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Design, Synthesis, and Evaluation of A/C/D-Ring Analogs of the Fungal Metabolite K-76 as Potential Complement Inhibitors

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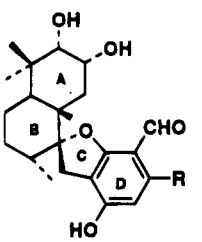
The terpenoid 6,7-diformyl-3′,4′,4a′,5′,6′,7′,8′,8a′-octahydro-4,6′,7′-trihydroxy-2′,5′,5′,8a′-tetramethylspiro[1′(2′*H*)-naphthalene-2(3*H*)-benzofuran] (**1a**; K-76), a natural product of fungal origin, and its monocarboxylate sodium salt **1c** (R = COONa; K-76COONa) inhibit the classical and alternative pathways of complement,[8] and **1c** was shown to inhibit the classical pathway at the C5 activation step. In an attempt to elucidate the essential pharmacophore of **1a,c**, the natural product was used as a "topographical model" for the design of partial analogs retaining the desired complement inhibiting potency. Therefore, A/C/D-ring analogs have been synthesized, as shown in Scheme 1 using 3-methoxyphenol (**3**) and limonene chloride (**5**) as starting materials, which contain functional groups similar to those found on the natural product. The use of (4*R*)-(+)- and (4*S*)-(−)-limonene chloride (**5a,b**, respectively) provided two series of compounds differing in the stereochemistry of the C-4 chiral center (limonene moiety numbering). The *in vitro* assay results of the inhibition of anaphylatoxin production and classical complement-mediated hemolysis revealed that 7-carboxy-2-(*R,S*)-methyl-2-(1′-methylcyclohexen-(4′*R*)-yl)-4-methoxybenzofuran (**13a**) and 7-carboxy-2-(*R,S*)-methyl-2-(1′-methylcyclohexen-(4′*S*)-yl)-4-methoxybenzofuran (**13b**) were active in the same range of concentrations as the natural product.

Introduction

The complement system is important to host defense against infectious pathogens[1] and serves to initiate the inflammatory response,[2] directly kill and promote the phagocytosis of invading microorganisms,[2,3] facilitate the primary and secondary antibody responses of B cells,[1,4] and effect the clearance of immune complexes.[1] The involvement of complement in the early recognition phases of the inflammatory response, as well as the wide array of proinflammatory consequences of complement activation, makes the complement system an attractive target for therapeutic intervention and has led to the isolation, design, and synthesis of numerous complement inhibitors.[5−7] To date, however, no specific inhibitors of complement have been approved for clinical use.

Natural product screening identified the fungal metabolite 6,7-diformyl-3′,4′,4a′,5′,6′,7′,8′,8a′-octahydro-4,6′,7′-trihydroxy-2′,5′,5′,8a′-tetramethylspiro [1′(2′*H*)-naphthalene-2(3*H*)-benzofuran] (**1a**; K-76) which was isolated from *Stachybotrys complementi* nov. sp. K-76 and shown to inhibit complement.[8] Both the compound **1a** and the partially oxidized derivative **1b** (R = COOH; K-76COOH) as well as its sodium salt **1c** (R = COONa; K-76COONa) inhibit the classical and alternative pathways of complement,[8] and the compound **1c** was shown to inhibit the classical pathway at the C5 activation step.[9] Compound **1c** was further shown to suppress Forssman shock in guinea pigs and mice, inhibit heterologous passive cutaneous anaphylaxis in guinea pigs, reduce protein excretion in rats with nephrotoxic nephritis, and prolong survival in a mouse model of lupus nephritis.[10]



1a: K-76; R = CHO
1b: K-76 COOH; R = COOH
1c: K-76COONa; R = COONa

In an attempt to elucidate the essential pharmacophore of compounds **1a,c**, the natural product was used as a "topographical model" for the design of partial analogs retaining the desired complement

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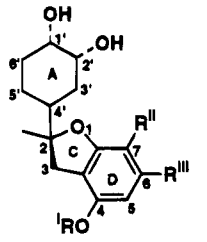
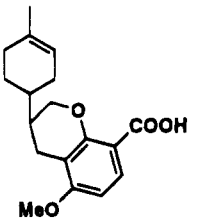
inhibiting potency. Therefore, A/C/D-ring analogs **2** have been synthesized which contain functional groups similar to those found on the natural product.



2
R^{I} = H, Me
R^{II} = CHO, COOH
R^{III} = H

Chemistry

The synthetic strategy is outlined in Scheme 1. The synthesis of compound **4**, which will constitute the skeleton of ring D of the proposed analogs of **1a**, was carried out starting with 3-methoxyphenol (**3**) and selecting the methoxymethyl ether functionality (MOM ether) as the protecting group for the free phenol. Compound **4** was then metalated with the TMEDA–*n*-BuLi complex in anhydrous tetrahydrofuran at room temperature. The addition of copper(I) iodide at −78 °C, followed by addition of limonene chloride (either **5a** or **5b**)[11] as the electrophile at the same temperature, provided compound **6a** or **6b**, respectively, as a colorless oil (Scheme 1). Both of the reactions were carried out at the same gram scale with the identical conditions giving similar product yields. This was also true for all subsequent reactions differing solely by the stereoisomer utilized. Each enantiomer of **6** was treated with the TMEDA–*n*-BuLi complex in hexane followed by reaction with anhydrous carbon dioxide at −78 °C which gave low yields (30–40%) of a mixture of expected intermediate **7** and deprotected derivative **10**, together with starting material and other difficult to isolate unidentified products. The mixture of **7** and **10** when treated with 4 N HCl in 2-propanol at room temperature exclusively provided crystalline **10** as the sole product. Compound **10** was then subjected to treatment with Amberlyst 15 in methylene chloride. Following chromatographic separation of the reaction mixture, three fractions were obtained. The least polar fraction (5%) was identified as recovered **10**, while the largest fraction, a solid obtained in 77% overall yield, was identified as the desired benzofuran **13**. The optical rotations (+4° and −1.5° for **13a,b**, respectively) were consistent with the expected mixtures of diastereomers in equimolar amounts. This was confirmed by the examination of the ^{13}C NMR spectra, where the spectrum of **13a,b** contained a duplication of signals. The fraction of intermediate polarity (11%) displayed a ^{1}H NMR spectrum that was consistent with structure **14**, likely resulting from phenolic oxygen atom attack at a secondary carbonium ion versus the expected tertiary carbonium ion formed during the protonation of **10**. Particularly decisive for the structural assignment were the absence of the C-2 methyl proton signals and the double doublet of the benzylic protons on the benzofuran.



