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Synthesis and Biological Activity of Optically Active Phenylbutenoid Dimers

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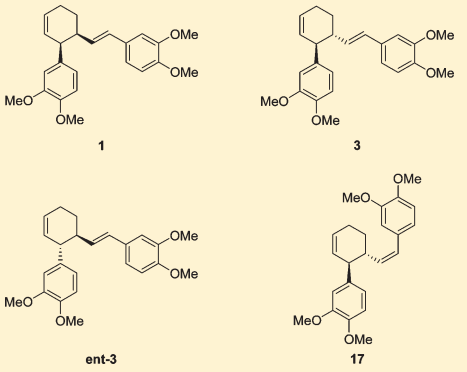
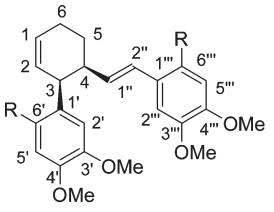
Supporting Information

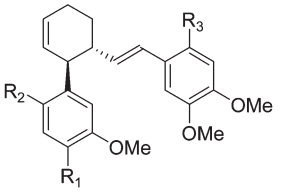
ABSTRACT: The total synthesis of optically active phenylbutenoid dimers **1**, **3**, and **ent-3** is described. The key step to access optically active cyclohexene rings was achieved by Diels−Alder reaction of chiral acryloyloxazolinone **9** and phenylbetadiene **10**.



Several phenylbutenoid dimers including 3-aryl-4-(*E*)-styrylcyclohexenes **1**−**6** and 1,2-bis((*E*)-styryl) cyclobutane were isolated from the rhizomes of a tropical *Zingiber cassumunar*, which has been used for the treatment of asthma, gastrointestinal distress, and motion sickness.[1] All of the phenylbutenoid dimers were isolated as racemic mixtures, implying that they were produced by a nonenzymatic process. Structure elucidations of these compounds were based on analyses of their 1D and 2D NMR and mass spectroscopic data, and configurations of the groups attached to the cyclohexene ring of **1** and **3** were deduced to be *cis* and *trans*, respectively, on the basis of the ^{1}H NMR coupling constants.[1d]



1: R = H
2: R = OMe



3: R_1 = OMe R_2 = H R_3 = H
4: R_1 = OMe R_2 = OMe R_3 = H
5: R_1 = OMe R_2 = H R_3 = OMe
6: R_1 = OH R_2 = H R_3 = H

There has been a growing interest in phenylbutenoid dimers due to their diverse biological activities. For example, (±)-3-(3,4-dimethoxyphenyl)-4-[(*Z*)-3,4-dimethoxystyryl]cyclohex-1-ene (**1**) has inhibitory activity for NO production induced by LPS in mouse macrophages.[2] Also, (±)-3-(3,4-dimethoxyphenyl)-4-[(*E*)-3,4-dimethoxystyryl]cyclohex-1-ene (**3**) has been reported to inhibit P-glycoprotein (P-gp) activity in a P-gp-overexpressing human breast cancer cell line,[3] cyclooxygenase-2 (COX-2) activity,[1f,4] and cell proliferation in several human tumor cell lines.[5] One limitation in biological evaluation of phenylbutenoid dimers is their low natural abundance. Thus, for precise analysis of structure−activity relationships, a new and general synthesis of optically active phenylbutenoid dimers became necessary. In an effort to further study the biological activities of these molecules, we were interested in the synthesis of optically active 3*S*-(3,4-dimethoxyphenyl)-4*S*-{(*E*)-3,4-dimethoxystyryl}cyclohex-1-ene (**1**), 3*S*-(3,4-dimethoxyphenyl)-4*R*-{(*E*)-3,4-dimethoxystyryl}cyclohex-1-ene (**3**), and 3*R*-(3,4-dimethoxyphenyl)-4*S*-{(*E*)-3,4-dimethoxystyryl}cyclohex-1-ene (**ent-3**).

