 (CH), 112.5 (C), 107.1 (CH), 104.5 (CH), 99.2 (CH), 96.3 (CH), 79.8(CH_2), 60.9 (OCH_3), 56.3(OCH_3), 55.4(OCH_3), 55.1(OCH_3), 35.6 (CH_2) ppm; HRMS: Found 501.1897; $C_{28}H_{30}O_7Na$ requires 501.1889.

7,8-Dimethoxy-5-(4-methoxy-phenyl)-4-(3,4,5-trimethoxy-phenyl)-2,3-dihydro-benzo[b]oxepin ***11l*** Preparation as described above for **11a** from 4-bromo-7,8-dimethoxy-5-(4-methoxy-phenyl)-2,3-dihydrobenzo[*b*]oxepin **10k** (100 mg, 0.256 mmol) and 3,4,5-trimethoxyphenyl boronic acid (70 mg, 0.332 mmol). The crude product was purified using column chromatography over silica gel (eluent 5% diethylether in hexane) to give the product as a pale yellow solid, (0.141 g, 100%). M.P. 81–90°C. IR ν_{max} (KBr): 2933, 1607, 1577, 1244 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 6.91 (2 H, d, *J* = 8.56 Hz, Ar-H), 6.71 (1 H, s, Ar-H), 6.67 (2 H, d, *J* = 6.76 Hz, Ar-H), 6.38 (3 H, m, Ar-H), 4.62 (2 H, t, *J* = 6.76 Hz, CH_2), 3.91 (3 H, s, OCH_3), 3.82 (3 H, s, OCH_3), 3.76 (3 H, s, OCH_3), 3.64 (9 H, s, OCH_3), 2.75 (2H, t, *J* = 6.76 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.1 (C), 152.6 (C), 150.2 (C), 148.9 (C), 145.0 (C), 138.0 (C), 137.1 (C), 137.0 (C), 133.5 (C), 132.3 (CH), 128.0 (C), 113.0 (CH), 113.0 (CH), 106.8 (CH), 105.5 (CH), 81.1 (CH_2), 60.9 (OCH_3), 56.1 (OCH_3), 55.9 (OCH_3), 55.1 (OCH_3), 35.5 (CH_2) ppm; HRMS: Found 501.1877; $C_{28}H_{30}O_7Na$ requires 501.1889.

5-Naphthalen-1-yl-2,3-dihydrobenzoxepin ***12*** Preparation as described above for **9a** from 3,4-dihydro-2*H*-1-benzoxepin-5-one **7a** (0.40 g, 2.50 mmol) and 1-naphthylboronic acid (493 mg, 2.86 mmol) via the triflate **8a**. The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give a yellow solid which was used in subsequent reactions without further purification, (497 mg, 73%). M.P. 68°C. IR ν_{max} (KBr): 2961.4, 1587.8, 1479.3, 1429.5, 1261.3, 799.7 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ 7.92 (2 H, t, *J* = 7.54 Hz, Ar-H), 7.84 (1 H, d,

J = 8.56 Hz, Ar-H), 7.57 (1 H, t, *J* = 7.54 Hz, Ar-H), 7.51–7.48 (1 H, m, Ar-H) 7.43-7.39 (2 H, m, Ar-H), 7.19 (2 H, *J* = 7.54 Hz, Ar-H), 6.84–6.79 (1 H, m, Ar-H), 6.74 (1 H, d, *J* = 8.04 Hz, Ar-H), 6.25 (1 H, t, *J* = 5.02 Hz, CH), 4.57 (2 H, t, *J* = 5.20 Hz, OCH_2), 2.86 (2 H, q, *J* = 5.52 Hz, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.4 (C), 141.7 (C), 138.2 (C), 133.6 (C), 132.2 (C), 131.7 (CH), 131.6 (CH), 130.6 (C), 128.1 (CH), 128.0 (CH), 127.8 (C), 127.5 (C), 127.5 (CH), 127.1 (CH), 126.3 (CH), 125.9 (CH), 125.8 (CH), 125.6 (CH), 122.8 (CH), 120.9 (CH), 73.1 (CH_2), 32.8 (CH_2) ppm.

4-Bromo-5-naphthalen-1-yl-2,3-dihydrobenzoxepin ***13*** Pyridinium tribromide (339 mg, 0.96 mmol) was added to a solution of 5-naphthalen-1-y-2,3-dihydrobenzoxepin **12** (262 mg, 0.96 mmol) in dichloromethane (20 mL) at 0°C and stirred for 5 min. Water (20 mL) was added and the aqueous layer extracted with dichloromethane (2 × 20 mL). The organic layer was washed with a saturated solution of sodium hydrogen carbonate (20 mL), brine (20 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was purified by chromatography (silica, 5% diethyl ether/hexane) to give the product as an orange solid which was used in subsequent reactions without further purification, (284 mg, 84 %). M.P. 107°C. IR ν_{max} (KBr): 2962.1, 1429.3, 1260.4, 1125.9, 1027.9, 801.3 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.92 (2 H, d, *J* = 8.00 Hz, Ar-H), 7.88 (1 H, d, *J* = 8.00 Hz, Ar-H), 7.60 (1 H, t, *J* = 7.78 Hz, Ar-H), 7.54–7.48 (3 H, m, Ar-H), 7.24–7.17 (2 H, m, Ar-H), 6.87–6.79 (2 H, m, Ar-H), 4.79–4.75 (1 H, m, OCH_2), 4.74–4.62 (1 H, m, OCH_2), 3.37–3.32 (1 H, m, CH_2), 3.30–3.18 (1 H, m, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.2 (C), 140.2 (C), 137.7 (C), 133.4 (C), 132.9 (C), 131.5 (C), 130.7 (CH), 130.5 (C), 128.5 (CH), 127.9 (CH), 127.6 (C), 127.5 (C), 127.2 (CH), 126.8 (CH), 126.1 (CH), 125.6 (CH), 125.0 (CH), 124.9 (CH), 123.0 (CH), 121.5 (CH), 75.7 (CH_2), 41.5 (CH_2) ppm.

