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Bioorganic & Medicinal Chemistry 14 (2006) 4627–4638
Bioorganic & Medicinal Chemistry

4,5-Diaryl-1*H*-pyrrole-2-carboxylates as combretastatin A-4/lamellarin T hybrids: Synthesis and evaluation as anti-mitotic and cytotoxic agents

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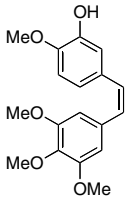
Received 10 January 2006; revised 8 February 2006; accepted 8 February 2006
Available online 28 February 2006

Abstract—The 4,5-diarylated-1*H*-pyrrole-2-carboxylates **3**–**8** have each been prepared as hybrids of the potent anti-mitotic agent combretastatin A-4 (**1**) and the similarly active marine alkaloid lamellarin T (**2**). The key steps involved selective lithium-for-halogen exchange at C5 within the N-PMB protected 4,5-dibromopyrrole **22** and Negishi cross-coupling of the derived zincated species with the relevant aryl iodide. The ensuing 5-aryl-4-bromopyrrole then engaged in Suzuki–Miyaura cross-coupling with the appropriate arylboronic acid to give the 4,5-diarylated pyrroles **4**, **6** and **8**. TFA-promoted removal of the *N*-PMB group within these last compounds then gave the N-unsubstituted congeners **3**, **5** and **7**. Compounds **3**–**8** have all been evaluated for their anti-mitotic and cytotoxic properties and two of them, **3** and **5**, display useful activities although they are less potent than combretastatin A-4.

1. Introduction

Combretastatin A-4 (**1**) is arguably the most biologically significant member of a group of *cis*-stilbenes isolated from a range of sources including the deciduous African willow tree *Combretum caffrum* Kuntz, extracts of which have been used in traditional medicines.[1–3] As a result of its capacity to dock in the colchicine binding site of tubulin, compound **1** displays potent anti-mitotic properties that have led to its examination as an anti-angiogenic agent. Indeed, a prodrug form of this natural product is now undergoing (phase I/II) clinical evaluation in the US and Europe as an agent for the treatment of various multi-drug resistant solid tumors.[1–3] Clearly then, combretastatin A-4 displays some highly attractive biological properties. However, its limited solubility in water and its potential to isomerise to the thermodynamically more stable (and essentially inactive) *trans*-isomer have prompted an extensive effort to find analogues that might display greater stability and/or improved solubilities in therapeutically relevant solvent systems.[1–6] Many structural variations have been investigated including those wherein there is a one-, two- or three-atom bridge linking the two multiply oxygenated and *cis*-related aromatic rings that appear to be essential requirements for biological activity.[1–6] The many efforts in this area have been summarised in two recent reviews.[1,2]



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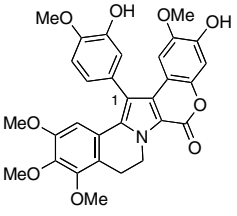

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Another intriguing class of natural product displaying related structural and biological properties is the lamellarins, a family of pyrrolic marine alkaloids.[7]

Keywords: Anti-mitotic; *cis*-Stilbene; Combretastatin A-4; Cytotoxic; Lamellarin T.
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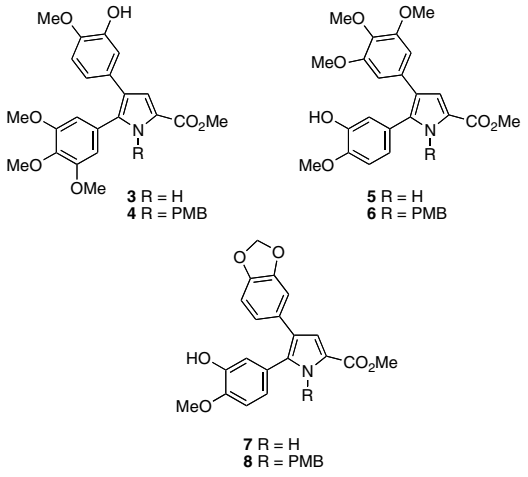
0968-0896/$ - see front matter
doi:10.1016/j.bmc.2006.02.018

Compound **2** (lamellarin T) is a representative example of that subclass incorporating a 6*H*-[1]-benzopyrano-[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolinone core and carrying an aryl substituent at C1 and which display topoisomerase I inhibitory, cytotoxic, reversion of multi-drug resistance, anti-bacterial and/or anti-mitotic properties. As such they are regarded as important new lead compounds in a number of therapeutic areas.[7] Inspection of the structure of compound **2** reveals that it incorporates a combretastatin A4-like substructure and, furthermore, because the two relevant aryl rings are attached at the C4- and C5-positions (pyrrole numbering) of the central pyrrole ring they are necessarily rigidly maintained in a *cis*-relationship to one another. On this basis, and as part of a program underway in our laboratories[8] to identify natural product-inspired anti-mitotic and anti-angiogenic agents, we saw merit in preparing a series of combretastatin A-4/lamellarin T hybrids for biological evaluation, particularly as anti-mitotic agents.[7] Specifically, we sought to prepare examples of the title compounds wherein each aryl unit would incorporate the same or closely related oxygenation patterns to those seen in compounds **1** and **2**. This type of study would allow for an assessment of the utility, or otherwise, of the methyl 4,5-diaryl-1*H*-pyrrole-2-carboxylate unit as a scaffold for preparing new anti-mitotic agents.[9,10] The presence of the carbomethoxy group within such constructs was of particular interest because of the possibility such a unit, or derivatives thereof, might offer for addressing the solubility issues associated with the development of compound **1** as a drug candidate. A further intriguing possibility associated with the target scaffold arises because of the presence of the embedded pseudo-α-amino acid residue and the capacity, therefore, to use this as a site for conjugation with specific peptide sequences that might deliver constructs likely to be more cytoselective anti-tumour agents.[11]

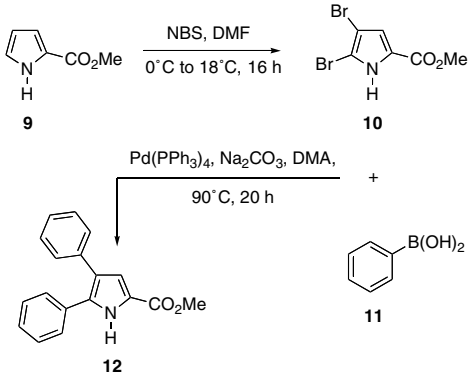
On the basis of the foregoing, we viewed the 4,5-diaryl-1*H*-pyrrole-2-carboxylates **3**–**8** as representing prototypical hybrids of the title natural products. In particular, compounds **3** and **5** seem the best amalgamation of structures **1** and **2**, while congener **7** represents an analogue lacking the 3,4,5-trimethoxyaryl residue often regarded as essential for the anti-mitotic properties of combretastatin A-4.[1,2] The *N*-*p*-methoxybenzyl- or N-PMB-substituted systems **4**, **6** and **8**, which were necessary precursors (see below) to their N-unsubstituted counterparts **3**, **5** and **7**, were also evaluated for their anti-mitotic and cytotoxic properties. The following section details the methodologies developed for the purposes of preparing the target hybrids **3**–**8**.

2. Synthetic studies

In connection with studies directed towards the synthesis of the simpler lamellarins, we established that Suzuki–Miyaura cross-coupling reactions between 3,4-dihalogenated pyrroles and the relevant arylboronic acid provided a useful method for assembling the 3,4-diarylpyrrole substructures of these natural products.[9,12] Consequently, the application of such protocols seemed an obvious first approach to the target compounds **3**–**8**. The lack of symmetry in the requisite 4,5-dihalogeno-1*H*-pyrrole-2-carboxylate coupling partner and the similar nature of the target hybrids immediately raised issues about whether a regioselective reaction of such a substrate with the appropriate arylboronic acid was possible. Our initial efforts, therefore, were focused on investigating such matters. To these ends (Scheme 1), methyl 1*H*-pyrrole-2-carboxylate (**9**)[13] was treated with two equivalents of *N*-bromosuccinimide (NBS) and the required coupling partner **10**[14] obtained, but in only 29% yield. It was hoped that by using carefully controlled conditions and equimolar quantities of phenylboronic acid (**11**) a regioselective Suzuki–Miyaura cross-coupling reaction with dibromide **10** might occur. Unfortunately, when equimolar quantities of the substrates were heated at 90 °C in dimethylacetamide (DMA), and in the presence of sodium carbonate and $Pd(PPh_3)_4$, a complex mixture of reaction products was produced. Mass spectrometric analysis of this mixture indicated the presence of the diphenylated product **12** but not of any monoarylated precursors. As a result, this approach to the target compounds was abandoned.