Thermal [4 + 2] dimerization of 1-phenylbutadiene was reported to give *cis*- and *trans*-phenylbutenoid dimers in ratio of 1.4:1,[6] and dimerization of 1-(3,4-dimethoxyphenyl)butadiene (**10**) yielded the *cis*-isomer (**1**) in 23% yield as a racemic mixture.[7] In our synthetic strategy, optically active phenylbutenoid dimers **1** and **3** would arise from (1*R*,2*S*)-2-(3,4-dimethoxyphenyl)

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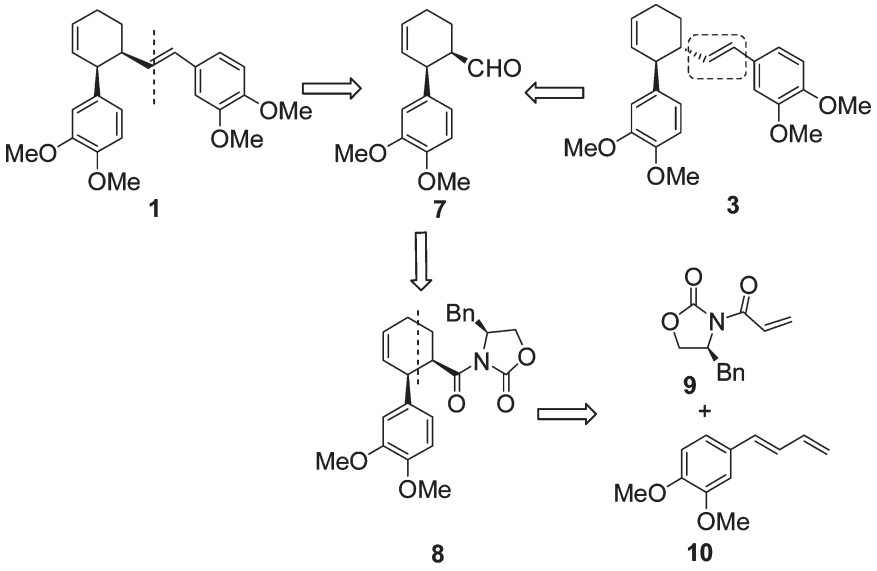
Scheme 1. Retrosynthetic Analysis



cyclohex-3-enecarbaldehyde (**7**), which in turn could be derived from the optically active compound (*S*)-4-benzyl-3-((1*R*,2*S*)-2-(3,4-dimethoxy phenyl)cyclohex-3-enecarbonyl)oxazolidin-2-one (**8**) via Diels−Alder cyclization between (*S*)-3-acryloyl-4-benzyloxazolidin-2-one (**9**) and 1-(3,4-dimethoxyphenyl)butadiene (**10**) with high regio- and stereoselectivity (Scheme 1).[8] The starting compound **9** was prepared from (*S*)-4-benzyloxazolidin-2-one by the reported method.[9] Compound **10** was obtained from 3,4-dimethoxyaldehyde by modification of the previous method in 57% yield.[1c] Diethylaluminum chloride mediated asymmetric cycloaddition reaction of **9** and **10** afforded **8** as a single stereoisomer in quantitative yield. Reduction of **8** with lithium borohydride in THF afforded the corresponding compound ((1*R*,2*S*)-2-(3,4-dimethoxyphenyl)cyclohex-3-enyl)methanol (**11**), which was then oxidized to **7** by Swern oxidation. Compound **7** was converted to 3*S*-(3,4-dimethoxyphenyl)-4*S*-{(*E*)-3,4-dimethoxystyryl}cyclohex-1-ene (**1**) by Horner−Wadsworth−Emmons condensation with dimethyl 3,4-dimethoxybenzylphosphonate (**12**) in 50% yield with high *E*/*Z*-selectivity in a ratio of 95:5 (Scheme 2).[10] For the synthesis of *trans*-isomer **3**, compound **7** was epimerized with K_2CO_3 in MeOH to thermodynamically more stable **13**. However, when olefination of **13** was carried out under the same conditions, 3*S*-(3,4-dimethoxyphenyl)-4*R*-{(*E*)-3,4-dimethoxystyryl}cyclohex-1-ene (**3**) was obtained in 24% yield.