5-Naphthalen-1-yl-4-(3,4,5-trimethoxyphenyl)-2,3-dihydro-benzoxepin ***14*** 4-Bromo-5-naphthalen-1-yl-2,3-dihydrobenzoxepin **13** (139 mg, 396 µmol) was dissolved in THF (30 mL). 3,4,5-Trimethoxyphenylboronic acid (109 mg, 515 µmol) and 2 M sodium carbonate (0.99 mL) were added and the mixture stirred under nitrogen for 10 min. $Pd(PPh_3)_4$ (30 mg, 0.025 m mol) was added and the reaction refluxed for 6 h at 85°C. The solution was cooled to room temperature and acidified with 2 M hydrochloric acid. The aqueous layer was extracted with dichloromethane (3 × 30 mL) and the combined organic layers were washed with water (30 mL), brine (30 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The product was purified by column chromatography (silica, 5% diethyl ether/hexane) to give the product as a yellow solid, (134 mg, 77%). M.P. 124°C. IR ν_{max} (KBr): 2930.3, 1579.3, 1505.7, 1462.2, 1246.5, 1123.7, 1019.5 cm^{-1}; ^1H (400 MHz, $CDCl_3$): δ 7.87 (1 H, m, Ar-H), 7.74 (1 H, m, Ar-H), 7.67 (1 H, m, Ar-H), 7.33 (4 H, m, Ar-H), 7.16 (3 H, m, Ar-H), 6.85 (2 H, m, Ar-H), 6.24 (2 H, s, Ar-H), 4.83 (1 H, m, OCH_2), 4.73 (1 H, m, OCH_2), 3.68 (3 H, s, OCH_3), 3.29 (6 H, s, 2 × OCH_3,), 2.98 (1 H, m, CH_2), 2.77 (1 H, m, CH_2) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.1 (C), 152.2 (C), 140.5 (C), 137.3 (C), 136.5 (C), 133.5 (C), 129.6 (CH), 128.4 (CH), 128.2 (CH), 128.0 (CH), 127.2 (CH), 126.3 (CH), 126.2 (CH), 125.5 (CH), 125.4 (CH), 123.6 (CH), 122.1 (CH), 105.7 (CH), 80.4 (CH_2), 60.7 (CH_3), 55.5 (CH_3), 34.8 (CH_2) ppm; HRMS: Found 461.1725; $C_{29}H_{26}O_4Na$ requires 461.1729.

Biochemical evaluation of activity

Antiproliferation studies

All assays were performed in triplicate for the determination of mean values reported. Compounds were assayed as the free bases isolated from reaction. The human breast tumor cell line MCF-7 was cultured in Eagle's minimum essential medium in a 95% O_2/5% CO_2 atmosphere with 10% fetal calf serum. The medium was supplemented with 1% non-essential amino acids. MDA-MB 231 cells were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum (FBS), 2mM L-glutamine and 100 µg/mL penicillin/streptomycin (complete medium). Cells were trypsinized and seeded at a density of 2.5×10^4 cells/mL (or 1.0×10^4 cells/well) into a 96-well plate and incubated at 37°C, 95%O_2/5% CO_2 atmosphere, for 24 h. After this time they were treated with 2 µL volumes of test compound which had been pre-prepared as stock solutions in ethanol to furnish the concentration range of study, 1 nM–100 µM, and re-incubated for a further 72 h. Control wells contained the equivalent volume of the vehicle ethanol (1%, v/v). The culture medium was then removed and the cells washed with 100 µL phosphate buffered saline (PBS) and 50 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) added, to reach a final concentration of 1 mg/mL MTT. Cells were incubated for 2 h in darkness at 37°C. At this point, solubilization was begun through the addition of 200 µL dimethylsulfoxide (DMSO) and the cells maintained at room temperature in darkness for 20 min to ensure thorough color diffusion before reading the absorbance. The absorbance value of control cells (no added compound) was set to 100% cell viability, and, from this, graphs of absorbance versus cell density per well were prepared to assess cell viability, and, from these, graphs of percentage cell viability versus concentration of subject compound added were drawn.

Analysis of pro-apoptotic effects

Flow cytometry The MCF-7 cells were seeded at a density of 18×10^4 cells/mL in 5 mL of medium (900,000 cells per flask). After 24 h, cells were treated with either vehicle (50 µL of ethanol; 1%, v/v) or selected compound (1 µM) and incubated for 72 h. Following incubation, the cells were removed from the bottom of the flask by scraping and the medium placed in a 20 mL Sterilin tube. They were centrifuged for 10 min at 600 × *g*. The supernatant was decanted and the pellet resuspended in 1 mL of ice-cold PBS; cells were again centrifuged for 10 min at 600 × *g*. The supernatant was decanted and the pellet resuspended in 200 µL of ice-cold phosphate buffered saline (PBS). Subsequently ice-cold 70% ethanol (2 mL) was slowly added to the tube as it was gently vortexed. The cells were kept at–20°C for at least 1 h (could