14a,b

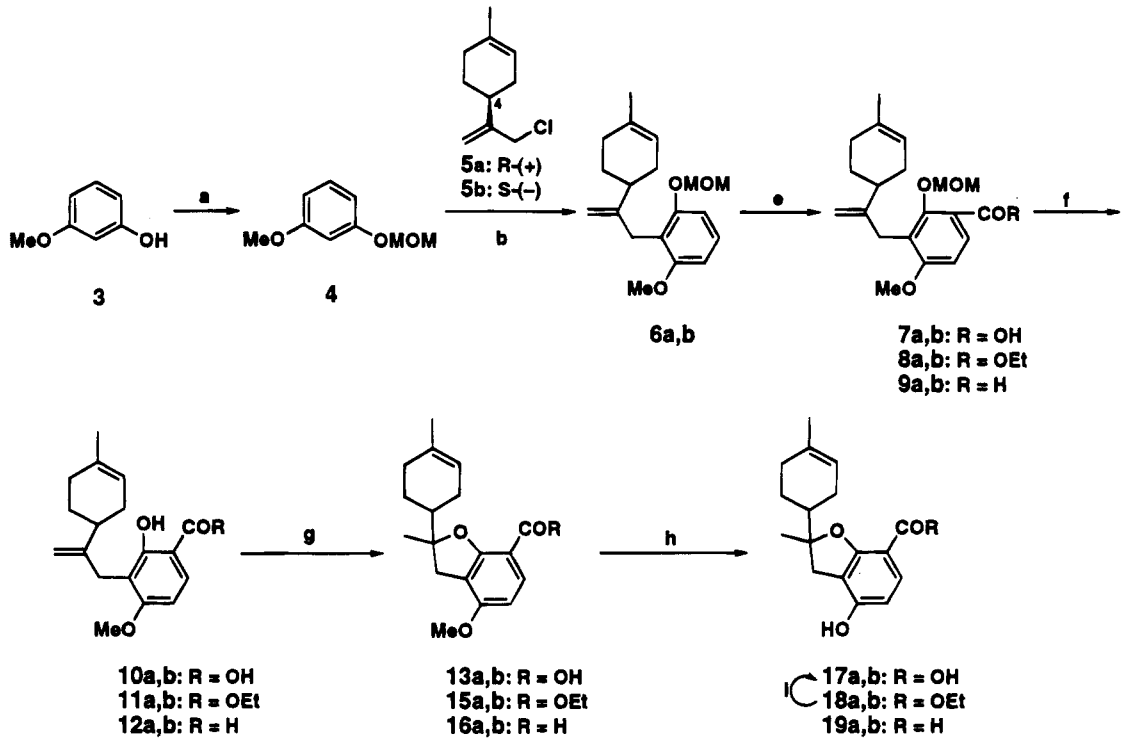
The final step of the synthesis of this series of aromatic carboxylic acids required the demethylation of **13**. The specific reagent selected was lithium *tert*-butylthiolate in a strict analogy to a previous report.[12] However, several experiments in which **13** and its sodium salt (formed by reaction of sodium hydride with **13** in anhydrous ether) were reacted with lithium *tert*-butylthiolate in anhydrous ether/HMPA at −22 °C did not yield the desired product **17**. Therefore, the ester **15** and the aldehyde **16** have been utilized for the demethylation reaction. These compounds have been synthesized by the same sequence of reactions (Scheme 1) as carboxylic acid **13**. The treatment of each enantiomer of **6** in anhydrous ether at low temperature with the TMEDA–*n*-BuLi complex, followed by addition of ethyl carbonate or dimethylformamide, gave compound **8** or **9**, respectively, in 60–64% yield. The reaction of **8** with aqueous potassium hydroxide in ethanol followed by neutralization and acidification with 6 N HCl to pH = 1 successfully gave compound **10** in 92% yield, which was confirmed by chromatographic comparison with an authentic sample and the analysis of its ^{1}H NMR spectrum. The treatment of **8** or **9** with 3 N HCl in 2-propanol resulted exclusively in the formation of compound **11** or **12**, as expected, in 88–92% yield. After stirring **11** or **12** with Amberlyst 15 resin in methylene chloride, compounds **15** or **16** was obtained in good yield. Again, the ^{13}C NMR spectrum displayed a duplication of the signals which indicated that both diastereomers in either **15** or **16** were generated in approximately equimolar quantities. In the next step, compounds **15** and **16** were treated with lithium *tert*-butylthiolate in HMPA/ether at low temperature, following the same procedure used in the attempted demethylation of **13**, and yielded white crystalline solids, which were identified as **18** and the easily oxidizable **19**, respectively. The treatment of **18** with aqueous sodium hydroxide in ethanol under reflux for 8 h gave the corresponding acid **17** in good yield.

Biological Studies

The target compounds described above were assayed for their ability to inhibit complement activation and inhibit the proliferation of activated lymphocytes. However, compounds **16a,b** and **19a,b** were not tested because of their very poor solubility in aqueous media.

C3a and C5a Production by Serum Complement. The capacity of the compounds to inhibit the production of the anaphylatoxins C3a and C5a by activated human serum complement was measured as previously described.[13] Aliquots of human serum (400 μL) were equilibrated at 37 °C with varying concentrations of the compounds dissolved in 100 μL of 0.1 M Hepes, 0.15 N sodium chloride, pH 7.4. Complement activation was initiated by the addition of 25 μL of heat-aggregated IgG

Scheme 1[a]



[a] Reagents: (a) MOMCl, NaH, DMF; (b) **5**, *n*-BuLi, CuI, TMEDA; (c) *n*-BuLi, TMEDA, O_2, Na_2SO_3; (d) Ph_3P, CCl_4, CH_2Cl_2; (e) *n*-BuLi, TMEDA, ether, CO_2 (R = OH), Co_3Et_2 (R = OEt), DMF (R = H); (f) 3 N HCl, 2-PrOH; (g) Amberlyst 15, CH_2Cl_2; (h) *t*-BuSLi, HMPA; (i) 30% NaOH, EtOH.

at 14 mg/mL[14] and incubatation at 37 °C for a fixed reaction time of 10 min (predetermined to yield >90% maximal C3a and C5a production). The C3a[desArg] and C5a[desArg] concentrations were measured using a commercially available radioimmunoassay kit (Amersham, Chicago, IL). C3a[desArg] and C5a[desArg] lack the carboxy-terminal arginine residues of C3a and C5a, respectively, which are rapidly removed by serum proteases. Samples were run in triplicate and averaged. The fractional inhibition was determined relative to the uninhibited sample (no added compound) and the background serum level of anaphylatoxin (no aggregated IgG).

The results shown in Figure 1A indicate that the natural product **1c** inhibits C5a production with 50% maximal inhibition (IC_{50}) at 3 mM but with little inhibition of C3a production over the concentration range tested. Thus complement inhibition by **1c** occurs predominantly at the C5 activation step as reported previously[9] because inhibition is more effective for C5a than for C3a production and because C3 activation immediately precedes C5 activation in both complement pathways. In a similar fashion, compounds **13a,b** preferentially inhibit the production of C5a relative to C3a (Figure 1B,C) with IC_{50} values (8 and 6 mM, respectively) for the inhibition of C5a production which are comparable to those of the compound **1c** itself. Interestingly, the replacement of the 4-methoxy group in **13** with the 4-hydroxy group of **17b** resulted in no observable inhibition of C3a and C5a production over the concentration range tested (Figure 1D).