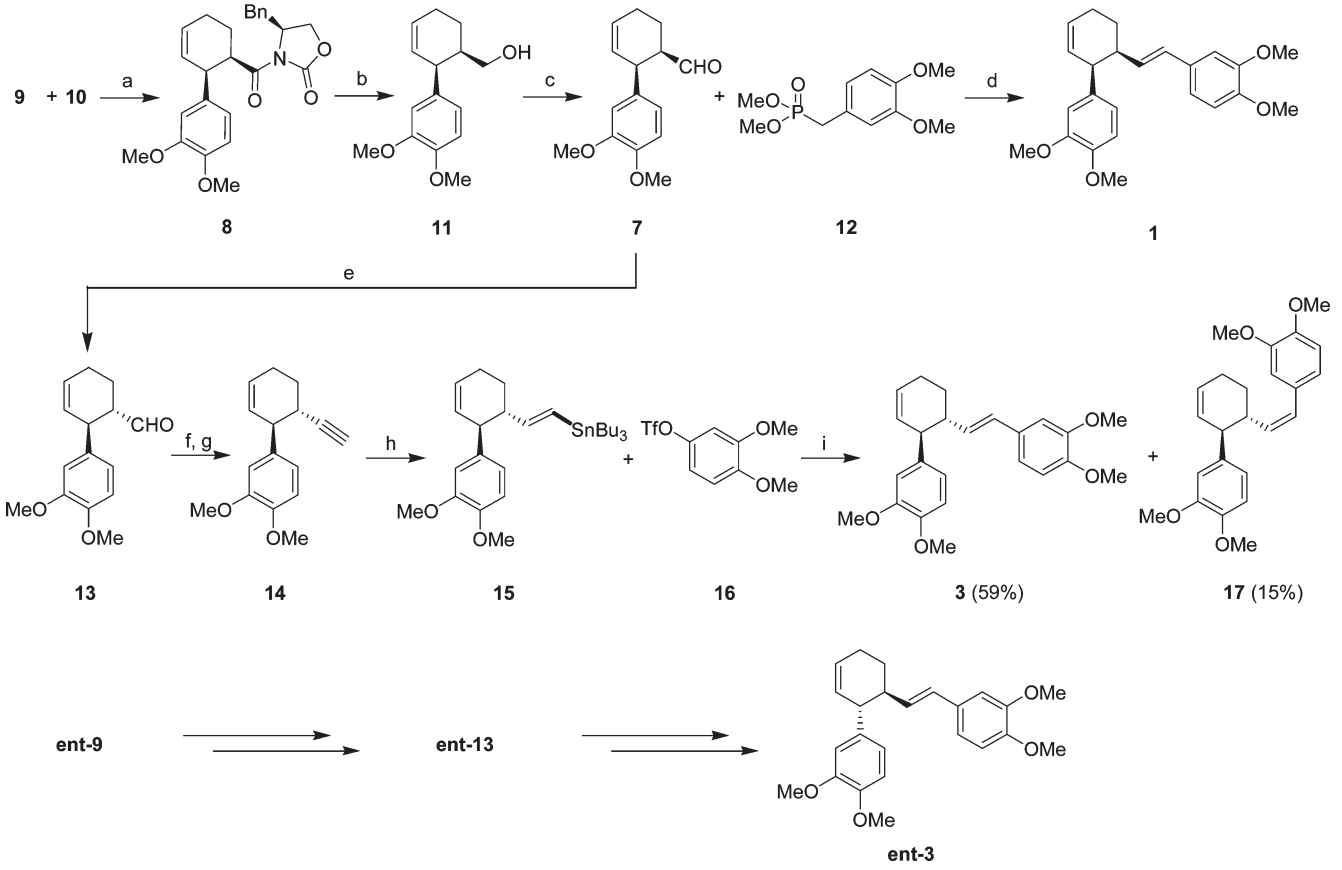
Therefore, an alternative route involving stereoselective vinylstannylation from **13** and Stille coupling to improve the yield of **3** was attempted. Compound **13** was treated with PPh_3 and carbon tetrabromide to afford the dibromide, then converted to 4-((1*S*,6*S*)-6-ethynylcyclohex-2-enyl)-1,2-dimethoxybenzene (**14**) by Corey Fuch alkyne synthesis.[11] Hydrostannylation of **14** with tributyltin hydride led to an 80% *E* and 20% *Z* mixture of tributyl((*E*/*Z*)-2-((1*S*,2*S*)-2-(3,4-dimethoxyphenyl)cyclohex-3-enyl)vinyl)stannane (**15**).[12] The *E*/*Z* mixture (**15**) was used directly without separation, as a single purification was planned for the final step of the synthesis. Stille coupling of **15** with 3,4-dimethoxyphenyl triflate (**16**) led to a 4:1 mixture of 3*S*-(3,4-dimethoxyphenyl)-4*R*-{(*E*)-3,4 dimethoxystyryl}cyclohex-1-ene (**3**) and 3*S*-(3,4-dimethoxyphenyl)-4*R*-{(*Z*)-3,4 dimethoxystyryl}cyclohex-1-ene (**17**) in 59% and 15% isolated yields, respectively, after separation by HPLC using a chiral OD-H column.[13] With the same synthetic route described above, 3*R*-(3,4-dimethoxyphenyl)-4*S*-{(*E*)-3,4-dimethoxystyryl}cyclohex-1-ene (**ent-3**) was prepared starting with (*R*)-3-acryloyl-4-benzyloxazolidin-2-one (**ent-7**) obtained by modification of the previous method.[8] Optically active phenylbutenoid dimers **3**, **ent-3**, and **17** were evaluated for their P-gylocoprotein inhibitory effect in a P-gp-overexpressing multidrug-resistant (MDR) human breast cancer cell line, MCF-7/ADR, using a previously reported protocol.[3] Phenylbutenoids **3** and **17**, with 3*S*, 4*R* configurations, were found to have more potent P-gp inhibitory activity compared to **ent-3**, with 3*R*, 4*S* configuration (Table 1). Further biological studies of these optically active phenylbutenoid dimers will be reported elsewhere.