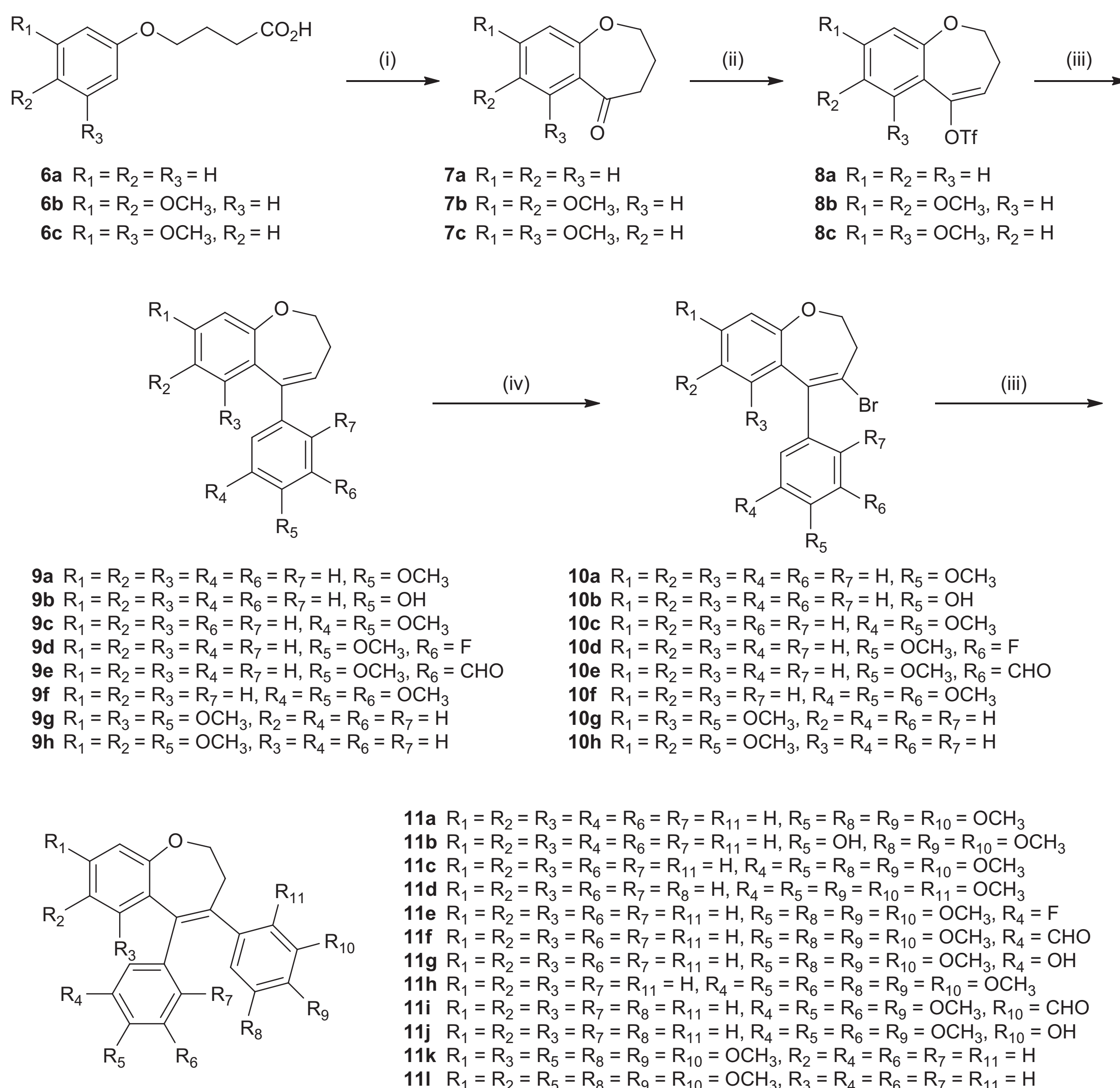
be left overnight). After the fixation, 5 µL of FBS was added to the samples. The cells were harvested by centrifugation at 600 × *g* for 10 min. The ethanol was carefully removed and the pellet resuspended in 400 µL of PBS and transferred to FACS (fluorescence-activated cell sorting) microtubes. A 25 µL aliquot of RNase A (1 mg/mL) and 75 µL of propidium iodide (PI) 1 mg/mL, a DNA binding fluorescent dye, were added to each tube. The samples were wrapped in aluminum foil and incubated for a minimum of 30 min at 37°C. The samples were read at 488 nM using a FACscalibur flow cytometer from Becton Dickinson. The FACS data for 10,000 cells were analyzed using the Macintosh-based application Cellquest and the data were stored as frequency histograms. An increase in the percentage of cells in the pre-G1 peak is indicative of apoptosis.

Computational procedure

Docking was carried out using FREDv2.2.3 software in conjunction with Chemgauss3.

Ligand preparation

All compounds were drawn using ACD/Chemsketch v10[26] and SMILES strings generated. A single conformer was generated using Corina v3.4 and ensuring Omega v2.2.1 was subsequently employed to generate a maximum of 1000 conformations of each compound.



Scheme 1. Synthesis of benzoxepins **11a–l**. Scheme reagents and conditions: (i) PPA, 110°C, 4 h, or Eaton's reagent, 80°C, 2 h; (ii) $(TfO)_2O$, Na_2CO_3, 18 h, rt; (iii) $Pd(PPh_3)_4$, $ArB(OH)_2$, Na_2CO_3(aq), THF; (iv) $PyHBr_3$, $CHCl_2$, 20°C, 18 h.

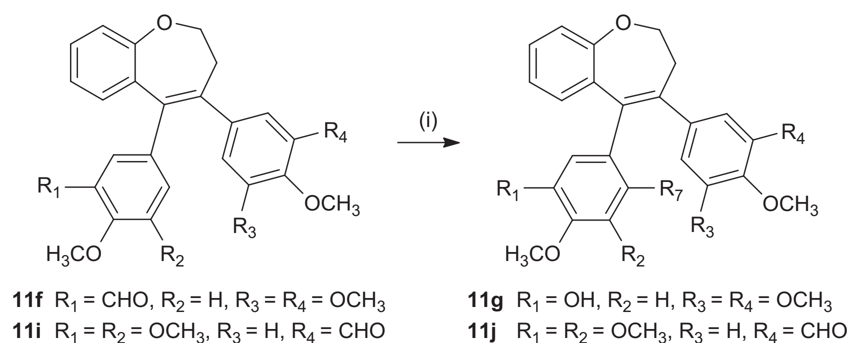
Receptor preparation

PDB entries 1SA0 and 1SA1 were downloaded from the Protein Data Bank (PDB). All waters were retained in both isoforms. Addition and optimization of hydrogen positions for these waters was carried out using MOE 2007.09[27] ensuring that all other atom positions remained fixed.