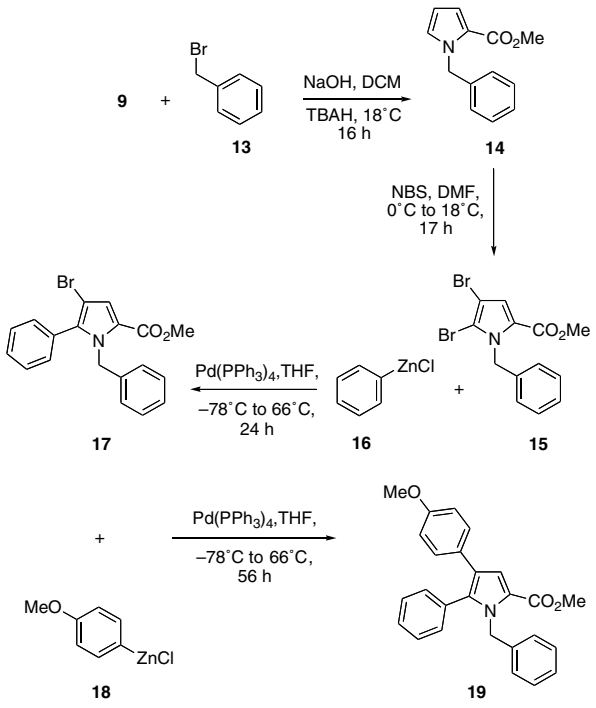


In earlier work,[9] we had established that methyl 3,4-dibromo-1-(triisopropylsilyl)-1*H*-pyrrole-2-carboxylate engaged in carbomethoxy-group directed lithium-for-C3-bromine exchange on exposure to phenyllithium. The ensuing lithio-species could then be transmetallated,



Scheme 1.

using $ZnCl_2$, to give the corresponding zincated species. This last compound was shown to engage in smooth Negishi cross-coupling reactions with various aryl halides to give, in a completely regioselective manner, the corresponding 3-aryl-4-bromo-1*H*-pyrrole-2-carboxylate. By repeating this sort of reaction sequence on the last compound, the C4-bromine could be replaced by an aryl unit and so provide various methyl 3,4-diaryl-1*H*-pyrrole-2-carboxylates.[9] We sought to apply this approach as a route to compounds **3**–**8**. This required working with an N-protected form of the methyl 4,5-dibromo-1*H*-pyrrole-2-carboxylate because the organozinc species used as a reaction partner in the initial Negishi cross-coupling reaction would be basic. Thus (Scheme 2), compound **9**[13] was N-benzylated using benzyl bromide (**13**) in the presence of base. Product **14**[15] (95%) was treated with two equivalents of NBS to afford compound **15** (93%), which would serve as the substrate in the pivotal Negishi reaction. Initially phenylzinc chloride (**16**) was used as the reaction partner for investigating the selectivity of cross-coupling processes involving dibromide **15**. The monoarylated and crystalline pyrrole **17** (74%) was the only reaction product and its structure was obtained from a single-crystal X-ray analysis (see Fig. 1 and Section 5). Selective formation of product **17** is consistent with other observations[12] that, in the absence of other directing groups, metallation (including palladation) of pyrroles takes place preferentially adjacent to the ring nitrogen. When the monoarylated system **17** was subjected to cross-coupling with *p*-methoxyphenylzinc chloride (**18**) the anticipated product **19** was obtained in 81% yield. Clearly, then, such cross-coupling processes offer considerable potential for the assembly of the desired methyl 4,5-diarylated-1*H*-pyrrole-2-carboxylates. However, we



Scheme 2.

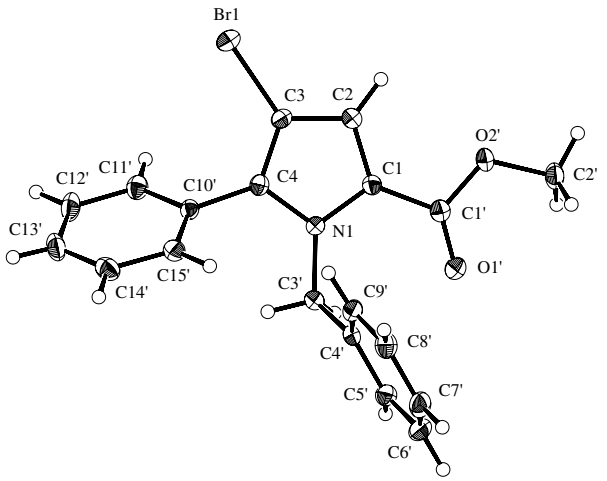
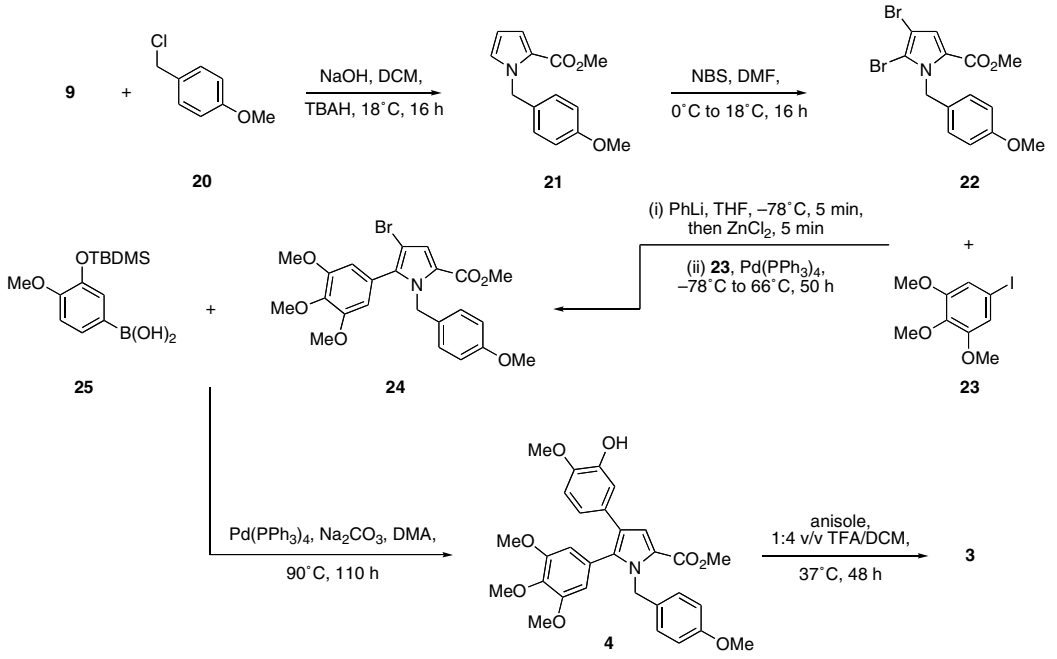


Figure 1. ORTEP derived from a single-crystal X-ray analysis of compound **17**. (Non-hydrogen atoms have been labelled using a crystallographic numbering scheme).

were unable to effect the cleavage of the *N*-benzyl group of **19** with a wide variety of agents. Thus, an alternate nitrogen-protecting group was required for the synthesis of compounds **3**, **5** and **7**. The use of the PMB-group to protect the pyrrole nitrogen met all synthetic requirements.

The successful syntheses of compounds **3** and **4** are shown in Scheme 3. The reaction sequence began with the N-protection of pyrrole **9** using *p*-methoxybenzyl chloride (PMB-Cl, **20**). The N-substituted **21** (75%) was brominated with NBS to give the dibromide **22** in 83% yield. To explore further the utility of the Negishi-coupling reaction, compound **22** was subjected to a lithium-for-bromine exchange, followed by transmetallation with $ZnCl_2$. The ensuing zincated species was cross-coupled with aryliodide **23**[16] and the C5-arylated pyrrole **24** was thereby obtained in 82% yield. The structure of this last compound follows from a single-crystal X-ray analysis of derivative **3** (see below). Suzuki–Miyaura cross-coupling of bromide **24** with the previously unreported but readily prepared arylboronic acid **25** (see Section 5) gave, after extended reaction times (110 h), compound **4** in 14% yield. The cause of the inefficient nature of this second cross-coupling step is unclear. Such difficulties were not encountered while carrying out the analogous steps associated with the preparation of the structurally related compounds **6** and **8**. The spectral data derived from compound **4** were in full accord with the assigned structure but final confirmation followed removal of the PMB-group by treatment with TFA/DCM in the presence of anisole (as cation scavenger), as described by Evans et al.[17] The structure of the resulting congener **3** (99%) was determined from a single-crystal X-ray analysis (see Fig. 2 and Section 5).

The reaction sequence leading to hybrids **5** and **6** is shown in Scheme 4, and used the same strategy as just described for the preparation of congeners **3** and **4**. Thus, the C5-zincated species derived from dibromide **22** was crossed-coupled with iodide **26**[18] to give the



Scheme 3.

