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Tetrahedron
Tetrahedron 63 (2007) 12986–12993

Synthesis and biological evaluation of isoflavone analogues from *Dalbergia oliveri*

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Received 9 August 2007; revised 2 October 2007; accepted 8 October 2007
Available online 10 October 2007

Abstract—Mucronulatol **1** and violanone **2** isolated from *Dalbergia oliveri* Gamble, and the corresponding isoflavone **3** were prepared by ligand coupling reactions involving organolead reagent. Biological studies have shown a significant cytotoxic effect against an HBL100 leukemia cell line only for isoflavan **1** with an IC_{50} value amounting to 5.7 μM. All the drugs modestly inhibit the in vitro microtubule assembly, independently of the cytotoxic ability. Natural compounds **1** and **2** have no antibacterial activity, but are potent inhibitors of the *Fusarium oxysporum* phytopathogenic fungal growth.

1. Introduction

Dalbergia oliveri Gamble belongs to the family of Leguminosae-Papilionoideae and grows in Thailand and Burma. This plant has been used in traditional Thai medicine for the treatment of chronic ulcer. Some preliminary activity screening showed that crude extracts of *D. oliveri* exhibit significant biological activities, such as antibacterial and anti-inflammatory effects. In previous investigations, many isoflavonoids and neoflavonoids have been isolated by extraction and fully characterized.[1,2] Flavonoid derivatives, either naturally occurring or from synthetic origin, are well known to exhibit a wide range of biological activities.[3] Some of them, belonging to the structural groups of flavones,[4–6] neoflavones,[7,8] isoflavone, and open-chain analogue chalcones[9,10] possess cytotoxicity against cancer cell lines, acting as tubulin polymerization inhibitors. This activity on the protein stabilization is frequently associated with the antimitotic properties.[11]

Due to the presence of mucronulatol **1** and violanone **2** in *D. oliveri* extracts, respectively, an isoflavan compound and an isoflavanone compound, we decided to synthesize these natural products as well as the isoflavone analogue **3** to investigate their activity as potential mitosis inhibitor agents (Fig. 1).

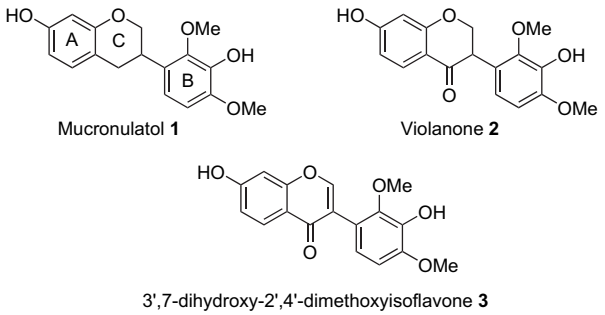

Figure 1. Structures of compounds **1–3**.

2. Chemistry

Because of their remarkably rich spectrum of biological activities,[12] isoflavones have been the topic of a number of studies toward their synthesis. Almost all published synthetic methods used the cyclization of 2-hydroxyarylalkylketones under acidic or basic condition as the key step.[13] However, in recent years, convergent approaches were developed taking advantage of the ligand coupling concept[14] via a Suzuki reaction[15,16] or using organolead chemistry.[17]

Keywords: Isoflavone; Ligand coupling; Cytotoxicity; Antimitotic; Antimicrobial.
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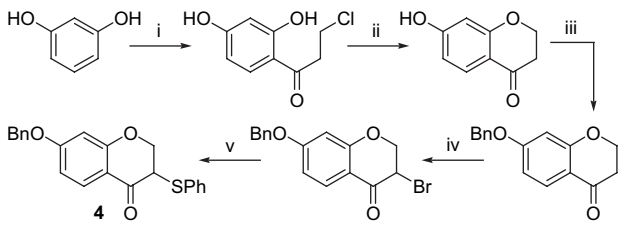
doi:10.1016/j.tet.2007.10.030

The synthesis of natural compounds **1** and **2** have previously been addressed by the oxidative rearrangement of 2′-hydroxychalcone with TTN in MeOH followed by cyclization to give isoflavone derivative. Finally, deprotection and subsequently catalytic hydrogenation in the presence of AcOH yielded mucronulatol **1** or hydrogenation in acetone on Pd–C catalyst furnished violanone **2**.[18] However, this method was unsatisfactory since the reactions proceeded in very poor yield with these chalcones. In addition, this method suffered from requiring the use of stoichiometric quantities of highly toxic thallium salts.

The method utilizing aryllead (IV) chemistry for the synthesis of isoflavones, isoflavanones, and neoflavonoids including naturally occurring products has been reported by Donnelly and colleagues,[19–22] the coupling reactions proceeding smoothly to give 7,4′-dimethoxyisoflavone, 2′,4′-dimethoxyisoflavone, 5,7,4′-trimethoxyisoflavone, 2′,4′-dimethoxyisoflavanone, 5,7-dimethoxyisoflavanone, and 3-hydroxy-8-methoxycoumarin in high yields, and by others for the synthesis of 3-arylflavanones.[23] Thus, aryllead-mediated route offered a direct, efficient, and selective entry into the synthesis of isoflavanones and isoflavones and their derivatives.

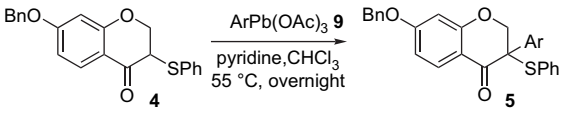
Here, we describe the synthesis of **1**–**3** via an aryllead-mediated coupling reaction using 3-phenylthio-chroman-4-one **4** as the common intermediate. The required 3-phenylthio-chroman-4-one **4** was prepared in five steps from resorcinol as outlined in Scheme 1.



Scheme 1. Synthesis of the chromanone intermediates. Reagents and conditions: (i) 3-chloropropionyl chloride, $AlCl_3$, Et_2O, 0 °C, 1 h; (ii) aq NaOH, rt, 2 h; (iii) BnBr, K_2CO_3, acetone, reflux, overnight; (iv) $CuBr_2$, EtOAc–$CHCl_3$, reflux, overnight; (v) PhSH, NaH, THF, 0 °C, 1 h.