Complement-Mediated Hemolysis. The capacity of the compounds to inhibit complement-mediated erythrocyte lysis (hemolysis) was assessed as previously described.[13,15] Antibody-sensitized sheep erythrocytes (Diamedix Corp., Miami, FL) were lysed using human serum as a complement source, diluted in 0.1 M Hepes, 0.15 N sodium chloride, pH 7.4. Sensitized sheep cells, the compounds to be tested, and human serum at a dilution previously determined to lyse 80–90% of the erythrocytes were incubated for 60 min at 37 °C. Cells were separated by centrifugation, and the absorbance at 410 nm of the supernatants was measured to quantify hemoglobin release. Samples were paired with identical controls lacking human serum (complement-independent lysis). Both samples and controls were run in triplicate. Values for complement-independent lysis were subtracted from sample values, and the fractional inhibition was determined relative to the uninhibited (no added compound) sample.

As shown in Figure 2A, the natural product **1c** inhibited hemolysis with an IC_{50} value of 0.30 mM, in agreement with earlier results in a similar assay.[8] Multiple assays of the inhibition of hemolysis by **1c** yielded an average IC_{50} of 0.57 (± 0.02; standard deviation, $n = 9$) mM. Compounds **13a,b** and **17a,b** are comparable to **1c** in the capacity to inhibit hemolysis with compounds **17a,b** being somewhat less effective (Figure 2).

***In Vitro* Lymphocyte Proliferation.** In addition to inhibiting complement, **1c** was also shown to inhibit a number of lymphocyte functions *in vitro*.[16,17] In particular, **1c** was shown to inhibit lectin-stimulated T cell proliferation.[17] Therefore, compounds **1c**, **13a**, and **17b** were tested for their ability to inhibit the proliferation of peripheral blood lymphocytes (PBL) activated by T cell mitogens. The proliferation of human PBL in response to phytohemagglutinin (PHA; Wellcome) or a murine anti-CD3 monoclonal antibody (OKT-3; Ortho) was assessed by the incorporation of [^{3}H]thymidine to quantitate DNA synthesis. Test compounds were di-

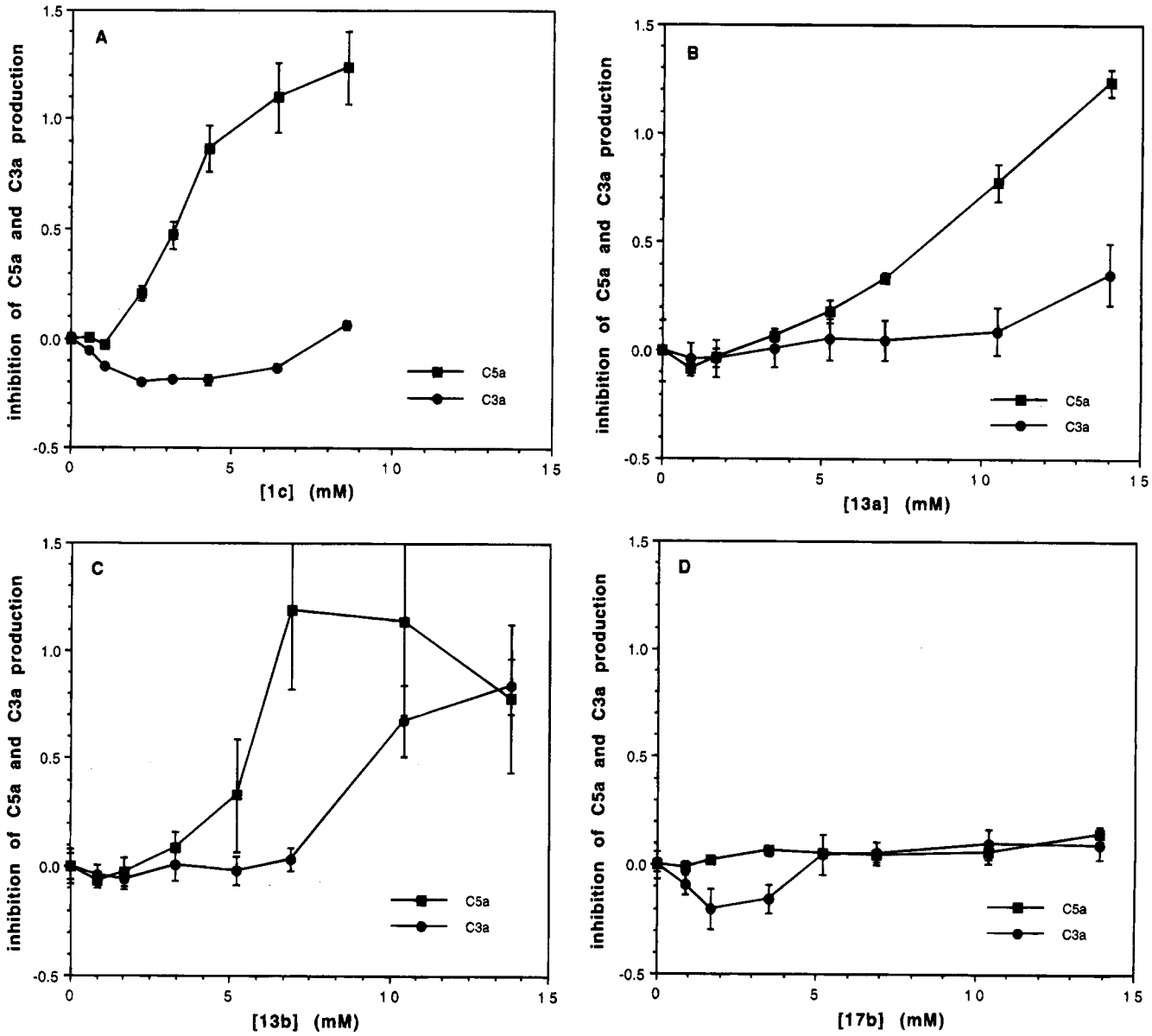


Figure 1. Inhibition of the generation of anaphylatoxins C3a (circles) and C5a (squares) in human serum activated by heat-aggregated IgG as a function of compound concentration. Error bars represent standard errors ($n = 3$) propagated in the normal manner.

luted in culture media to the desired concentrations, human PBL's were added to a final concentration of 10^6 cells/mL, and then either PHA or anti-CD3 antibody (final concentration of 1 mg/mL) was added to initiate proliferation. The final sample volume was 100 μL. The cells were incubated for 72 h after stimulation, pulsed with 1 μCi of [^{3}H]thymidine/sample for 4 h, harvested, and counted in a scintillation counter. Separate experiments, conducted on samples exposed only to varying amounts of test compounds without the mitogen, showed that the PBL's were viable over the concentration ranges used above, as determined visually by trypan blue exclusion (data not shown).