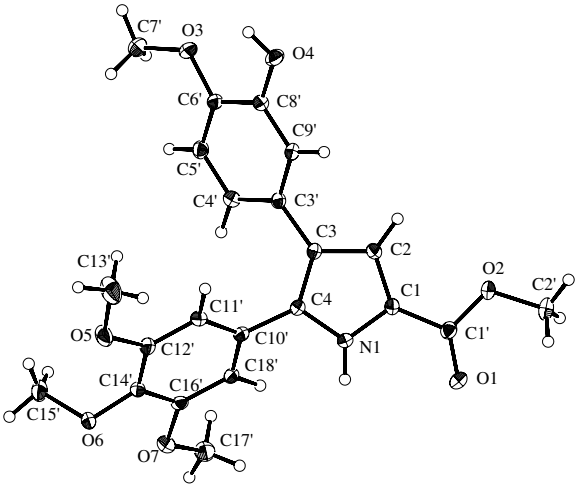
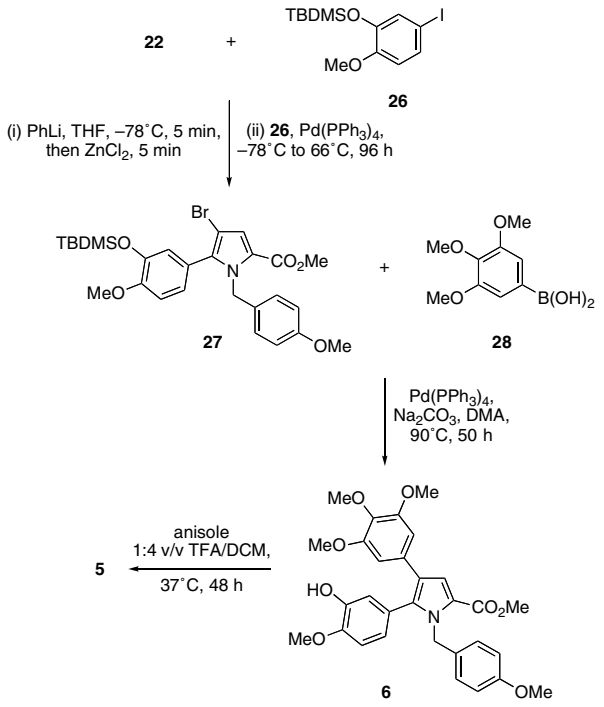


Figure 2. ORTEP derived from a single-crystal X-ray analysis of compound **3**. (Non-hydrogen atoms have been labelled using a crystallographic numbering scheme.)

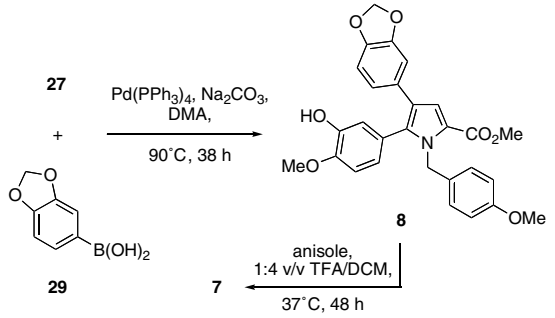
anticipated product **27** in 56% yield. Suzuki–Miyaura cross-coupling of compound **27** with arylboronic acid **28**,[19] readily prepared from iodide **23**, proceeded slowly (50 h) but afforded the N-benzylated and 4,5-diarylated pyrrole **6** in 52% yield. Removal of the PMB-group within pyrrole **6** was again achieved using the Evans' protocol[17] and compound **5** thus obtained in 62% yield. The spectral data derived from product **5** were closely related to but not identical with those obtained from isomer **3**, the structure of which was obtained by X-ray analysis. These similarities, together with the synthetic routes employed, provide strong evidence in support of the structure assigned to target **5**.

The preparation of the final pair of target hybrids, compounds **7** and **8**, proceeded in much the same way as



Scheme 4.

detailed above. Specifics are presented in Scheme 5. Thus, Suzuki–Miyaura cross-coupling of bromopyrrole **27** with commercially available boronic acid **29** afforded, after 38 h, product **8** in 87% yield. Removal of the PMB-group[17] gave compound **7** in 60% yield. The spectral data derived from these compounds were in full accord with the proposed structures, but final confirmation of the structure of pyrrole **7** was obtained from a single-crystal X-ray analysis (see Fig. 3 and Section 5). This



Scheme 5.

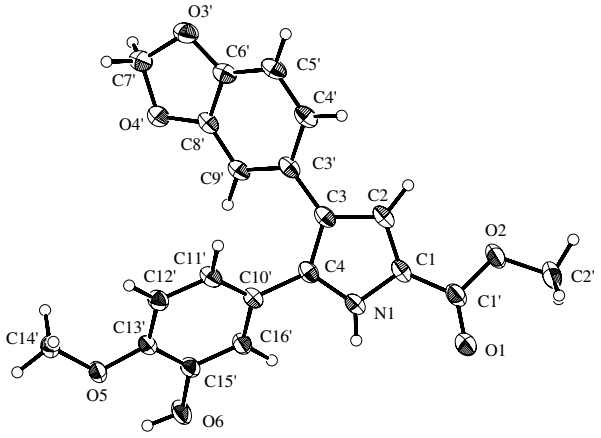


Figure 3. ORTEP derived from a single-crystal X-ray analysis of compound **7**. (Non-hydrogen atoms have been labelled using a crystallographic numbering scheme).

analysis provides additional evidence that the assigned structures of compounds **27**, **5** and **6** are correct.

3. Biological studies

The evaluation of the title hybrids **3**–**8** (Table 1) as potential anti-mitotic agents was undertaken using well-established assays (see Section 5), with direct comparisons with authentic samples of combretastatin A-4 (**1**), generously provided by G. R. Pettit, Arizona State University. Hybrids **3** and **5** most closely resembled the natural product **1** in terms of inhibitory effects on the binding of [^{3}H]colchicine to bovine brain tubulin. As a consequence, these compounds also proved to be potent inhibitors of tubulin polymerisation, with IC_{50}s essentially identical with that of combretastatin A-4 (**1**). Compound **7** was significantly less active while the three N-PMB-substituted systems, **4**, **6** and **8**, were essentially inactive. These biochemical results were mirrored in the effects of the various compounds on the growth of CA46 Burkitt lymphoma cells. Thus, the IC_{50} values obtained for compounds **3** and **5** in this assay were 29 and 31 nM, respectively, while hybrids **4**, **6** and **8** displayed values >1000. These values compare with an IC_{50} of 3.2 nM observed for combretastatin A-4 (**1**). The increased mitotic indices obtained for the same cells, treated with concentrations of **1**, **3** and **5** 10-fold higher than the IC_{50}s, further confirm that tubulin is the probable target of **3** and **5**, as well as of **1**.

4. Conclusions

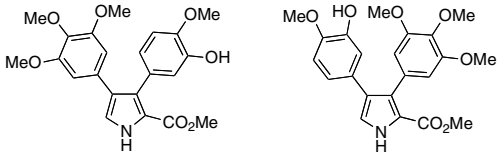
Compounds **3** and **5**, the hybrids most closely resembling combretastatin A-4 in structure, have potent anti-mitotic and cytotoxic properties, owing to their binding to the colchicine site on tubulin. Compound **7**, lacking the 3,4,5,-trimethoxyaryl residue, showed a 4-fold loss of activity relative to compounds **3** and **5** as an inhibitor of tubulin assembly. This result is consistent with other reports.[1,2] The lack of activity displayed by the N-PMB-substituted systems **4**, **6** and **8** suggests that contiguous substitution at four adjacent positions on the pyrrole backbone disrupts the relevant ligand–protein binding. Such extensive substitution may prevent the two vicinally related aryl units from adopting the necessary conformation for binding at the colchicine site on tubulin. The removal of the N-substituent in compounds **3** and **5** would reduce such restrictions so that appropriate binding conformations can occur. It is particularly interesting that hybrids **3** and **5** are equally active, thus showing that either aryl unit could be at positions C4 and C5 on the pyrrole backbone. In such constructs each unit is 2-atoms removed from the carbon bearing the carbomethoxy group. In contrast, we previously[9] found that the isomeric compounds **30** and **31** are essentially inactive as anti-mitotic agents.[20] This lack of activity may arise because of conformational impediments imposed by the C2-carbomethoxy group associated with

Table 1. Evaluation of 4,5-diaryl-1*H*-pyrrole-2-carboxylates **3**–**8** as anti-mitotic and cytotoxic agents

Compound	%Inhibition of colchicine binding[a]	Inhibition of tubulin polymerisation[b] IC_{50} (μM)	Inhibition of CA46 Burkitt lymphoma cell growth[c] IC_{50} (nM)	Mitotic index[d] % (drug concn.)
1	95	1.1 (±0.1)	3.2	24 (30 nM)
3	74	1.4 (±0.1)	29	15 (300 nM)
4	—	>40	>1000	—
5	74	1.3 (±0.2)	31	19 (300 nM)
6	—	>40	>5000	—
7	19	5.8 (±1.0)	—	—
8	—	>40	>1000	—

[a] Results of two experiments—[tubulin] = 1 μM, [^{3}H]colchicine and inhibiting drug at 5 μM.
[b] Results of three experiments.
[c] Results of two experiments.
[d] 400 cells counted (one experiment). Cells examined for growth after 24 h. Without drug, 2% of the cells were in mitosis.

the latter compounds. Clearly, the position of the carbomethoxy group will be of critical importance in the design, for example, of compounds linked to short peptide sequences that might facilitate tumour recognition and provide less toxic, more selective anti-cancer agents.[11] Work directed towards examining such possibilities is now underway in our laboratories.