The chromanone ring was prepared efficiently by the method of Naylor et al.[24] As the selective monoarylation of ketonic substrates with aryllead reagents requires activation to the enol form, the phenylthio group was introduced by reaction of sodium phenylthiolate with the required α-bromo-ketone.[20]

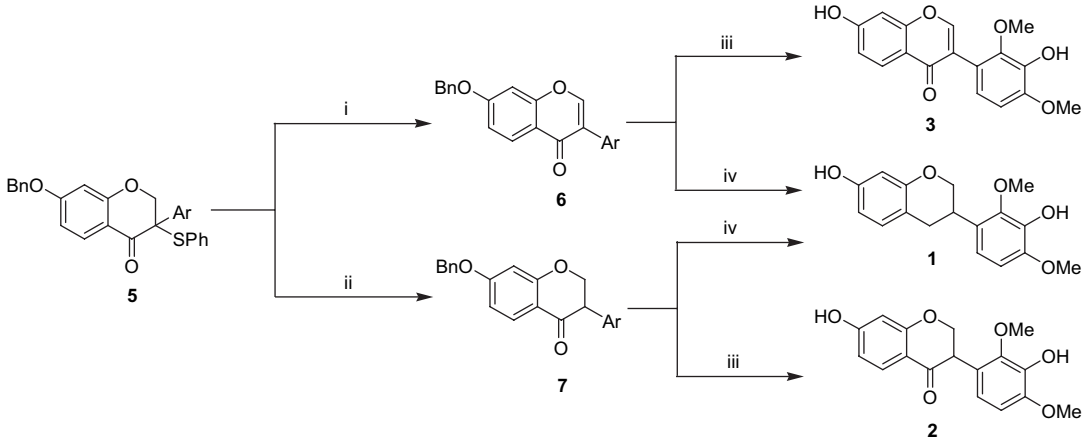
The aryllead triacetate **9**, prepared in good yield by tin–lead exchange,[25] was used in the coupling reaction to afford the protected 3-aryl-3-phenylthiochroman-4-one **5** in 51% yield (Scheme 2).



Scheme 2. Ligand coupling reaction with activated chroman-4-one.

Removal of the phenylthio group by mild oxidation of **5** with *m*-chloroperbenzoic acid (MCPBA) followed by thermal elimination led to isoflavone **6** in 86% yield (Scheme 3). Reduction of **5** with an excess of in situ generated nickel boride led to the corresponding isoflavanone **7** in 59% yield. Mucronulatol **1** was prepared from either **6** or **7** by hydrogenation in ethanol in the presence of catalytic amounts of palladium on charcoal. Violanone **2** and 3′,7-dihydroxy-2′,4′-dimethoxyisoflavone **3** were obtained in excellent yields by hydrolysis of the benzyloxy protecting group using a 47% HBr aqueous solution.

The molecular structures of mucronulatol **1** and the corresponding isoflavone **3** have been determined by single-crystal X-ray crystallography. The ORTEP diagrams of **1** and **3** are presented in Figures 2 and 4, while selected parameters are listed in Table 1.



Scheme 3. Synthesis of isoflavonoid derivatives. Reagents and conditions: (i) MCPBA, EtOAc, 0 °C, then toluene, reflux; (ii) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, EtOH–H_2O, reflux, 4 h; (iii) 47% aq HBr, 50 °C, overnight; (iv) H_2, Pd–C, EtOH, rt.

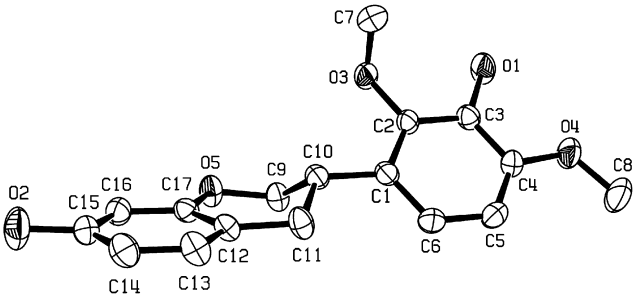


Figure 2. ORTEP drawing of mucronulatol **1** (all hydrogen atoms are omitted for clarity).

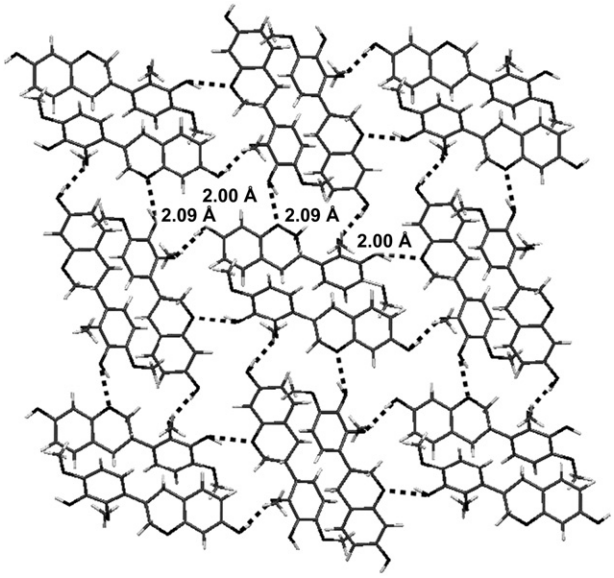


Figure 3. Mucronulatol **1** intermolecular hydrogen bond.

Both structures revealed a conformation in which the two aromatic rings (A and B) are not coplanar with significant differences in the dihedral angles of 45.2° and 85.4°, respectively. The crystal structure in both cases reveals a network formed by chains of intermolecular hydrogen bonds with distances in the range of 1.868–2.088 Å (Figs. 3 and 5). If several pharmacophore groups are essential for the tubulin polymerization inhibition, hydrogen bond acceptor and hydrogen bond donor form the critical interactions with the protein and convey specificity to the binding. The

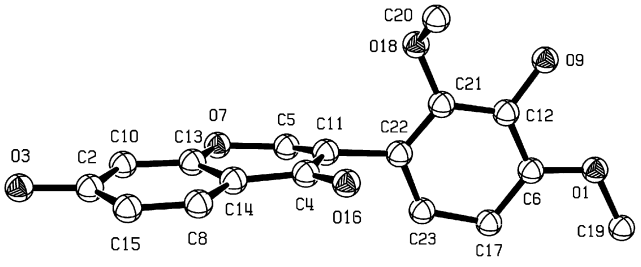


Figure 4. ORTEP drawing of isoflavone **3** (all hydrogen atoms are omitted for clarity).