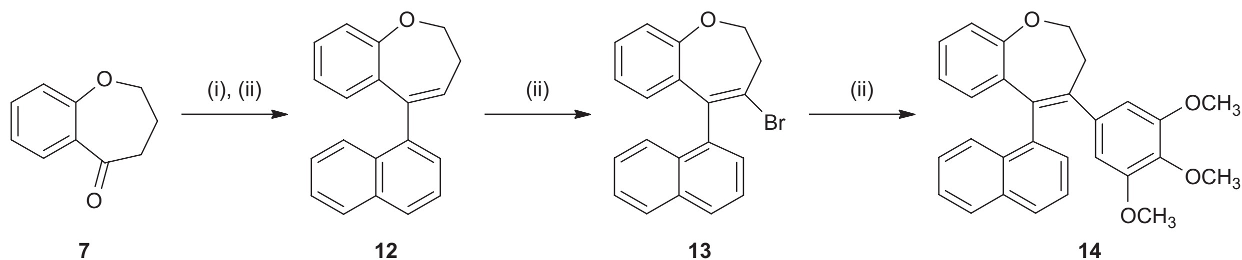
Results and discussion

Chemistry

The synthesis of the benzoxepin analogs of combretastatin CA-4 is illustrated in Scheme 1 and is achieved by ligand coupling reactions of polymethoxylated 5-arylated benzoxepins with arylboronic acids in the presence of tetrakis(triphenylphosphine)palladium(0) catalyst. The target benzoxepin compounds chosen for synthesis were designed to have comparable structures to the reported combretastatin analogs in ring A and ring B substitution pattern[11]. The trimethoxy substitution pattern was retained in all compounds, which is a common structural feature of colchicines, CA-4, and many other tubulin inhibitors[11,28]. The required 2,3,4,5-tetrahydro-1-benzoxepin-2-ones **7a–c** were obtained by acid-promoted cyclization of the appropriate 4-phenoxybutyric acids **6a–c** (obtained by alkylation of the appropriate phenols with ethyl bromobutyrate). Polyphosphoric acid was found to be the most efficient reagent for the preparation of the unsubstitued benzoxepin **7a**, R = H, while Eaton's reagent was found to be superior for **7b** and **7c**, with yields of up to 67% achieved for **7c**. To facilitate the subsequent arylation reaction, the 2,3,4,5-tetrahydro-1-benzoxepin-2-ones **7a–c** were first converted to the triflates **8a–c** by treatment with triflic anhydride and then subsequently coupled *in situ* with a number of substituted arylboronic acids in a Suzuki reaction[29] promoted by $Pd(PPh_3)_4$ to afford the 5-arylbenzoxepins **9a–h** in moderate yield. These products were converted to the vinylic bromides **10a–h** on treatment with pyridine hydrobromide perbromide. This reaction was completed rapidly and required careful monitoring to prevent the subsequent aryl bromination reaction. A second Suzuki reaction promoted by $Pd(PPh_3)_4$ was then completed on compounds **10a–h** to afford the required products **11a–l** in relatively good yield for most of the products (Table 1). For synthesis of the phenolic products **11g** and **11j**, the direct coupling of the vinyl triflate **8a** or the vinyl bromide **10f** with the protected 3-benzyloxy-4-methoxyboronic acid was first attempted, but was not found to be successful. An alternative approach was necessary to obtain these products in which the aldehyde compounds **11f** and **11i** were treated with hydrogen peroxide in a Baeyer–Villiger oxidation reaction to afford the phenols **11g** and **11j** (Scheme 2). The 5-naphthylbenzoxepin product **14** was synthesized in a similar synthetic sequence to compounds **9a–h**, by first arylation of the benzoxepin **7a** with 1-naphthylboronic acid to afford the product **12** in 73% yield. Subsequent bromination of **12** proceeded in high yield (84%) to afford the vinyl



Scheme 2. Synthesis of benzoxepins **11g** and **11j**. Scheme reagents and conditions: (i) H_2O_2, H_2SO_4, CH_3OH, 20°C, 6 h.



Scheme 3. Synthesis of benzoxepin **14**. Scheme reagents and conditions: (i) $(TfO)_2O$, Na_2CO_3, 18 h, rt; (ii) $Pd(PPh_3)_4$, $ArB(OH)_2$, Na_2CO_3(aq), THF; (iii) $PyHBr_3$, $CHCl_2$, 20°C, 18 h.

bromide **13**. Suzuki reaction of the bromide **13** promoted by $Pd(PPh_3)_4$ afforded the required product **14**, again in high yield (Scheme 3). The ^1H NMR spectra of the products **11a–l** and **14** revealed the characteristic methylene protons at C-2 and C-3 of the benzoxepin ring as coupled triplet signals, e.g. for compound **11g** these signals were observed at δ 4.63 and δ 2.71, respectively, with J = 6.02 Hz.

Biochemistry

The benzoxepin compounds prepared above were evaluated in a series of *in vitro* assays which determined their antiproliferative activity in MCF-7 and MDA-MB 231 breast cancer cell lines and also their pro-apoptotic effects in MCF-7 cells by flow cytometry.

Antiproliferative activity in MCF-7 and MDA-MB 231 breast cancer cells

Compounds **11a–l** and **14** were initially screened for their antiproliferative activity using the ER expressing (ER dependent) MCF-7 human breast cancer cell line by means of the MTT (tetrazolium) based assay. The drug concentration required to inhibit the cell growth by 50% (IC_{50}) following incubation of the cells in the culture medium for 72 h was determined and the results are displayed in Table 1. The IC_{50} values obtained for combretastatin CA-4 were 0.0031 μM for MCF-7 and 0.043 μM for MDA-MB 231, which are in good agreement with the reported values for combretastatin CA-4 using the MTT assay on human MCF-7 and MDA-MB 231 breast cancer cell lines[11,20,30].