30 31

5. Experimental

5.1. Synthetic studies

5.1.1. General procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer using deuterochloroform as solvent. Infrared spectra were recorded using KBr plates on either a Perkin-Elmer 683 or 1800 FTIR instrument. Unless otherwise specified, mass spectral analyses were carried out in electron-impact mode and on a VG Micromass 7070F Double-Focusing Spectrometer. Thin layer chromatographic analyses were carried out on aluminium-backed 0.2 mm thick silica gel 60 GF_{254} plates supplied by Merck while flash chromatographic purifications were conducted according to the method of Still et al.[21] using Merck silica gel 60 (230–400 mesh) as adsorbent. All solvents and common reagents were purified by established procedures.[22]

5.1.2. Methyl 1*H*-pyrrole-2-carboxylate (9). The title compound was prepared according to literature procedures[13] and obtained in 87% yield as a pale-pink solid, mp 70–73 °C (lit. mp 73–74 °C).[13] ^{1}H NMR (300 MHz) δ 9.15 (br s, 1H), 6.98 (m, 1H), 6.95 (m, 1H), 6.27 (m, 1H), 3.88 (s, 3H).

5.1.3. Methyl 4,5-dibromo-1*H*-pyrrole-2-carboxylate (10). A solution of NBS (1.47 g, 8.3 mmol) in DMF (15 mL) was added, dropwise, to a magnetically stirred solution of pyrrole **9** (516 mg, 4.1 mmol) in DMF (30 mL) maintained at 0 °C under a nitrogen atmosphere. The mixture was allowed to warm to 18 °C over 1 h, and stirring continued at this temperature for 15 h. The mixture was poured into diethyl ether (150 mL) and water (150 mL) and the separated aqueous phase extracted with diethyl ether (3× 150 mL). The combined organic extracts were washed with water (3× 500 mL) and brine (1× 500 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a pale-yellow oil. Subjection of this material to flash chromatography (1:5 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f 0.3) afforded a pale-yellow oil that crystallised upon trituration (ether/hexane) to give the title methyl ester **10**[14] (333 mg, 29%) as colourless needles, mp 158–160 °C (lit. mp 158–159 °C).[14] ^{1}H NMR (500 MHz) δ 9.60 (br s, 1H), 6.89 (d, *J* 2.7 Hz, 1H), 3.88 (s, 3H). ^{13}C NMR (75 MHz) δ 160.4 (C), 123.6 (C), 118.0 (CH), 107.3 (C), 100.6 (C), 52.2 (CH_3); *m*/*z* (70 eV) 285, 283 and 281 (42%, 71% and 44%, all $M^{+\cdot}$), 253, 251 and 249 (61, 100 and 61), 226, 224 and 222 (8, 15 and 8).

5.1.4. Methyl 1-benzyl-1*H*-pyrrole-2-carboxylate (14). Benzyl bromide (**13**) (3.14 g, 18.4 mmol) was added dropwise to a magnetically stirred mixture of pyrrole **9** (2.09 g, 16.7 mmol), sodium hydroxide (150 mL of a 50% w/v aqueous solution) and tetra-*n*-butylammonium hydroxide (1 mL of a 40% w/v aqueous solution) in DCM (150 mL) maintained at 0 °C. The ensuing mixture was allowed to warm to 18 °C over 1 h, stirred at this temperature for 15 h then acidified with 3 M HCl to pH 1. The separated aqueous phase was extracted with DCM (3× 150 mL) and the combined organic extracts dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a tan-coloured oil. Subjection of this material to flash chromatography (silica, 1:5 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f 0.8) furnished the title pyrrole **14**[15] (3.39 g, 95%) as a pale-green solid, mp 31–32 °C (lit. mp 31–32 °C).[15] ^{1}H NMR (300 MHz) δ 7.34–7.25 (complex m, 1H), 7.30 (d, *J* 7.6 Hz, 2H), 7.11 (d, *J* 7.6 Hz, 2H), 7.01 (m, 1H), 6.90 (m, 1H), 6.20 (m, 1H), 5.58 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (75 MHz) δ 161.2 (C), 138.1 (C) 128.9 (CH), 128.4 (2× CH), 127.2 (CH), 126.6 (2× CH), 121.7 (C), 118.2 (CH), 108.3 (CH), 51.8 (CH_3), 50.8 (CH_2); *m*/*z* (70 eV) 215 (77%, $M^{+\cdot}$), 183 (32), 156 (19), 91 (100, $C_7H_7^+$).

5.1.5. Methyl 4,5-dibromo-1-benzyl-1*H*-pyrrole-2-carboxylate (15). A solution of NBS (5.39 g, 30.3 mmol) in DMF (20 mL) was added dropwise to a magnetically stirred solution of methyl 1-benzyl-1*H*-pyrrole-2-carboxylate (**14**) (3.26 g, 15.2 mmol) in DMF (50 mL) maintained at 0 °C. The mixture was allowed to warm to 18 °C over ca. 1 h, stirred at this temperature for 16 h then partitioned between diethyl ether (150 mL) and cold water (150 mL). The separated aqueous phase was extracted with diethyl ether (3× 100 mL) and the combined organic phases washed with water (10× 100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a pale-yellow solid. Recrystallisation of this material (diethyl ether/hexane) afforded methyl 4,5-dibromo-1-benzyl-1*H*-pyrrole-2-carboxylate (**15**) (5.20 g, 93%) as a colourless solid, mp 111–113 °C (Found: C, 42.00; H, 2.98; N, 3.68; Br, 42.52. $C_{13}H_{11}Br_2NO_2$ requires C, 41.86; H, 2.97; N, 3.75; Br, 42.84%). ^{1}H NMR (500 MHz) δ 7.36–7.21 (complex m, 3H), 7.09 (s, 1H), 7.02 (d, *J* 7.1 Hz, 2H), 5.73 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (75 MHz) δ 159.7 (C), 136.6 (C) 128.5 (2× CH), 127.4 (CH), 126.3 (2× CH), 123.5 (C), 120.0 (CH), 113.6 (C), 99.6 (C), 51.5 (CH_3), 51.1 (CH_2); ν_{max} (KBr) 3150, 2943, 1701, 1391, 1254 cm^{-1}; *m*/*z* (70 eV) 375, 373 and 371 (6, 12 and 7%, all $M^{+\cdot}$), 91(100, $C_7H_7^+$).

5.1.6. Methyl 4-bromo-1-benzyl-5-phenyl-1*H*-pyrrole-2-carboxylate (17). *tert*-Butyllithium (9.60 mL of a 1.7 M solution in pentane, 16.4 mmol) was added dropwise

to a magnetically stirred solution of bromobenzene (1.29 g, 8.2 mmol) in dry THF (10 mL) maintained at −78 °C. Stirring was continued for a further 0.2 h, and anhydrous zinc chloride (1.12 g, 8.2 mmol) was added and the cold bath removed. Whilst warming, methyl 4,5-dibromo-1-benzyl-2-pyrrolecarboxylate (**15**) (509 mg, 1.4 mmol) and $Pd(PPh_3)_4$ (473 mg, 0.4 mmol) were added to the reaction mixture, which was then stirred at reflux for 22 h. After this time, the reaction mixture was cooled, treated with flash chromatography-grade silica (2.31 g) and concentrated under reduced pressure. The resulting free-flowing solid was added to the top of a flash chromatography column filled with silica, and this was eluted with 2:3 v/v DCM/hexane. Concentration of the appropriate fractions (R_f 0.5) afforded a pale-yellow oil trituration (diethyl ether/hexane) of which afforded methyl 4-bromo-1-benzyl-5-phenylpyrrole (**17**) (370 mg, 74%) as colourless needles, mp 78–80 °C (Found: C, 61.45; H, 4.39; N, 3.61; Br, 21.34. $C_{19}H_{16}BrNO_2$ requires C, 61.64; H, 4.36; N, 3.78; Br, 21.58%). ^{1}H NMR (500 MHz) δ 7.35 (m, 3H), 7.26 (m, 2H), 7.21–7.16 (complex m, 4H), 6.80 (d, *J* 7.0 Hz, 2H), 5.52 (s, 2H), 3.73 (s, 3H); ^{13}C NMR (75 MHz) δ 160.5 (C), 139.1 (C), 138.4 (C), 130.6 (2× CH), 129.6 (C), 129.0 (CH), 128.4 (4× CH), 127.0 (CH), 125.7 (2× CH), 122.2 (C), 120.1 (CH), 97.1 (C), 51.3 (CH_3), 49.8 (CH_2); ν_{max} (KBr) 3565, 3319, 1710, 1453, 1258, 1190 cm^{-1}; *m*/*z* (70 eV) 371 and 369 (both 47%, $M^{+\cdot}$), 340 and 338 (both 7), 91 (100, $C_7H_7^+$).