Table 1. X-ray crystallographic data of **1** and **3**

Compound **1**		Compound **3**	
Bond or torsion angle	Distance (Å) or angle (°)	Bond or torsion angle	Distance (Å) or angle (°)
Ring (A)–ring (B)	45.19	Ring (A)–ring (B)	85.37
Ring (B)–O3–C7	76.25	Ring (B)–O18–C20	69.00
Ring (B)–O4–C8	6.22	Ring (B)–O1–C19	3.08

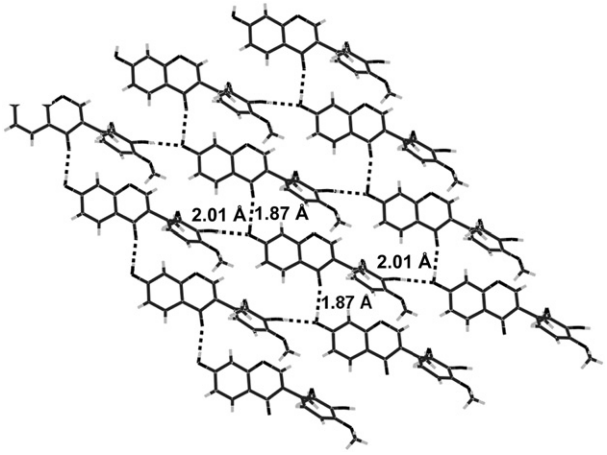
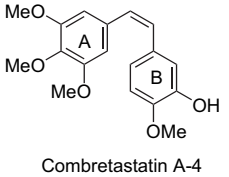


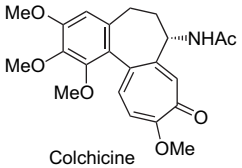
Figure 5. Isoflavone **3** intermolecular hydrogen bond.

hydrophobic center, such as methyl group, and planar group, that form the basis for a diaryl system, serve as the rigid portion of the molecular scaffold that satisfy the overall geometric and steric requirements of binding. The different pharmacophoric groups can be divided among two planes (A and B) having a dihedral angle close to 45° and match the shape of the colchicine site.[26]

Isoflavonoids **1**–**3** are structurally similar to colchicine and combretastatin, possessing the three classic features, a non-coplanar diaryl system, a polyoxygenated moiety, and a constrained conformation. These similarities can be expected to lead to a potent activity on the colchicine site of tubulin polymerization.



Combretastatin A-4



Colchicine

3. Biology

3.1. Cytotoxicity

A tetrazolium-based assay was applied to determine the drug concentration required to inhibit the cell growth by 50% after incubation in the culture medium for 72 h. The calculated IC_{50} values with the HBL_{100} human leukemia cell line are collected in Table 2.

Table 2. Cytotoxicity of compounds **1–3** toward HBL_{100} human leukemia cell line

Compounds	Mucronulatol **1**	Violanone **2**	Isoflavone **3**
IC_{50}[a] (μM)	5.7	>50	>50

[a] Drug concentration that inhibits the growth of HBL_{100} by 50% after incubation in liquid medium for 72 h. Each drug concentration was tested in triplicate, SE of each point is <10%.

Compounds **2** and **3** were found to be inactive. In contrast, mucronulatol **1** exhibited a significant antiproliferative activity with an IC_{50} value of 5.7 μM.

3.2. Effects on tubulin polymerization

Figure 6 shows the effects of **1** on the DAPI fluorometric monitoring time course of microtubule assembly from pure tubulin.[27] A significant inhibition was noted, and the rate of assembly as well as the final amount of microtubules was lower in the presence of drug than in the control experiment. Compared to violanone **2** and isoflavone derivative **3** (data not shown), mucronulatol **1** had the highest effect on tubulin polymerization.

The inset shows that the extent of inhibition increased monotonically with the molar ratio of the total ligand to total tubulin (*R*) greater than the stoichiometric value. In this figure, 50% inhibition occurred at a mole ratio of **1**, per mole of tubulin, greater than the highest drug concentration used. The low solubility of these compounds didn't allow measurements at a drug concentration greater than 60 μM. Thus, at an arbitrarily fixed molar ratio of 3, **1** inhibited the microtubule assembly by 26%, **2** by 16%, and **3** by 21%.

We also noted that the most potent compound against tubulin assembly is also the most cytotoxic agent. However, the two inactive compounds **2** and **3** inhibit tubulin assembly approximately in the same range as the cytotoxic mucronulatol **1**. So it seems that tubulin is not the main target for the action of this compound.

3.3. Antimicrobial activity

Natural mucronulatol **1** and violanone **2**, isolated from *D. oliveri* were investigated for their antimicrobial activity against several yeast strains, Gram-positive and Gram-negative bacteria strains.[28] Both compounds tested in the present study were found to be inactive against the micro-organisms listed in Table 3, using streptomycin and econazole as reference compounds.

When screening was performed by direct bioautographic assay[29] on TLC with phytopathogenic fungi a clear growth inhibition was observed. Detection limits were determined against *Fusarium oxysporum* and *Alternaria brassicicola* fungi, using iprodion and captan as reference compounds. The results are listed in Table 4.

Table 3. Antimicrobial activity of compounds **1** and **2**

Compounds	Antimicrobial activity (IC_{80})[a] (μg/mL)		
	Saccharomyces cerevisiae (ATCC 28383)	*Staphylococcus aureus* (CIP 53154)	*Escherichia coli* (CIP 54127)
Mucronulatol **1**	>100	>100	>100
Violanone **2**	>100	>100	>100
Streptomycin	—	6–12	6–12
Econazole	<3	—	—

[a] Drug concentration that inhibits the growth of fungi by 80% after incubation in liquid medium.