As shown in Figure 3A,B, the natural product **1c** inhibited mitogen-stimulated PBL proliferation with IC_{50} values of 0.5 mM, in agreement with the published inhibition of PHA-stimulated PBL for this compound.[17] Compounds **13a** and **17b** inhibited mitogen-stimulated PBL proliferation at similar but somewhat higher concentrations than compound **1c** (IC_{50} values ranging from 1.7 to 2.8 mM, Figure 3).

Conclusion

The synthesis of simplified analogs of **1a** (K-76) retaining complement inhibitory activity has been accomplished. The *in vitro* inhibition of anaphylatoxin production and of classical complement-mediated hemolysis indicates that compounds **13a,b** are active at similar concentrations as the natural product **1c**, which is promising because modifications of these compounds may further lower the concentrations required for activity. These results strongly suggest that the entire **1a** structure is not essential for potent complement inhibitory activity. In addition, the improved capacity to inhibit complement by the 4-methoxybenzofuran derivatives **13a,b** relative to the 4-hydroxy derivatives **17a,b** suggests the potential for non-native substitutions at this position to further improve the potency of future analogs. However, the magnitude of these differences in potency is small, and the testing of additional analogs will be required to confirm the proposed structural requirements for complement inhibition.

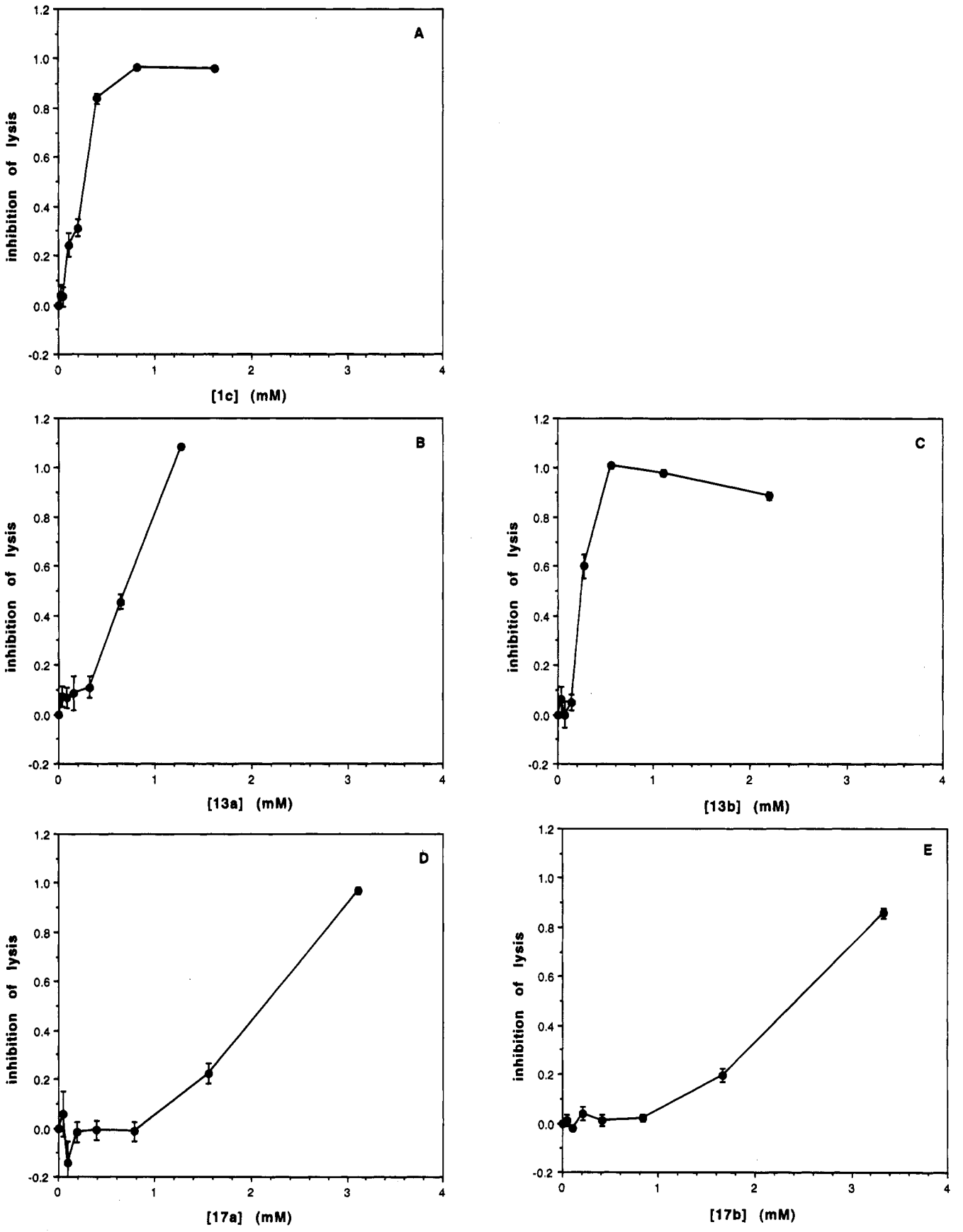


Figure 2. Inhibition of complement-mediated erythrocyte lysis as a function of compound concentration. Error bars represent propagated standard errors ($n = 3$).

Small differences were also observed between the anticomplement activities of **13a,b** which suggest that the stereochemistry at C-4′ may play a role in the biological activity. Because these compounds are diastereomeric mixtures, however, the actual differences due to C-4′ stereochemistry may be obscured. Attempts