5.1.7. Methyl 1-benzyl-4-(4-methoxyphenyl)-5-phenyl-1*H*-pyrrole-2-carboxylate (19). *tert*-Butyllithium (5.50 mL of a 1.7 M solution in pentane, 9.4 mmol) was added dropwise to a magnetically stirred solution of *p*-methoxybromobenzene (880 mg, 4.7 mmol) in dry THF (10 mL) maintained at −78 °C. Stirring was continued for 0.2 h at this temperature, and anhydrous zinc chloride (641 mg, 4.7 mmol) was added to the reaction mixture and the cold bath removed. Whilst warming, compound **17** (290 mg, 0.8 mmol) and $Pd(PPh_3)_4$ (91 mg, 0.1 mmol) were added to the reaction mixture, which was then stirred at reflux for 56 h. After this time, the reaction mixture was cooled, treated with flash chromatography-grade silica (480 mg) and concentrated under reduced pressure. The resulting free-flowing solid was added to the top of a flash chromatography column filled with silica, and this was eluted with 2:3 v/v DCM/hexane. Concentration of the appropriate fractions (R_f 0.2) afforded a pale-yellow oil. Crystallisation (diethyl ether/hexane) of this material afforded methyl 1-benzyl-4-(4-methoxyphenyl)-5-phenyl-1*H*-pyrrole-2-carboxylate (**19**) (225 mg, 81%) as colourless needles, mp 131–132 °C (Found: C, 78.47; H, 5.76; N, 3.30. $C_{26}H_{23}NO_3$ requires C, 78.57; H, 5.83; N, 3.52%). ^{1}H NMR (500 MHz) δ 7.32–7.27 (complex m, 4H), 7.22–7.15 (complex m, 5H), 7.07–7.05 (complex m, 2H), 6.84 (d, *J* 7.5 Hz, 2H), 6.72–6.70 (complex m, 2H), 5.50 (s, 2H), 3.76 (s, 3H), 3.72 (s, 3H); ^{13}C NMR (125 MHz) δ 161.4 (C), 157.7 (C), 139.1 (C), 137.7 (C), 131.6 (C), 131.0 (2× CH), 128.8 (2× CH), 128.6 (2× CH), 128.5 (CH), 128.3 (2× CH), 127.5 (C), 126.7 (CH), 125.8 (2× CH), 123.2 (C), 121.8 (C), 117.5 (CH), 113.6 (2× CH), 55.1 (CH_3), 51.1 (CH_3), 48.9 (CH_2); ν_{max} (KBr) 3313, 1736, 1700, 1455, 1247, 1175 cm^{-1}; *m*/*z* (70 eV) 397(100%, $M^{+\cdot}$), 382(5), 366(7), 306(14), 91(82, $C_7H_7^+$).

5.1.8. Methyl 1-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxylate (21). A magnetically stirred mixture of aqueous sodium hydroxide (150 mL of a 50% w/v solution) and methyl 1*H*-pyrrole-2-carboxylate (**9**) (4.41 g, 35.2 mmol) in DCM (50 mL) maintained at 18 °C was treated with PMB-Cl (**20**) (6.07 g, 38.8 mmol). The mixture was stirred for 16 h then neutralised with 3 M HCl. The resulting mixture was extracted with DCM (3× 150 mL) then dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a brown oil. Subjection of this material to flash chromatography (silica, 3:2 v/v DCM/hexane elution) and concentration of the appropriate fractions (R_f 0.3) afforded methyl 1-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxylate (**21**) (6.50 g, 75%) as a pale-yellow oil (Found: $M^{+\cdot}$, 245.1049. $C_{14}H_{15}NO_3$ requires $M^{+\cdot}$, 245.1052). ^{1}H NMR (300 MHz) δ7.08 (d, *J* 8.5 Hz, 2H), 6.98(m, 1H), 6.86 (m, 1H), 6.83 (d, *J* 8.5 Hz, 2H), 6.15 (m, 1H), 5.48 (s,2H), 3.77 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (75 MHz) δ 161.3 (C), 158.8 (C), 130.1 (C), 128.6 (CH), 128.3 (2× CH), 121.6 (C), 118.2 (CH), 113.8 (2× CH), 108.2 (CH), 55.0 (CH_3), 51.3 (CH_2), 50.8 (CH_3); ν_{max} (KBr) 3000, 2950, 1704, 1612, 1514, 1437, 1107 cm^{-1}; *m*/*z* (70 eV) 245 (30%, $M^{+\cdot}$), 230 (12), 213 (7), 121 [100, $(C_7H_6OCH_3)^+$].

5.1.9. Methyl 4,5-dibromo-1-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxylate (22). A solution of NBS (4.61 g, 25.9 mmol) in DMF (15 mL) was added dropwise to a magnetically stirred solution of methyl 1-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxylate (**21**) (3.68 g, 13.0 mmol) in DMF (50 mL) maintained at 0 °C. The mixture was allowed to warm to 18 °C over ca. 1 h, stirred for 16 h then partitioned between diethyl ether (100 mL) and cold water (150 mL). The aqueous phase was extracted with diethyl ether (3× 100 mL), and the combined organic phases were washed with water (10× 100 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a pale-yellow oil. Crystallisation of this material (diethyl ether/hexane) then afforded methyl 4,5-dibromo-1-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxylate (**22**) (4.33 g, 83%) as a clear, colourless solid, mp 66–67 °C (Found: C, 41.42; H, 3.03; N, 3.56; Br, 39.64. $C_{14}H_{13}Br_2NO_3$ requires C, 41.72; H, 3.25; N, 3.48; Br, 39.65%). ^{1}H NMR (300 MHz) δ 7.08 (s, 1H), 7.06 (d, *J* 8.7 Hz, 2H), 6.83 (d, *J* 8.7 Hz, 2H), 5.66 (s, 2H), 3.78 (s, 3H), 3.77 (s, 3H); ^{13}C NMR (75 MHz) δ 159.8 (C), 158.8 (C), 128.8 (C), 128.0 (2× CH), 123.4(C), 120.0 (CH), 113.9 (2× CH), 113.4 (C), 99.6 (C), 55.1 (CH_3), 51.5 (CH_3), 50.6 (CH_2); ν_{max} (KBr) 2952, 1712, 1613, 1514, 1436, 1250 cm^{-1}; *m*/*z* (70 eV) 405, 403 and 401 (15, 24 and 16%, all $M^{+\cdot}$), 121 [100, $(C_7H_6OCH_3)^+$].

5.1.10. 5-Iodo-1,2,3-trimethoxybenzene (23). Sodium nitrite (6.78 g, 98.3 mmol) was added in portions to a magnetically stirred solution of 3,4,5-trimethoxyaniline (15.0 g, 81.9 mmol, ex. Aldrich Chemical Co.) in H_2SO_4 (150 mL of a 6% w/v aqueous solution, 91.8 mmol) maintained at 0 °C. After 1 h, the yellow

solution was added dropwise over 1 h to a magnetically stirred solution of potassium iodide (16.3 g, 98.2 mmol) in water (50 mL) maintained at 0 °C. The mixture was stirred for a further 2 h at 0 °C then poured into ethyl acetate (200 mL). The separated aqueous phase was extracted with ethyl acetate (3× 200 mL), and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a dark-yellow oil. Subjection of this material to flash chromatography (1:10 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f 0.2) furnished 5-iodo-1,2,3-trimethoxybenzene (**23**)[16] (11.5 g, 48%) as a pale-yellow solid, mp 83–85 °C (lit. mp 85–87 °C).[16] ^{1}H NMR (300 MHz) δ 6.89 (s, 2H), 3.84 (s, 6H), 3.82 (s, 3H); ^{13}C NMR (75 MHz) δ 153.7 (2× C), 138.0 (C), 114.7 (2× CH), 86.0 (C), 60.6 (CH_3), 56.1 (2× CH_3); *m*/*z* (70 eV) 294 (100%, $M^{+\cdot}$), 279 (56).

