

Tubulin-binding dibenz[*c,e*]oxepines as colchinel analogues for targeting tumour vasculature†David J. Edwards,^{a,b} John A. Hadfield,^{b,c} Timothy W. Wallace^{*a} and Sylvie Ducki^{‡c}

Received 27th July 2010, Accepted 12th October 2010

DOI: 10.1039/c0ob00500b

Various methoxy- and hydroxy-substituted dibenz[*c,e*]oxepines were prepared *via* the copper(I)-induced coupling of ether-tethered arylstannanes or the dehydrative cyclisation of 1,1'-biphenyl-2,2'-dimethanols, assembled using the Ullmann cross-coupling of *ortho*-bromoaryl carbonyl compounds. The dibenzoxepines were screened for their ability to inhibit tubulin polymerisation and the *in vitro* growth of K562 human chronic myelogenous leukemia cells. The most active was 5,7-dihydro-3,9,10,11-tetramethoxydibenz[*c,e*]oxepin-4-ol, whose tubulin inhibitory and cytotoxicity (IC₅₀) values were 1 µM and 40 nM, respectively.

Introduction

Tumour growth requires the support of an associated blood supply, making tumour vasculature a potential target for anti-cancer therapy. This principle has inspired decades of research into the pathways of angiogenesis (the formation of new blood vessels), leading to the identification of a family of vascular endothelial growth factors (VEGFs) that stimulate this process.¹ The subsequent search for VEGF inhibitors yielded *inter alia* the monoclonal antibody bevacizumab (Avastin®), which in 2004 became the first angiogenesis inhibitor to be approved in the USA for clinical use in the treatment of cancer.² While the underlying principles and clinical practices of anti-angiogenic therapy continue to evolve,³ an alternative antivasculature strategy is emerging. Tumour vasculature is structurally and functionally abnormal, being disorganised and prone to inefficiencies caused by branching, uneven diameter, shunts, *etc.*, rendering it susceptible to the effects of vascular disrupting agents (VDAs).⁴ Various small molecules have come under scrutiny in this context,⁵ the most prominent being combretastatin A-4 (CA-4) **1**, which was isolated by Pettit and coworkers from the African bush willow *Combretum caffrum*⁶ and shown to induce shutdown of tumour vasculature within minutes, while leaving normal vasculature intact.⁷ Clinical trials of the water-soluble prodrug CA-4P **2** (Zybrestat) were initiated in 1998, and the congener combretastatin A-1 **3** is also

under development as an antitumour agent in the form of the phosphate prodrug **4** (OXi4503).⁸ Other structures undergoing evaluation as VDAs include the combretastatin analogue **5** (AVE8062), which abruptly and irreversibly stops tumour blood flow in a range of cancer cell lines,⁹ the analogue **6** of the marine natural product dolastatin,¹⁰ the xanthenylacetic acid **7**,¹¹ the substituted heterocycles **8**,¹² **9**,¹³ **10**,¹⁴ **11**,¹⁵ **12**¹⁶ and **13**,¹⁷ and the phosphate prodrug **14** of *N*-acetylcolchinel.¹⁸ With the notable exception of **7**, which appears to act both through the host immune system, *e.g.* by stimulating the production of tumour necrosis factor (TNF),¹⁹ and directly by inducing vascular endothelial cell apoptosis,²⁰ the biological target of these potential VDAs is tubulin.

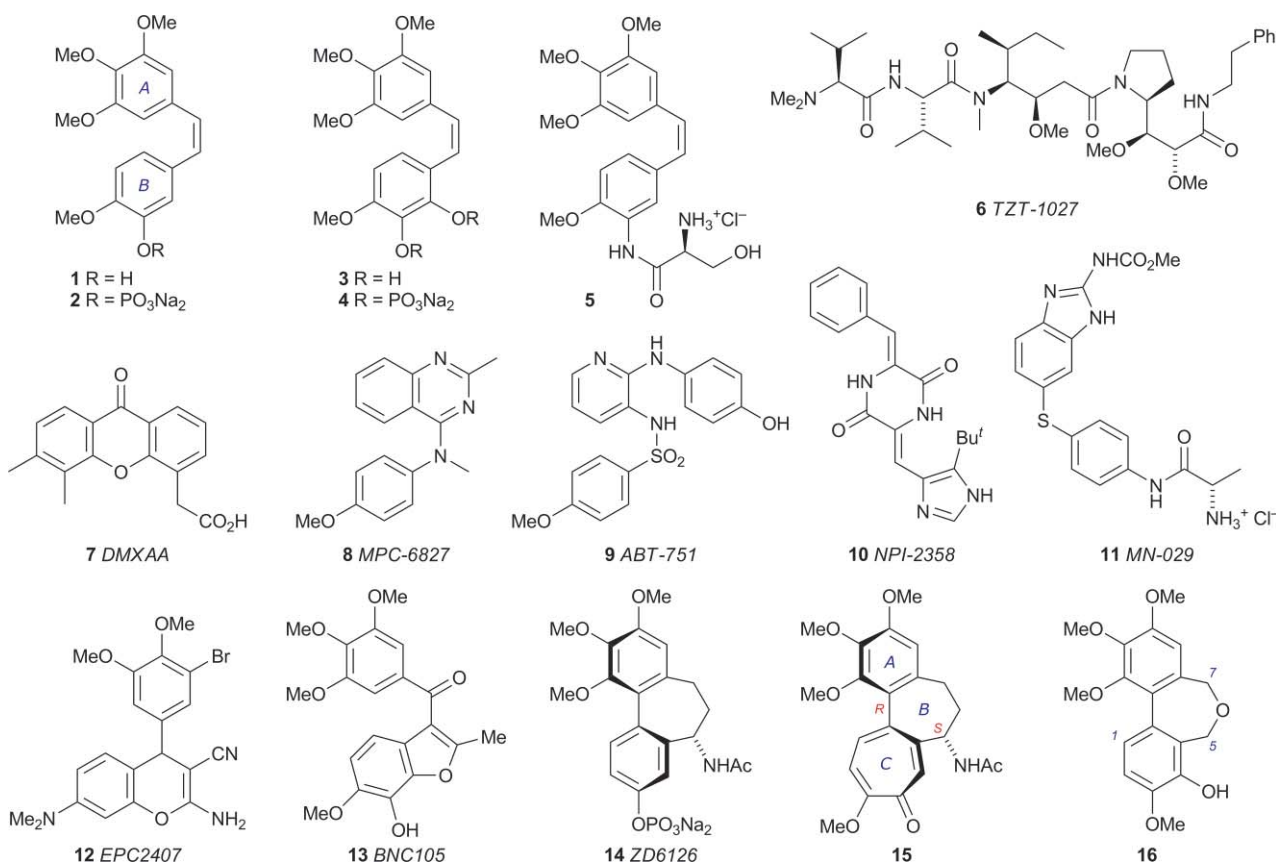
Tubulin, in the form of various isotypes, is an abundant component of the cytoplasm of animal cells. Two GTP-binding monomers, α- and β-tubulin, form a heterodimer whose polymerisation generates microtubules, which occupy a pivotal role in a range of intracellular processes involving structure, shape, signalling and transport, including chromosome segregation and positioning during mitosis. The dynamics of microtubule assembly and disassembly are responsive to the identity of the nucleotide bound to the β-tubulin of its terminal heterodimer unit, the microtubule being viable when this is GTP but prone to rapid shrinkage when it is exchanged for GDP.²¹ The dynamics of tubulin–microtubule interconversion are delicately poised and can be disrupted by coordinating species: some of the tubulin binders in clinical use (taxanes, epothilones) stabilise the GDP-bound tubulin in the microtubule, thereby inhibiting its disassembly,²² while others (vincristine, etoposide) bind to the αβ-tubulin heterodimer and inhibit microtubule assembly.²³ The origin and subtleties of the latter type of inhibition have become more apparent since the acquisition of high-resolution crystallographic structures of tubulin–drug complexes by Knossow and coworkers.^{24,25} Tubulin binding agents can exert a direct cytotoxic effect by perturbing microtubule dynamics, thereby undermining

^aSchool of Chemistry, The University of Manchester, Oxford Road, Manchester, UK M13 9PL. E-mail: tim.wallace@manchester.ac.uk; Fax: +44 (0) 161 275 4939

^bDrug Development Group, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester, UK M20 4BX

^cKidsCan Laboratories, Centre for Molecular Drug Design, School of Environment and Life Sciences, University of Salford, Salford, UK M5 4WT
† Electronic supplementary information (ESI) available: Further experimental details. See DOI: 10.1039/c0ob00500b

‡ Present address: Laboratoire de Chimie des Hétérocycles et des Glucides, ENSCCF, Clermont Université, 63174 Aubière, France.



mitosis (which requires the rapid turnover of microtubules at all stages) and bringing about cell death. Microtubules also play a prominent role in maintaining the physical structure of the endothelial cells lining new tumour vasculature, which lack the well-defined actin cytoskeleton and other strengthening features of mature endothelial cells, and it has been established that some tubulin-binding VDAs induce morphological changes in the endothelial cells of immature tumour vasculature, *e.g.* rounding and detachment, leading to reduced blood flow and tumour necrosis.^{4,18b,26} Significantly, vascular shutdown by CA-4 **1** is achieved using substantially less than the maximum tolerated dose (MTD), illustrating one of the potential advantages of targeting tumour vasculature rather than the mitotic apparatus, which generally involves less favourable therapeutic margins.

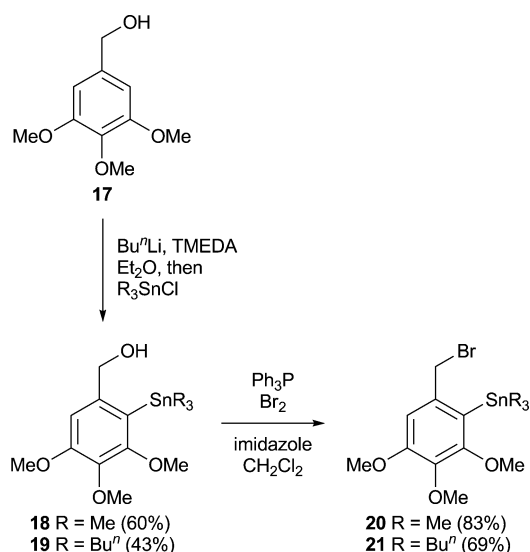
In common with CA-4 **1**,²⁷ the structures **8–14** bind to tubulin at or close to the same site as colchicine **15**, the best known tubulin-binding agent and 'spindle toxin'.²⁸ The toxicity of colchicine **15** precludes its clinical use as an antimetabolic agent, but it is clear that the colchicine binding site of tubulin can accommodate a diverse range of structures and so offers considerable scope for the design of binding agents with minimised pharmacokinetic half-lives and cardiostimulatory effects,²⁹ the latter being a potentially generic problem with tubulin-targeting VDAs.^{18d,30} Our interest in the axial chirality of colchicine **15** led us to analyse the variation of the interaryl dihedral angle in a series of heterocyclic variants of the bridged biaryl core,³¹ and we observed that the degree of helicity in dibenz[*c,e*]oxepines closely matches that found in colchicine **15**. In pursuing this line we synthesised a series of new dibenz[*c,e*]oxepines and assessed their ability to inhibit tubulin polymerisation, which led to the identification of 5,7-dihydro-

3,9,10,11-tetramethoxydibenz[*c,e*]oxepin-4-ol **16** as a new lead in the search for effective VDAs.³² Our results, herein described in detail, suggest that a dibenz[*c,e*]oxepine unit may be capable of providing the helical core of a new series of tubulin-binding small molecules.³³

Synthesis of materials

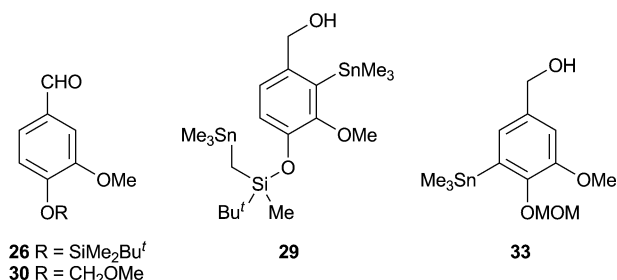
Initially we assessed the methods available for biaryl synthesis, seeking to identify those suited for use in approaches to polysubstituted dibenz[*c,e*]oxepines. Although not ideal from an environmental viewpoint, the copper(I)-mediated intramolecular coupling of tethered arylstannane units had been shown by Piers *et al.*³⁴ to be an effective source of the target heterocyclic system, and we therefore chose to apply this method to our first targets. To prepare the required series of doubly-stannylated dibenzyl ethers, we first acquired stocks of the stannylated trimethoxybenzyl bromides **20**³⁴ and **21** (Scheme 1) and then used analogous lithiation–transmetalation sequences to convert benzyl alcohol **22** and veratryl alcohol **24** into the respective tributyltin derivatives **23** and **25** (Scheme 2).

To extend the series, the protected vanillin **26** was reduced to **27** for use in the lithiation–stannylation sequence. However, in this case the yield of the desired alcohol **28** was poor (28%) and the by-products included the doubly-stannylated species **29** (11%). The formation of the latter, which was characterised *inter alia* by the ¹H NMR signals from its diastereotopic SnCH₂Si group [δ_{H} (400 MHz, CDCl₃) 0.00 (1 H, d, *J* 19.4 Hz), –0.07 (1 H, d, *J* 19.4 Hz)], serves as a reminder³⁵ of the increased susceptibility of a *t*-butyldimethylsilyloxy group towards deprotonation by an



Scheme 1

alkyllithium when a second lithium coordination site, in this case the methoxy group, is located nearby. Repeating the sequence with the MOM-protected vanillin **31** also proved troublesome, with poor conversion to the desired alcohol **32** (23%) and the formation of a comparable amount of the regioisomeric product **33** (19%). Using an alternative stannylation procedure based on lithium–bromine exchange, the bromo alcohol **34** provided a fair yield of **35** in straightforward fashion.