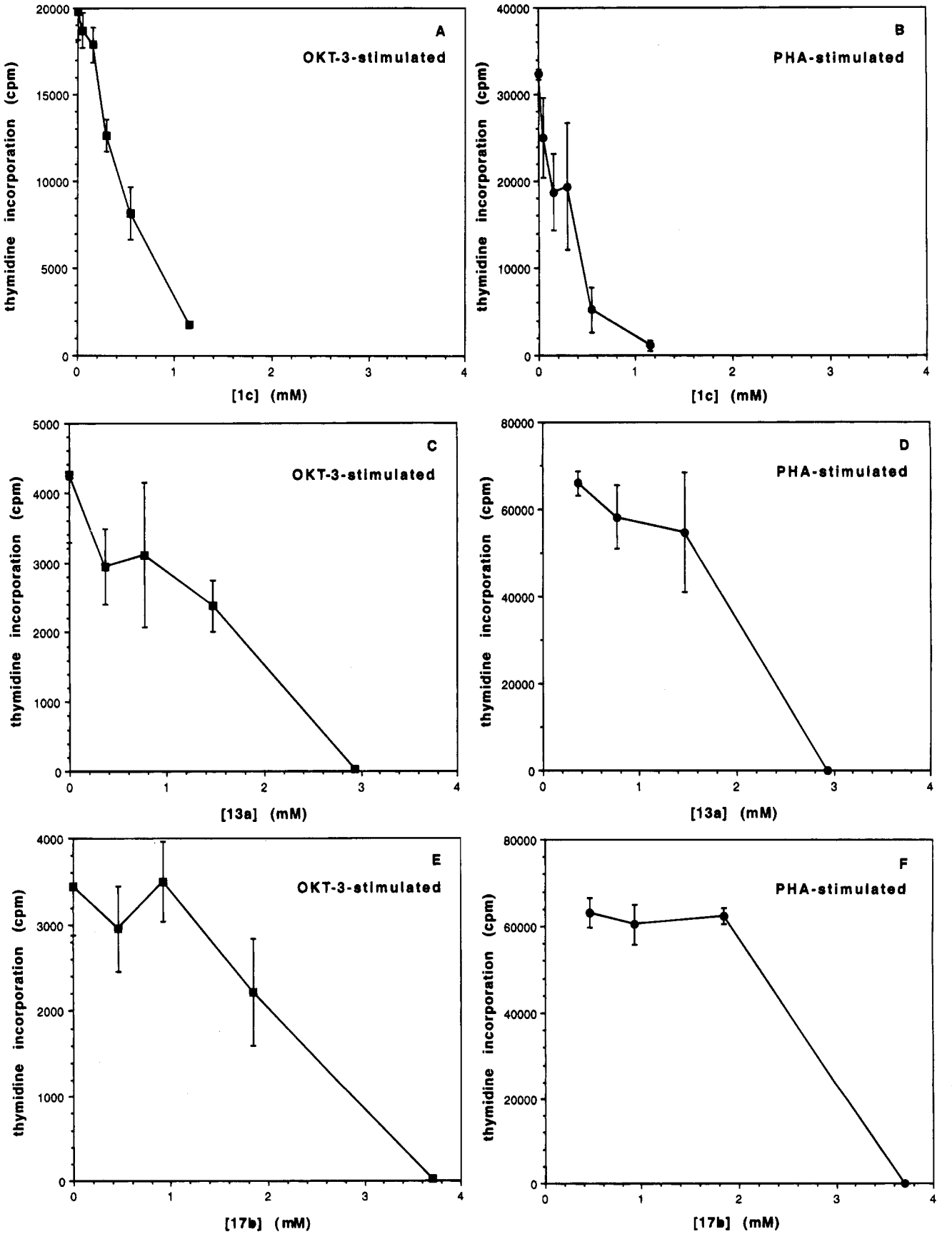


Figure 3. *In vitro* lymphocyte proliferation as a function of compound concentration when stimulated either with the monoclonal antibody OKT-3 or with the lectin PHA. Proliferation was assessed by the incorporation of [^{3}H]thymidine into cellular DNA. Error bars represent standard errors (n = 3).

to resolve these diastereomers by HPLC using different mixtures of solvents proved unsuccessful. The analysis of models of **10**–**12**, the mechanistic study of a stereospecific cyclization, and further investigations of the structure–activity relationships of new A/C/D-ring analogs of the compound **1a** are in progress.

Experimental Section

Melting points were determined on a Thomas-Hover capillary melting-point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded at 300 MHz on a Varian VXR 300 instrument in deuterochloroform except where noted. Chemical shifts are reported as δ units (ppm) relative to tetramethylsilane as internal standard, and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or b (broad). IR spectra were obtained with a Perkin Elmer 281B spectrophotometer, while the optical rotations observed at the Na D line were determined at 25° with a Perkin-Elmer 141 polarimeter. Mass spectra were recorded on a Finnigan 3221-F200 mass spectrometer in either EI or fast-atom bombardment mode. A Hewlett Packard 5890 gas chromatograph, with a 30 m DB-5 column, in the isothermic mode (160 °C) using helium (1 mL/min) as carrier, was employed for purity analysis. The elemental analysis (±0.4%) was performed by Atlantic Microlab, Norcross, GA. Thin layer chromatography (TLC) was performed on Merck 0.25 mm glass plates of silica gel 60 F_{254}, and visualization was achieved with UV light. All extracted solutions were dried over anhydrous sodium sulfate unless otherwise noted and concentrated to dryness on a rotary evaporator under reduced pressure.

3-(Methoxymethoxy)anisole (4). NaH (50% in mineral oil; 4.37 g, 91.13 mmol) was washed with anhydrous hexane (2 × 20 mL). Dry DMF (40 mL) was added, and the mixture was cooled to 0 °C. A solution of **3** (10 g, 80.65 mmol) in dry DMF (30 mL) was added slowly to the mixture and then the mixture was stirred for 2 h at 25 °C. The mixture was recooled to 0 °C, and a solution of MOMCl (7.17 g, 88.72 mmol) in dry DMF (38 mL) was added dropwise. The mixture was stirred at 25 °C overnight. Ether (200 mL) was added to the mixture, and it was washed with H_2O (4 × 200 mL). The ether portion was dried and concentrated to give a pale yellow liquid which was distilled under reduced pressure to furnish **4** as a clear colorless oil (12.15 g, 89%): bp 45 °C (0.15 mmHg); ^1H NMR 3.49 (s, 3H), 3.79 (s, 3H), 5.16 (s, 2H), 6.61 (m, 3H), 7.16 (m, 1H); ^{13}C NMR 55.1, 55.9, 94.3, 102.5, 107.3, 108.2 129.7 158.2, 160.5. Anal. ($C_9H_{12}O_3$) C,H.

3-(Methoxymethoxy)-2-[2′-(1″-methylcyclohexen-4″-yl)propen-3′-yl]anisole (6a,b). To a stirred solution of **4** (2.0 g, 11.9 mmol) in THF (90 mL), under a nitrogen atmosphere, was slowly added TMEDA (1.97 mL, 13.1 mmol). The system was cooled to 0 °C, and *n*-BuLi (6.1 mL, 13.1 mmol) was added dropwise. After stirring for another 45 min at room temperature, the mixture was cooled to −78 °C, and copper (I) iodide (2.7 g 14.3 mmol) was added, all at once. The temperature was allowed to reach −45 °C for 1 h, and then the system was recooled to −78 °C, and either **5a** [(4*R*)-(+)] or **5b** [(4*S*)-(−)][11] (2.5 g, 14.9 mmol) was added dropwise. The heterogeneous system was stirred at room temperature for 3 days, and then an excess of a saturated solution of sodium bicarbonate was added. After stirring for 3 h, the organic layer was separated and the aqueous phase was extracted with ether (3 × 80 mL). The organic fractions were dried and concentrated under reduced pressure, the remaining oil was filtered through a short column of silica gel, and the filtrate was purified by fractional distillation. Part of the excess of **5a** or **5b** was recovered, followed by **6a** or **6b** (3.3 g, 93%), as an oil: bp: 132 °C (0.3 mmHg), >99% pure according to GC; $[\alpha]^{25}_D$ = +57.5° (*c* = 0.16, $CHCl_3$) and −56.56° (*c* = 0.64, $CHCl_3$) for **6a,6b**, respectively; IR (neat) 3000−2820, 1635, 1590, 1470, 1430, 1400, 1320, 1270, 1250, 1200, 1150, 1100, 1070, 1020, 920, 890, 800, 780, 735 cm^{-1}; ^1H NMR 1.66 (s, 3H), 3.40 (s, 2H), 3.43 (s, 3H), 3.78 (s, 3H), 4.34 and 4.67 (bs, 1H ea.), 5.14 (s, 2H) 5.43 (bs, 1H), 6.58 (d, 1H, *J* = 8.1 Hz), 6.73 (d, 1H, *J* = 8.1 Hz), 7.13 (t, 1H, *J* = 8.1 Hz); ^{13}C NMR 23.51, 28.19, 28.27, 30.81, 31.30, 40.46, 55.87, 56.01, 94.44, 104.72, 106.43, 107.07, 118.03, 120.97, 127.11, 133.71, 153.03, 156.02, 158.65. Anal. ($C_{19}H_{26}O_3$) C,H.