5.1.11. Methyl 1-(4-methoxybenzyl)-4-bromo-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (24). Phenyllithium (1.7 mL of a 1.8 M solution in cyclohexane/diethyl ether, 3.02 mmol) was added dropwise to a magnetically stirred solution of dibromopyrrole **22** (1.11 g, 2.7 mmol) in dry THF (20 mL) maintained at −78 °C under a nitrogen atmosphere. Stirring was continued for a further 0.2 h then anhydrous zinc chloride (411 mg, 3.0 mmol) was added in one portion and the cold bath removed. Whilst the reaction mixture was warming up, 3,4,5-trimethoxyiodobenzene (**23**) (1.29 g, 4.4 mmol) and $Pd(PPh_3)_4$ (317 mg, 0.3 mmol) were added, and the solution was then stirred at reflux for 50 h. After this time, the reaction mixture was cooled, treated with flash chromatography-grade silica (2.60 g) and concentrated under reduced pressure. The resulting free-flowing solid was added to the top of a flash chromatography column filled with silica, and the column was eluted with 1:4 v/v ethyl acetate/hexane. Concentration of the appropriate fractions (R_f 0.3) afforded methyl 1-(4-methoxybenzyl)-4-bromo-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (**24**) (1.10 g, 82%) as a viscous yellow gum (Found: $M^{+\cdot}$, 489.0787. $C_{23}H_{24}{}^{79}BrNO_6$ requires $M^{+\cdot}$, 489.0787). ^{1}H NMR (300 MHz) δ 7.14 (s, 1H), 6.83–6.76 (complex m, 4H), 6.43 (s, 2H), 5.46 (s, 2H), 3.88 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.66 (s, 6H); ^{13}C NMR (75 MHz) δ 160.6 (C), 158.5 (C), 152.9 (2× C), 138.9 (C), 130.9 (2× C), 126.9 (2× CH), 124.7 (C), 122.2 (C), 120.0 (CH), 113.8 (2× CH), 107.7 (2× CH), 96.8 (C), 60.8 (CH_3), 55.9 (2× CH_3), 55.2 (CH_3), 51.4 (CH_3), 49.5 (CH_2) (one signal obscured or overlapping); ν_{max} (KBr) 1709, 1584, 1513, 1473, 1444, 1252, 1127, 1090 cm^{-1}; *m*/*z* (70 eV) 491 and 489 (both 20%, $M^{+\cdot}$), 121 [100, $(C_7H_6OCH_3)^+$].

5.1.12. 3-{[(*tert*-Butyl)dimethylsilyl]oxy}-4-methoxyphenylboronic acid (25) and (5-iodo-2-methoxyphenoxy)(*tert*-butyl)dimethylsilane (26). *Step (i)*: Acetyl chloride (7.33 g, 93.4 mmol) was added dropwise to a magnetically stirred solution of 2-methoxyphenol (guaicol) (10.0 g, 80.6 mmol) and pyridine (14.4 mL, 178 mmol) in DCM (200 mL) maintained at 0 °C under a nitrogen atmosphere. The mixture was allowed to warm to 18 °C over 1 h, and stirring continued for 23 h. The resulting solution was poured into ice-cold H_3PO_4 (200 mL of a 1 M aqueous solution) and the separated aqueous phase extracted with DCM (3× 200 mL). The combined organic extracts were washed with brine (1× 500 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to furnish 2-methoxyphenyl acetate[18] (13.4 g, 99%) as a pale-yellow oil.

Step (ii): A solution of 2-methoxyphenyl acetate (13.4 g, 80.6 mmol) in chloroform (200 mL) maintained at 0 °C under a nitrogen atmosphere was treated, in one portion, with silver trifluoroacetate (23.1 g, 105 mmol). To this mixture was added, dropwise over 1 h, a solution of molecular iodine (20.5 g, 80.8 mmol) in chloroform (500 mL). The mixture was allowed to warm to 18 °C over 1 h, stirred for 4 h then washed with sodium thiosulfate (1× 500 mL of a 20% w/v aqueous solution), dried ($MgSO_4$), filtered and concentrated under reduced pressure to furnish 5-iodo-2-methoxyphenyl acetate[18] (17.2 g, 73%) as a light-brown and photosensitive solid.

Step (iii): A solution of 5-iodo-2-methoxyphenyl acetate (17.2 g, 58.9 mmol) in methanol (500 mL) maintained at 18 °C under an atmosphere of nitrogen was treated in one portion with potassium carbonate (40.7 g, 294 mmol). The mixture was stirred at 18 °C for 16 h then filtered through a sintered-glass funnel. The filtrate was concentrated under reduced pressure to furnish 5-iodo-2-methoxyphenol[18] (14.7 g, 99%) as a tan-coloured solid, mp 85–87 °C (lit. mp 87–88 °C).[18] ^{1}H NMR (300 MHz) δ 7.23 (d, *J* 2.2 Hz, 1H), 7.17 (dd, *J* 8.5 and 2.2 Hz, 1H), 6.60 (d, *J* 8.5 Hz, 1H), 5.55 (br s, 1H), 3.87 (s, 3H).

Step (iv): TBDMSCl (143 mg, 0.95 mmol) was added dropwise to a magnetically stirred solution of imidazole (186 mg, 2.7 mmol) and 5-iodo-2-methoxyphenol (215 mg, 0.9 mmol) in DMF (5 mL) maintained at 0 °C under a nitrogen atmosphere. The mixture was allowed to warm to 18 °C over 1 h, stirred for 15 h then partitioned between ice-cold H_3PO_4 (10 mL of a 1 M aqueous solution) and diethyl ether (10 mL). The separated aqueous phase was extracted with diethyl ether (3× 10 mL) and the combined organic extracts washed with water (3× 50 mL) and brine (1× 50 mL) then dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a pale-yellow oil. Subjection of this material to flash chromatography (1:10 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f 0.7) furnished (5-iodo-2-methoxyphenoxy)(*tert*-butyl)dimethylsilane (**26**)[18] (264 mg, 84%) as a clear, colourless solid, mp 32–34 °C (lit. mp 36 °C).[18] ^{1}H NMR (300 MHz) δ 7.20 (dd, *J* 8.8 and 2.1 Hz, 1H), 7.14 (d, *J* 2.1 Hz, 1H), 6.59 (d, *J* 8.8 Hz, 1 H), 3.77 (s, 3H, OCH_3), 0.99 (s, 9H, 3× CH_3), 0.15 (s, 6H, 2× CH_3); *m*/*z* (70 eV) 364 (9%, $M^{+\cdot}$), 307 (91), 292 (100).

Step (v): *tert*-Butyllithium (711 μL of a 1.7 M solution in pentane, 1.21 mmol) was added dropwise to a magnetically stirred solution of (5-iodo-2-methoxyphenoxy)(*tert*-butyl)dimethylsilane (**26**) (200 mg, 0.6 mmol) in toluene (5 mL) maintained at −78 °C under a nitrogen atmosphere. After 30 min, trimethylborate (152 mg, 1.5 mmol) was added to the reaction mixture and the resulting solution allowed to warm to 18 °C over

1 h and, after 17 h, it was poured into brine-water (30 mL of a 1:1 v/v mixture). The mixture was acidified to pH 3 with 3 M HCl. The separated aqueous phase was extracted with DCM (3× 100 mL), and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford 3-{[(*tert*-butyl)dimethylsilyl]oxy}-4-methoxyphenylboronic acid (**25**) (123 mg, 80%) as a tan-coloured oil, and containing of a variable mixture of anhydrides. Due to the presence of these anhydrides, this material gave rise to highly variable and complex NMR spectra. Acid **25** was used without purification in the next step of the reaction sequence.

5.1.13. Methyl 1-(4-methoxybenzyl)-4-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (4). Na_2CO_3 (8.4 mL of a 2.0 M aqueous solution, 16.8 mmol) was added to a solution of bromopyrrole **24** (330 mg, 0.7 mmol), arylboronic acid (**25**) (800 mg, 2.8 mmol) and $Pd(PPh_3)_4$ (80 mg, 0.1 mmol) in DMA (20 mL). The solution was deoxygenated (by subjecting it to six cycles of exposure to 16 mm Hg pressure then back filling with nitrogen) and stirred rapidly while heating at 90 °C for 110 h. The reaction mixture was cooled to 18 °C, acidified with 3 M HCl and extracted with diethyl ether (4× 50 mL). The combined organic extracts were washed with H_2O (12× 60 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a dark-coloured oil. This material was subjected to flash chromatography (silica, 2:5 v/v ethyl acetate/hexane elution), and concentration of the appropriate fractions (R_f 0.4) afforded methyl 1-(4-methoxybenzyl)-4-(3-hydroxy-4-methoxyphenyl)-5-3,4,5-trimethoxyphen-yl)-1*H*-pyrrole-2-carboxylate (**4**) (14%) as a pale-yellow oil (Found: $M^{+\cdot}$, 533.2047. $C_{30}H_{31}NO_8$ requires $M^{+\cdot}$, 533.2050). ^1H NMR (300 MHz) δ 7.25 (s, 1H), 6.85 (d, *J* 2.1 Hz, 1H), 6.80–6.77 (complex m, 4H), 6.67 (d, *J*, 8.4 Hz, 1H), 6.62 (dd, *J* 8.4 and 2.1 Hz, 1H), 6.32 (s, 2H), 5.48 (br s, 1H), 5.42 (br s, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.75 (s, 3H), 3.54 (s, 6H); ^{13}C NMR (75 MHz) δ 161.4 (C), 158.4 (C), 153.0 (2× C), 145.2 (C), 144.9 (C), 138.1 (C), 137.5 (C), 131.6 (C), 128.4 (C), 127.0 (2× CH), 126.7 (C), 122.8 (C), 121.8 (C), 119.3 (CH), 117.3 (CH), 113.9 (CH), 113.7 (2× CH), 110.4 (CH), 108.0 (2× CH), 60.9 (CH_3), 55.9 (3× CH_3), 55.2 (CH_3), 51.2 (CH_3), 48.6 (CH_2); ν_{max} (KBr) 2925, 1703, 1583, 1513, 1248, 1095 cm^{-1}; *m*/*z* (70 eV) 533 (43%, $M^{+\cdot}$), 412 (8), 121 [100, $(C_7H_6OCH_3)^+$].