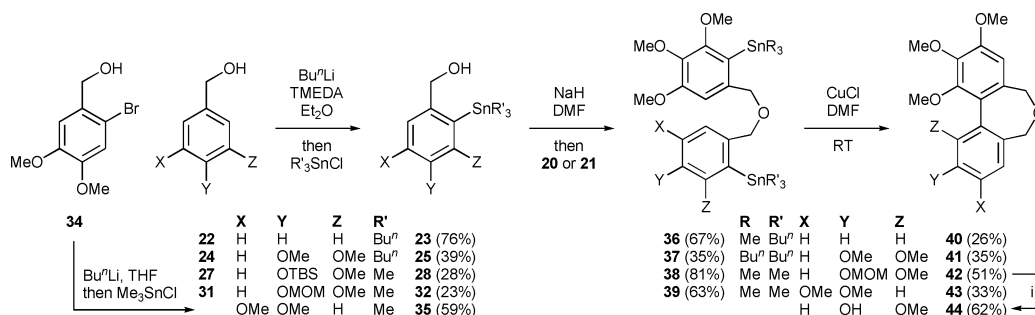
The alcohols **23**, **25**, **32** and **35** were converted into the respective dibenzyl ethers **36–39** using the appropriate aryl bromide **20** or **21**. These etherifications were more efficient when using the trimethylstannyl bromide **20**, presumably for steric reasons. Subjecting the ethers **36–39** to the conditions of the cyclisation process³⁴ gave the desired dibenz[*c,e*]oxepines **40–43** in yields that were, at best, modest, but allowed the isolation of sufficient material

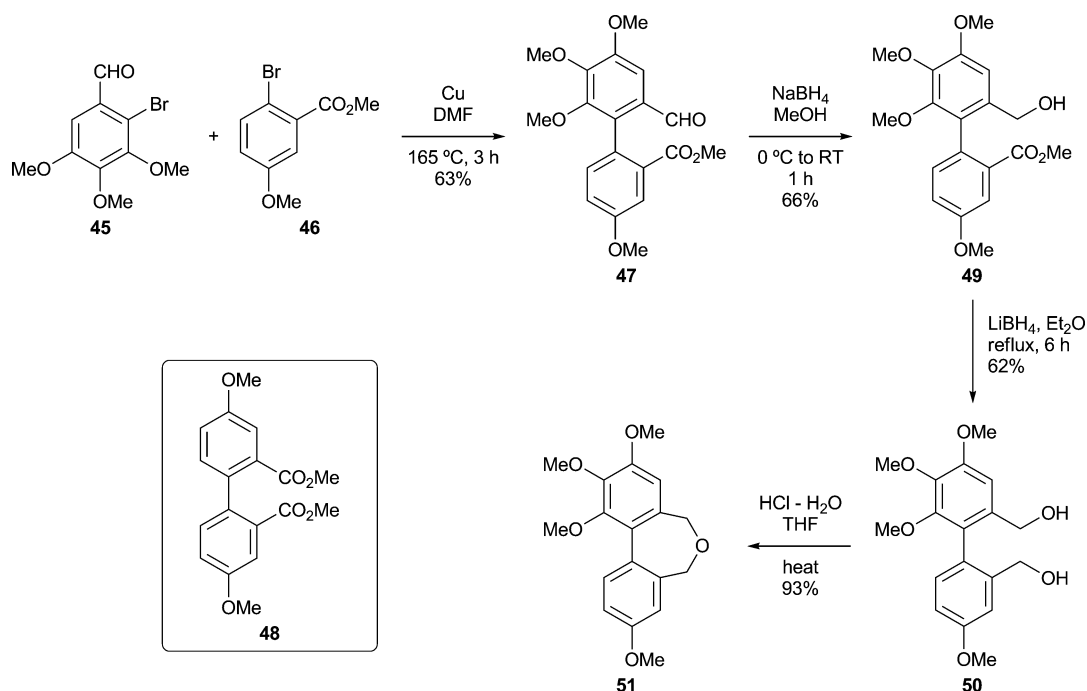
for testing purposes and they therefore remain to be optimised. A sample of the MOM-protected variant **42** was transformed into the corresponding phenol **44** using a mild procedure.³⁶

Other dibenz[*c,e*]oxepines were synthesised using the Ullmann reaction³⁷ to effect the coupling of appropriate substituted haloarenes. Although conventional Ullmann cross-couplings tend to give mixtures containing homocoupled products, they offer rapid access to certain types of target structure and can be optimised by modifying the reaction conditions and stoichiometry so as to inhibit homocoupling,^{38,39} or by the quantitative formation of the intermediate arylcopper species from one of the reaction partners prior to its exposure to the other.⁴⁰ In our first Ullmann approach (Scheme 3), we found that combining mole equivalents of the bromoaldehyde **45** and the bromoester **46** gave an acceptable yield of **47**, although chromatography was required to isolate it from the homocoupled product **48** (12%). Reduction of the carbonyl functions of **47** provided, successively, the ester-alcohol **49** and the diol **50**. The latter was transformed into the oxepine **51** upon treatment with aqueous acid.⁴¹

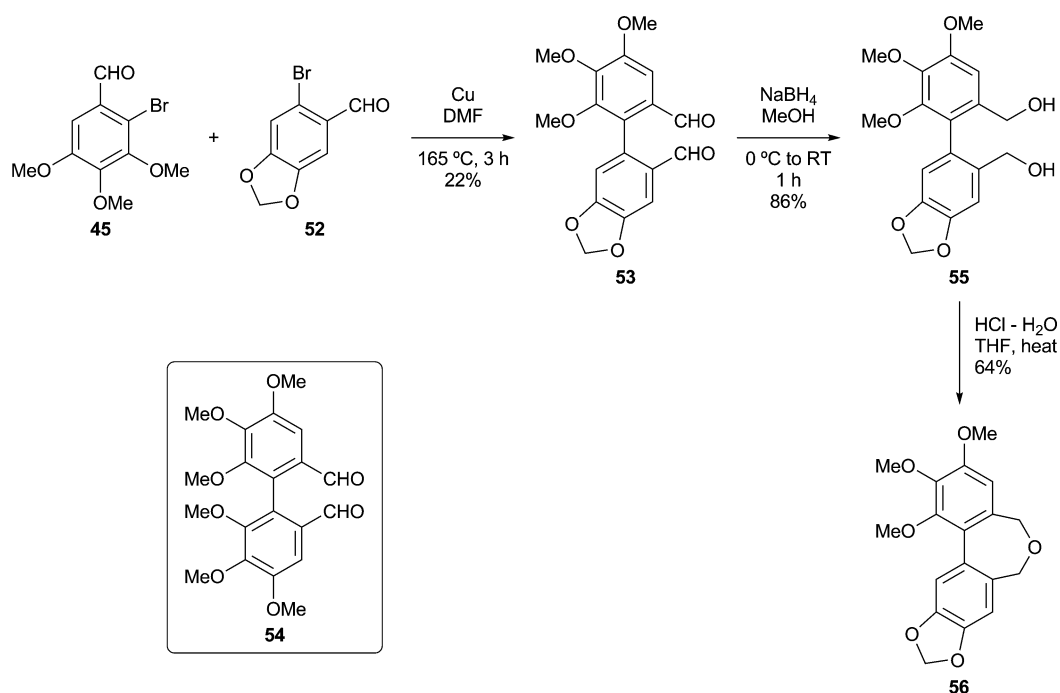
In a second Ullmann cross-coupling (Scheme 4), reacting **45** with 6-bromopiperonal **52** gave the dialdehyde **53** (22%), the low isolated yield in this case being partly due to the use of crystallisations to remove the contaminating dialdehyde **54**. The dialdehyde **53**³⁸ and related structures are useful lignan precursors that can also be prepared *via* Suzuki–Miyaura coupling protocols.⁴² Reduction of **53** to the diol **55**, followed by ring closure as before, gave the dibenzoxepine **56** as a crystalline solid.

Based on our analysis of the structure–activity relationships in known colchicine, allocolchicine and combretastatin derivatives, we surmised that the substitution pattern present in the dibenzoxepine **16** was particularly worthy of study. The proposed route to this compound required a hydroxyl protecting group that would withstand the conditions of the Ullmann coupling reaction, and encouraged by a literature precedent⁴³ we elected to use a methanesulfonyl (mesyl) group in this capacity. The route to the target therefore began with the mesylation of the commercially available bromoaldehyde **57** under standard conditions (Scheme 5). This provided a good yield of the mesylate **58**, together with a by-product identified as the sultone **59** (22%) derived from **58** *via* the base-induced condensation of the mesylate and aldehyde groups. We surmise that the formation of **59**, whose heterocyclic core is rare, is assisted by the buttressing effect of the flanking OMe and Br functions. Ullmann coupling of the mesylate **58** with the bromoaldehyde **45** (3 equiv.), followed by chromatography, gave an acceptable yield of the dialdehyde **60**,

Scheme 2 Reagents: i, Montmorillonite K10, CH₂Cl₂, RT, 3 h.



Scheme 3

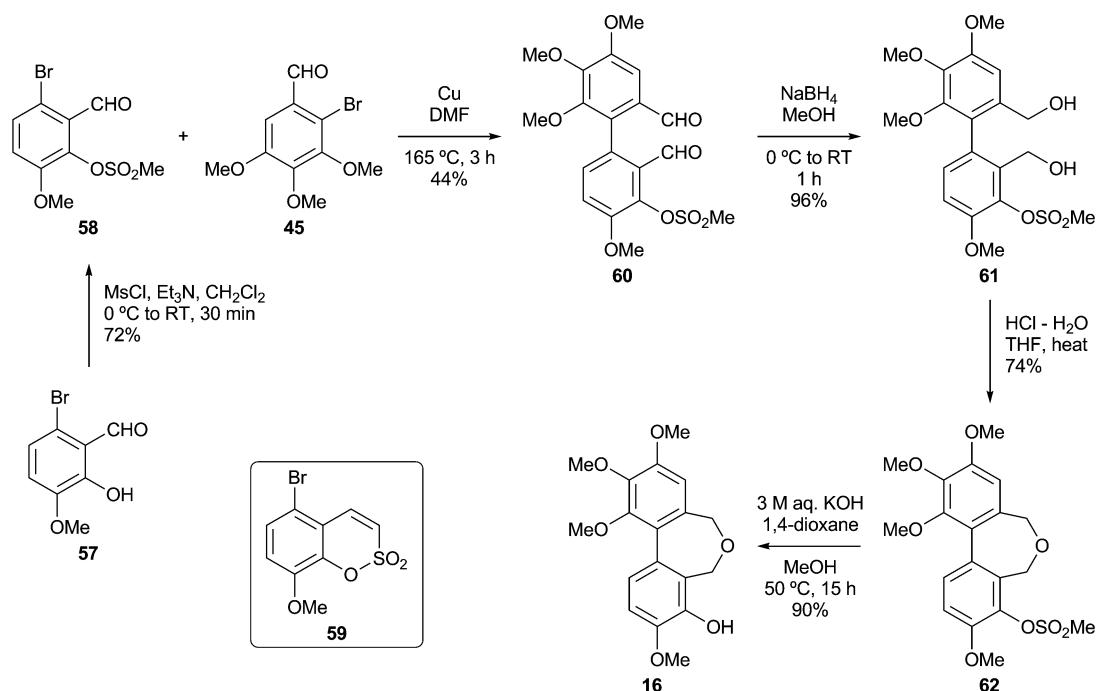


Scheme 4

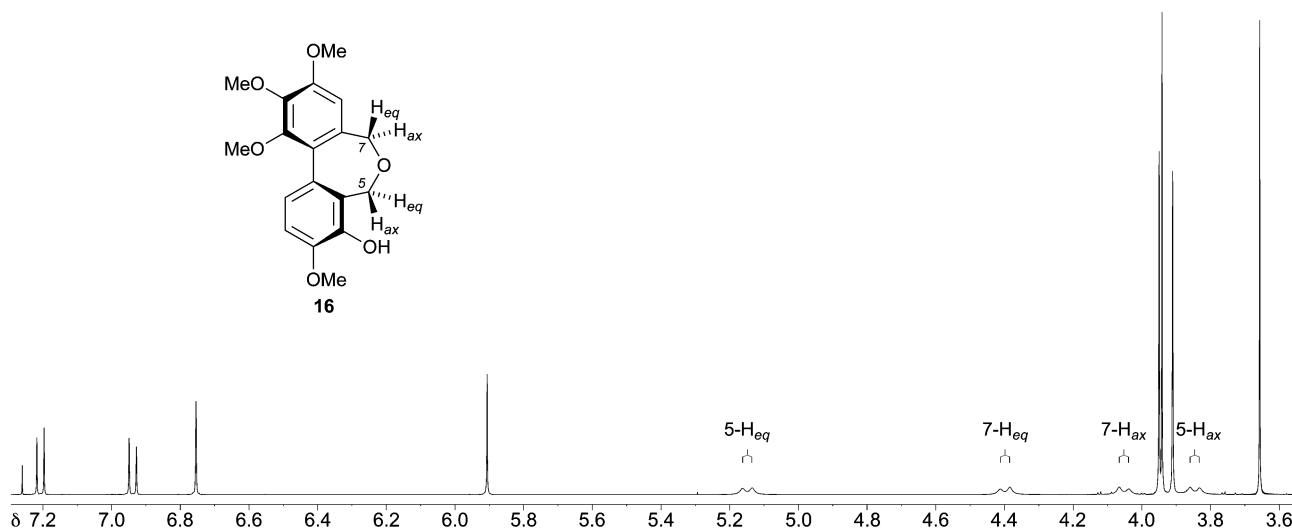
and subsequent reduction followed by acid-induced cyclisation gave the desired mesylate **62**. Alkaline hydrolysis of **62** provided the target dibenzoxepine **16** as a white crystalline solid, m.p. 145–147 °C (MeOH). The ¹H NMR spectrum of **16** (Fig. 1) illustrates the line broadening for the diastereotopic methylene signals that is typical of compounds of this type, in which the biaryl unit is non-planar and fluctional, undergoing axis inversion slowly on the NMR time scale.⁴⁴

Compound evaluation

New materials were screened for their ability to inhibit microtubule assembly⁴⁵ and for growth inhibitory activity (IC₅₀) against the K562 human chronic myelogenous leukemia cell-line.⁴⁶ Both of these assays are routinely used for the evaluation of test compounds and provide a useful comparison with benchmarks such as CA-4 **1** and colchicine **15**. The results (Table 1) show



Scheme 5

Fig. 1 ^1H NMR spectrum (400 MHz, CDCl_3 , 25°C) of the dibenzoxepine **16**.