Table 4. Antifungal activity of compounds **1** and **2**

Compounds	Antifungal activity[a] (μg)	
	Fusarium oxysporum	*Alternaria brassicicola*
Mucronulatol **1**	0.5	>10
Violanone **2**	1.0	>10
Iprodion	0.1	0.1
Captan	0.1	0.1

[a] Minimal amount (μg) of compounds spotted on a silica gel TLC plate that produces detectable growth inhibition.

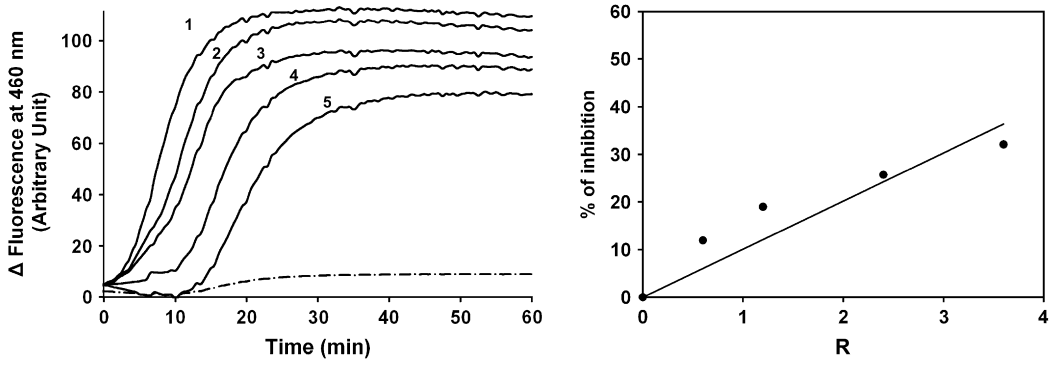


Figure 6. Effect of **1** on the 4′,6-diamidino-2-phenylindole (DAPI) fluorimetric time course of in vitro microtubule assembly. The reaction was started by warming the solution to 37 °C. Panel shows tubulin at 15 μM (line 1) and aliquots of the same solution with 9, 18, 36, and 54 μM (lines 2, 3, 4, and 5) of **1**. As a positive control inhibitor, the dashed dotted line shows 15 μM of tubulin in the presence of 3 μM of combretastatin A-4. The inset shows the percentage of fluorescence inhibition as a function of the mole ratio of total ligand to total tubulin in the solution (*R*).

The two isoflavonoids displayed a significant activity against *F. oxysporum* and only a marginal or no activity against *A. brassicicola* even at 10 μg by spot.

4. Conclusion

Natural mucronulatol **1** and violanone **2**, constituents of the Thai plant, *D. oliveri*, and a synthetic isoflavone analogue **3** were prepared by aryllead-mediated coupling reaction. Compound **1** has been demonstrated to have significant potency as a cytotoxic agent against human breast leukemia cell line. By comparison with combretastatin A-4, an 80-fold higher concentration of **1** was necessary to obtain a similar tubulin assembly inhibitory effect. In addition, compounds **1** and **2** were selective inhibitors of the phytopathogenic fungus *F. oxysporum*, when compared with other micro-organisms.

5. Experimental

5.1. Chemistry

Melting points were taken on a Büchi capillary apparatus and are uncorrected. NMR spectra were obtained on a Bruker AC 300 spectrometer. Chemical shifts (δ) are reported in parts per million for a solution of the compound in $CDCl_3$ with Me_4Si as internal reference and J values in hertz. Separations by column chromatography were performed using Merck Kieselgel 60 (70–230 mesh). Ether refers to diethyl ether and light petroleum refers to the fraction with distillation range 40–65 °C. All solvents were purified by standard techniques.

5.1.1. 3-Bromo-7-benzyloxychroman-4-one. Under an atmosphere of argon, to a refluxing mixture of copper (II) bromide (7.81 g, 33.46 mmol) in ethyl acetate (15 mL) was added a solution of 7-benzyloxychroman-4-one[30] (5 g, 19.46 mmol) in anhydrous chloroform (30 mL). The resulting mixture was refluxed overnight with vigorous stirring. The precipitate was filtered off and washed with ethyl acetate. The solvent was distilled off under reduced pressure and the residue was purified by column chromatography (eluent CH_2Cl_2) to afford 3-bromo-7-benzyloxychroman-4-one (4.23 g, 66%) as a white solid, mp 108.9–109.8 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 4.55–4.66 (3H, m, 2- and 3-H), 5.29 (2H, s, OCH_2Ph), 6.54 (1H, d, J 2.3, 8-H), 6.72 (1H, dd, J 8.9 and 2.3, 6-H), 7.34–7.41 (5H, m, Ph), and 7.89 (1H, d, J 8.9, 5-H).

5.1.2. 7-Benzyloxy-3-phenylthiochroman-4-one (4). At 0 °C, to a solution of thiophenol (1.29 mL, 12.64 mmol) in dry tetrahydrofuran (30 mL) was added sodium hydride 60% in oil (0.51 g, 12.64 mmol). After standing for 30 min at 0 °C, a solution of 3-bromo-7-benzyloxychroman-4-one (4.13 g, 12.39 mmol) in dry tetrahydrofuran (60 mL) was added dropwise over a period of 30 min. The reaction mixture was allowed to warm at room temperature and filtered through Celite. The filtrate was washed with 5% aqueous hydrochloric acid and water successively, and dried over anhydrous Na_2SO_4. The solvent was distilled off under reduced pressure and the residue was purified by column chromatography (eluent Et_2O–pentane 3:7) to afford 7-benzyloxy-3-phenylthiochroman-4-one **4** (3.98 g, 89%) as a white solid, mp 79 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 4.02 (1H, dd, J 6.4 and 3.9, 3-H), 4.50 (1H, dd, J 11.7 and 6.4, 2-H_{ax} or 2-H_{eq}), 4.61 (1H, dd, J 11.7 and 3.9, 2-H_{eq} or 2-H_{ax}), 5.09 (2H, s, OCH_2Ph), 6.49 (1H, d, J 2.3, 8-H), 6.69 (1H, dd, J 8.9 and 2.3, 6-H), 7.29–7.54 (10H, m, 2×Ph), and 7.87 (1H, d, J 8.9, 5-H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C 50.9 (C-3), 70.1 (C-2), 70.4 (OCH_2Ph), 101.4 (C-8), 110.8 (C-10), 113.8 (C-6), 127.3, 127.9, 128.1, 128.5, 128.9, 129.8 (C-5), 131.9, 132.8, 135.6, 162.5 (C-9), 165.0 (C-7), and 186.5 (C-4).