In conclusion, phenylbutenoid dimer **1** and the structurally related congeners **3**, **17**, and **ent-3** have been successfully synthesized from key intermediate **8** by employing asymmetric Diels−Alder reactions with Evans chiral oxazolidinone **9**. The successful synthesis of phenylbutenoid dimers via a route that readily gives entry to analogues will allow for further investigations of their pharmacological properties and structure−activity relationships.

EXPERIMENTAL SECTION

General Experimental Procedures. All reagents were purchased from Aldrich Chemical Co. and Alfa Aesar Chemical Co. and used as obtained unless otherwise noted. RPMI 1640 cell culture medium and antibiotic-antimycotic agent were obtained from Invitrogen (Carlsbad, CA, USA), and fetal bovine serum (FBS) was from Hyclone (South Logan, UT, USA). Daunomycin, verapamil, and sulfo-rhodamine B (SRB) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Anhydrous THF and CH_2Cl_2 were prepared by a solvent purification system. Melting points were obtained on a Thomas Scientific melting point apparatus and are uncorrected. Flash chromatography was carried out using silica gel

Scheme 2. Synthesis of Optically Active Phenybutenoid Dimers 1, 3, and ent-3[a]



[a] Reagents and conditions: (a) Et_2AlCl, CH_2Cl_2, −78 °C, 99%; (b) $LiBH_4$, H_2O, THF, 0 °C 78%; (c) DMSO, $(ClCO)_2$, Et_3N, CH_2Cl_2, −78 °C to rt, 94%; (d) **12**, *n*-BuLi, THF, −78 °C, 10 min, then **7**, −78 °C to rt, 2 h, 50%; (e) K_2CO_3, MeOH, rt, 73%; (f) PPh_3, CBr_4, CH_2Cl_2, 0 °C, 99%; (g) *n*-BuLi, THF, −78 °C, 90%; (h) Bu_3SnH, AIBN, toluene, 80 °C, 83%; (i) $Pd(PPh_3)_4$, NMP, 90 °C, 74%.

Table 1. Effects of Phenylbutenoid Dimers on Daunomycin (DNM) Cytotoxicity[a]

compound	IC_{50} (μM)
daunomycin	14.33 ± 1.39
verapamil	1.90 ± 0.30
3*S*-(3,4-dimethoxyphenyl)-4*R*-{(*E*)-3, 4-dimethoxystyryl}cyclohex-1-ene (**3**)	1.44 ± 0.48
3*S*-(3,4-dimethoxyphenyl)-4*R*-{(*Z*)-3, 4-dimethoxystyryl}cyclohex-1-ene (**17**)	1.74 ± 0.33
3*R*-(3,4-dimethoxyphenyl)-4*S*-{(*E*)-3, 4-dimethoxystyryl}cyclohex-1-ene (**ent-3**)	3.19 ± 1.08

[a] IC_{50} values of DNM were determined in MCF-7/ADR cells after 2 h of incubation with three phenylbutenoids and verapamil at a final concentration of 50 μM. Each data point is expressed as mean ± SD from three different experiments. Daunomycin: negative control; verapamil: positive control.

60 (230−400 mesh) using various solvent mixtures. High-performance liquid chromatography (HPLC) was carried out on an Acme 9000 HPLC system (Younglin, Korea) equipped with a Chiragel OD-H column (250 mm × 10 mm i.d., Daicel Chemical Industries, Tokyo, Japan). Optical rotation measurements were performed on a Rudolph Autopol IV (automatic polarimeter). NMR spectra were recorded at 300 or 400 MHz (^{1}H NMR) and 75 or 100 MHz (^{13}C NMR), referenced to an internal standard (TMS) or residual solvent protons, and signals are reported in ppm (δ). High-resolution mass spectra (HRMS) were recorded on an AEI MS3074 spectrometer and Agilent 6220 Accurate-Mass TOF LC/MS system. Circular dichroism measurements were performed using a Jasco J-715 CD/ORD spectropolarimeter.