5.1.14. Methyl 4-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (3). A solution of pyrrole **4** (50 mg, 0.1 mmol), anisole (101 mg, 0.9 mmol) and trifluoroacetic acid (1.0 mL) in DCM (4.0 mL) was stirred at 37 °C for 48 h, at which time the excess trifluoroacetic acid was removed under a stream of nitrogen. The residue was then concentrated further under reduced pressure. The resulting dark-brown oil was subjected to flash chromatography (silica, 3:2 v/v ethyl acetate/hexane elution), and concentration of the appropriate fractions (R_f 0.4) afforded a colourless solid. Recrystallisation (diethyl ether/hexane) of this material afforded methyl 4-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (**3**) (39 mg, 99%) as colourless crystals, mp 168–170 °C (Found: $M^{+\cdot}$, 413.1472. $C_{22}H_{23}NO_7$ requires $M^{+\cdot}$, 413.1475). ^1H NMR (300 MHz) δ 9.02 (br s, 1H), 6.99 (d, *J* 2.8 Hz, 1H), 6.95 (m, 1H), 6.80 (m, 2H), 6.57 (s, 2H), 5.56 (s, 1H), 3.88 (s, 6H), 3.87 (s, 3H), 3.72 (s, 6H); ^{13}C NMR (75 MHz) δ 161.8 (C), 153.2 (2× C), 145.3 (2× C), 137.8 (C), 133.0 (C), 128.7 (C), 127.2 (C), 123.7 (C), 121.5 (C), 120.4 (CH), 116.8 (CH), 115.0 (CH), 110.5 (CH), 105.1 (2× CH), 60.9 (CH_3), 56.1 (3× CH_3), 51.6 (CH_3) (one signal obscured or overlapping); ν_{max} (KBr) 2926, 1736, 1699, 1244, 1126 cm^{-1}; *m*/*z* (70 eV) 413 (100%, $M^{+\cdot}$), 398(25), 381(42), 366(50).

5.1.15. Methyl 4-bromo-5-{[(*tert*-butyl)dimethylsilyloxy]-4-methoxyphenyl}-1-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxylate (27). Phenyllithium (1.8 mL of a 1.8 M solution in cyclohexane/diethyl ether, 3.3 mmol) was added dropwise to a magnetically stirred solution of compound **22** (1.32 g, 3.3 mmol) in dry THF (10 mL) maintained at −78 °C. Stirring continued for a further 0.2 h then anhydrous zinc chloride (491 mg, 3.6 mmol) was added in one portion and the cold bath removed. Whilst warming, compound **26** (1.20 g, 3.6 mmol) and $Pd(PPh_3)_4$ (378 mg, 0.3 mmol) were added to the reaction mixture which was then heated and stirred at reflux for 96 h. After this time, the reaction mixture was cooled, treated with flash chromatography-grade silica (3.6 g) and concentrated under reduced pressure. The resulting free-flowing solid was added to the top of a flash chromatography column filled with silica, and the column was eluted with 1:9 v/v ethyl acetate/hexane. Concentration of the appropriate fractions (R_f 0.2) afforded methyl 4-bromo-5-{[(*tert*-butyl)dimethylsilyloxy]-4-methoxyphenyl}-1-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxylate (**27**) (1.03 g, 56%) as a colourless and viscous gum (Found: $M^{+\cdot}$, 559.1382. $C_{27}H_{34}{}^{79}BrNO_5Si$ requires $M^{+\cdot}$, 559.1390). ^1H NMR (300 MHz) δ 7.10 (s, 1H), 6.85–6.84 (complex m, 2H), 6.76–6.75 (complex m, 5H), 5.46 (s, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 3.74 (s, 3H), 0.95 (s, 9H), 0.10 (s, 6H); ^{13}C NMR (75 MHz) δ 160.7 (C), 158.5 (C), 151.7 (C), 144.7 (C), 138.9 (C), 130.7 (C), 127.1 (2× CH), 124.3 (CH), 123.2 (CH), 122.1 (C), 121.8 (C), 120.1 (CH), 113.8 (2× CH), 111.6 (CH), 97.1 (C), 55.4 (CH_3), 55.1 (CH_3), 51.3 (CH_3), 49.2 (CH_2), 25.6 (3× CH_3), 18.4 (C), −4.7 (2× CH_3); ν_{max} (KBr) 2953, 2930, 2856, 1711, 1612, 1576, 1470, 1252, 1173 cm^{-1}; *m*/*z* (70 eV) 561 and 559 (6 and 5%, $M^{+\cdot}$), 504 and 502 (21 and 19), 121[100, $(C_7H_6OCH_3)^+$].

5.1.16. 3,4,5-Trimethoxyphenylboronic acid (28). The title compound was prepared, in 84% yield, from iodide **23** using the same protocol as employed for the conversion **26** → **25**. Like boronic acid **25**, congener **28**[19] was obtained as a tan-coloured oil and as a variable admixture with various anhydride forms. Due to the presence of these anhydrides, this material gave rise to highly variable and complex NMR spectra. Acid **28** was used without purification in the next step of the reaction sequence.

5.1.17. Methyl 1-(4-methoxybenzyl)-5-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (6). The title compound was prepared by

cross-coupling precursors **27** and **28** using essentially the same protocol as employed for the conversion **24** + **25** → **4**, but with a reaction time of 50 h. In this manner methyl 1-(4-methyoxybenzyl)-5-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (**6**) (52%) was obtained as pale-yellow crystals, mp 47–49 °C (Found: C, 67.59; H, 5.89; N, 2.44. $C_{30}H_{31}NO_8$ requires C, 67.53; H, 5.86; N, 2.63%) (R_f 0.5 in 3:2 v/v ethyl acetate/hexane). ^{1}H NMR (300 MHz) δ 7.27 (s, 1H), 6.84–6.73 (complex m, 6H), 6.69 (dd, *J* 2.1 and 7.1 Hz, 1H), 6.40 (s, 2H), 5.62 (br s, 1H), 5.44 (br s, 2H), 3.89 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.63 (s, 6H); ^{13}C NMR (75 MHz) δ 161.3 (C), 158.3 (C), 152.7 (2× C), 146.9 (C), 145.6 (C), 137.7 (C), 135.9 (C), 131.0 (C), 130.6 (C), 127.1 (2× CH), 124.5 (C), 123.2 (CH), 123.0 (C), 121.4 (C), 117.2 (CH), 117.0 (CH), 113.6 (2× CH), 110.6 (CH), 104.5 (2× CH), 60.7 (CH_3), 55.9 (CH_3), 55.6 (2× CH_3), 55.1 (CH_3), 51.1 (CH_3), 48.2 (CH_2); ν_{max} (KBr) 3318, 2923, 1699, 1385, 1251, 1033 cm^{-1}; *m*/*z*(70 eV) 533 (72%, $M^{+\cdot}$), 121 [100, $(C_7H_6OCH_3)^+$].

5.1.18. Methyl 5-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (5). The title compound was prepared from precursor **6** using the same protocol as employed in the conversion **4** → **3**. In this manner methyl 5-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (**5**) (62%) was obtained as colourless needles, mp 172–174 °C (Found: C, 63.73; H, 5.74; N, 3.65. $C_{22}H_{23}NO_7$ requires C, 63.92; H, 5.61; N, 3.39%) (R_f 0.5 in 3:2 v/v ethyl acetate/hexane). ^{1}H NMR (300 MHz) δ 9.04 (br s, 1H), 7.04 (d, J 2.8 Hz, 1H), 7.02 (d, *J* 2.2 Hz, 1H), 6.90 (dd, *J* 2.2 and 8.4 Hz, 1H), 6.81 (d, *J* 8.4 Hz, 1H), 6.54 (s, 2H), 5.72 (s, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.72 (s, 6H); ^{13}C NMR (75 MHz) δ 161.8 (C), 153.0 (2× C), 146.6 (C), 145.6 (C), 136.5 (C), 133.2 (C), 131.0 (C), 125.0 (C), 123.4 (C), 121.4 (C), 120.5 (CH), 116.6 (CH), 113.9 (CH), 110.7 (CH), 105.5 (2× CH), 60.9 (CH_3), 56.0 (CH_3), 55.9 (2× CH_3), 51.7 (CH_3); *m*/*z* (70 eV) 413(100%, $M^{+\cdot}$), 398(77), 381(42), 366(57).