5.1.3. (3-Benzyloxy-2,4-dimethoxyphenyl)tributylstannane. Under an atmosphere of argon, at −78 °C, to a stirred solution of 3-benzyloxy-2,4-dimethoxybromobenzene[31] (13.56 g, 41.96 mmol) in dry tetrahydrofuran (80 mL) was added a solution of butyllithium 2.5 M in hexane (20.14 mL, 50.35 mmol) over 15 min. After stirring at this temperature for 30 min, tributylchlorostannane (13.66 mL, 50.35 mmol) was added over 5 min and the resulting mixture was stirred for 20 min at −78 °C. The reaction mixture was poured into a saturated aqueous solution of ammonium chloride (30 mL) and then to water (200 mL) and extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to afford a yellow oil (27.13 g, crude) which upon distillation gave (3-benzyloxy-2,4-dimethoxyphenyl)tributylstannane (20.14 g, 90%) as a yellow oil (bp 180 °C at 10^{-3} mmHg). ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 0.89 (9H, t, J 7.4, 3×CH_3), 1.00–1.24 (6H, m, 3×CH_2), 1.27–1.39 (6H, m, 3×CH_2), 1.47–1.53 (6H, m, 3×CH_2), 3.82 (3H, s, OCH_3), 3.88 (3H, s, OCH_3),5.00 (2H, s, OCH_2Ph), 6.69 (2H, d, J 7.9, 4- and 6-H), 7.01 (1H, d, J 7.9, 5-H), and 7.32–7.50 (5H, m, Ph). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C 9.8 (3×CH_3), 13.6 (3×CH_2), 27.3 (3×CH_2), 29.1 (3×CH_2), 55.8 (OCH_3), 60.7 (OCH_3), 74.7 (OCH_2Ph), 108.0 (C-5), 125.8 (C-1), 127.4 (C-4′), 128.1 (C-2′ and C-6′), 128.2 (C-3′ and C-5′), 131.0 (C-6), 137.7 (C-3), 140.0 (C-1′), 154.7 (C-4), and 158.1 (C-2).

5.1.4. 3-Benzyloxy-2,4-dimethoxyphenyllead triacetate (9). Under an atmosphere of argon, to a mixture of lead tetraacetate (3.90 g, 8.80 mmol) and mercuric acetate (0.13 g, 0.40 mmol) in dry chloroform (50 mL) was quickly added dropwise a solution of (3-benzyloxy-2,4-dimethoxyphenyl)-tributylstannane (4.27 g, 8.00 mmol) in dry chloroform (30 mL). The reaction mixture was stirred at 40 °C for 3 h, filtered through Celite, and the solvent was concentrated under reduced pressure to a small volume. Light petroleum was added and the solution was kept overnight at −15 °C. The precipitate was collected by filtration, washed with light petroleum to give 3-benzyloxy-2,4-dimethoxyphenyllead triacetate **9** (4.14 g, 82%) as a brown powder, mp 161–162 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 2.11 (9H, s, 3×$OCOCH_3$), 3.88 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 5.03 (2H, s, OCH_2Ph), 6.84 (1H, d, J 9.1, 6-H), 7.33–7.44 (5H, m, Ph), and 7.49 (1H, d, J 9.1, 5-H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C 20.9 (3×$OCOCH_3$), 56.9 (OCH_3), 62.5 (OCH_3), 75.8, 109.3, 126.7, 128.6, 128.7, 128.8, 137.2, 141.4, 147.3, 152.9, 157.8, and 180.0 (3×$OCOCH_3$).

5.1.5. 7-Benzyloxy-3-(3-benzyloxy-2,4-dimethoxyphenyl)-3-phenylthiochroman-4-one (5). Under an atmosphere of argon, to a stirred mixture of **4** (1.06 g, 2.92 mmol) and **9** (2.20 g, 3.51 mmol) in dry chloroform

(30 mL) was injected dry pyridine (0.78 mL, 9.64 mmol). The resulting mixture was stirred at 55 °C overnight, allowed to warm at room temperature, and successively diluted with chloroform (150 mL), washed with 6% aqueous sulfuric acid (150 mL) and extracted with chloroform. The combined organic layers were filtered through Celite, dried over anhydrous Na_2SO_4, and the solvent was evaporated to lead to a mixture of the expected 3-arylated substrate and the unexpected 3-acetylated substrate. The residue was dissolved in hot ethanol and 10% aqueous sodium hydroxide was added under stirring. After extraction with dichloromethane, the organic layers were dried over anhydrous Na_2SO_4 and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (eluent Et_2O–pentane 4:6) to afford 7-benzyloxy-3-(3-benzyloxy-2,4-dimethoxyphenyl)-3-phenylthiochroman-4-one **5** (0.89 g, 51%) as a yellow solid, mp 50.7–52.8 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 3.67 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 4.15 (1H, d, *J* 12.1, 2-H_{ax} or 2-H_{eq}), 4.84 (1H, d, *J* 12.1, 2-H_{eq} or 2-H_{ax}), 4.90 (1H, d, *J* 10.9, OCH_{2A}Ph), 4.94 (1H, d, *J* 10.9, OCH_{2B}Ph), 5.04 (2H, s, OCH_2Ph), 6.47 (1H, d, *J* 2.3, 8-H), 6.64 (1H, dd, *J* 8.7 and 2.3, 6-H), 6.67 (1H, d, *J* 8.9, 5′-H), 7.10–7.40 (15H, m, 3×Ph), 7.74 (1H, d, *J* 8.7, 5-H), and 7.86 (1H, d, *J* 8.9, 6′-H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C 55.9 (OCH_3), 60.0 (OCH_3), 62.6 (C-3), 70.2 (O*C*H_2Ph), 73.4 (C-2), 74.6 (O*C*H_2Ph), 101.5 (C-8), 106.4 (C-5′), 110.5 (C-6), 114.3 (C-1′), 122.0 (C-10), 125.3, 127.5, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.6, 129.8 (C-5), 130.5 (C-6′), 135.4, 136.0, 137.2, 141.1 (C-3′), 151.7(C-4′), 154.3 (C-2′), 161.6 (C-9), 164.4 (C-7), and 185.2 (C-4).