(*S*)-4-Benzyl-3-[(1*R*,2*S*)-2-(3,4-dimethoxyphenyl)cyclohex-3-enecarbonyl)]oxazolidin-2-one (8). To a solution of (*S*)-3-acryloyl-4-benzyloxazolidin-2-one (**9**)[9] (1.30 g, 5.62 mmol) in CH_2Cl_2 (50 mL) was added sequentially 1-(3,4-dimethoxyphenyl)butadiene (**10**) (1.50 g, 7.87 mmol) and diethylaluminum chloride (4.37 mL, 7.87 mmol) at −78 °C. The resulting solution was stirred for an additional 5 min at −78 °C and then poured into a stirred solution of 1 N HCl (40 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layer was washed with H_2O (50 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by crystallization from EtOAc and hexane to give **8** (2.37 g, 99%) as a white solid: mp 132 °C; $[\alpha]^{25}_D$ +307 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31−7.23 (3H, m), 7.12−7.09 (2H, m), 6.78−6.75 (3H, m), 5.98−5.94 (1H, m,), 5.78−5.73 (1H, m), 4.55−4.47 (1H, m), 4.15−4.03 (4H, m), 3.86 (3H, s), 3.77 (3H, s), 2.98−2.92 (1H, m), 2.35−2.05 (4H, m), 1.81−1.75 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 150.8, 146.0, 145.7, 133.2, 130.8, 126.6, 126.4, 126.1, 124.7, 124.6, 119.1, 110.5, 108.2, 74.9, 74.5, 74.1, 53.4, 53.4, 53.0, 41.2, 39.0, 35.7, 21.5, 18.0; HREIMS *m*/*z* 421.1888 $[M]^+$ (calcd for $C_{25}H_{27}NO_5$, 421.1889).

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(1*R*,2*S*)-2-[(3,4-Dimethoxyphenyl)cyclohex-3-enyl)]methanol (11). To a suspension of $LiBH_4$ (535 mg, 24.6 mmol) in THF (28 mL) and H_2O (7 mL) was added **8** (3.45 g, 8.2 mmol) at −20 °C. The reaction mixture was stirred for 5 h at 0 °C, diluted with 0.1 N HCl (100 mL), and extracted with EtOAc (2 × 100 mL). The combined organic layer was washed with H_2O (100 mL × 2), dried over $MgSO_4$, and concentrated. The crude product was purified by flash column chromatography (EtOAc/hexane, 1:3−1:2) to give **11** (1.6 g, 78%) as a colorless oil: $[\alpha]^{25}_D$ +242 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83−6.77 (3H, m), 5.94−5.90 (1H, m), 5.77−5.72 (1H, m), 3.86 (6H, s), 3.58 (1H, br s), 3.30−3.26 (2H, m), 2.20−2.15 (2H, m), 2.12−2.07 (1H, m), 1.60−1.41 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.4, 147.6, 133.6, 129.1, 127.8, 121.6, 113.0, 110.7, 77.4, 77.0, 76.6, 65.1, 55.8, 42.1, 40.8, 24.7, 20.7; HREIMS *m*/*z* 248.1413 $[M]^+$ (calcd for $C_{15}H_{20}O_3$, 248.1412).

(1*R*,2*S*)-2-(3,4-Dimethoxyphenyl)cyclohex-3-enecarbaldehyde (7). To a solution of dimethyl sulfoxide (1.3 mL, 18.3 mmol) in CH_2Cl_2 (20 mL) was added dropwise oxalyl chloride (1.6 mL, 18.3 mmol) at −78 °C. The reaction mixture was stirred for 1 h, and **11** (1.5 g, 6.1 mmol) in CH_2Cl_2 (10 mL) was added. After stirring for 1 h at the same temperature, triethylamine was added. The reaction mixture was allowed to warm to 0 °C, stirred for an additional 30 min, diluted with H_2O (50 mL), and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layer was washed with H_2O (100 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane, 1:4) to give **8** (1.41 g, 94%) as a white solid: mp 74 °C; $[\alpha]^{25}_D$ +247 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (1H, d, *J* = 2.1 Hz), 6.81−7.72 (3H, m), 6.00−5.97 (1H, m), 5.84−5.79 (1H, m), 3.92 (1H, s), 3.85 (6H, s), 2.77−2.70 (1H, m), 2.33−2.09 (2H, m), 1.89−1.83 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.9, 148.7, 148.0, 132.5, 128.5, 128.0, 121.3, 122.5, 111.0, 55.8, 50.8, 41.1, 23.6, 18.8; HREIMS *m*/*z* 246.1255 $[M]^+$ (calcd for $C_{15}H_{18}O_3$, 246.1256).