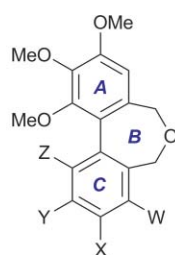
that, as might be expected, biological activity is dependent on the aromatic substitution pattern, and reveal useful levels of activity in the dibenzoxepines **16**, **51** and **56** (entries 3, 9 and 10). In particular, the phenol **16** inhibits tubulin assembly to the same extent as CA-4 **1**, whose substituent array is the same, and returned an IC_{50} value of 40 nM against the K562 cell-line. None of the other compounds inhibited tubulin polymerisation to a significant degree, although **43** and **44** (entries 7 and 8) displayed some *in vitro* cytotoxicity.

Discussion

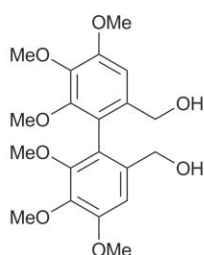
The difference in potency of **40** and **51** as tubulin polymerisation inhibitors mirrors that of their respective carbocyclic analogues **71**

($\text{IC}_{50} > 50 \mu\text{M}$)⁴⁸ and **72** (IC_{50} 1.5 μM),⁴⁹ and indicates that at least one oxygen substituent in the C-ring is a prerequisite for tubulin-binding activity. However, the hexamethoxy series comprising **63** and **66–70**, which had been prepared from the diol **64** for our crystallographic study,³¹ was almost devoid of activity. The cytotoxicity of the dibenzazepine **66** (entry 13) is intriguing, given that its C-ring oxygenation pattern cannot be viewed as optimal, although it is recognised that the link between cytotoxicity and the ability to inhibit tubulin polymerisation is potentially complex.

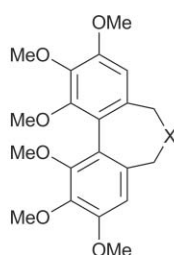
The properties of the dibenzoxepine **16** are consistent with its structural analogy to the colchicinoids, e.g. **14** and **15**. The interaction of colchicine **15** with tubulin has long been under intense scrutiny, now informed by the 1SA0 crystal structure,



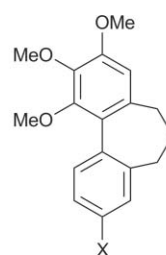
	W	X	Y	Z
16	OH	OMe	H	H
40	H	H	H	H
41	H	H	OMe	OMe
42	H	H	OMOM	OMe
43	H	OMe	OMe	H
44	H	H	OH	OMe
51	H	OMe	H	H
56	H	O-CH ₂ -O	H	H
62	OMs	OMe	H	H
63	H	OMe	OMe	OMe



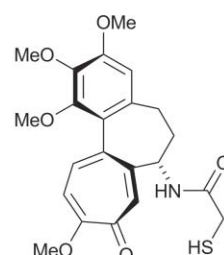
64



66 X = NBn
 67 X = NMe
 68 X = S
 69 X = SO
 70 X = SO₂



71 X = H
 72 X = OMe



73

Table 1 Activities (IC₅₀) of substituted dibenzoxepines, dibenzazepines and dibenzothiepienes in the microtubule assembly and K562 cytotoxicity assays

Entry	Compound	Tubulin assembly IC ₅₀ /μM ^a	K562 MTT assay IC ₅₀ /μM ^b
1	1	1.3	0.0010 ^c
2	15	3.9	0.0022 ^d
3	16	1.0	0.04
4	40	>10	>20
5	41	>10	>20
6	42	>10	>20
7	43	>10	3
8	44	>10	19
9	51	7.4	0.13
10	56	>10	0.10
11	62	>10	>20
12	63	>10	>400
13	66	>10	73
14	67	>10	360
15	68	>10	>400
16	69	>10	>400
17	70	>10	>400

^a Concentration required for 50% inhibition of tubulin assembly.

^b Concentration that inhibits the growth of the K562 cell line by 50% after incubation for 5 days. Each drug concentration was tested in triplicate, and the standard error of each value is <10%. ^c All entries in this column are normalised to this value for **1**, which varied over the range 0.0010–0.0022 between batches. ^d Value taken from ref. 47.

which gives a detailed picture of the interaction of *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine (DAMA-colchicine) **73** with the αβ-tubulin heterodimer.²⁴ A comparison of the tubulin-bound **73** with a model of the dibenzoxepine **16** (Fig. 2) reveals some clear parallels. We speculate that, as has been proposed for CA-4 **1**,⁵⁰ the phenolic hydroxyl of **16** is suitably placed to emulate the side-chain N–H of the colchicinoids in H-bonding to the carbonyl oxygen of the residue Thr179 located on the adjacent α-tubulin chain, while the 3-methoxy group of **16** can interact with the side-chain nitrogen of the β-tubulin residue Lys352. In the absence of substituents at C(1), C(5) and C(7), the biaryl unit of **16** has a configurationally unbiased axis of the *tropos* type,⁵¹ i.e. whose low inversion barrier renders it free to adopt the (*aR*) arrangement required for binding. It is therefore proposed that, while chemically distinct from CA-4

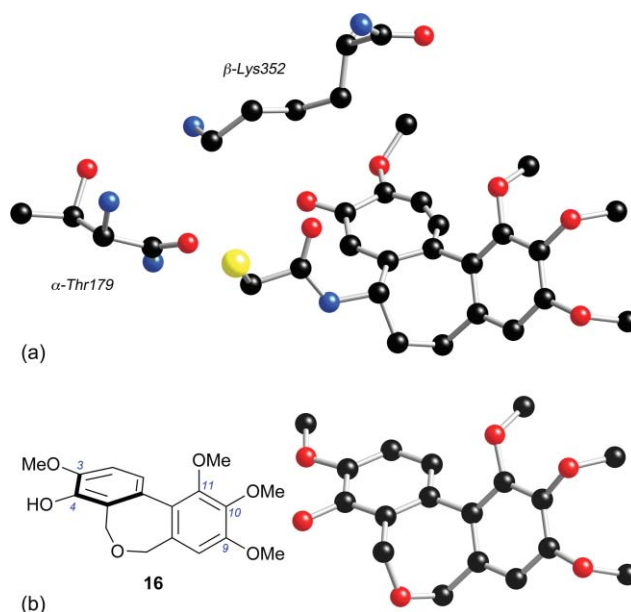


Fig. 2 (a) The tubulin-binding conformation of DAMA-colchicine **73**, extracted from the crystal structure of the DAMA-colchicine/tubulin:RB3 conjugate (ref. 24), together with the nearby β-Lys352 and α-Thr179 residues (numbering system from ref. 24). (b) The energy-minimised structure of dibenzoxepine **16** (MacroModel 8, MM3 force field).

1 and the colchicinoids **14** and **15**, the dibenzoxepine **16** binds to tubulin in a similar manner.

Conclusion

In the course of this study we have synthesised a series of heterocyclic colchicinol analogues and monitored their ability to inhibit microtubule assembly and the *in vitro* growth of K562 human chronic myelogenous leukemia cells, leading us to identify 5,7-dihydro-3,9,10,11-tetramethoxydibenz[*c,e*]oxepin-4-ol **16** as a potent tubulin-binding and cytotoxic agent. Our results suggest that replacing the central methylene group in the three-atom bridge of colchicinol analogues with an oxygen atom does not diminish the ability of suitably substituted derivatives of the system to bind to tubulin and, by inference, interfere with cell

division processes and the integrity of tumour neovasculature. We consider the structure **16** to be the leading member of a new series of potential therapeutic agents based on the hitherto unexploited dibenz[*c,e*]oxepine pharmacophore, and are currently pursuing this principle in the context of a search for clinically useful antitumour agents and VDAs.

Experimental

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 CaCl₂ drying tube and freed from traces of oxygen using an Oxysept cartridge (both Aldrich). Tetrahydrofuran (THF) and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) were dried using sodium benzophenone ketyl under argon. Organic solutions were usually dried using anhydrous MgSO₄ and concentrated by rotary evaporation under reduced pressure. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 on aluminium plates containing a 254 nm fluorescent indicator. The chromatograms were visualised by the use of UV light or the following developing agents; ethanolic vanillin or potassium permanganate. Unless otherwise indicated, preparative (column) chromatography was carried out using the flash technique⁵³ 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 **16**, **51** and **56** have also been described in a patent application.³² Compounds **18**,³⁴ **20**,³⁴ **63**³¹ and **67–70**³¹ were prepared using published procedures.

General procedure for alcohols **19**, **25**, **28** and **32**

To a stirred solution of *n*-BuLi (2.5 eq.) in dry ether (10 mL mmol⁻¹ of alcohol) at –78 °C was added TMEDA (2.5 eq.) and stirring was continued for 10 min. The neat alcohol was added and the solution was allowed to warm to RT. After stirring for 3 h, the reaction was cooled to –78 °C and the trialkyltin chloride (1.5 eq.) was added. The reaction was warmed to RT and stirred for 2 h. Water (10 mL mmol⁻¹ of alcohol) was added and the mixture was extracted with ether (3 × 10 mL mmol⁻¹ of alcohol). The combined

organic extract was washed with brine (20 mL/mmol of alcohol), dried, evaporated, and the residue purified by chromatography.

3,4,5-Trimethoxy-2-tributylstannylbenzyl alcohol 19 was prepared from **17** (4.00 g, 20.2 mmol). Chromatography (hexane–ethyl acetate, 6:1) gave the *title compound 19* (4.20 g, 43%) as a colourless oil. Elemental analysis, ¹H NMR spectroscopy and MS indicated the presence of residual Bu₃SnCl. Other data: $\nu_{\max}/\text{cm}^{-1}$ 3464, 2955, 2928, 2866, 2854, 1584, 1561, 1460, 1379, 1313, 1196, 1157, 1099; δ_{H} (400 MHz, CDCl₃) 6.81 (1 H, s, 6-H), 4.54 (2 H, d, *J* 5.7 Hz, CH₂O), 3.85 (3 H, s, OMe), 3.84 (3 H, s, OMe), 3.80 (3 H, s, OMe), 1.71 (1 H, br t, *J* 5.7 Hz, OH), 1.52 (6 H, m, 3 × CH₂CH₂Sn), 1.34 (6 H, m, 3 × CH₂CH₃), 1.09 (6 H, t, *J* 8.3 Hz, 3 × CH₂Sn), 0.90 (9 H, t, *J* 7.3 Hz, 3 × CH₂CH₃); δ_{C} (100 MHz, CDCl₃, DEPT-135) 12.1 (CH₂), 14.1 (CH₃), 27.8 (CH₂), 29.6 (CH₂), 56.3 (CH₃), 60.8 (CH₃), 61.0 (CH₃), 67.4 (CH₂), 108.4 (CH); *m/z* (ES) 489 (MH⁺, 100%), 332, 291; *R*_f 0.30 (hexane–ethyl acetate, 6:1).

3,4-Dimethoxy-2-(tributylstannyl)benzyl alcohol 25 was prepared from **24** (3.40 g, 20.2 mmol). Chromatography (hexane–ethyl acetate, 6:1) gave the *title compound 25* (3.60 g, 39%) as a colourless viscous oil (Found: C, 55.0; H, 8.5; Sn, 25.9. C₂₁H₃₈O₃Sn requires C, 55.16; H, 8.38; Sn, 25.96%); $\nu_{\max}/\text{cm}^{-1}$ 3367, 2951, 2924, 2870, 2850, 1592, 1464, 1386, 1293, 1269, 1200, 1138, 1037, 870; δ_{H} (400 MHz, CDCl₃) 7.14 (1 H, d, *J* 8.1 Hz, 5-H), 6.89 (1 H, d, *J* 8.1 Hz, 6-H), 4.55 (2 H, d, *J* 5.7 Hz, CH₂O), 3.88 (3 H, s, OMe), 3.85 (3 H, s, OMe), 1.71 (1 H, br t, *J* 5.7 Hz, OH), 1.52 (6 H, m, 3 × CH₂CH₂Sn), 1.34 (6 H, m, 3 × CH₂CH₃), 1.09 (6 H, t, *J* 8.3 Hz, 3 × CH₂Sn), 0.90 (9 H, t, *J* 7.3 Hz, 3 × CH₂CH₃); *R*_f 0.40 (hexane–ethyl acetate, 4:1).