5.1.6. 7-Benzyloxy-3-(3-benzyloxy-2,4-dimethoxyphenyl)chromen-4-one (6). MCPBA (67 mg, 0.387 mmol) in dry EtOAc (10 mL) was added to a solution of **5** (78 mg, 0.129 mmol) in dry EtOAc (8 mL) at 0 °C. The solution was stirred at room temperature and the reaction evolution was monitored by TLC. The solvent was distilled under reduced pressure yielding a crude product that was purified by preparative chromatography using CH_2Cl_2–EtOH (98:2) as eluent. The product obtained was then boiled in toluene. The reaction was checked by TLC until the intermediate sulfoxide completely disappeared. The solvent was distilled off under reduced pressure to give **6** (38 mg, 59%) as a yellow solid, mp 143–145 °C (lit.,[18] mp 145–147 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 3.81 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.07 (2H, s, OCH_2Ph), 5.18 (2H, s, OCH_2Ph), 6.75 (1H, d, *J* 8.7, 6′-H), 6.95 (1H, d, *J* 2.3, 8-H), 7.07 (1H, dd, *J* 8.9 and 2.3, 6-H), 7.08 (1H, d, *J* 8.7, 5′-H), 7.31–7.54 (10H, m, 2×Ph), 7.91 (1H, s, 2-H), and 8.23 (1H, d, *J* 8.9, 5-H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C 55.1 (OCH_3), 60.3 (OCH_3), 69.5 (O*C*H_2Ph), 74.2 (O*C*H_2Ph), 100.3 (C-8), 106.5 (C-5′), 113.9 (C-6), 117.5 (C-1′), 117.6 (C-10), 121.2 (C-6′), 125.2 (C-3), 126.5, 126.9, 127.3, 127.4, 127.8, 134.8 (OCH_2*Ph*), 136.7 (OCH_2*Ph*), 140.4 (C-3′), 151.5 (C-2′), 152.7 (C-4′), 153.3 (C-2), 156.9 (C-9), 162.0 (C-7), and 174.8 (C-4).

5.1.7. 7-Benzyloxy-3-(3-benzyloxy-2,4-dimethoxyphenyl)chroman-4-one (7). To a stirred mixture of **5** (0.60 g, 0.99 mmol) and nickel chloride hexahydrate (5.66 g, 23.81 mmol) in ethanol (150 mL) was added dropwise a solution of sodium borohydride (0.75 g, 19.84 mmol) in water (10 mL). The mixture was refluxed for 4 h and allowed to warm at room temperature, then filtered through Celite, and washed with ethanol. The filtrate was concentrated to a small volume and extracted with ether. The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (eluent Et_2O–pentane 4:6) to afford 7-benzyloxy-3-(3-benzyloxy-2,4-dimethoxyphenyl)-chroman-4-one **7** (0.42 g, 86%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 3.83 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.18 (1H, dd, *J* 11.7 and 5.6, 3-H), 4.47 (1H, dd, *J* 10.9 and 5.6, 2-H_{ax} or 2-H_{eq}), 4.59 (1H, dd, *J* 11.7 and 10.9, 2-H_{eq} or 2-H_{ax}), 5.01 (2H, s, OCH_2Ph), 5.11 (2H, s, OCH_2Ph), 6.53 (1H, d, *J* 2.3, 8-H), 6.65 (1H, d, *J* 8.7, 5′-H), 6.70 (1H, dd, *J* 8.7 and 2.3, 6-H), 6.83 (1H, d, *J* 8.7, 6′-H), 7.31–7.50 (10H, m, 2×Ph), and 7.94 (1H, d, *J* 8.7, 5-H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C 48.1 (3-C), 56.0 (OCH_3), 61.0 (OCH_3), 70.2 (O*C*H_2Ph), 71.4 (C-2), 74.9 (O*C*H_2Ph), 101.7 (C-8), 107.5 (C-5′), 110.4 (C-6), 115.5 (C-10), 121.4 (C-1′), 124.6 (C-6′), 127.4, 127.8, 128.2, 128.6, 129.3 (C-5), 135.9, 137.5, 141.2 (C-3′), 152.4 (C-4′), 153.7 (C-2′), 163.7 (C-9), 164.8 (C-7), and 191.5 (C-4).

5.1.8. Mucronuatol (1). A mixture of **7** (78 mg, 0.159 mmol) and 10% palladium on charcoal (20 mg) in absolute ethanol (10 mL) was stirred under an atmosphere of hydrogen during 24 h. The reaction mixture was filtered through Celite and washed with acetone. The solvent was distilled off under reduced pressure to give **1** (47 mg, quantitative yield) as white crystals, mp 221–224 °C (lit.,[32] mp 227 °C). ^{1}H NMR (acetone-d_6, 300 MHz): δ_H 2.80 (1H, ddd, *J* 15.7, 5.4, and 1.9, 4-H_{eq}), 2.92 (1H, dd, *J* 15.7 and 10.6, 4-H_{ax}), 3.46 (1H, tdd, *J* 10.6, 5.4, and 3.6, 3-H), 3.84 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.95 (1H, t, *J* 10.6, 2-H_{ax}), 4.20 (1H, ddd, *J* 10.6, 3.6, and 1.9, 2-H_{eq}), 6.31 (1H, d, *J* 2.3, 8-H), 6.39 (1H, dd, *J* 8.3 and 2.3, 6-H), 6.67 (1H, d, *J* 8.5, 6′-H),), 6.74 (1H, d, *J* 8.5, 5′-H), and 6.90 (1H, d, *J* 8.3, 5-H). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ_C 32.3 (C-4), 33.0 (C-3), 56.7 (OCH_3), 61.1 (OCH_3), 71.2 (C-2), 103.9 (C-8), 108.2 (C-5′), 109.1 (C-6), 114.5 (C-10), 117.6 (C-6′), 128.4 (C-1′), 131.2 (C-5), 140.6 (C-3′), 147.0 (C-4′), 148.7 (C-2′), 156.3 (C-9), and 157.8 (C-7).