3*S*-(3,4-Dimethoxyphenyl)-4*S*-[(*E*)-3,4-dimethoxystyryl]-cyclohex-1-ene (1). To a solution of dimethyl 3,4-dimethoxybenzylphosphonate (**12**)[10] (137 mg, 0.53 mmol) in dry THF (3 mL) was added *n*-butyllithium (1.6 M in hexanes, 0.291 mL, 0.467 mmol) at −78 °C. The reaction mixture was allowed to warm to 0 °C and stirred for 20 min, and **7** (100 mg, 0.406 mmol) in THF (1 mL) was then added. The mixture was stirred for an additional 2 h, diluted with saturated NH_4Cl (20 mL), and extracted with EtOAc (20 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane, 1:5) to give **1** (71 mg, 50%) as a white solid: mp 107 °C (lit.[1d] mp 99−99.5 °C); $[\alpha]^{25}_D$ +103 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82−6.69 (6H, m), 6.25 (1H, d, *J* = 15.7 Hz), 6.02−5.93 (1H, m), 5.84−5.76 (1H, m), 5.58 (1H, dd, *J* = 15.7, 9.1 Hz), 3.86 (3H, s) 3.85 (3H, s), 3.83 (3H, s), 3.75 (3H, s), 3.54−3.48 (1H, m), 2.96−2.64 (1H, m), 2.32−2.11 (2H, m), 1.72−1.57 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.1, 148.4, 148.2, 147.7, 133.9, 132.6, 131.2, 129.3, 128.6, 128.2, 122.0, 118.9, 113.7, 111.3, 110.5, 108.8, 56.0, 55.9, 55.9, 45.9, 42.7, 24.9, 24.4; HREIMS *m*/*z* 380.1988 $[M]^+$ (calcd for $C_{245}H_{28}O_4$, 380.1988).

(1*S*,2*S*)-2-(3,4-Dimethoxyphenyl)cyclohex-3-enecarbaldehyde (13). Potassium carbonate (493 mg, 3.57 mmol) was added to a solution of **7** (0.8 g, 3.24 mmol) in MeOH (15 mL), and the reaction mixture was stirred for 36 h at room temperature and then concentrated. The residue was diluted with H_2O (50 mL), extracted with EtOAc (50 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane, 1:10) to give **13** (650 mg, 81%) as a colorless oil: $[\alpha]^{25}_D$ +168 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.69 (1H, d, *J* = 1.2 Hz), 6.82−6.74 (3H, m), 5.92−5.86 (1H, m), 5.70−5.65 (1H, m), 3.86 (6H, d, *J* = 3 Hz), 3.74−3.69 (1H, m), 2.61−2.55 (1H, m), 2.18−2.17 (2H, m), 1.98−1.93 (1H, m), 1.81−1.71 (1H, m), ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.0, 149.0, 147.8, 136.1, 129.1, 127.6, 120.2, 111.3, 111.2, 55.9, 55.8, 54.0, 41.0, 23.4, 21.0; HREIMS *m*/*z* 246.1255 $[M]^+$ (calcd for $C_{15}H_{18}O_3$, 246.1256).

4-[(1*S*,6*S*)-6-Ethynylcyclohex-2-enyl]-1,2-dimethoxybenzene (14). Compound **14** was prepared from **13** in two steps (81%) by a reported method:[11] white solid; mp 55 °C; $[\alpha]^{25}_D$ −187 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (3H, d, *J* = 9.3 Hz), 5.91−5.87 (1H, m), 5.66−5.62 (1H, m), 3.87 (6H, d, *J* = 3 Hz), 3.40−3.36 (1H, m), 2.55−2.47 (1H, m), 2.20−2.18 (2H, m), 2.06−1.95 (2H, m), 1.82−1.70 (1H, m); ^{13}C NMR (300 MHz, $CDCl_3$) δ 148.6, 147.7, 136.4, 128.9, 127.6, 120.2, 111.4, 110.7, 87.4, 77.4, 77.2, 77.0, 76.6, 69.3, 55.8, 47.1, 34.5, 27.3, 23.8; HREIMS *m*/*z* 242.1304 $[M]^+$ (calcd for $C_{16}H_{18}O_2$, 242.1307).