5.1.19. Methyl 1-(4-methoxybenzyl)-4-(benzo[*d*][1,3]dioxol-6-yl)-5-(3-hydroxy-4-methoxy-phenyl)-1*H*-pyrrole-2-carboxylate (8). The title compound was prepared by cross-coupling precursors **27** and **29** using the same protocol as employed for the conversion **24** + **25** → **4**, but with a reaction time of 38 h. In this manner methyl 1-(4-methoxybenzyl)-4-(benzo-[*d*][1,3]dioxol-6-yl)-5-(3-hydroxy-4-methoxyphenyl)-1*H*-pyrrole-2-carboxylate (**8**) (87%) was obtained as a pale-yellow oil (Found: $M^{+\cdot}$, 487.1632. $C_{28}H_{25}NO_7$ requires $M^{+\cdot}$, 487.1631), (R_f 0.3 in 1:5 v/v ethyl acetate/hexane). ^{1}H NMR (300 MHz) δ 7.19 (s, 1H), 6.80–6.63 (complex m, 10H), 5.88 (s, 2H), 5.61 (br s, 1H), 5.42 (s, 2H), 3.90 (s, 3H), 3.77 (s, 3H), 3.75 (s, 3H); ^{13}C NMR (75 MHz) δ 161.4 (C), 158.3 (C), 147.2 (C), 146.8 (C), 145.5 (2× C), 137.4 (C), 131.1 (C), 129.3 (C), 127.1 (2× CH), 124.3 (C), 123.1 (CH), 123.0 (C), 121.4 (C), 121.0 (CH), 117.6 (CH), 117.0 (CH), 113.6 (2× CH), 110.5 (CH), 108.3 (CH), 108.1 (CH), 100.6 (CH_2), 55.7 (CH_3), 55.1 (CH_3), 51.1 (CH_3), 48.2 (CH_2) (one signal obscured or overlapping); ν_{max} (KBr) 1703, 1513, 1459, 1444, 1249, 1219, 1176 cm^{-1}; *m*/*z* (70 eV) 487 ($M^{+\cdot}$, 61%), 121[100, $(C_7H_6OCH_3)^+$].

5.1.20. Methyl 4-(benzo[*d*][1,3]dioxol-6-yl)-5-(3-hydroxy-4-methoxyphenyl)-1*H*-pyrrole-2-carboxylate (7). The title compound was prepared from precursor **8** using the same protocol as employed in the conversion **4** → **3**. In this manner methyl 4-(benzo[*d*][1,3]dioxol-6-yl)-5-(3-hydroxy-4-methoxyphenyl)-1*H*-pyrrole-2-carboxylate (**7**) (60%) was obtained as colourless crystals, mp 145–147 °C (Found: C, 65.55; H, 4.98; N, 4.14. $C_{20}H_{17}NO_6$ requires C, 65.39; H, 4.66; N, 3.81%), (R_f 0.4 in 2:5 v/v ethyl acetate/hexane). ^{1}H NMR (500 MHz) δ 9.27 (br s, 1H), 7.00 (d, *J* 2.0 Hz, 1H), 6.96 (d,*J* 3.0 Hz, 1H), 6.85 (m, 1H), 6.81–6.75 (complex m, 4H), 5.94 (s, 2H), 3.90 (s, 3H), 3.88 (s, 3H) (signal due to OH not observed); ^{13}C NMR (75 MHz) δ 161.7 (C), 147.5 (C), 146.5 (C), 146.1 (C), 145.7 (C), 132.9 (C), 129.4 (C), 125.1 (C), 123.3 (C), 121.9 (CH), 121.3 (C), 120.0 (CH), 116.9 (CH), 113.9 (CH), 110.8 (CH), 109.1 (CH), 108.3 (CH), 100.8 (CH_2), 55.9 (CH_3), 51.6 (CH_3); ν_{max} (KBr) 1697, 1684, 1464, 1259, 1085, 1038 cm^{-1}; *m*/*z*367 (70 eV) (100%, $M^{+\cdot}$), 335 (77).

5.2. X-ray crystallographic studies

5.2.1. Data collection. Intensity data were collected on a Rigaku AFC6R diffractometer using the $\omega - 2\theta$ scan technique to a maximum 2θ value of 120°. The weak reflections [$I < 10.0\sigma(I)$] were rescanned (maximum of four scans), and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak to background counting time was 2:1.

5.2.2. Crystal data. *Compound* ***3***: $C_{22}H_{23}NO_7$, $M = 413.43$, $T = -80$ °C, triclinic, space group $P\bar{1}$, $Z = 2$, $a = 8.767(1)$, $b = 9.197(2)$, $c = 13.617(1)$ Å, $\alpha = 98.23(2)°$, $\beta = 106.90(1)°$, $\gamma = 99.86(1)°$, $V = 1013.2(4)$ Å^3, $D_x = 1.355$ Mg m^{-3}, 3011 unique data ($2\theta_{max} = 120.1°$), 2665 with $I > 2.0\sigma(I)$; $R = 0.037$, $R_w = 0.053$, $S = 2.352$.

Compound ***7***: $C_{20}H_{17}NO_6$, $M = 367.36$, $T = -80$ °C, triclinic, space group $P\bar{1}$, $Z = 2$, $a = 8.8204(6)$, $b = 9.433(1)$, $c = 11.803(1)$ Å, $\alpha = 111.838(8)°$, $\beta = 91.606(8)°$, $\gamma = 100.445(8)°$, $V = 891.6(2)$ Å^3, $D_x = 1.368$ Mg m^{-3}, 2654 unique data ($2\theta_{max} = 120.1°$), 2259 with $I > 2.0\sigma(I)$; $R = 0.035$, $R_w = 0.041$, $S = 2.677$.

Compound ***17***: $C_{19}H_{16}BrNO_2$, $M = 370.24$, $T = -80$ °C, monoclinic, space group $P2_1/c$, $Z = 4$, $a = 9.382(2)$, $b = 19.034(3)$, $c = 10.135(2)$ Å, $\beta = 116.37(1)°$, $V = 1621.5(5)$ Å^3, $D_x = 1.517$ Mg m^{-3}, 2425 unique data ($2\theta_{max} = 120.1°$), 2098 with $I > 3.0\sigma(I)$; $R = 0.027$, $R_w = 0.028$, $S = 2.424$.

5.2.3. Data reduction. Absorption corrections were applied. The data were corrected for Lorentz and polarisation effects.

Structure solution and refinement: The structures were solved by direct methods[23] and expanded using Fourier

techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atom positions were refined for compounds **7** and **17**. All calculations were performed using the teXsan[24] crystallographic software package of Molecular Structure Corporation.

Data deposition: Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC reference numbers 289493, 289494 and 289495 for compounds **3**, **7** and **17**, respectively). These data can be obtained free-of-charge via the Internet at www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

5.3. Biological studies

5.3.1. Tubulin assays. Bovine brain tubulin was purified as described previously.[25] Assays for the evaluation of tubulin assembly[26] and for the binding of [^{3}H]colchicine to tubulin[27] were performed as described previously. The tubulin assembly assay included a preincubation of the potential inhibitor, at varying concentrations, with 10 μM tubulin prior to addition of the GTP required for assembly to occur. After GTP addition, reactions were followed turbidimetrically for 20 min at 30 °C, and the IC_{50} value was defined as the compound concentration, obtained by interpolation between the data points, that inhibited the extent of assembly by 50%. In the colchicine binding assay, reaction mixtures contained 1.0 μM tubulin, 5.0 μM [^{3}H]colchicine, and the potential inhibitor at 5 μM. Reaction mixtures were incubated for 10 min at 37 °C, a time point at which the colchicine binding reaction is about 50% complete.

5.3.2. Cytotoxicity and mitotic index assays. The Burkitt lymphoma CA46 cells were grown in 5-mL flasks, and IC_{50} values were determined by counting the cells in a Coulter counter (Beckman Coulter, Fullerton, CA), with the IC_{50} value defined as the drug concentration that reduced increase in cell number by 50% at 24 h after drug addition. The cells were grown in RPMI 1640 medium supplemented with 17% fetal bovine serum and 2 mM L-glutamine at 37 °C in a 5% CO_2 atmosphere. The dimethyl sulfoxide concentration was 0.1% (v/v) in all culture flasks.

The mitotic index in the Burkitt cell cultures was also determined at 24 h. About 4.5 mL of cell culture medium was centrifuged at 1000 rpm for 1 min. The pelleted cells were resuspended in 5 mL of phosphate-buffered saline at room temperature, and the cells were harvested by centrifuging the suspension as before. The cell pellet was suspended in 0.5 mL of half-strength phosphate-buffered saline, and the cells were allowed to swell for 10 min. The cells were then fixed by adding 6 mL of 0.5% acetic acid/1.5% ethanol. After 30 min, the cells were harvested by centrifuging as before. The cells were resuspended in 1:3 v/v acetic acid/ethanol, and a droplet of the cell suspension was spread on the slide. The slide was air-dried and stained with Giemsa. The slide was examined under a light microscope, with mitotic cells defined as those with condensed chromosomes and no nuclear membrane. Four hundred cells were counted for each condition examined.

Acknowledgments

We thank the Australian Research Council for financial support including the provision of a PhD Scholarship to D.J.W., Mr. Tony Herlt (RSC, ANU) for expert assistance with the operation of HPLC instrumentation and Mr. Chris Blake (RSC, ANU) for help with the acquisition of certain NMR data.

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