4-Methoxy-3-[2′-(1″-methylcyclohexen-4″-yl)propen-3′-yl]-2-hydroxybenzoic Acid (10a,b). To a stirred solution of either **6a** or **6b** (2.0 g, 6.6 mmol) in anhydrous hexane (80 mL) was added dropwise TMEDA (1.2 mL, 7.95 mmol). After cooling at 0 °C, *n*-BuLi (3.7 mL, 7.95 mmol) was added. After stirring at 0 °C for a 15 min period, the ice bath was removed and stirring was continued for another 2h. The system was then cooled at −78 °C, and anhydrous CO_2 (obtained from dry ice and passed through $CaCl_2$ tubes) was bubbled for 1 h and then for another 1 h at room temperature. The organic phase was extracted with 5% NaOH (3 × 40 mL), and the aqueous phases were mixed, acidified with 6 N HCl, and extracted with ether (3 × 150 mL). After washing, drying, and concentrating the organic material, the remaining oil was chromatographed, giving a mixture of **7a** or **7b** and **10a** or **10b** (0.95 g), respectively.

The above mixture was dissolved in 2-propanol (20 mL), and to the resulting solution was added dropwise 3 N HCl (9 mL, 27 mmol). After the mixture had stirred overnight at room temperature, solid NaCl and brine were added. The organic compounds were extracted with ether (3 × 60 mL), and the mixed organic phases were washed, dried, concentrated, and chromatographed to yield **10a** or **10b** (0.79 g, 40%) as a solid: mp 163−163.5 and 163−164 °C for **10a,10b**, respectively; $[\alpha]^{25}_D$ = +54.3° (*c* = 0.20, $CHCl_3$) and −52.8° (*c* = 0.54, $CHCl_3$), for **10a,10b**, respectively; IR (KBr) 3150−2500, 3000−2810, 1640, 1610, 1495, 1455, 1420, 1265, 1180, 1090, 885 cm^{-1}; ^1H NMR 1.67 (s, 3H), 3.41 (s, 2H), 3.87 (s, 3H), 4.39 and 4.70 (s, 1H ea.), 5.09 (s, 2H), 5.44 (bs, 1H), 6.52 (d, 1H, *J* = 9 Hz), 7.85 (d, 1H, *J* = 9 Hz), 10.72 (s, 1H); ^{13}C NMR 23.50, 27.65, 28.27, 30.78, 31.27, 40.38, 55.89, 102.84, 104.78, 106.43, 115.76, 120.91, 130.62, 133.74, 152.18, 161.45, 164.18, 175.05; MS (*m*/*e*, rel intensity) 302 (M^+, 39), 284 (17), 269 (10), 255 (15), 243 (12), 216 (38), 201 (24), 191 (23), 181 (34), 163 (85), 145 (17), 133 (30), 121 (100), 104 (40), 93 (34), 77 (29), 67 (15). Anal. ($C_{18}H_{22}O_4$) C,H.

7-Carboxy-2-(*R*,*S*)-methyl-2-(1′-methylcyclohexen-4′-yl)-4-methoxybenzofuran (13a,b). To a gently stirred solution of either **10a** or **10b** (0.47 g, 1.56 mmol) in anhydrous methylene chloride (32 mL) was added Amberlyst 15, and the reaction was monitored by TLC. After stirring at room temperature for 30 min, the resin was decanted and washed several times with ether. The organic fractions were concentrated under reduced pressure, and the resulting oil was chromatographed, giving starting material (0.02 g, 5%), benzopyran **14a** or **14b** (0.05 g, 11%), and acid **13a** or **13b** (0.36 g, 77%).

8-Carboxy-3-(1′-methylcyclohexen-4′-yl)-5-methoxybenzopyran (14a,b): ^1H NMR 1.71 (s, 3H), 3.90 (s, 3H), 5.35 (bs, 1H), 6.55 (d, 1H, *J* = 8 Hz), 8.05 (d, 1H, *J* = 8 Hz).

Acids 13a,b: $[\alpha]^{25}_D$ = +4° (*c* = 0.2, $CHCl_3$) and −1.56° (*c* = 0.44, $CHCl_3$) for **13a,b**, respectively; mp 142−144 and 131−135 °C for **13a,b**, respectively; IR (KBr) 3300−2500, 3000−2820, 1670, 1620, 1440, 1270, 1100, 770 cm^{-1}; ^1H NMR 1.48 (s, 3H), 1.65 (s, 3H), 2.79−3.17 (m, 2H), 3.89 (s, 3H), 5.38 (bs, 1H), 6.52 (d, 1H, *J* = 9 Hz), 7.86 (d, 1H, *J* = 9 Hz); ^{13}C NMR 23.33, 23.87, 24.51, 26.57, 30.39, 36.16, 43.30, 55.70, 96.76, 104.25, 105.69, 114.02, 119.62, 132.83, 134.16, 159.44, 160.33, 165.88; MS (*m*/*e*, rel intensity) 302 (M^+, 55), 284 (17), 207 (22), 191 (58), 181 (19), 163 (68), 147 (14), 135 (40), 121 (100), 105 (31), 93 (25), 77 (26). Anal. ($C_{18}H_{22}O_4$) C,H.