Compounds **11a–e** and **11k**, which display polymethoxylated aryl substitution, were found to exert modest antiproliferative effects on MCF-7 cells, with IC_{50} in the range 4.86–10.70 μM, when compared with our determined values for combretastatin CA-4 (IC_{50} = 0.0031 μM). A common substitution pattern of 3,4,5-trimethoxy or 2,3,4-trimethoxyphenyl for the aryl ring located on position 4 of the benzoxepin scaffold was present in these compounds, together with 4-methoxy, 4-hydroxy, 4,5-dimethoxy, and 4-fluoro-5-methoxyphenyl substitution at C-5. Compound **11e** was designed to mimic a fluorinated combretastatin CA-4 analog in which the hydroxy group on ring B was replaced with a fluorine without substantial loss of activity[31,32]. However, compound **11e** was found to exhibit only moderate antiproliferative activity, with IC_{50} = 10.7 μM. Compound **14**, containing the 1-naphthyl substituent at C-4 of the benzoxepin scaffold, was found to have poor antiproliferative activity, with IC_{50} = 25.9 μM. This compound could be considered as an analog of the known naphthylcombretastatins, in which the 1-naphthyl ring mimics the 3-hydroxy-4-methoxy ring B of combretastatin A4[33]. A possible explanation for the low activity of compound **14** is the steric hindrance caused by the presence of the 1-naphthyl substituent in the conformationally restricted benzoxepin analog, which hinders the required favorable aryl alignment predicted for ring B in the binding site. The most active compound in the series was identified as **11g** (IC_{50} = 850 nM), in which the aromatic rings located at C-5 of the benzoxepin ring contain a similar substitution pattern to that found for ring A (3,4,5-trimethoxyphenyl) of the combretastatin CA-4, and the aromatic rings located at C-4 of the benzoxepin ring contain a similar substitution pattern to that found for ring B (3-hydroxy-4-methoxyphenyl) of the combretastatin CA-4 molecule. Compound **11h**, also containing the C-5 trimethoxyphenyl ring substituent together with 3,4-dimethoxyphenyl substitution at C-4, was active (IC_{50} = 2.55 μM). Compound **11j** (IC_{50} = 1.635 μM), in which the aryl substitution pattern for the aromatic rings located at C-4 and C-5 is also similar to combretastatin CA-4, reversed in orientation from compound **11g**, is slightly less active than **11g**, indicating that the most favorable orientation of aryl substitution on the benzoxepin scaffold is for the 3,4,5-trimethoxyaryl ring A to be positioned at C-4 and the 3-hydroxy-4-methoxyaryl ring B to be located at C-5. The aldehyde compounds **11f** and **11i**, which are synthetic precursors of the active compounds **11g** and **11j**, were found to be considerably less active than their phenolic products **11g** and **11j**, with IC_{50} values of 6.24 μM and 37.19 μM, respectively. Compounds **11k** and **11l**, which contain the 6,8- and 6,7-dimethoxy substitution pattern, respectively, on the benzoxepin aryl ring, are less active (IC_{50} = 8.25, 12.79 μM) than the unsubstituted analog **11a** (IC_{50} = 4.86 μM).

The compounds were also evaluated for antiproliferative activity in the MDA-MB 231 human breast cancer cell line and the results are displayed in Table 1. The compounds were shown to have moderate activity when compared with combretastatin CA-4, IC_{50} = 0.043 μM, with **11k**, **11j** being the most active, displaying IC_{50} values of 5.33 μM, 4.57 μM respectively.

Table 1. Yield and antiproliferative activity for benzoxepins **11a–l** and **14**.

Compound number	Yield (%)	Antiproliferative activity, IC_{50} (μM)	
		MCF-7 cells[a]	MDA-MB 231 cells[a]
11a	73	4.86 ± 0.559	12.60 ± 0.141
11b	67	8.83 ± 2.79	13.04 ± 4.06
11c	52	5.26 ± 1.08	7.51 ± 1.14
11d	90	9.84 ± 1.80	10.50 ± 1.12
11e	94	10.70 ± 5.92	12.40 ± 1.27
11f	96	6.24 ± 1.33	17.99 ± 6.40
11g	58	0.85 ± 0.09	11.56 ± 2.51
11h	83	2.55 ± 3.15	20.40 ± 14.4
11i	69	37.19 ± 27.23	23.42 ± 9.95
11j	50	1.635 ± 1.393	4.57 ± 0.363
11k	19	8.25 ± 3.05	5.33 ± 2.38
11l	100	12.79 ± 0.50	46.43 ± 3.26
14	77	25.9 ± 20.7	96.43 ± 9.66

[a]Experimental values represent the average for experiments performed in triplicate along with the standard deviation (SD) between the assay values. IC_{50} values are half maximal inhibitory concentrations required to block the growth stimulation of MCF-7 cells. The IC_{50} values obtained for combretastatin CA-4 are 0.0031 μM for MCF-7 and 0.043 μM for MDA-MB 231, which are in good agreement with the reported values for combretastatin CA-4 using the MTT assay on human MCF-7 and MDA-MB 231 breast cancer cell lines, respectively.

Preliminary apoptosis experiments were performed by flow cytometry analysis on propidium iodide labeled MCF-7 cells, and showed that compound **11g** caused an increase in the pre-G1 peak from 11.1% (control) to 19.8%, indicating a possible pro-apoptotic effect for the compound.

To assess the similarity in structure of combretastatin CA-4 and the most potent benzoxepin synthesized **11g**, an overlay of the two structures was first examined, as illustrated in Figure 2. The MOE flexible alignment tool[27] was used for the above illustration, retaining default settings. It can be confirmed that the aryl rings A and B of the benzoxepin **11g** structure are aligned in an identical orientation to that of the combretastatin CA-4, in which they are not coplanar, thus indicating that the presence of the seven-membered ring on the alkene bridge in compound **11g** facilitates the constraint of the two aryl rings in the required Z configuration.