2-Trimethylstannyl-4-(*O*-silyldimethyl-*t*-butyl)-3-methoxybenzyl alcohol 28 was prepared from **27**⁵⁵ (2.00 g, 7.45 mmol). Chromatography (hexane–ethyl acetate, 10:1) gave the crude stannane **28** (0.90 g, 28%) as a yellow viscous oil which was used without further purification; $\nu_{\max}/\text{cm}^{-1}$ 3309, 2955, 2928, 2854, 1581, 1460, 1383, 1282, 1196, 1134, 1002, 948, 893, 819; δ_{H} (400 MHz, CDCl₃) 7.01 (1 H, d, *J* 8.0 Hz, 5-H), 6.79 (1 H, d, *J* 8.0 Hz, 6-H), 4.53 (2 H, d, *J* 5.4 Hz, CH₂), 3.76 (3 H, s, OMe), 1.48 (1 H, br t, *J* 5.4 Hz, OH), 1.00 (9 H, s, CMe₃), 0.38 (9 H, SnMe₃), 0.18 (6 H, s, SiMe₂); δ_{C} (100 MHz, CDCl₃, DEPT-135) –5.7 (CH₃), –4.1 (CH₃), 26.2 (CH₃), 60.8 (CH₃), 66.8 (CH₂), 122.1 (CH), 124.8 (CH); *m/z* (ES) 415 (MH⁺–OH, 100%) (HRMS could not be performed due to low ionisation levels); *R*_f 0.30 (hexane–ethyl acetate, 8:1). Other fractions provided a sample of the ether **29** (0.50 g, 11%) as a colourless solid, m.p. 54–56 °C, which was identified from the following data: $\nu_{\max}/\text{cm}^{-1}$ 3320, 2963, 2928, 2854, 1581, 1464, 1386, 1285, 1196, 1134, 1006, 948, 893, 831, 769; δ_{H} (400 MHz, CDCl₃) 6.97 (1 H, d, *J* 8.0 Hz, 5-H), 6.79 (1 H, d, *J* 8.0 Hz, 6-H), 4.53 (2 H, d, *J* 4.6 Hz, OCH₂), 3.77 (3 H, s, OMe), 1.51 (1 H, br m, OH), 0.97 (9 H, s, CMe₃), 0.33 (9 H, s, ArSnMe₃), 0.22 (3 H, s, SiMe), 0.05 (9 H, s, CH₂SnMe₃), 0.00 (1 H, d, *J* 19.4 Hz, CHSnMe₃), –0.07 (1 H, d, *J* 19.4 Hz, CHSnMe₃); δ_{C} (100 MHz, CDCl₃, DEPT-135) –8.4 (CH₂), –7.3 (CH₃), –5.7 (CH₃), –2.5 (CH₃), 26.6 (CH₃), 60.8 (CH₃), 66.8 (OCH₂), 121.9 (CH), 124.7 (CH); *m/z* (ES) 577 (MH⁺–OH, 100%) (HRMS could not be performed due to low ionisation levels); *R*_f 0.35 (hexane–ethyl acetate, 8:1).

2-Trimethylstannyl-3-methoxy-4-methoxymethylbenzyl alcohol 32 was prepared from **31**^{56,57} (3.80 g, 19.2 mmol). Chromatography (hexane–ethyl acetate, 5:1 to 3:1) gave the *title compound 32* (1.60 g, 23%) as a colourless viscous oil (Found: C, 43.5; H, 6.4;

Sn, 32.7. $C_{13}H_{22}O_4Sn$ requires C, 43.25; H, 6.14; Sn, 32.88%; $\nu_{\max}/\text{cm}^{-1}$ 3421, 2971, 2936, 2909, 1464, 1394, 1262, 1192, 1157, 1134, 1080, 1002, 773; δ_H (400 MHz, $CDCl_3$) 7.12 (1 H, d, J 8.2 Hz, 5-H), 7.07 (1 H, d, J 8.2 Hz, 6-H), 5.20 (2 H, s, OCH_2O), 4.55 (2 H, s, $ArCH_2O$), 3.88 (3 H, s, $ArOMe$), 3.52 (3 H, s, CH_2OMe), 1.70 (1 H, br s, OH), 0.38 (9 H, $SnMe_3$); δ_C (100 MHz, $CDCl_3$, DEPT-135) -5.6 (CH_3), 56.6 (CH_3), 61.3 (CH_3), 66.7 (CH_2), 95.3 (CH_2), 117.3 (CH), 124.8 (CH); m/z (ES) 345 ($MH^+ - OH$, 70%), 315 (55), 206 (100); R_f 0.35 (hexane-ethyl acetate, 4:1). Other fractions provided a sample of the isomeric stannane **33** (1.30 g, 19%) as a colourless oil, δ_H (400 MHz, $CDCl_3$) 6.96 (1 H, d, J 1.9 Hz, ArH), 6.94 (1 H, d, J 1.9 Hz, ArH), 5.20 (2 H, s, OCH_2O), 4.55 (2 H, s, $ArCH_2O$), 3.85 (3 H, s, $ArOMe$), 3.50 (3 H, s, CH_2OMe), 1.65 (1 H, br s, OH), 0.35 (9 H, $SnMe_3$); δ_C (75 MHz, $CDCl_3$, DEPT-135) -8.1 (CH_3), 55.95 (CH_3), 58.0 (CH_3), 65.6 (CH_2), 99.1 (CH_2), 112.6 (CH), 126.8 (CH); m/z (ES) 345 ($MH^+ - OH$, 70%), 315 (55), 206 (100); R_f 0.18 (hexane-ethyl acetate, 4:1).

3,4,5-Trimethoxy-2-tributylstannylbenzyl bromide **21**

A solution of triphenylphosphine (1.62 g, 6.2 mmol) in dry DCM (50 mL) at 0 °C was treated dropwise with bromine (0.32 mL, 1.0 g, 6.2 mmol). The yellow colour that persisted was discharged by the addition of a few more crystals of triphenylphosphine, and the solution was stirred for a further 20 min. Imidazole (450 mg, 6.6 mmol) was then added in one portion and the stirring continued for a further 20 min. A solution of the alcohol **19** (2.31 g, 4.74 mmol) in DCM (10 mL) was added and the mixture was stirred at 0 °C for 20 min and RT for 1 h. Pentane (7 mL) was added and the white suspension filtered through a cake of silica gel (*ca.* 10 g), rinsing with ether (100 mL). Evaporation of the filtrate and chromatography of the residue (110 g silica gel, hexane-ethyl acetate, 6:1) gave the *title compound* **21** (1.80 g, 69%) as a colourless oil which was used without further purification; δ_H (400 MHz, $CDCl_3$) 6.68 (1 H, s, 6-H), 4.37 (2 H, s, CH_2Br), 3.81 (3 H, s, OMe), 3.79 (3 H, s, OMe), 3.75 (3 H, s, OMe), 1.45 (6 H, m, $3 \times CH_2CH_2Sn$), 1.27 (6 H, m, $3 \times CH_2CH_3$), 1.09 (6 H, t, J 8.3 Hz, $3 \times CH_2Sn$), 0.90 (9 H, t, J 7.3 Hz, $3 \times CH_2CH_3$); δ_C (100 MHz, $CDCl_3$, DEPT-135) 12.2 (CH_2), 14.0 (CH_3), 27.8 (CH_2), 29.5 (CH_2), 37.9 (CH_2), 56.4 (CH_3), 60.8 (CH_3), 61.0 (CH_3), 110.3 (CH); R_f 0.50 (hexane-ethyl acetate, 8:1).

4,5-Dimethoxy-2-trimethylstannylbenzyl alcohol **35**

A stirred solution of 2-bromo-4,5-dimethoxybenzyl alcohol **34** (2.90 g, 11.7 mmol) in THF (10 mL) at -78 °C was treated dropwise with *n*-BuLi (2.5 M in hexanes, 11.3 mL, 28.3 mmol). The solution was stirred for 1 h at -78 °C, treated dropwise with a solution of trimethyltin chloride (3.50 g, 17.6 mmol) in THF (4 mL), stirred at -78 °C for a further 1 h and then allowed to warm to RT and stirred overnight. The mixture was poured into 1 M sulfuric acid (50 mL), extracted with ether (3×50 mL), and the combined extracts dried and evaporated. Chromatography of the residue (ethyl acetate-hexane, 8:1 to 1:1) gave the *title compound* **35** (2.30 g, 59%) as a colourless oil (Found: C, 43.6; H, 6.3; Sn, 35.8. $C_{12}H_{20}O_3Sn$ requires C, 43.54; H, 6.09; Sn, 35.86%); $\nu_{\max}/\text{cm}^{-1}$ 3499, 2959, 1577, 1495, 1452, 1324, 1289, 1251, 1045, 866; δ_H (300 MHz, $CDCl_3$) 6.97 (1 H, s, ArH), 6.89 (1 H, s, ArH), 4.97 (2 H, d, J 5.7 Hz, CH_2O), 3.87 (3 H, s, OMe), 3.84 (3 H, s, OMe), 1.91 (1 H, t, J

5.7 Hz, OH), 0.28 (9 H, s, $SnMe_3$); m/z (FAB) 332 (M^+ , 5%), 315 ($M^+ - OH$, 100); R_f 0.40 (hexane-ethyl acetate, 1:1).

General procedure for bis(trialkylstannylbenzyl) ethers **36–39**

To a suspension of NaH [1.3 eq., pre-washed with pentane (6 mL mmol^{-1}) and THF (6 mL mmol^{-1})] in anhydrous DMF (1 mL mmol^{-1} of alcohol) at 0 °C was added the alcohol (1.1 eq.) as a solution in anhydrous DMF (1 mL mmol^{-1}) dropwise and the mixture was stirred for 10 min. The bromide was added as a solution in DMF (1 mL mmol^{-1}) and the solution was stirred at 0 °C for 20 min, then at RT for 16 h. DCM (10 mL mmol^{-1}) and water (10 mL mmol^{-1}) was added and the mixture was extracted with DCM (2×5 mL mmol^{-1} of alcohol). The combined extract was washed with brine (10 mL/ mmol of alcohol), dried, evaporated, and the residue purified by chromatography.

Tributyl(2-((3,4,5-trimethoxy-2-(trimethylstannyl)benzyloxy)-methyl)phenyl)stannane **36** was prepared from alcohol **23**⁵⁴ (960 mg, 2.42 mmol) and bromide **20**³⁴ (930 mg, 2.19 mmol). Chromatography (hexane-ethyl acetate, 10:1) gave the *title compound* **36** (1.08 g, 67%) as a clear oil (Found: C, 51.9; H, 7.4; Sn, 31.8. $C_{32}H_{54}O_4Sn_2$ requires C, 51.92; H, 7.35; Sn, 32.08%); $\nu_{\max}/\text{cm}^{-1}$ 3048, 2955, 2924, 2847, 1584, 1561, 1480, 1464, 1375, 1351, 1313, 1192, 1161, 1049, 1017; δ_H (300 MHz, $CDCl_3$) 7.48 (1 H, d, J 6.8 Hz, ArH), 7.30 (1 H, t, J 6.7 Hz, ArH), 7.31–7.25 (2 H, m, ArH), 6.76 (1 H, s, 5-H), 4.46 (2 H, s, CH_2O), 4.39 (2 H, s, CH_2O), 3.67 (3 H, s, OMe), 3.65 (6 H, s, $2 \times OMe$), 1.52 (6 H, m, $3 \times CH_2CH_2Sn$), 1.31 (6 H, m, $3 \times CH_2CH_3$), 1.07 (6 H, t, J 8.2 Hz, $3 \times CH_2Sn$), 0.90 (9 H, t, J 7.3 Hz, $3 \times CH_2CH_3$), 0.26 (9 H, s, $SnMe_3$); δ_C (100 MHz, $CDCl_3$, DEPT-135) -6.3 (CH_3), 10.7 (CH_2), 14.1 (CH_3), 27.8 (CH_2), 29.6 (CH_2), 56.4 (CH_3), 60.9 (CH_3), 61.2 (CH_3), 73.2 (CH_2), 74.0 (CH_2), 109.0 (CH), 127.4 (2 $\times$ CH), 128.4 (CH), 137.3 (CH); m/z (ES) 741 (MH^+ , 100%); R_f 0.55 (hexane-ethyl acetate, 6:1).

Tributyl(6-((3,4-dimethoxy-2-(tributylstannyl)benzyloxy)methyl)-2,3,4-trimethoxyphenyl)stannane **37** was prepared from alcohol **25** (1.60 g, 3.50 mmol) and bromide **21** (1.70 g, 3.09 mmol). Chromatography (hexane-ethyl acetate, 10:1) gave the *title compound* **37** (1.00 g, 35%) as a clear oil (Found: C, 55.9; H, 8.4; Sn, 25.9. $C_{43}H_{76}O_6Sn_2$ requires C, 55.74; H, 8.27; Sn, 25.63%); $\nu_{\max}/\text{cm}^{-1}$ 2951, 2928, 2847, 1584, 1565, 1464, 1375, 1317, 1262, 1103, 1041, 1013; δ_H (400 MHz, $CDCl_3$) 7.10 (1 H, d, J 8.1 Hz, 6'-H), 6.86 (1 H, t, J 8.1 Hz, 5'-H), 6.85 (1 H, s, 5-H), 4.40 (2 H, s, OCH_2), 4.33 (2 H, s, OCH_2), 3.88 (9 H, br s, $3 \times OMe$), 3.85 (6 H, br s, $2 \times OMe$), 1.52 (12 H, m, $6 \times CH_2CH_2Sn$), 1.31 (12 H, m, $6 \times CH_2CH_3$), 1.10 (6 H, t, J 8.5 Hz, $3 \times CH_2Sn$), 1.04 (6 H, t, J 8.2 Hz, $3 \times CH_2Sn$), 0.89 (18 H, m, $6 \times CH_2CH_3$); δ_C (100 MHz, $CDCl_3$, DEPT-135) 12.1 (CH_2), 12.15 (CH_2), 14.1 (CH_3), 14.12 (CH_3), 27.8 (CH_2), 27.9 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 55.8 (CH_3), 56.3 (CH_3), 60.8 (CH_3), 61.0 (CH_3), 61.0 (CH_3), 73.1 (CH_2), 73.4 (CH_2), 108.2 (CH), 112.6 (CH), 125.4 (CH); R_f 0.45 (hexane-ethyl acetate, 6:1).