Ethyl 4-Methoxy-3-[2′-(1″-methylcyclohexen-4″-yl)propen-3′-yl]-2-(methoxymethoxy)benzoate (8a,b). To a cooled (0 °C), stirred solution of either **6a** or **6b** (3.8 g, 12.58 mmol) in anhydrous ether (100 mL) was added TMEDA (2.49 mL, 16.55 mmol) followed by *n*-BuLi (8.07 mL, 16.55 mmol). After 15 min, the ice was removed and the reaction mixture was stirred at room temperature for 90 min. It was then cooled at −50 °C and transferred, via double needle, into a solution of ethyl carbonate (6.41 mL, 52.96 mmol) in ether (50 mL). After the mixture had stirred overnight at room temperature, brine (50 mL) was added and the aqueous phase was extracted with ether (3 × 50 mL). The organic phases were mixed, washed with brine (1 × 10 mL), dried, concentrated under reduced pressure, and finally chromatographed to furnish **8a** or **8b** as an oil (2.8 g, 60%): $[\alpha]^{25}_D$ = +38.6° (*c* = 0.48, $CHCl_3$) and −36.7° (*c* = 0.56, $CHCl_3$) for **8a,b**, respectively; IR (neat) 3020−2820, 1715, 1640, 1590, 1480−1430, 1260, 1140, 1070, 990, 940, 840 cm^{-1}; ^1H NMR 1.36 (t, 3H), 1.66 (s, 3H), 3.46 (s, 2H), 3.53 (s, 3H), 3.85 (s, 3H), 4.33 (q, 2H, *J* = 4 Hz), 4.20 and

4.69 (bs, 1H ea.), 5.03 (s, 2H), 5.43 (bs, 1H), 6.68 (d, 1H, J = 8 Hz), 7.82 (d, 1H, J = 8 Hz); ^{13}C NMR 14.37, 23.48, 28.28, 28.30, 30.79, 31.29, 55.87, 57.62, 60.63, 76.63, 101.25, 106.14, 106.89, 117.23, 120.85, 123.43, 131.02, 133.74, 152.43, 157.32, 161.94, 165.93. Anal. ($C_{22}H_{30}O_5$) C,H.

Preparation of 10a,b from 8a,b. To a stirred solution of either **8a** or **8b** (0.38 g, 1.02 mmol) in ethanol (5 mL), under a nitrogen atmosphere, was added dropwise 40% aqueous KOH (1 mL). After stirring overnight at room temperature, the solution was acidified (pH = 1) with 6 N HCl and stirred for an additional 3 h. The reaction mixture was extracted with ether (4 × 50 mL) and washed with brine (2 × 10 mL). The organic phases were combined, dried, concentrated, and chromatographed to provide **10a** or **10b** (0.28 g, 92%). The spectral data of the products were superimposable on those described earlier.

The following compounds were similarly prepared.

Ethyl 4-Methoxy-3-[2′-(1″-methylcyclohexen-4″-yl)propen-3′-yl]-2-hydroxybenzoate (11a,b): from **8a,b** (88%), oil; $[\alpha]^{25}_D$ = +43.6° (c = 0.36, $CHCl_3$) and −41.5° (c = 0.48, $CHCl_3$) for **11a,b**, respectively; IR (neat) 3080, 2980−2810, 1660, 1610, 1500, 1370, 1275, 1180, 1095, 1030, 890, 785, 755 cm^{-1}; ^{1}H NMR 1.43 (t, 3H, J = 3.6 Hz), 1.66 (s, 3H), 3.34 (s, 2H), 3.88 (s, 3H), 4.39 and 4.63 (bs, 1H ea.), 4.42 (q, 2H, J = 3.6 Hz), 5.10 (s, 2H), 5.40 (bs, 1H), 6.38 (d, 1H, J = 8 Hz), 7.70 (d, 1H, J = 8 Hz); MS (m/e, rel intensity) 330 (M$^+$, 16), 284 (12), 216 (20), 209 (53), 191 (24), 188 (27), 163 (90), 133 (22), 121 (100), 105 (26), 91 (18), 77 (17), 67 (12). Anal. ($C_{20}H_{26}O_4$·0.5 H_2O) C,H.

7-Carbethoxy-2-(*R,S*)-methyl-2-(1′-methylcyclohexen-4′-yl)-4-methoxybezofuran (15a,b): from **11a,b** (81%), oil; IR (neat) 3000−2815, 1715, 1690, 1650, 1610, 1495, 1430, 1270, 1100, 1030, 890, 770 cm^{-1}; ^{1}H NMR 1.34 (t, 3H, J = 4 Hz), 1.42 (s, 3H), 1.67 (s, 3H), 2.69−3.14 (m, 2H), 3.86 (s, 3H), 4.31 (m, 2H), 5.38 (bs, 1H), 6.38 (d, 1H, J = 8 Hz), 7.74 (d, 1H, J = 8 Hz); ^{13}C NMR 14.41, 23.36, 24.25, 26.54, 30.59, 36.37, 43.47, 55.44, 60.04, 93.36, 107.03, 115.03, 120.26, 131.95, 133.92, 159.78, 161.42, 165.29; MS (m/e, rel intensity) 330 (M$^+$, 20), 285 (14), 273 (18), 209 (37), 191 (29), 163 (100), 147 (13), 133 (13), 121 (68), 105 (18), 77 (13); FABMS calcd for $C_{20}H_{26}O_4$ 330.426, found 330.4259.

7-Carboxy-2-(*R,S*)-methyl-2-(1′-methylcyclohexen-4′-yl)-4-hydroxybenzofuran (17a,b). A solution (9 mL) prepared with HMPA (15 mL), *tert*-butyl mercaptan (0.9 mL), and n-BuLi (3.1 mL) was added to a stirred solution of either **15a** or **15b** (0.6 g, 1.82 mmol) in anhydrous ether/HMPA (9 mL, 2:1) at −22 °C under nitrogen atmosphere. After the mixture had stirred at room temperature for 3 days, ether and brine were added and it was stirred for an additional 2 h. The product was extracted with ether (3 × 75 mL), and the organic extracts were combined, dried, and concentrated. The crude product was chromatographed to yield 7-carbethoxy-2-(*R,S*)-methyl-2-(1′-methylcyclohexen-4′-yl)-4-hydroxybenzofuran (**18a,b**; 0.53 g, 97%) as an oil which crystallized in the refrigerator: mp: 53−57 and 53.5−57 °C for **18a,b** respectively; IR (KBr) 3200, 2990−2840, 1680, 1600, 1445, 1270, 1050, 890, 780 cm^{-1}; ^{1}H NMR 1.34 (t, 3H, J = 4 Hz), 1.39 (s, 3H), 1.63 (s, 3H), 2.62−3.11 (m, 2H), 4.32 (m, 2H), 5.36 (bs, 1H), 6.38 (d, 1H, J = 8 Hz), 7.32 (bs, 1H), 7.61 (d, 1H, J = 8 Hz); ^{13}C NMR 14.36, 23.36, 23.76, 24.48, 26.37, 30.55, 35.71, 43.37, 60.30, 93.79, 105.48, 108.02, 113.71, 120.13, 131.56, 133.96, 157.52, 162.35, 166.02.