Molecular modeling studies of novel benzoxepin compounds

To investigate the possible tubulin binding mode of these benzoxepin compounds, a docking study was carried out to examine the docked orientations of the most potent benzoxepins **11g** and **11j** in the colchicine binding site of tubulin, using the reported X-ray structures of tubulin co-crystallized with a colchicine derivative, DAMA-colchicine (PDB entry 1SA0) and also podophyllotoxin (PDB entry 1SA1)[34]. Figure 3 illustrates the docked positions for each benzoxepin **11g** and **11j** in tubulin, together with DAMA-colchicine and combretastatin CA-4, and importantly shows that **11g** and **11j** should exhibit similar tubulin binding modes when compared with both CA-4 and colchicine. To dock the benzoxepins, PDB entry 1SA0 (tubulin–colchicine: RB3-SLD) was selected, as it can be seen from Figure 3 that steric hindrance from Thr179 and movement of Asn249 would have rendered the benzoxepins in a docking pose unattainable in reality for PDB entry 1SA1 (tubulin–podophyllotoxin: RB3-SLD[34]). For the most active benzoxepin **11g**, it can be seen that the trimethoxy ring A is located in the colchicine binding site in the region of Cys241, and with a very similar orientation to the trimethoxy ring of the DAMA-colchicine in the reported co-crystallized structure. There is also clear interaction between the benzoxepin aryl ring and the Thr179, and also between the benzoxepin heterocyclic and Asn249. Additional hydrophobic contacts are also observed, which stabilize the binding of compounds

Figure 2. Overlay of combretastatin CA-4 (blue) with benzoxepin **11g** (gray).

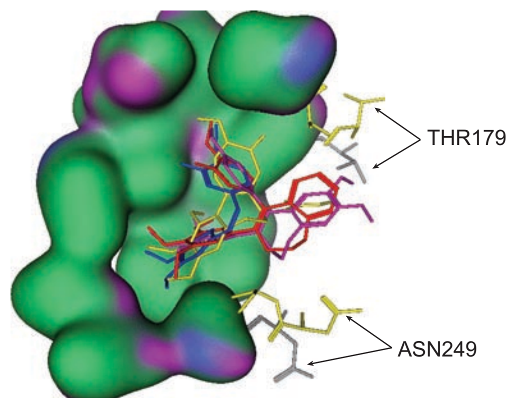


Figure 3. Docked poses of benzoxepins **11g** (red) and **11j** (pink) in tubulin overlayed by backbone with docked poses of CA-4 (blue) and colchicine (yellow). 1SA0, yellow; 1SA1, gray.

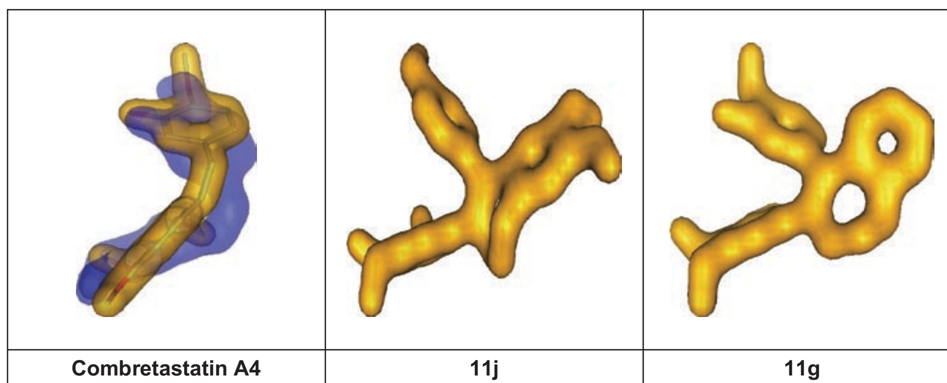
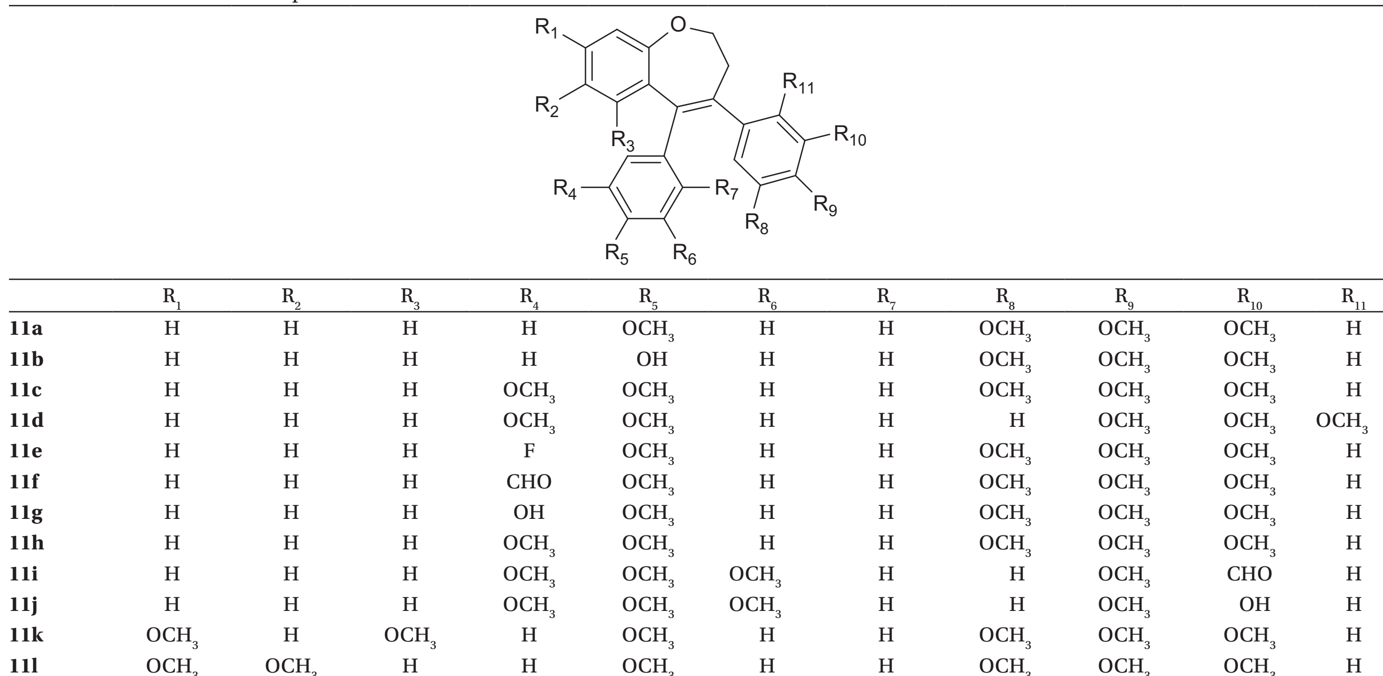


Figure 4. PostDOCK view of docked compounds combretstatin A-4, **11j**, and **11g** using FREDv2.2.1 combined with Chemgauss3 as a scoring function.