5.1.9. Removal of the benzyl group with HBr—general procedure. A suspension of the substrate (0.5 mmol) in 47% hydrobromic acid aqueous solution was stirred at 50 °C overnight. The mixture was then poured into water (50 mL) and extracted with dichloromethane (4×20 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, and the solvent was distilled off under reduced pressure to give the corresponding isoflavone derivative.

5.1.9.1. Violanone (2). Purified by column chromatography (Et_2O–pentane 9:1) as white crystals (149 mg, 94%), mp 200–202 °C (lit.,[1] 200–202 °C). ^{1}H NMR (acetone-d_6, 300 MHz): δ_H 3.81 (3H, s, OMe), 3.86 (3H, s, OMe), 4.15 (1H, dd, *J* 11.5 and 5.5, 3-H), 4.48 (1H, dd, *J* 11.0 and 5.5, 2-H_{eq}), 4.61 (1H, dd, *J* 11.5 and 11.0, 2-H_{ax}), 6.44 (1H, d, *J* 2.3, 8-H), 6.62 (1H, dd, *J* 8.5 and 2.3, 6-H), 6.64 (1H, d, *J* 8.1, 5′-H), 6.71 (1H, d, *J* 8.5, 6′H), and 7.81 (1H, d, *J* 8.5, 5-H). ^{13}C NMR (acetone-d_6): δ_C 48.9 (3-C), 56.5 (OMe), 60.1 (OMe), 72.1 (C-2), 103.5 (C-8), 107.4

(C-5′), 111.2 (C-6), 115.8 (C-10), 120.4 (C-6′), 123.1 (C-1′), 130.0 (C-5), 140.3 (C-3′), 146.9 (C-4′), 149.1 (C-2′), 164.7 (C-9), 164.9 (C-7), and 191.3 (C-4).

5.1.9.2. 7-Hydroxy-3-(3-hydroxy-2,4-dimethoxyphenyl)chromen-4-one (3). Yellow solid (149 mg, 95%), mp 253 °C (lit.,[18] 251–252 °C). ^{1}H NMR (acetone-d_6, 300 MHz): δ_H 3.77 (3H, s, OMe), 3.89 (3H, s, OMe), 6.78 (2H, s, 5′-H and 6′-H), 6.93 (1H, d, *J* 2.3, 8-H), 7.03 (1H, dd, *J* 2.3 and 8.7, 6-H), 7.53 (1H, s, OH), 8.00 (1H, s, 2-H), 8.07 (1H, d, *J* 8.7, 5-H), and 9.60 (1H, s, OH). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ_C 56.4 (OMe), 60.1 (OMe), 103.0 (C-8), 107.1 (C-5), 115.3 (C-6), 118.3 (C-10), 120.0 (C-1′), 121.6 (C-6′), 123.4 (C-3), 128.2 (C-5), 140.2 (C-3′), 147.0 (C-2′), 149.5 (C-4′), 154.0 (C-2), 158.7 (C-9), 163.0 (C-7), and 175.5 (C-4).

5.2. Cell cultures and survival assay

Human HB_{100} leukemia cells were obtained from the American Tissue Culture Collection. Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in DMEM medium, supplemented with 10% fetal bovine serum, L-glutamine (2 mM), 1.5 g L^{-1} sodium bicarbonate, 4.5 g L^{-1} glucose, 10 mM HEPES, 1 mM sodium pyruvate, penicillin (100 IU mL^{-1}), and streptomycin (100 μg mL^{-1}). The cytotoxicity of the test compounds was assessed using a cell proliferation assay developed by Promega (CellTiter 96® aqueous one solution cell proliferation assay). Briefly, 6×10^3 exponentially growing cells were seeded in 96-well microculture plates with various drug concentrations in a volume of 150 μL. After 72 h incubation at 37 °C, 150 μL of the MTT dye was added to each well and the samples were incubated for further 2 h at 37 °C. Plates were analyzed on a Labsystems Multiskan MS (type 352) reader at 492 nm.

5.3. Preparation of lamb brain tubulin

Tubulin was purified from lamb brain by ammonium sulfate fractionation and ion-exchange chromatography. The protein was stored in liquid nitrogen and prepared as described.[33–35] Protein concentrations were determined spectrophotometrically with a Perkin Elmer spectrophotometer Lambda 800 and an extinction coefficient at 275 nm of 1.07 L g^{-1} cm^{-1} in neutral aqueous buffer or 1.09 L g^{-1} cm^{-1} in 6 M guanidine hydrochloride.

5.4. Microtubule assembly monitored by fluorescence

Microtubule assembly was monitored on a Fluoroscan Ascent FL spectrofluorometer (Labsystems) using a 96-well plate. The excitation wavelength was set at 355 nm and the emission wavelength was set at 460 nm. Experiments were carried out at 37 °C and performed with 7.5 μM Dapi, 15 μM tubulin in 20 mM sodium phosphate buffer, 1 mM EGTA, 10 mM $MgCl_2$, and 3.4 M glycerol, pH 6.5. Under these conditions, the Dapi fluorescence enhancement is directly proportional to the concentration of polymerized tubulin[27] and was monitored as a function of time. DMSO concentration was maintained below 4% in all samples and controls. Experiments were done in triplicate.