(2-Methoxy-3-(methoxymethoxy)-6-((3,4,5-trimethoxy-2-(trimethylstannyl)benzyloxy)methyl)phenyl)trimethylstannane **38** was prepared from alcohol **32** (1.52 g, 4.21 mmol) and bromide **20**³⁴ (2.40 g, 5.7 mmol). Chromatography (hexane-ethyl acetate, 6:1) gave the *title compound* **38** (2.40 g, 81%) as a clear oil (Found: C, 44.3; H, 6.2; Sn, 34.0. $C_{26}H_{42}O_7Sn_2$ requires C, 44.36; H, 6.01; Sn, 33.72%); $\nu_{\max}/\text{cm}^{-1}$ 2932, 2850, 1584, 1561, 1468, 1375, 1317, 1262, 1192, 1161, 1103, 1014, 767; δ_H (300 MHz, $CDCl_3$) 7.11 (1 H, d,

J 8.7 Hz, 6-H), 7.03 (1 H, d, J 8.7 Hz, 5-H), 6.75 (1 H, s, 6'-H), 5.23 (2 H, s, OCH₂O), 4.40 (2 H, s, ArCH₂O), 4.35 (2 H, s, ArCH₂O), 3.85–3.75 (12 H, 4 × s, 4 × OMe), 3.54 (3 H, s, OCH₂OMe), 0.32 (9 H, s, SnMe₃), 0.26 (9 H, s, SnMe₃); δ_{C} (100 MHz, CDCl₃, DEPT-135) –7.7 (CH₃), –5.7 (CH₃), 56.2 (CH₃), 56.4 (CH₃), 56.6 (CH₃), 60.9 (CH₃), 61.3 (CH₃), 72.7 (CH₂), 73.2 (CH₂), 95.4 (CH₂), 109.0 (CH), 117.0 (CH), 125.7 (CH); m/z (ES) 727 ($M\text{Na}^+$, 100%); R_{f} 0.38 (hexane–ethyl acetate, 5 : 1).

(4,5-Dimethoxy-2-((3,4,5-trimethoxy-2-(trimethylstannyl)benzyloxy)methyl)phenyl)-trimethylstannane **39** was prepared from alcohol **35** (1.00 g, 3.02 mmol) and bromide **20**³⁴ (1.20 g, 2.83 mmol). Chromatography (hexane–ethyl acetate, 6 : 1) gave the *title compound* **39** (1.20 g, 63%) as a clear oil (Found: C, 44.7; H, 6.1; Sn, 35.2. C₂₅H₄₀O₆Sn₂ requires C, 44.55; H, 5.98; Sn, 35.23%); $\nu_{\text{max}}/\text{cm}^{-1}$ 2932, 2909, 2839, 1584, 1557, 1499, 1460, 1379, 1313, 1250, 1161, 1045, 1014, 909, 773, 730; δ_{H} (300 MHz, CDCl₃) 6.97 (1 H, s, ArH), 6.87 (1 H, s, Ar), 6.71 (1 H, s, ArH), 4.40 (2 H, s, CH₂), 4.37 (2 H, s, CH₂), 3.90 (3 H, s, OMe), 3.85 (6 H, s, 2 × OMe), 3.83 (3 H, s, OMe), 3.80 (3 H, s, OMe), 0.26 (9 H, s, SnMe₃), 0.25 (9 H, s, SnMe₃); δ_{C} (100 MHz, CDCl₃, DEPT-135) –7.7 (CH₃), –5.7 (CH₃), 56.2 (CH₃), 56.4 (CH₃), 56.4 (CH₃), 60.9 (CH₃), 61.3 (CH₃), 73.1 (CH₂), 73.4 (CH₂), 109.3 (CH), 112.4 (CH), 119.1 (CH); m/z (ES) 697 ($M\text{Na}^+$, 100%); R_{f} 0.38 (hexane–ethyl acetate, 6 : 1).

General procedure for dibenz[c,e]oxepines 40–43

To a suspension of CuCl (5 eq.) in anhydrous DMF (18 mL mmol^{–1} of ether) under argon was added dropwise the ether (1 eq.) as a solution in anhydrous DMF (30 mL mmol^{–1}) and the mixture was stirred for 2 h. Saturated NH₄Cl solution (pH 8, *ca.* 20 mL mmol^{–1}) was added and the mixture was stirred in an open vessel until deep blue. The mixture was extracted with ether (2 × 40 mL mmol^{–1} of ether). The combined extract was washed with brine (10 mL/mmol of ether), dried, evaporated, and the residue purified by chromatography.

5,7-Dihydro-1,2,3-trimethoxydibenz[c,e]oxepine **40** was prepared from the ether **36** (1.00 g, 1.35 mmol). Chromatography (hexane–ethyl acetate, 6 : 1) gave a sample of **40** (320 mg, 83%) as a clear oil which crystallised on standing and was shown by ¹H NMR spectroscopy to contain a co-eluting by-product. Crystallisation (ether–hexane) gave the pure *title compound* **40** (100 mg, 26%), m.p. 79–80 °C (Found: C, 71.4; H, 6.3. C₁₇H₁₈O₄ requires C, 71.31; H, 6.34%); $\nu_{\text{max}}/\text{cm}^{-1}$ 2955, 2932, 2854, 1596, 1487, 1460, 1452, 1402, 1332, 1254, 1231, 1192, 1146, 1118, 1091, 1052, 1005; δ_{H} (300 MHz, CDCl₃) 7.64 (1 H, d, J 7.8 Hz, 11-H), 7.40–7.27 (3 H, m, 8,9,10-H), 6.70 (1 H, s, 4-H), 4.42 (1 H, br m, 5-H), 4.31 (1 H, br m, 5-H), 4.10 (1 H, br m, 7-H), 3.96 (1 H, br m, 7-H), 3.88 (3 H, s, OMe), 3.86 (3 H, s, OMe), 3.60 (3 H, s, OMe); δ_{C} (100 MHz, CDCl₃, DEPT-135) 56.5 (CH₃), 61.3 (CH₃), 61.5 (CH₃), 67.91 (CH₂), 67.93 (CH₂), 109.1 (CH), 128.1 (CH), 128.4 (CH), 129.9 (CH), 130.0 (CH); m/z (ES) 287 ($M\text{H}^+$, 23%), 257 (100), 224 (30); R_{f} 0.18 (hexane–ethyl acetate, 6 : 1).

5,7-Dihydro-1,2,3,10,11-pentamethoxydibenz[c,e]oxepine **41** was prepared from the ether **37** (1.00 g, 1.08 mmol). Chromatography (hexane–ethyl acetate, 6 : 1) followed by crystallisation (ethyl acetate) gave the *title compound* **41** (130 mg, 35%) as colourless prisms, m.p. 159–160 °C (Found: C, 66.0; H, 6.6. C₁₉H₂₂O₆ requires C, 65.88; H, 6.40%); $\nu_{\text{max}}/\text{cm}^{-1}$ 2994, 2963, 2940, 2858, 1600, 1577, 1487, 1460, 1410, 1324, 1274, 1196, 1157, 1111, 1060, 1010; δ_{H}

(400 MHz, CDCl₃) 7.11 (1 H, d, J 8.2 Hz, 9-H), 6.97 (1 H, d, J 8.2 Hz, 8-H), 6.75 (1 H, s, 4-H), 4.43 (1 H, d, J 11.3 Hz, 5-H), 4.38 (1 H, d, J 11.3 Hz, 5-H), 4.07 (1 H, d, J 11.2 Hz, 7-H), 4.06 (1 H, d, J 11.2 Hz, 7-H), 3.94 (9 H, s, 3 × OMe), 3.78 (3 H, s, OMe), 3.65 (3 H, s, OMe); δ_{C} (100 MHz, CDCl₃, DEPT-135) 56.3 (CH₃), 56.4 (CH₃), 60.8 (CH₃), 61.2 (CH₃), 61.3 (CH₃), 67.4 (CH₂), 67.6 (CH₂), 108.1 (CH), 112.4 (CH), 124.8 (CH); m/z (ES) 410 [$M(\text{MeCN})\text{Na}^+$, 100%], 369 ($M\text{Na}^+$, 12), 347 ($M\text{H}^+$, 25), 317 (32); R_{f} 0.24 (hexane–ethyl acetate, 4 : 1).

5,7-Dihydro-1,2,3,11-tetramethoxy-10-methoxymethoxydibenz[c,e]oxepine **42** was prepared from the ether **38** (2.40 g, 3.41 mmol). Chromatography (ether–petroleum, 1 : 1) followed by crystallisation (ethyl acetate–hexane) gave the *title compound* **42** (650 mg, 51%) as colourless crystals, m.p. 76–77 °C (Found: C, 63.6; H, 6.6. C₂₀H₂₄O₇ requires C, 63.82; H, 6.43%); $\nu_{\text{max}}/\text{cm}^{-1}$ 2944, 2854, 1596, 1487, 1464, 1406, 1332, 1266, 1161, 1115, 1068; δ_{H} (300 MHz, CDCl₃) 7.17 (1 H, d, J 8.2 Hz, 9-H), 7.04 (1 H, d, J 8.2 Hz, 8-H), 6.71 (1 H, s, 4-H), 5.28 (1 H, d, J 6.7 Hz, OCHOMe), 5.25 (1 H, d, J 6.7 Hz, OCHOMe), 4.40 (1 H, d, J 11.3 Hz, 5-H), 4.35 (1 H, d, J 11.3 Hz, 5-H), 4.05 (1 H, d, J 11.2 Hz, 7-H), 4.01 (1 H, d, J 11.2 Hz, 7-H), 3.91 (3 H, s, ArOMe), 3.91 (3 H, s, ArOMe), 3.73 (3 H, s, ArOMe), 3.63 (3 H, s, ArOMe), 3.54 (3 H, s, OCH₂OMe); δ_{C} (100 MHz, CDCl₃, DEPT-135) 56.4 (CH₃), 56.7 (CH₃), 61.0 (CH₃), 61.2 (CH₃), 61.3 (CH₃), 67.4 (CH₂), 67.6 (CH₂), 95.8 (CH₂), 108.2 (CH), 116.9 (CH), 124.9 (CH); m/z (ES) 440 [$M(\text{MeCN})\text{Na}^+$, 100%], 399 ($M\text{Na}^+$, 54); R_{f} 0.25 (hexane–ethyl acetate, 4 : 1).

5,7-Dihydro-1,2,3,9,10-pentamethoxydibenz[c,e]oxepine **43** was prepared from the ether **39** (1.10 g, 1.63 mmol). Chromatography (hexane–ethyl acetate, 6 : 1) followed by crystallisation (ethyl acetate–hexane) gave the *title compound* **43** (186 mg, 33%) as colourless crystals, m.p. 135–136 °C (lit.⁵⁸ 124–125 °C) (Found: C, 66.1; H, 6.6. C₁₉H₂₂O₆ requires C, 65.88; H, 6.40%); $\nu_{\text{max}}/\text{cm}^{-1}$ 2936, 2854, 1608, 1515, 1491, 1460, 1410, 1375, 1328, 1247, 1122, 1091, 1049, 1014, 990, 854, 734; δ_{H} (300 MHz, CDCl₃) 7.30 (1 H, s, 11-H), 6.93 (1 H, s, 8-H), 6.78 (1 H, s, 4-H), 4.42 (2 H, br m, 5-H₂), 4.17–4.07 (1 H, br m, 7-H), 4.05–3.95 (1 H, br m, 7-H), 3.99–3.93 (12 H, 4 × s, 4 × OMe), 3.68 (3 H, s, ArOMe); δ_{C} (100 MHz, CDCl₃, DEPT-135) 56.3 (CH₃), 56.4 (CH₃), 61.2 (CH₃), 61.6 (CH₃), 67.7 (CH₂), 68.0 (CH₂), 109.2 (CH), 112.5 (CH), 112.9 (CH) (in accord with published data⁵⁸); m/z (ES) 410 [$M(\text{MeCN})\text{Na}^+$, 100%], 371 (45), 347 ($M\text{H}^+$, 5), 317 (94), 302 (20); R_{f} 0.20 (hexane–ethyl acetate, 4 : 1).

5,7-Dihydro-10-hydroxy-1,2,3,11-tetramethoxydibenz[c,e]oxepine **44**

To a solution of the oxepine **42** (100 mg, 0.27 mmol) in dry DCM (2 mL) was added Montmorillonite clay K10 (100 mg, washed with dry DCM, dried *in vacuo*).³⁶ The mixture was stirred at RT for 3 h and then concentrated *in vacuo*. Chromatography of the residue (10 g silica gel, hexane–ethyl acetate, 3 : 2) gave the *title compound* **44** (55 mg, 62%) as a colourless solid, m.p. 166–167 °C (Found: C, 65.2; H, 6.0. C₁₈H₂₀O₆ requires C, 65.05; H, 6.07%); $\nu_{\text{max}}/\text{cm}^{-1}$ 3379, 2941, 2862, 1598, 1489, 1465, 1406, 1328, 1259, 1194, 1150, 1112, 1064; δ_{H} (300 MHz, CDCl₃) 7.09 (1 H, d, J 8.1 Hz, 9-H), 7.00 (1 H, d, J 8.1 Hz, 8-H), 6.78 (1 H, s, 4-H), 6.00 (1 H, s, OH), 4.43 (1 H, d, J 11.4 Hz, 5-H), 4.39 (1 H, d, J 11.3 Hz, 7-H), 4.09 (1 H, d, J 11.3 Hz, 7-H), 4.02 (1 H, d, J 11.4 Hz, 5-H), 3.95 (3 H, s, OMe), 3.95 (3 H, s, OMe), 3.70 (3 H, s, OMe), 3.43 (3 H, s,

OMe); δ_c (75 MHz, CDCl_3) 56.4 (CH_3), 60.8 (CH_3), 61.4 (CH_3), 61.5 (CH_3), 67.4 (CH_2), 67.6 (CH_2), 108.5 (CH), 115.1 (CH), 123.1 (CH), 125.6 (C), 128.5 (C), 129.1 (C), 131.3 (C), 142.8 (C), 144.7 (C), 149.4 (C), 151.5 (C), 154.1 (C); m/z (ES) 396 [$M(\text{MeCN})\text{Na}^+$, 100%], 303 (25), 286 (23); R_f 0.23 (hexane–ethyl acetate, 7 : 1).