Table 2. Structures of benzoxepins **11a–l**.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}
11a	H	H	H	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H
11b	H	H	H	H	OH	H	H	OCH_3	OCH_3	OCH_3	H
11c	H	H	H	OCH_3	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H
11d	H	H	H	OCH_3	OCH_3	H	H	H	OCH_3	OCH_3	OCH_3
11e	H	H	H	F	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H
11f	H	H	H	CHO	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H
11g	H	H	H	OH	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H
11h	H	H	H	OCH_3	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H
11i	H	H	H	OCH_3	OCH_3	OCH_3	H	H	OCH_3	CHO	H
11j	H	H	H	OCH_3	OCH_3	OCH_3	H	H	OCH_3	OH	H
11k	OCH_3	H	OCH_3	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H
11l	OCH_3	OCH_3	H	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3	H

11g and **11j** to the protein. Computational analysis by ter Haar *et al.* has shown that the colchicine binding site of tubulin can accommodate structurally diverse ligands[5], suggesting a high plasticity of the tubulin protein at this site[35].

To assess the propensity of each ligand to dock in different manners using FREDv2.2.3 in combination with Chemgauss3, an analysis of the docked poses was carried out using PostDOCK. A new visualization tool—PostDOCK[36]—was utilized to examine the resulting docked poses for **11g** and **11j** generated by FREDv2.2.3. The program was developed using SVL (Scientific Vector Language) to integrate within MOE and analyzes a set of docked poses for a given compound whereby a pseudo-3D snapshot is generated, representing the conformations and energies of the docked set. Docking energies are represented by a transparency scale whereas the poses themselves are represented by color. Briefly, the Boltzmann population of each pose is calculated whereby a high Boltzmann is equivalent to an opaque surface and a low Boltzmann is related to a transparent surface. A color scale is produced, and the more yellow the structural representation, the closer the total population is in conformation to the lowest energy binding pose. Importantly we have added confidence that our docking protocol is working effectively, as a significant population of poses exhibited a low rmsd (root mean square deviation) with the lowest energy pose for each. The Boltzmann distributions were as follows: 55.22% of docked structures have an rmsd of 0 with the lowest energy pose of combretastatin docked in tubulin, 95.57% for **11j** and 97.61% for **11g**, indicating that the conformations depicted for **11g** and **11j** in Figure 4 are accurate representations of the lowest energy conformations for the docked molecules **11g** and **11j**.

Conclusion

A series of polymethoxylated rigid analogs of combretastatin which contain a benzoxepin ring in place of the usual ethylene bridge present in the natural combretastatin products have been synthesized. The compounds displayed moderate antiproliferative activity when evaluated against the MCF-7 and MDA-MB 231 human breast carcinoma cell lines. 5-(3-Hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,3-dihydro-benzoxepin (**11g**) was found to be the most potent derivative and showed an IC_{50} value of 850 nM when evaluated against the MCF-7 breast cancer cell line. From molecular modeling studies, the structures of the active compounds **11g** and **11j** were shown to adopt a conformation in which the two aromatic rings are not coplanar, and can align within the colchicine binding site of tubulin. The conformationally restricted 4,5-diarylbenzoxepins **11a–l** (Table 2), structurally similar to colchicines and combretastatin, are shown to be potentially useful scaffolds for the further development of antitumor agents which are designed to target tubulin polymerization.

Declaration of interest

This work was supported through funding from the Trinity College IITAC research initiative (HEA PRTLI), Enterprise Ireland (EI), Science Foundation Ireland (SFI), and the Health Research Board (HRB), with additional support for computational facilities from the Wellcome Trust. A post-

graduate research award from Trinity College is gratefully acknowledged.