To a solution of either **18a** or **18b** in ethanol (5 mL) was added dropwise 30% aqueous NaOH (1 mL). After heating under reflux overnight, the reaction mixture was cooled to 0 °C and acidified with 3 N HCl until pH = 1. Brine (10 mL) was added, and the solution was extracted with ether (3 × 50 mL). The combined ether fractions were dried, concentrated, and chromatographed yielding **17a** or **17b** (0.17 g, 85% from **15a** or **15b**) as a crystalline solid: mp 228−231 and 227−230 °C dec for **17a,b**, respectively; IR (KBr) 3380, 3500−2500, 1660, 1630, 1595, 1490, 1455, 1280, 1040, 880, 780 cm^{-1}; ^{1}H NMR (acetone-d_6) 1.36 (s, 3H), 1.60 (s, 3H), 2.70−3.33 (m, 2H), 5.32 (bs, 1H), 6.38 (d, 1H, J = 8 Hz), 7.50 (d, 1H, J = 8 Hz); FABMS calcd for $C_{17}H_{20}O_4$ 288.346, found 288.345. Anal. ($C_{17}H_{20}O_4$·0.25 H_2O) C,H.

4-Methoxy-3-[2′-(1″-methylcyclohexen-4″-yl)propen-3′-yl]-2-(methoxymethoxy)benzaldehyde (9a,b). To a stirred solution of either **6a** or **6b** (1.23 g, 4.07 mmol) in anhydrous ether (25 mL) was added dropwise n-BuLi (3 mL, 5.29 mmol) at −45 °C under nitrogen atmosphere. After the mixture was stirred for 30 min, the cold bath was removed and the reaction mixture was stirred at room temperature for another 1.5 h. The system was recooled to 0 °C, and anhydrous DMF (12.59 mL, 16.28 mL) was added in one portion. The reaction mixture was then stirred at room temperature for 24 h, and brine (30 mL) was added. It was extracted with ether (4 × 50 mL), and the combined organic extracts were washed one time with brine (50 mL) and dried over magnesium sulfate. The ether solution was evaporated, and the remaining crude product was chromatographed to furnish starting material (**6a** or **6b**, 0.19 g, 15%) and **9a** or **9b** (0.85 g, 64%) as an oil: $[\alpha]^{25}_D$ = +48.46° (c = 0.35, $CHCl_3$) and −43.96° (c = 0.53, $CHCl_3$) for **9a,b**, respectively; IR (neat) 3020−2815, 1675, 1590, 1430, 1380, 1275, 1255, 1160, 1065, 985, 940, 810, 760 cm^{-1}; ^{1}H NMR 1.65 (s, 3H), 3.45 (s, 2H), 3.52 (s, 3H), 3.88 (s, 3H), 4.25 and 4.66 (bs, 1H ea.), 5.05 (s, 2H), 5.39 (bs, 2H), 6.80 (d, 1H, J = 8 Hz), 7.86 (d, 1H, J = 8 Hz), 10.16 (s, 1H); ^{13}C NMR 23.48, 28.27, 28.78, 30.73, 31.28, 40.79, 56.05, 57.89, 101.29, 107.25, 120.67, 122.39, 128.87, 133.85, 152.27, 160.06, 163.88, 189.83; MS (m/e, rel intensity) 330 (M$^+$, 15), 298 (14), 285 (79), 257 (12), 217 (12), 203 (39), 193 (52), 191 (44), 179 (20), 175 (22), 165 (100), 149 (13), 133 (63), 121 (21), 119 (33), 105 (56), 91 (38), 77 (32), 67 (13); FABMS calcd for $C_{20}H_{26}O_4$ 330.426, found 330.426.

The following compounds were similarly prepared.

4-Methoxy-3-[2′-(1″-methylcyclohexen-4″-yl)propen-3′-yl]-2-hydroxy benzaldehyde (12a,b): from **9a,b** (92%), oil; $[\alpha]^{25}_D$ = +52.8° (c = 0.50, $CHCl_3$) and −51.6° (c = 0.45, $CHCl_3$) for **12a,b** respectively; IR (neat) 3000−2820, 1640, 1620, 1495, 1425, 1250, 1150, 1015, 890, 795, 640 cm^{-1}; ^{1}H NMR 1.66 (s, 3H), 3.88 (s, 3H), 4.39 and 4.69 (s, 1H ea.), 5.10 (s, 2H), 5.42 (bs, 1H), 6.58 (d, 1H, J = 8 Hz), 7.42 (d, 1H, J = 8 Hz), 9.72 (s, 1H); ^{13}C NMR 23.50, 27.22, 28.24, 30.75, 31.23, 40.32, 55.98, 103.10, 106.60, 115.57, 115.79, 120.85, 133.74, 134.06, 151.91, 161.26, 164.48, 194.70; MS (m/e, rel intensity) 286 (M$^+$, 28), 268 (11), 203 (26), 191 (13), 189 (13), 177 (15), 165 (73), 153 (15), 135 (27), 121 (100), 105 (20), 93 (29), 91 (24), 79 (26), 77 (29), 67 (11). Anal. ($C_{18}H_{22}O_3$) C,H.

7-Formyl-2-(*R,S*)-methyl-2-(1′-methylcyclohexen-4′-yl)-4-methoxybenzofuran (16a,b): from **12a,b** (73%), oil; IR (neat) 3000−2800, 1675, 1610, 1500, 1430, 1390, 1285, 1270, 1205, 1100, 1060, 885, 790 cm^{-1}; ^{1}H NMR 1.44 (s, 3H), 1.65 (s, 3H), 2.69−3.11 (m, 2H), 3.88 (s, 3H), 5.40 (bs, 1H), 6.45 (d, 1H, J = 8 Hz), 7.64 (d, 1H, J = 8 Hz), 10.34 (s, 1H); ^{13}C NMR 23.35, 23.74, 24.64, 26.55, 30.49, 35.91, 43.41, 55.63, 94.74, 103.58, 113.25, 114.57, 119.92, 129.18, 134.10, 161.36, 168.38, 187.47. Anal. ($C_{18}H_{22}O_3$) C,H.

7-Formyl-2-(*R,S*)-methyl-2-(1′-methylcyclohexen-4′-yl)-4-hydroxybenzofuran (19a,b): from **16a,b** (95%), mp 73−75 and 72−74 °C for **19a,b**, respectively; IR (KBr) 3500−3150, 2980−2820, 1640, 1590, 1445, 1255, 1210, 1160, 1040, 800 cm^{-1}; ^{1}H NMR 1.44 (s, 3H), 1.64 (s, 3H), 2.74−3.19 (m, 2H), 5.37 (bs, 1H), 6.49 (d, 1H, J = 8 Hz), 7.52 (d, 1H, J = 8 Hz), 8.91 (bs, 1H), 9.94 (s, 1H); ^{13}C NMR 23.35, 23.73, 24.67, 26.55, 30.46, 35.51, 43.37, 95.25, 109.72, 112.97, 113.31, 119.89, 129.50, 134.10, 160.48, 165.02, 188.84; MS (m/e, relative intensity) 272 (M$^+$, 52), 254 (7), 204 (9), 189 (26), 177 (42), 165 (15), 151 (71), 139 (17), 121 (100), 105 (23), 93 (53), 77 (34), 67 (14). Anal. ($C_{17}H_{20}O_3$) C,H.

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