Tributyl{(*E*)-2-[(1*S*,2*S*)-2-(3,4-dimethoxyphenyl)cyclohex-3-enyl]vinyl}stannane (15). 2,2′-Azobisisobutyronitrile (3 mg) was added to a solution of **14** (107 mg, 0.44 mmol) and tributyltin hydride (0.185 mL, 0.70 mmol) in toluene (3 mL). The mixture was heated to reflux for 6 h, diluted with EtOAc (10 mL), and washed with H_2O (10 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane, 1:10) to give **15** (197 mg, 83%) as an inseparable *E*/*Z* mixture (*E*/*Z* = 4:1): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.77−6.62 (2.8H, m), 6.51−6.44 (0.2H m), 5.96−5.78 (2H, m), 5.74−5.59 (2H, m), 3.86 (2.4H, s), 3.85 (3H, s), 3.83 (0.6H, s), 3.20−3.1.7(0.2H, m, for *Z*-17), 3.15−3.09 (0.8H, m, for *E*-17), 2.28−2.14 (3H, m), 1.90−1.82 (1H, m), 1.68−1.61 (14H, m), 0.91−0.62 (14H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.3, 148.6, 147.2, 137.8, 130.5, 127.4, 126.3, 120.6, 111.3, 110.6, 55.8, 49.5, 47.4, 29.1, 27.3, 24.6, 13.7, 9.3; HREIMS *m*/*z* 478.1891 $[M - C_4H_8 + 1]^+$ (calcd for $C_{24}H_{38}O_2Sn$, 478.1894).

3*S*-(3,4-Dimethoxyphenyl)-4*R*-[(*E*)-3,4-dimethoxystyryl]-cyclohex-1-ene (3) and 17. To a solution of **15** (780 mg, 1.45 mmol), **16**[13] (504 mg, 1.75 mmol), and LiCl (74 mg, 1.75 mmol) in *N*-methylpyrrolidone (5 mL) was added $Pd(PPh_3)_4$ (340 mg, 0.31 mmol). The reaction mixture was heated for 3 h, diluted with H_2O (30 mL), and extracted with EtOAc (30 mL). The organic layer was washed with H_2O (2 × 20 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc/hexane, 1:5) to give a mixture of **3** and **17** (430 mg, 74%). The mixture of **3** and **17** (430 mg) was subjected to semipreparative HPLC, using an isocratic mixture of hexane/2-propanol (9:1, 1.5 mL/min) as solvent system, to afford **17** (16 mg) and **3** (226 mg).

3*S*-(3,4-Dimethoxyphenyl)-4*R*-[(*E*)-3,4-dimethoxystyryl]-cyclohex-1-ene (3): yellow gum; $[\alpha]^{25}_D$ +260 (*c* 0.13, MeOH); CD (*c* 0.34 mM, MeOH) $\Delta\varepsilon_{230}$ +7.4, $\Delta\varepsilon_{255}$ +10.9, $\Delta\varepsilon_{267}$ +11.6, $\Delta\varepsilon_{283}$ +10.0; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.81 (1H, br s, H-2‴), 6.78 (1H, dd, *J* = 9.1, 2.4 Hz, H- 6‴), 6.77 (2H, d, *J* = 9.1 Hz, H-5′ and H-5‴), 6.72 (1H, dd, *J* = 9.1, 2.0 Hz, H-6′), 6.70 (1H, d, *J* = 2.0 Hz, H-2′), 6.10 (1H, d, *J* = 16.0 Hz, H-2″), 6.02 (1H, dd, *J* = 16.0, 6.8 Hz, H-1″), 5.89 (1H, br d, *J* = 10.0 Hz, H-1), 5.68 (1H, dd, *J* = 10.0, 2.6 Hz, H-2), 3.87 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.18 (1H, dd, *J* = 8.4, 2.6 Hz, H-3), 2.35 (1H, br d, *J* = 8.4 Hz, H-4), 2.21 (2H, m, H-6), 1.93 (1H, dd, *J* = 12.8, 3.2 Hz, H-5), 1.66 (1H, m, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.0 (C-4′), 148.6 (C-4‴), 148.3 (C-3‴), 147.4 (C-3′), 137.6 (C-1′), 132.2 (C-1″), 131.0 (C-1‴), 130.3 (C-2), 129.0 (C-2″), 127.6 (C-1), 120.5 (C-6′), 118.9 (C-6‴), 111.8 (C-2′), 111.3 (C-5‴), 111.0 (C-5′), 108.8 (C-2‴), 56.0 (OCH_3), 55.9 (OCH_3), 55.8 (OCH_3), 55.8 (OCH_3), 48.1 (C-3), 45.5 (C-4), 28.0 (C-5), 24.6 (C-6); HRESIMS (positive mode) *m*/*z* 381.2060 $[M + H]^+$ (calcd for $C_{24}H_{29}O_4$, 381.2060).