Dibenz[*c,e*]oxepines **16**, **51** and **56** via Ullmann cross-coupling reactions

The sequences leading to **16**, **51** and **56** proceeded along conventional lines, as illustrated by the route to **16** (Scheme 5) below. Full details of Schemes 3 and 4 are provided in the ESI.†

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 (2×10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. Chromatography (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. $\text{C}_{18}\text{H}_{20}\text{O}_5$ requires C, 68.34; H, 6.37%); $\nu_{\text{max}}/\text{cm}^{-1}$ 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) [one CH_2 signal obscured], 68.1 (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 [$M(\text{MeCN})\text{Na}^+$, 100%], 287 ($M\text{H}^+ - \text{CH}_2\text{O}$, 100); R_f 0.39 (hexane–ethyl acetate, 3 : 1).

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 (2×10 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. $\text{C}_{18}\text{H}_{18}\text{O}_6$ requires C, 65.45; H, 5.49%); $\nu_{\text{max}}/\text{cm}^{-1}$ 2967, 2932, 2866, 1600, 1484, 1460, 1414, 1324, 1239, 1146, 1107, 1045; 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 ($M\text{H}^+ - \text{CH}_2\text{O}$, 100%); R_f 0.28 (hexane–ethyl acetate, 4 : 1).

3-Bromo-2-formyl-6-methoxyphenyl methanesulfonate **58**³²

To a stirred solution of 6-bromo-2-hydroxy-3-methoxybenzaldehyde **57** (0.33 g, 1.43 mmol) and triethylamine (0.17 g, 1.71 mmol) in DCM (5 mL) was added methanesulfonyl chloride (0.19 g, 2.3 mmol) at 0 °C. The reaction was stirred at 0 °C for 10 min and then at RT for 30 min, by which time a brown colour had developed. Water (20 mL) was added, the mixture was extracted with ethyl acetate (2×20 mL). The combined extract was washed with brine, dried and evaporated to give a crude solid (0.42 g). Chromatography (60 g silica gel, hexane–ethyl acetate, 3 : 1) gave the mesylate **58** (320 mg, 72%) and 5-bromo-8-methoxybenzo[*e*][1,2]oxathiine 2,2-dioxide **59** (90 mg, 22%) as white solids. The *title compound* **58** had m.p. 95–97 °C; $\nu_{\text{max}}/\text{cm}^{-1}$ 1705, 1565, 1468, 1399, 1363, 1293, 1219, 1165, 1130, 971, 878, 800; δ_{H} (400 MHz, CDCl_3) 10.28 (1 H, s, CHO), 7.55 (1 H, d, J 8.9 Hz, 4-H), 7.09 (1 H, d, J 8.9 Hz, 5-H), 3.93 (3 H, s, OMe), 3.38 (3 H, s, SMe); δ_c (100 MHz, CDCl_3) 40.1 (CH_3), 56.9 (CH_3), 115.2 (C), 118.1 (CH), 128.9 (C), 133.9 (CH), 138.6 (C), 152.6 (C), 189.8 (CH); m/z (ES) 374/372 [$M(\text{MeCN})\text{Na}^+$, 100%], 333/331 ($M\text{Na}^+$, 80); R_f 0.35 (hexane–ethyl acetate, 1 : 1). 5-Bromo-8-methoxybenzo[*e*][1,2]oxathiine 2,2-dioxide **59** had m.p. 186–188 °C (Found: C, 37.3; H, 2.2; S, 10.9; Br, 27.2. $\text{C}_9\text{H}_7\text{BrO}_4\text{S}$ requires C, 37.13; H, 2.42; S, 11.01; Br, 27.45%); $\nu_{\text{max}}/\text{cm}^{-1}$ (nujol mull) 3091, 2959, 2924, 2854, 1608, 1569, 1468, 1367, 1309, 1270, 1169, 1080, 909; δ_{H} (300 MHz, CDCl_3) 7.62 (1 H, d, J 10.5 Hz, 4-H), 7.47 (1 H, d, J 10.5 Hz, 6-H), 6.96 (1 H, d, J 10.5 Hz, 7-H), 6.88 (1 H, d, J 10.5 Hz, 3-H), 3.88 (3 H, s, OMe); δ_c (75 MHz, CDCl_3) 57.0 (CH_3), 113.7 (C), 116.2 (CH), 120.3 (C), 123.9 (CH), 129.9 (CH), 135.8 (CH), 142.0 (C), 149.1 (C); R_f 0.60 (hexane–ethyl acetate, 1 : 1).

2,6'-Diformyl-4,2',3',4'-tetramethoxybiphenyl-3-yl methanesulfonate **60**

To a suspension of copper bronze (0.646 g, 10 mmol) in anhydrous DMF (2 mL) was added a solution of 2-bromo-3,4,5-trimethoxybenzaldehyde **45**³⁸ (0.80 g, 2.91 mmol) and **58** (0.30 g, 0.97 mmol) in anhydrous DMF (1 mL) and the suspension was stirred at 165 °C for 3 h. TLC showed the presence of unreacted **58** and more **45** (100 mg) was added. After a further 1 h at 165 °C the reaction, now complete, was cooled and diluted with ethyl acetate (20 mL). The resulting suspension was filtered through Celite® (3 g) and the filtrate concentrated *in vacuo*. Chromatography (70 g silica gel, hexane–ethyl acetate, 2 : 1 to 1 : 1) and crystallisation from ethyl acetate yielded the *title compound* **60** (180 mg, 44%), m.p. 131–132 °C (Found: C, 53.7; H, 4.8; S, 7.8. $\text{C}_{19}\text{H}_{20}\text{O}_9\text{S}$ requires C, 53.77; H, 4.75; S, 7.56%); $\nu_{\text{max}}/\text{cm}^{-1}$ 2934, 2843, 1701, 1682, 1588, 1561, 1480, 1371, 1336, 1285, 1169, 1111, 1072; δ_{H} (400 MHz, CDCl_3) 10.15 (1 H, s, 2-CHO), 9.61 (1 H, s, 6'-CHO), 7.34 (1 H, s, 5'-H), 7.26 (1 H, d, J 8.5 Hz, 6-H), 7.15 (1 H, d, J 8.5 Hz, 5-H), 4.00 (3 H, s, OMe), 3.958 (3 H, s, OMe), 3.955 (3 H, s, OMe), 3.55 (3 H, s, OMe), 3.41 (3 H, s, SMe); δ_c (100 MHz, CDCl_3) 40.0 (CH_3), 56.2 (CH_3), 56.6 (CH_3), 60.9 (CH_3), 61.2 (CH_3), 105.9 (CH), 116.4 (CH), 127.3 (C), 129.7 (C), 130.3 (C), 130.7 (C), 132.1 (CH), 139.5 (C), 147.3 (C), 150.8 (C), 152.3 (C), 153.8 (C), 189.0

(CH), 190.2 (CH); m/z (ES⁺) 447 (MNa^+ , 100%); R_f 0.20 (ethyl acetate–hexane, 2 : 1), 0.30 (ether).

2,6'-Bis(hydroxymethyl)-4,2',3',4'-tetramethoxybiphenyl-3-yl methanesulfonate **61**

To a solution of **60** (170 mg, 0.40 mmol) in methanol (4 mL) was added sodium borohydride (45 mg, 1.20 mmol) and the solution was stirred at RT for 1 h. Water (20 mL) and ethyl acetate (20 mL) were added to the reaction, the layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic extract was dried over Na_2SO_4 and filtered. The solvent was removed *in vacuo* and the residue purified by chromatography (25 g silica gel, hexane–ethyl acetate, 1 : 2), followed by crystallisation (ethyl acetate), which yielded the *title compound* **61** (164 mg, 96%) as a white crystals, m.p. 124–126 °C (Found: C, 53.30; H, 5.67; S, 7.26. $C_{19}H_{24}O_6S$ requires C, 53.26; H, 5.65; S, 7.48%); ν_{max}/cm^{-1} 3219, 2947, 1607, 1484, 1410, 1363, 1328, 1278, 1161, 1111, 1006, 889; δ_H (400 MHz, $CDCl_3$) 7.07 (1 H, d, J 8.5 Hz, 6-H), 7.02 (1 H, d, J 8.5 Hz, 5-H), 6.89 (1 H, s, 5'-H), 4.62 (1 H, dd, J 4.2, 12.0 Hz, $CHOH$), 4.26–4.15 (3 H, m, $CHOH$ and CH_2OH), 3.94 (3 H, s, OMe), 3.91 (3 H, s, OMe), 3.88 (3 H, s, OMe), 3.55 (3 H, s, OMe), 3.51–3.46 (2 H, m, 2 × OH), 3.40 (3 H, s, SMe); δ_C (100 MHz, $CDCl_3$) 39.3 (CH_3), 56.1 (CH_3), 56.2 (CH_3), 57.5 (CH_2), 61.0 (CH_3), 61.1 (CH_3), 62.5 (CH_2), 108.7 (CH), 112.2 (CH), 125.1 (C), 130.1 (CH), 130.2 (C), 135.0 (C), 136.0 (C), 137.8 (C), 141.5 (C), 150.9 (C), 151.3 (C), 153.6 (C); m/z (ES) 451 (MNa^+ , 100%); R_f 0.18 (hexane–ethyl acetate, 1 : 2).

5,7-Dihydro-3,9,10,11-tetramethoxydibenz[*c,e*]oxepin-4-yl methanesulfonate **62**

A solution of **61** (155 mg, 0.362 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 × 10 mL). The extracts were combined, dried over Na_2SO_4 , filtered and concentrated. Chromatography of the residue (20 g silica gel, hexane–ethyl acetate, gradient 3 : 1 to 1 : 1) gave the *title compound* **62** (110 mg, 74%) as a colourless solid, m.p. 158–161 °C (MeOH) (Found: C, 55.61; H, 5.34; S, 7.74. $C_{19}H_{22}O_8S$ requires C, 55.60; H, 5.40; S, 7.81%); ν_{max}/cm^{-1} 2940, 1604, 1573, 1484, 1460, 1367, 1282, 1161, 1118, 1072, 1060, 909, 831; δ_H (400 MHz, $CDCl_3$) 7.61 (1 H, d, J 8.7 Hz, 1-H), 7.09 (1 H, d, J 8.7 Hz, 2-H), 6.75 (1 H, s, 8-H), 5.01 (1 H, br d, J 11.3 Hz, 5-H), 4.42 (1 H, br d, J 11.3 Hz, 7-H), 4.01 (1 H, br d, J 11.3 Hz, 7-H), 3.96 (3 H, s, OMe), 3.94 (3 H, s, OMe), 3.92 (3 H, s, OMe), 3.90 (1 H, br d, J 11.3 Hz, 5-H), 3.68 (3 H, s, OMe), 3.39 (3 H, s, SMe); δ_C (100 MHz, $CDCl_3$) 39.5 (CH_3), 56.2 (CH_3), 56.3 (CH_3), 60.4 (5- CH_2), 61.1 (CH_3), 61.2 (CH_3), 67.9 (7- CH_2), 108.9 (CH), 112.3 (CH), 125.4 (C), 129.0 (CH), 130.2 (C), 130.8 (C), 131.0 (C), 137.1 (C), 142.8 (C), 150.6 (C), 151.2 (C), 153.3 (C); m/z (ES) 433 (MNa^+ , 100%), 411 (MH^+ , 58); R_f 0.31 (hexane–ethyl acetate, 1 : 1).

5,7-Dihydro-3,9,10,11-tetramethoxydibenz[*c,e*]oxepin-4-ol **16**

To a solution of **62** (85 mg, 0.231 mmol) in 1,4-dioxane (0.25 mL) and methanol (0.25 mL) was added aqueous 3 M potassium hydroxide (0.5 mL) and the mixture was stirred overnight at

50 °C. The solution was diluted with 2 M aqueous hydrochloric acid (2 mL) at 0 °C, extracted with DCM (3 × 5 mL), washed with saturated aqueous sodium hydrogen carbonate (5 mL), dried and evaporated. The residual white solid was chromatographed (30 g silica gel, ethyl acetate–hexane, 1 : 2) to obtain the *title compound* **16** (62 mg, 90%), m.p. 145–147 °C (Found: C, 64.8; H, 6.1. $C_{18}H_{20}O_6$ requires C, 65.05; H, 6.07%); ν_{max}/cm^{-1} 3394, 2932, 2858, 1600, 1480, 1344, 1270, 1250, 1150, 1115, 1087, 1056; δ_H (400 MHz, $CDCl_3$) 7.21 (1 H, d, J 8.4 Hz, 1-H), 6.94 (1 H, d, J 8.4 Hz, 2-H), 6.75 (1 H, s, 8-H), 5.91 (1 H, s, 4-OH), 5.15 (1 H, d, J 11.1 Hz, 5-CH), 4.40 (1 H, d, J 11.0 Hz, 7-CH), 4.05 (1 H, d, J 11.0 Hz, 7-CH), 3.95 (3 H, s, OMe), 3.94 (3 H, s, OMe), 3.91 (3 H, s, OMe), 3.85 (1 H, d, J 11.1 Hz, 5-CH), 3.66 (3 H, s, OMe); δ_C (100 MHz, $CDCl_3$) 56.1 (CH_3), 56.2 (CH_3), 59.4 (5- CH_2), 61.0 (CH_3), 61.2 (CH_3), 67.9 (7- CH_2), 108.9 (CH), 110.1 (CH), 120.9 (CH), 121.2 (C), 126.5 (C), 130.7 (C), 131.3 (C), 142.7 (C), 143.5 (C), 145.7 (C), 150.8 (C), 152.8 (C); m/z (ES) 396 [$M(MeCN)Na^+$, 55%], 355 (MNa^+ , 4), 315 ($MH^+ - H_2O$, 100), 303 ($MH^+ - CH_2O$, 17); R_f 0.16 (acetone–hexane, 1 : 4); R_f 0.41 (ethyl acetate–hexane, 1 : 1).