References

1. Jordan MA, Wilson L.Microtubules as a target for anticancer drugs.Nat Rev Cancer 2004, 4: 253–65.
2. Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. Curr Med Chem Anticancer Agents 2002;2:1–17.
3. Attard G, Greystoke A, Kaye S, De Bono J. Update on tubulin-binding agents. Pathol Biol (Paris) 2006;54:72–84.
4. Tron GC, Pagliai F, Del Grosso E, Genazzani AA, Sorba G. Synthesis and cytotoxic evaluation of combretafurazans. J Med Chem 2005;48:3260–8.
5. ter Haar E, Rosenkranz HS, Hamel E, Day BW. Computational and molecular modeling evaluation of the structural basis for tubulin polymerization inhibition by colchicine site agents. Bioorg Med Chem 1996;4:1659–71.
6. Rai SS, Wolff J. Localization of the vinblastine-binding site on beta-tubulin. J Biol Chem 1996;271:14707–11.
7. Pettit GR, Singh SB, Boyd MR, Hamel E, Pettit RK, Schmidt JM, et al. Antineoplastic agents. 291.*Isolation and synthesis of combretastatins A-4, A-5, and A*-6(1a). J Med Chem 1995;38:1666–72.
8. Andreu JM, Barasoain I. The interaction of baccatin III with the taxol binding site of microtubules determined by a homogeneous assay with fluorescent taxoid. Biochemistry 2001;40:11975–84.
9. Diaz JF, Barasoain I, Andreu JM. Fast kinetics of Taxol binding to microtubules. Effects of solution variables and microtubule-associated proteins. J Biol Chem 2003;278:8407–19.
10. Pettit GR, Rhodes MR, Herald DL, Hamel E, Schmidt JM, Pettit RK. Antineoplastic agents. 445. Synthesis and evaluation of structural modifications of (Z)- and (E)-combretastatin A-41. J Med Chem 2005;48:4087–99.
11. Pettit GR, Singh SB, Hamel E, Lin CM, Alberts DS, Garcia-Kendall D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. Experientia 1989;45:209–11.
12. Hori K. Antineoplastic strategy: irreversible tumor blood flow stasis induced by the combretastatin A-4 derivative AVE8062 (AC7700). Chemotherapy 2005;51:357–60.
13. Nam NH. Combretastatin A-4 analogues as antimitotic antitumor agents. Curr Med Chem 2003;10:1697–722.
14. Bailly C, Bal C, Barbier P, Combes S, Finet JP, Hildebrand MP, et al. Synthesis and biological evaluation of 4-arylcoumarin analogues of combretastatins. J Med Chem 2003;46:5437–44.
15. Ganina OG, Daras E, Bourgarel-Rey V, Peyrot V, Andresyuk AN, Finet JP, et al. Synthesis and biological evaluation of polymethoxylated 4-heteroarylcoumarins as tubulin assembly inhibitor. Bioorg Med Chem 2008;16:8806–12.
16. Kim Y, Nam NH, You YJ, Ahn BZ. *Synthesis and cytotoxicity of 3,4-diaryl-*2(5H)-furanones. Bioorg Med Chem Lett 2002;12:719–22.
17. Wang L, Woods KW, Li Q, Barr KJ, McCroskey RW, Hannick SM, et al. Potent, orally active heterocycle-based combretastatin A-4 analogues: synthesis, structure-activity relationship, pharmacokinetics, and in vivo antitumor activity evaluation. J Med Chem 2002;45:1697–711.
18. Pirali T, Busacca S, Beltrami L, Imovilli D, Pagliai F, Miglio G, et al. Synthesis and cytotoxic evaluation of combretafurans, potential scaffolds for dual-action antitumoral agents. J Med Chem 2006;49:5372–6.
19. Medarde M, Ramos A, Caballero E, de Clairac RPL, Lopez JL, Gravalos DG, et al. Synthesis and antineoplastic activity of combretastatin analogues: heterocombretastatins. Eur J Med Chem 1998;33:71–7.
20. De Martino G, La Regina G, Coluccia A, Edler MC, Barbera MC, Brancale A, et al. Arylthioindoles, potent inhibitors of tubulin polymerization. J Med Chem 2004;47:6120–3.
21. Lloyd DG, Hughes RB, Zisterer DM, Williams DC, Fattorusso C, Catalanotti B, et al. Benzoxepin-derived estrogen receptor modulators: a novel molecular scaffold for the estrogen receptor. J Med Chem 2004;47:5612–15.
22. Meegan MJ, Barrett I, Zimmermann J, Knox AJ, Zisterer DM, Lloyd DG. Benzothiepin-derived molecular scaffolds for estrogen receptor modulators: synthesis and antagonistic effects in breast cancer cells. J Enzyme Inhib Med Chem 2007;22:655–66.
23. Cushman M, Nagarathnam D, Gopal D, Chakraborti AK, Lin CM, Hamel E. Synthesis and evaluation of stilbene and dihydrostilbene derivatives as potential anticancer agents that inhibit tubulin polymerization. J Med Chem 1991;34:2579–88.
24. Freedman JS, Kenneth T. *The preparation of 3,4-dihydro-1-benzoxepin-*5(2H)-ones. J Heterocycl Chem 1989;26:1547–54.
25. Tandon VK, Khanna JM, Arand N, Srimal RC, Prasad CR. Agents acting on the central nervous system. XX.5-Subsitituted and 4,5-disubstituted 2,3,4,5-tetrahydro-1-benzoxepines. Indian J Chem 1975;13:1–8.
26. ACD/Chemsketch v10. Atlanta, GA: Advanced Chemistry Labs (http://www.acdlabs.com/download/chemsk.html).
27. Molecular Operating Environment (MOE). Montreal: Chemical Computing Group (http://www.chemcomp.com).
28. Zhang SX, Feng J, Kuo SC, Brossi A, Hamel E, Tropsha A, et al.Antitumor agents. 199. Three-dimensional quantitative structure-activity relationship study of the colchicine binding site ligands using comparative molecular field analysis. J Med Chem 2000;43:167–76.
29. Boland GM, Donnelly DMX, Finet JP, Rea MD. Synthesis of neoflavones by Suzuki arylation of 4-substituted coumarins. J Chem Soc Perkin Trans 1 1996;(21):2591–7.
30. Flynn BL, Flynn GP, Hamel E, Jung MK. The synthesis and tubulin binding activity of thiophene-based analogues of combretastatin A-4. Bioorg Med Chem Lett 2001;11:2341–3.
31. Gaukroger K, Hadfield JA, Lawrence NJ, Nolan S, McGown AT. Structural requirements for the interaction of combretastatins with tubulin: how important is the trimethoxy unit? Org Biomol Chem 2003;1:3033–7.
32. Lawrence NJ, Hepworth LA, Rennison D, McGown AT, Hadfield JA. Synthesis and anticancer activity of fluorinated analogues of combretastatin A-4. J Fluorine Chem 2003;123:101–8.
33. Maya AB, del Rey B, Lamamie de Clairac RP, Caballero E, Barasoain I, Andreu JM, et al. Design, synthesis and cytotoxic activities of naphthyl analogues of combretastatin A-4. Bioorg Med Chem Lett 2000;10:2549–51.
34. Ravelli RB, Gigant B, Curmi PA, Jourdain I, Lachkar S, Sobel A, et al. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. Nature 2004;428:198–202.
35. Jordan A, Hadfield JA, Lawrence NJ, McGown AT. Tubulin as a target for anticancer drugs: agents which interact with the mitotic spindle. Med Res Rev 1998;18:259–96.
36. Springer C, Adalsteinsson H, Young MM, Kegelmeyer PW, Roe DC. PostDOCK: a structural, empirical approach to scoring protein ligand complexes. J Med Chem 2005;48:6821–31.

Copyright of Journal of Enzyme Inhibition & Medicinal Chemistry is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.