5.5. Antimicrobial assays

Bioautography procedure[29] was performed to estimate the antifungal activity. Antimicrobial MICs of compounds were determined in a microdilution assay.[28]

Acknowledgements

The authors thank INTAS for financial support (Grant No. 03-514-915) and the Thailand Research Fund for the 2002 Royal Golden Jubilee Ph.D. research assistant fellowship.

References and notes

1. Donnelly, D. M. X.; Kavanagh, P. J. *Phytochemistry* **1974**, *13*, 2587–2591.
2. Ito, C.; Itoigawa, M.; Kanemastu, T.; Ruangrungsi, N.; Mukainaka, T.; Tokuda, H.; Nishino, H.; Furukawa, H. *Phytochemistry* **2003**, *64*, 1265–1268.
3. Harborne, J. B.; Williams, C. A. *Phytochemistry* **2000**, *55*, 481–504.
4. Lichius, J. J.; Thoison, O.; Montagnac, A.; Païs, M.; Guéritte-Voegelein, F.; Sévenet, T.; Cosson, J.-P.; Hadi, A. H. A. *J. Nat. Prod.* **1994**, *57*, 1012–1016.
5. Haïdara, K. Z.; Zamir, L.; Shi, Q.-W.; Batist, G. *Cancer Lett.* **2006**, *242*, 180–190.
6. Beutler, J. A.; Cardellina, J. H.; Lin, C. M.; Hamel, E.; Cragg, G. M.; Boyd, M. R. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 581–584.
7. Ito, A.; Chai, H. B.; Shin, Y. G.; Garcia, R.; Mejia, M.; Gao, Q.; Fairchild, C. R.; Lane, K. E.; Menendez, A. T.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **2000**, *56*, 6401–6405.
8. Bailly, C.; Bal, C.; Barbier, P.; Combes, S.; Finet, J.-P.; Hildebrand, M.-P.; Peyrot, V.; Wattez, N. *J. Med. Chem.* **2003**, *46*, 5437–5444.
9. Edwards, M. L.; Stemerick, D. M.; Sunkara, P. S. *J. Med. Chem.* **1990**, *33*, 1948–1954.
10. Lawrence, N. J.; McGown, A. T.; Ducki, S.; Hadfield, J. A. *Anticancer Drug Des.* **2000**, *15*, 135–141.
11. Hamel, E. *Med. Res. Rev.* **1996**, *16*, 207–231.
12. Ren, M. Q.; Kuhn, G.; Wegner, J.; Chen, J. *Eur. J. Nutr.* **2001**, *40*, 135–146.
13. Wahala, K.; Hase, T. A. *J. Chem. Soc., Perkin Trans. 1* **1991**, 3005–3008.
14. Finet, J.-P. *Polyphenols Actualites* **2000**, *19*, 18–25.
15. Pelter, A.; Ward, R. S.; Whalley, J. L. *Environ. Toxicol. Pharmacol.* **1999**, *7*, 217–220.
16. Ding, K.; Wang, S. *Tetrahedron Lett.* **2005**, *46*, 3707–3709.
17. Finet, J.-P. *Comprehensive Organometallic Chemistry III*; Crabtree, R. H., Mingos, D. M. P., Eds.; Elsevier: Oxford, 2006; Vol. 9, Chapter 9.9, pp 381–424.
18. Farkas, L.; Gottsegen, A.; Nogradi, M.; Antus, S. *J. Chem. Soc., Perkin Trans. 1* **1974**, 305–312.
19. Donnelly, D. M. X.; Finet, J.-P.; Guiry, P. J.; Hutchinson, R. M. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2851–2852.
20. Donnelly, D. M. X.; Fitzpatrick, B. M.; O'Reilly, B. A.; Finet, J.-P. *Tetrahedron* **1993**, *49*, 7967–7976.
21. Donnelly, D. M. X.; Finet, J.-P.; Rattigan, B. A. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1729–1735.

22. Donnelly, D. M. X.; Finet, J.-P.; Rattigan, B. A. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1679–1683.
23. Chen, H. Y.; Dykstra, K. D.; Birzin, E. T.; Frisch, K.; Chan, W.; Yang, Y. T.; Mosley, R. T.; DiNinno, F.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1417–1421.
24. Naylor, P.; Ramage, G. R.; Schofield, F. *J. Chem. Soc.* **1958**, 1190–1193.
25. Kozyrod, R. P.; Morgan, J.; Pinhey, J. T. *Aust. J. Chem.* **1985**, *38*, 1147–1153.
26. Nguyen, T. L.; McGrath, C.; Hermone, A. R.; Burnett, J. C.; Zaharevitz, D. W.; Day, B. W.; Wipf, P.; Hamel, E.; Gussio, R. *J. Med. Chem.* **2005**, *48*, 6107–6116.
27. Heusele, C.; Bonne, D.; Carlier, M. F. *Eur. J. Biochem.* **1987**, *165*, 613–620.
28. Dei-Cas, E.; Dujardin, L.; Ribeiro Pinto, M. E.; Ajana, F.; Fruit, J.; Poulain, D.; Camus, D.; Vernes, A. *Mycoses* **1991**, *34*, 167–172.
29. Hamburger, M. O.; Cordell, G. A. *J. Nat. Prod.* **1987**, *50*, 19–22.
30. Fitton, A. O.; Ramage, G. R. *J. Chem. Soc.* **1962**, 4870–4874.
31. Cooke, R. G.; McQuilkin, R. M. *Aust. J. Chem.* **1969**, *22*, 2395–2401.
32. Kurosawa, K.; Ollis, W. D.; Redman, B. T.; Sutherland, I. O. *J. Chem. Soc., Chem. Commun.* **1968**, 1263–1264.
33. Weisenberg, R. C.; Borisy, G. G.; Taylor, E. W. *Biochemistry* **1968**, *7*, 4466–4479.
34. Lee, J. C.; Frigon, R. P.; Timasheff, S. N. *J. Biol. Chem.* **1973**, *248*, 7253–7262.
35. Andreu, J. M.; Gorbunopff, M. J.; Lee, J. C.; Timasheff, S. N. *Biochemistry* **1984**, *23*, 1742–1752.