6-Benzyl-6,7-dihydro-1,2,3,9,10,11-hexamethoxy-5H-dibenz[*c,e*]azepine ($\pm$)-**66**

To a solution of the 6,6'-bis(bromomethyl)-2,2',3,3',4,4'-hexamethoxybiphenyl **65**, prepared from the diol **64** (134 mg, 0.34 mmol) as described,³¹ in dry DMF (1 mL) at 0 °C under argon was added benzylamine (146 mg, 1.36 mmol) and triethylamine (103 mg, 1.02 mmol) and the mixture was stirred overnight at RT. Water (10 mL) was added and the mixture extracted with ethyl acetate (3 × 10 mL). The combined organic extract was washed with brine (10 mL), dried and evaporated *in vacuo*. Chromatography of the residue (25 g silica gel, hexane–ethyl acetate, 3 : 2) gave the *title compound* **66** (155 mg, 98%) as a colourless crystalline solid, m.p. 117–118 °C, which darkened in air (Found: $M + H^+$, 466.2216; $C_{27}H_{32}O_6N$ requires 466.2225); ν_{max}/cm^{-1} 2940, 2835, 1600, 1577, 1495, 1456, 1406, 1317, 1231, 1130, 1099, 734, 699; δ_H (400 MHz, $CDCl_3$) 7.43 (2 H, d, J 7.1 Hz, 2',6'-H), 7.38–7.27 (3 H, m, 3',4',5'-H), 6.62 (2 H, s, 4,8-H), 3.91 (6 H, s, 2 × OMe), 3.90 (6 H, s, 2 × OMe), 3.73 (6 H, s, 2 × OMe), 3.75–3.65 (2 H, br m, 12- H_2), 3.45 (2 H, d, J 12.0 Hz, 5,7- H_A), 3.08 (2 H, d, J 12.0 Hz, 5,7- H_B); δ_C (100 MHz, $CDCl_3$) 55.4 (CH_2), 56.4 (CH_3), 60.0 (CH_2), 61.1 (CH_3), 61.4 (CH_3), 108.5 (CH), 123.2 (C), 127.7 (CH), 128.9 (CH), 129.7 (CH), 130.6 (C), 142.0 (C), 151.8 (C), 153.1 (C) (1 C coincident); m/z (ES) 466 (MH^+ , 100%); R_f 0.22 (hexane–ethyl acetate, 3 : 2).

Biological evaluation

Inhibition of tubulin assembly.⁴⁵ The assembly of microtubules from porcine tubulin was monitored spectrophotometrically by measuring the associated increase in solution turbidity. For each of the test compounds, six samples were prepared directly in quartz cuvettes at 0 °C and contained 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer [740 μ L (0.1 M MES, 1 mM EGTA, 0.5 mM $MgCl_2$, distilled water, pH 6.6)], GTP (100 μ L, 10 mM in MES buffer), tubulin^{45b} (1 mg, 100 μ L at 10 mg mL^{-1} in MES buffer) and the test compound (10 μ L, decreasing concentration starting at 10 mM in DMSO). The cells were immediately placed in a Varian

Cary 300 Bio UV/visible spectrophotometer, preheated at 37 °C, alongside six blank samples each containing MES buffer (1 mL) and the test compound. The absorbance at λ 350 nm was then recorded during a period of 30 min. The results were compared to the untreated control cells to evaluate the relative degree of change in optical density. From the data, an IC_{50} value (50% inhibition of tubulin assembly) was determined graphically.

K562 growth inhibition by MTT assay.⁴⁶ K562 cells were cultured in RPMI medium, free of antibiotics and containing 2-mercaptoethanol (2 μ M) and L-glutamine (2 mM), supplemented with 10% v/v foetal calf serum (FCS). The cells were adjusted to a concentration of 4000 cells mL^{-1} in RPMI medium supplemented with 10% v/v FCS, and the test compound was made up as a 10 mM stock solution in DMSO. Starting with eight sterile centrifuge tubes, a portion of cell solution (2 mL) was added to the first tube followed by 4 μ L of the test compound stock solution. A portion (1 mL) of the resulting solution was added to 1 mL of cell solution in the adjacent tube, giving a test compound concentration of one-half of that of the first tube. This series of dilutions was continued to afford seven samples at different concentrations. As a control, the eighth centrifuge tube contained 1 mL of cell solution only. Aliquots of 100 μ L of the treated cells were then pipetted in triplicate into a 96-well microtitre testplate and incubated (37 °C, 5% CO_2 in air) for 5 days. After this time the plate was removed from the incubator and 50 μ L of a solution of MTT (3 mg mL^{-1} in phosphate-buffered saline) was added to each well. After further incubation (37 °C, 5% CO_2 in air, 3 h) the medium was carefully removed from each well by suction and the resulting formazan precipitate was redissolved in 200 μ L of DMSO. The optical density of each well was then read at two wavelengths (λ 540 and 690 nm) using a plate reader. After processing and analysis through the application of an in-house software package, the results obtained enabled the calculation of the test compound dose required to inhibit cell growth by 50% (IC_{50} value), determined by graphical means as a percentage of the control growth. Each drug concentration was tested in triplicate, and the standard error of each value is <10%.

Molecular mechanics calculation (Fig. 2b)

The model of **16** (Fig. 2b) was generated on a Mac Mini 2.6 GHz Intel Core 2 Duo running Linux (Fedora Core 12, x86 64-bit), using MacroModel v. 8.0 (Maestro v. 9.0.211 interface) with the MM3 force field and Monte Carlo conformational search (*csearch*) method (1000 iterations). For the calculation, the dihedral angles ($C-O-C_n-C_{n-1}$) associated with the methoxy groups at $C(n)$ [where $n = 9, 10$ or 11] were constrained (force constant 5000) so as to match those of the DAMA-colchicine **73**. The set dihedral angles were: $n = 9$, 100.0° (99.7° after minimisation); $n = 10$, 79.1° (79.1° after minimisation); $n = 11$, 78.8° (78.6° after minimisation). The *csearch* parameters were: no solvent; PRCG method; convergence on gradient; max. number of iterations 3000; convergence threshold 0.0200.

Acknowledgements

We thank the Association for International Cancer Research (AICR) for financial support of this work (grant ref. 00-090). We are also grateful to Alan McGown (University of Salford)

for valuable discussions, Edward Piers (University of British Columbia) for his kind provision of experimental details, Darren Cook (University of Salford) for help with compound testing, and Val Boote and Steve Kelly (University of Manchester) for assistance with MS and NMR measurements. We also wish to acknowledge the use of the EPSRC's Chemical Database Service at Daresbury.⁵⁹

References

- (a) N. Ferrara, H.-P. Gerber and J. LeCouter, *Nat. Med.*, 2003, **9**, 669–676; (b) D. Ribattia, A. Vaccab and M. Presta, *Gen. Pharmacol.*, 2002, **35**, 227–231.
- (a) K. J. Kim, B. Li, J. Winer, M. Armanini, N. Gillett, H. S. Phillips and N. Ferrara, *Nature*, 1993, **362**, 841–844; (b) L. G. Presta, H. Chen, H., S. J. O'Connor, V. Chisholm, Y. G. Meng, L. Krummen, M. Winkler and N. Ferrara, *Cancer Res.*, 1997, **57**, 4593–4599; (c) N. Ferrara, K. J. Hillan, H.-P. Gerber and W. Novotny, *Nat. Rev. Drug Discovery*, 2004, **3**, 391–400.
- For pertinent discussions, see (a) P. Carmeliet and R. K. Jain, *Nature*, 2000, **407**, 249–257; (b) G. M. Tozer, *Br. J. Radiol.*, 2003, **76**, S23–S35; (c) R. K. Jain, *Science*, 2005, **307**, 58–62; (d) S. Yano, Y. Matsumori, K. Ikuta, H. Ogino, T. Doljinsuren and S. Sone, *Int. J. Clin. Oncol.*, 2006, **11**, 73–81; (e) S. C  be-Suarez, A. Zehnder-Fj  llman and K. Ballmer-Hofer, *Cell. Mol. Life Sci.*, 2006, **63**, 601–615; (f) A. Bozec, S. Lassalle, J. Gugenheim, J.-L. Fischel, P. Formento, P. Hofman and G. Milano, *Br. J. Cancer*, 2006, **95**, 722–728; (g) L. M. Ellis and D. J. Hicklin, *Nat. Rev. Cancer*, 2008, **8**, 579–591; (h) J. M. L. Roodhart, M. H. G. Langenberg, L. G. M. Daenen and E. E. Voest, *Biochim. Biophys. Acta Rev. Cancer*, 2009, **1796**, 41–49.
- For reviews, see (a) P. E. Thorpe, *Clin. Cancer Res.*, 2004, **10**, 415–427; (b) G. M. Tozer, C. Kanthou and B. C. Baguley, *Nat. Rev. Cancer*, 2005, **5**, 423–435; (c) C. Kanthou and G. M. Tozer, *International Journal of Experimental Pathology*, 2009, **90**, 284–294.
- J. W. Lippert, *Bioorg. Med. Chem.*, 2007, **15**, 605–615.
- G. R. Pettit, S. B. Singh, M. R. Boyd, E. Hamel, R. K. Pettit, J. M. Schmidt and F. Hogan, *J. Med. Chem.*, 1995, **38**, 1666–1672 and references cited therein.
- (a) G. G. Dark, S. A. Hill, V. E. Prise, G. M. Tozer, G. R. Pettit and D. J. Chaplin, *Cancer Res.*, 1997, **57**, 1829–1834; (b) S. M. Galbraith, R. J. Maxwell, M. A. Lodge, G. M. Tozer, J. Wilson, N. J. Taylor, J. J. Stirling, L. Sena, A. R. Padhani and G. S. Rustin, *J. Clin. Oncol.*, 2003, **21**, 2831–2842.
- (a) I. G. Kirwan, P. M. Loadman, D. J. Swaine, D. A. Anthoney, G. R. Pettit, J. W. Lippert, S. D. Shnyder, P. A. Cooper and M. C. Bibby, *Clin. Cancer Res.*, 2004, **10**, 1446–1453; (b) Y. Sheng, J. Hua, K. G. Pinney, C. M. Garner, R. R. Kane, J. A. Prezioso, D. J. Chaplin and K. Edvardsen, *Int. J. Cancer*, 2004, **111**, 604–610; (c) H. W. Salmon and D. W. Siemann, *Clin. Cancer Res.*, 2006, **12**, 4090–4094; (d) G. R. Pettit, A. J. Thornhill, B. R. Moser and F. Hogan, *J. Nat. Prod.*, 2008, **71**, 1561–1563.
- (a) K. Hori, S. Saito and K. Kubota, *Br. J. Cancer*, 2002, **86**, 1604–1614; (b) K. Hori and S. Saito, *Br. J. Cancer*, 2003, **89**, 1334–1344; (c) A. Delmonte and C. Sessa, *Expert Opin. Invest. Drugs*, 2009, **18**, 1541–1548.
- (a) K. Miyazaki, M. Kobayashi, T. Natsume, M. Gondo, T. Mikami, K. Sakakibara and S. Tsukagoshi, *Chem. Pharm. Bull.*, 1995, **43**, 1706–1718; (b) N. Yamamoto, M. Andoh, M. Kawahara, M. Fukuoka and H. Niitani, *Cancer Sci.*, 2009, **100**, 316–321 and references cited therein.
- (a) G. J. S. Rustin, C. Bradley, S. Galbraith, M. Stratford, P. Loadman, S. Waller, K. Bellenger, L. Gumbrell, L. Folkes and G. Halbert, *Br. J. Cancer*, 2003, **88**, 1160–1167; (b) M. B. Jameson, P. I. Thompson, B. C. Baguley, B. D. Evans, V. J. Harvey, D. J. Porter, M. R. McCrystal, M. Small, K. Bellenger, L. Gumbrell, G. W. Halbert and P. Kestell, *Br. J. Cancer*, 2003, **88**, 1844–1850; (c) B. G. Siim, A. E. Lee, S. Shalal-Zwain, F. B. Pruijn, M. J. McKeage and W. R. Wilson, *Cancer Chemother. Pharmacol.*, 2003, **51**, 43–52.
- (a) S. Kasibhatla, V. Baichwal, S. X. Cai, B. Roth, I. Skvortsova, S. Skvortsov, P. Lukas, N. M. English, N. Sirisoma, J. Drewe, A. Pervin, B. Tseng, R. O. Carlson and C. M. Pleiman, *Cancer Res.*, 2007, **67**, 5865–5871; (b) N. Sirisoma, A. Pervin, H. Zhang, S. Jiang, J. A. Willardsen, M. B. Anderson, G. Mather, C. M. Pleiman, S. Kasibhatla,