3*S*-(3,4-Dimethoxyphenyl)-4*R*-[(*Z*)-3,4-dimethoxystyryl]-cyclohex-1-ene (17): yellow gum; $[\alpha]^{25}_D$ −24.3 (*c* 0.18, MeOH); CD (*c* 0.47 mM, MeOH) $\Delta\varepsilon_{230}$ +4.4, $\Delta\varepsilon_{250}$ −6.6, $\Delta\varepsilon_{274}$ −4.4; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.73(1H, d, *J* = 8.0, H-5′), 6.70 (1H, d, *J* = 8.0 Hz, H-5‴), 6.65 (1H, dd, *J* = 8.0, 2.2 Hz, H-6′), 6.56 (1H, d, *J* = 2.2 Hz, H-2′), 6.43 (1H, br s, H-2‴), 6.42 (1H, dd, *J* = 8.0, 1.6 Hz, H-6‴), 6.27 (1H, d, *J* = 11.6 Hz, H-2″), 5.84 (1H, br dd, *J* = 10.0, 3.0 Hz, H-1), 5.65 (1H, dd, *J* = 10.0, 2.3 Hz, H-2), 5.51 (1H, dd, *J* = 11.6, 10.8 Hz, H-1″),

3.84 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.14 (1H, dd, J = 8.6, 2.3 Hz, H-3), 2.84 (1H, ddd, J = 10.8, 8.6, 2.8 Hz, H-4), 2.17 (2H, m, H-6), 1.83 (1H, br dd, J = 12.8, 2.8 Hz, H-5), 1.64 (1H, m, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.1 (C-3‴), 148.8 (C-4′), 148.5 (C-4‴), 147.5 (C-3′), 137.8 (C-1′), 135.9 (C-1″), 130.6 (C-1‴), 130.5 (C-2), 128.5 (C-2″), 127.3 (C-1), 120.8 (C-6‴), 120.3 (C-6′), 111.7 (C-2‴), 111.3 (C-2′), 110.8 (C-5′), 110.7 (C-5‴), 56.9 (OCH_3), 56.9 (OCH_3), 56.8 (OCH_3), 56.8 (OCH_3), 48.1 (C-3), 40.7 (C-4), 28.6 (C-5), 24.4 (C-6); ^{1}H−^{13}C HMBC correlations ($CDCl_3$, 400 MHz) H-5′/C-6′, C-1′; C-4′; H-5‴/C-1‴; H-6′/C-2′, C-4′, C-3; H-2′/C-3,C-6′, C-3′, C-4′; H-2‴/C-6‴, C-2″, C-1‴, C-3‴, C-4‴; H-6‴/ C-2‴, C-4‴; H-2″/C-4, C-1″, C-2‴, C-6‴, C-1‴; H-1/ C-2; H-2/C-6, C-4, C-3, C-1; H-1″/C-5, C-4, C-3, C-2, C-1‴; OCH_3/ C-4‴; OCH_3/C-3′; OCH_3/C-4′; OCH_3/C-3‴; H-3/C-1, C-2; H-4/C-2″, C-1″; H-6/C-1, C-2; H-5/C-1, C-1″; HRESIMS (positive mode) m/z 381.2060 $[M + H]^+$ (calcd for $C_{24}H_{29}O_4$, 381.2060).

Biological Assays. Biological assays were conducted according to published protocols.[3] In brief, MCF-7/ADR cells were cultured in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 10 mM HEPES, 24 mM $NaHCO_3$, and 1% antibiotic−antimycotic agent and maintained at 37 °C in a humidified 5% CO_2 atmosphere. In order to examine the effects of phenylbutenoid dimers on daunomycin cytotoxicity, the cells (5000 cells/well) were seeded in 96-well plates and incubated for 24 h at 37 °C. Then, daunomycin was added to each well to achieve final concentrations of 1.0×10^{-7} to 1.0×10^{-4} M with and without 50 μM phenylbutenoid dimers **3**, **ent-3**, and **17**, followed by incubation for 2 h. Daunomycin containing 50 μM verapamil was used as the positive control. After the cells were washed and maintained with 200 μL of fresh media for 72 h, the cytotoxicity of daunomycin was determined using a modification of a SRB staining assay.[14]

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures for the preparation of compounds **ent-3**, **10**, **12**, **14**, **15**, and **16**, copies of NMR spectra of **1**, **3**, **ent-3**, **7**, **8**, and **10**−**17**, and CD spectra of **3**, **ent-3**, and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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