- B. Tseng, J. Drewe and S. X. Cai, *J. Med. Chem.*, 2009, **52**, 2341–2351; (c) N. Sirisoma, A. Pervin, H. Zhang, S. Jiang, J. A. Willardsen, M. B. Anderson, G. Mather, C. M. Pleiman, S. Kasibhatla, B. Tseng, J. Drewe and S. X. Cai, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2330–2334.
- 13 (a) J. A. Segreti, J. S. Polakowski, K. A. Koch, K. C. Marsh, J. L. Bauch, S. H. Rosenberg, H. L. Sham, B. F. Cox and G. A. Reinhart, *Cancer Chemother. Pharmacol.*, 2004, **54**, 273–281; (b) Y. Luo, V. P. Hradil, D. J. Frost, S. H. Rosenberg, G. B. Gordon, S. J. Morgan, G. D. Gagne, B. F. Cox, S. K. Tahir and G. B. Fox, *Anti-Cancer Drugs*, 2009, **20**, 483–492.
- 14 B. Nicholson, G. K. Lloyd, B. R. Miller, M. A. Palladino, Y. Kiso, Y. Hayashi and S. T. C. Neuteboom, *Anti-Cancer Drugs*, 2006, **17**, 25–31.
- 15 (a) W. Shi and D. W. Siemann, *Anticancer Res.*, 2005, **25**, 3899–3904; (b) A. M. Traynor, M. S. Gordon, D. Alberti, D. S. Mendelson, M. S. Munsey, G. Wilding, R. E. Gammans and W. L. Read, *Invest. New Drugs*, 2010, **28**, 509–515.
- 16 (a) S. Kasibhatla, H. Gourdeau, K. Meerovitch, J. Drewe, S. Reddy, L. Qiu, H. Zhang, F. Bergeron, D. Bouffard, Q. Yang, J. Herich, S. Lamothe, S. X. Cai and B. Tseng, *Mol. Cancer Ther.*, 2004, **3**, 1365–1373; (b) H. Gourdeau, L. Leblond, B. Hamelin, C. Desputeau, K. Dong, I. Kianicka, D. Custeau, C. Boudreau, L. Geerts, S.-X. Cai, J. Drewe, D. Labrecque, S. Kasibhatla and B. Tseng, *Mol. Cancer Ther.*, 2004, **3**, 1375–1383; (c) W. Kemnitzer, J. Drewe, S. Jiang, H. Zhang, Y. Wang, J. Zhao, S. Jia, J. Herich, D. Labrecque, R. Storer, K. Meerovitch, D. Bouffard, R. Rej, R. Denis, C. Blais, S. Lamothe, G. Attardo, H. Gourdeau, B. Tseng, S. Kasibhatla and S. X. Cai, *J. Med. Chem.*, 2004, **47**, 6299–6310.
- 17 G. Kremmidiotis, A. F. Leske, T. C. Lavanos, D. Beaumont, J. Gasic, A. Hall, M. O'Callaghan, C. A. Matthews and B. Flynn, *Mol. Cancer Ther.*, 2010, **9**, 1562–1573.
- 18 (a) P. D. Davis, G. J. Dougherty, D. C. Blakey, S. M. Galbraith, G. M. Tozer, A. L. Holder, M. A. Naylor, J. Nolan, M. R. Stratford, D. J. Chaplin and S. A. Hill, *Cancer Res.*, 2002, **62**, 7247–7253; (b) D. C. Blakey, F. R. Westwood, M. Walker, G. D. Hughes, P. D. Davis, S. E. Ashton and A. J. Ryan, *Clin. Cancer Res.*, 2002, **8**, 1974–1983; (c) L. V. Beerepoot, S. A. Radema, E. O. Witteveen, T. Thomas, C. Wheeler, S. Kempin and E. E. Voest, *J. Clin. Oncol.*, 2006, **24**, 1491–1498; (d) S. Gould, F. R. Westwood, J. O. Curwen, S. E. Ashton, P. D. Roberts, S. C. Lovick and A. J. Ryan, *J. Natl. Cancer Inst.*, 2007, **99**, 1724–1728.
- 19 (a) Z. J. Roberts, N. Goutagny, P.-Y. Perera, H. Kato, H. Kumar, T. Kawai, S. Akira, R. Saven, D. van Echo, K. A. Fitzgerald, H. A. Young, L.-M. Ching and S. N. Vogel, *J. Exp. Med.*, 2007, **204**, 1559–1569; (b) A. Wallace, D. F. LaRosa, V. Kapoor, J. Sun, G. Cheng, A. Jassar, A. Blouin, L.-M. Ching and S. M. Albelda, *Cancer Res.*, 2007, **67**, 7011–7019; (c) S. Gobbi, F. Belluti, A. Bisi, L. Piazzi, A. Rampa, A. Zampiron, M. Barbera, A. Caputo and M. Carrara, *Bioorg. Med. Chem.*, 2006, **14**, 4101–4109.
- 20 L.-M. Ching, Z. Cao, C. Kieda, S. Zwain, M. B. Jameson and B. C. Baguley, *Br. J. Cancer*, 2002, **86**, 1937–1942.
- 21 For reviews, see (a) K. H. Downing, *Annu. Rev. Cell Dev. Biol.*, 2000, **16**, 89–111; (b) L. A. Amos, *Org. Biomol. Chem.*, 2004, **2**, 2153–2160.
- 22 (a) M. A. Jordan, R. J. Toso, D. Thrower and L. Wilson, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 9552–9556 and references cited therein; (b) C. Elie-Caille, F. Severin, J. Helenius, J. Howard, D. J. Muller and A. A. Hyman, *Curr. Biol.*, 2007, **17**, 1765–1770; (c) S. Goodin, M. P. Kane and E. H. Rubin, *J. Clin. Oncol.*, 2004, **22**, 2015–2025.
- 23 B. Bhattacharyya, D. Panda, S. Gupta and M. Banerjee, *Med. Res. Rev.*, 2008, **28**, 155–183.
- 24 R. B. G. Ravelli, B. Gigant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel and M. Knossow, *Nature*, 2004, **428**, 198–202.
- 25 (a) B. Gigant, C. Wang, R. B. G. Ravelli, F. Roussi, M. O. Steinmetz, P. A. Curmi, A. Sobel and M. Knossow, *Nature*, 2005, **435**, 519–522; (b) A. Dorléans, B. Gigant, R. B. G. Ravelli, P. Mailliet, V. Mikol and M. Knossow, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 13775–13779.
- 26 S. M. Galbraith, D. J. Chaplin, F. Lee, M. R. L. Stratford, R. J. Locke, B. Vojnovic and G. M. Tozer, *Anticancer Res.*, 2001, **21**, 93–102.
- 27 (a) C. M. Lin, S. B. Singh, P. S. Chu, R. O. Dempcy, J. M. Schmidt, G. R. Pettit and E. Hamel, *Mol. Pharmacol.*, 1988, **34**, 200–208; (b) C. M. Lin, H. H. Ho, G. R. Pettit and E. Hamel, *Biochemistry*, 1989, **28**, 6984–6991.
- 28 For a review, see B. Bhattacharyya, D. Panda, S. Gupta and M. Banerjee, *Med. Res. Rev.*, 2008, **28**, 155–183.
- 29 T. J. Lampidis, D. Kolonias, N. Savaraj and R. W. Rubin, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 1256–1260.
- 30 (a) W. J. van Heeckeren, S. Bhakta, J. Ortiz, J. Duerk, M. M. Cooney, A. Dowlati, K. McCrae and S. C. Remick, *J. Clin. Oncol.*, 2006, **24**, 1485–1488; (b) For a study of the cardiovascular profile of CA-4P 2, see M. M. Cooney, T. Radivoyevitch, A. Dowlati, B. Overmoyer, N. Levitan, K. Robertson, S. L. Levine, K. DeCaro, C. Buchter, A. Taylor, B. S. Stambler and S. C. Remick, *Clin. Cancer Res.*, 2004, **10**, 96–100.
- 31 D. J. Edwards, R. G. Pritchard and T. W. Wallace, *Acta Crystallogr., Sect. B: Struct. Sci.*, 2005, **61**, 335–345.
- 32 T. W. Wallace, D. J. Edwards, and J. A. Hadfield, PCT Int. Appl. WO 2008075048, 2008.
- 33 For independent studies along related lines, see (a) V. Colombel, A. Joncour, S. Thoret, J. Dubois, J. Bignon, J. Wdzieczak-Bakala and O. Baudoin, *Tetrahedron Lett.*, 2010, **51**, 3127–3129; (b) A. Joncour, J.-M. Liu, A. Décor, S. Thoret, J. Wdzieczak-Bakala, J. Bignon and O. Baudoin, *ChemMedChem*, 2008, **3**, 1731–1739; (c) A. Joncour, A. Décor, S. Thoret, A. Chiaroni and O. Baudoin, *Angew. Chem., Int. Ed.*, 2006, **45**, 4149–4152; (d) N. Nicolaus, S. Strauss, J.-M. Neudörfl, A. Prokop and H.-G. Schmalz, *Org. Lett.*, 2009, **11**, 341–344.
- 34 E. Piers, J. G. K. Yee and P. L. Gladstone, *Org. Lett.*, 2000, **2**, 481–484.
- 35 H. Imanieh, P. Quayle, M. Voaden, J. Conway and S. D. A. Street, *Tetrahedron Lett.*, 1992, **33**, 543–546.
- 36 J. P. Deville and V. Behar, *J. Org. Chem.*, 2001, **66**, 4097–4098.
- 37 For reviews, see (a) T. D. Nelson and R. D. Crouch, *Org. React.*, 2004, **63**, 265–555; (b) J. Hassan, M. Sévignon, C. Gozzi, E. Schulz and M. Lemaire, *Chem. Rev.*, 2002, **102**, 1359–1469; (c) P. E. Fanta, *Synthesis*, 1974, 9–21.
- 38 E. Brown, J.-P. Robin and R. Dhal, *Tetrahedron*, 1982, **38**, 2569–2579.
- 39 H. Suzuki, T. Enya and Y. Hisamatsu, *Synthesis*, 1997, 1273–1276.
- 40 (a) F. E. Ziegler, I. Chliwner, K. W. Fowler, S. J. Kanfer, S. J. Kuo and N. D. Sinha, *J. Am. Chem. Soc.*, 1980, **102**, 790–798; (b) F. E. Ziegler, K. W. Fowler, W. B. Rodgers and R. T. Wester, *Org. Synth.*, 1987, **65**, 108–118.
- 41 J. M. Insole, *J. Chem. Res. (S)*, 1990, 378–379; J. M. Insole, *J. Chem. Res. (M)*, 1990, 2831–2867.
- 42 (a) G. Wu, H.-F. Guo, K. Gao, Y.-N. Liu, K. F. Bastow, S. L. Morris-Natschke, K.-H. Lee and L. Xie, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5272–5276; (b) L. G. Monovich, Y. Le Huérou, M. Rönn and G. A. Molander, *J. Am. Chem. Soc.*, 2000, **122**, 52–57.
- 43 I. Lantos and B. Loev, *Tetrahedron Lett.*, 1975, **16**, 2011–2014.
- 44 M. Oki, H. Iwamura and T. Nishida, *Bull. Chem. Soc. Jpn.*, 1968, **41**, 656–660.
- 45 (a) N. J. Lawrence, A. T. McGown, S. Ducki and J. A. Hadfield, *Anti-Cancer Drug Des.*, 2000, **15**, 135–141; (b) J. A. Woods, J. A. Hadfield, G. R. Pettit, B. W. Fox and A. T. McGown, *Br. J. Cancer*, 1995, **71**, 705–711.
- 46 (a) J. M. Edmondson, L. S. Armstrong and A. O. Martinez, *J. Tissue Cult. Methods*, 1988, **11**, 15–17; (b) T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55–63.
- 47 Y. Kataoka, M. Ishikawa, M. Miura, M. Takeshita, R. Fujita, S. Furusawa, M. Takayanagi, Y. Takayanagi and K. Sasaki, *Biol. Pharm. Bull.*, 2001, **24**, 612–617.
- 48 O. Boye, A. Brossi, H. J. C. Yeh, E. Hamel, B. Wegrzynski and V. Toome, *Can. J. Chem.*, 1992, **70**, 1237–1249.
- 49 M. G. Banwell, J. M. Cameron, M. Corbett, J. R. Dupuche, E. Hamel, J. N. Lambert, C. M. Lin and M. F. Mackay, *Aust. J. Chem.*, 1992, **45**, 1967–1982.
- 50 S. Ducki, G. Mackenzie, B. Greedy, S. Armitage, J. Fournier, D. Chabert, E. Bennett, J. Nettles, J. P. Snyder and N. J. Lawrence, *Bioorg. Med. Chem.*, 2009, **17**, 7711–7722.
- 51 For a discussion, see K. Mikami, K. Aikawa, Y. Yusa, J. J. Jodry and M. Yamanaka, *Synlett*, 2002, 1561–1578.
- 52 D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, *Purification of Laboratory Chemicals, 2nd Edition*, Pergamon, Oxford, 1980.
- 53 W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923–2925.
- 54 N. Meyer and D. Seebach, *Chem. Ber.*, 1980, **113**, 1304–1319.
- 55 S. B. Singh and G. R. Pettit, *Synth. Commun.*, 1987, **17**, 877–892.
- 56 S. Mabic, L. Vaysse, C. Benezra and J.-P. Lepoittevin, *Synthesis*, 1999, 1127–1134.
- 57 Y. Wang, C. A. Mathis, G. Huang, D. P. Holt, M. L. Debnath and W. E. Klunk, *J. Labelled Compd. Radiopharm.*, 2002, **45**, 647–664.
- 58 T. Takada, M. Arisawa, M. Gyoten, R. Hamada, H. Tohma and Y. Kita, *J. Org. Chem.*, 1998, **63**, 7698–7706.
- 59 D. A. Fletcher, R. F. McMeeking and D. Parkin, *J. Chem. Inf. Comput. Sci.*, 1996, **36**, 746–749.