



Synthesis and biological activity of a fluorescent schweinfurthin analogue

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ABSTRACT

Most of the natural schweinfurthins are potent and selective inhibitors of cell growth as measured by the National Cancer Institute's 60-cell line screen. Due to the limited supply of these natural products, we have initiated a program aimed at their synthesis. To date, this effort has led to the preparation of three natural schweinfurthins and more than 40 analogues, and assays on these compounds have afforded some understanding of structure–activity relationships in this family. Further development of schweinfurthins as chemotherapeutic agents would benefit from characterization of their mechanism(s) of action. This perspective led to development of a fluorescent schweinfurthin analogue that retains the differential activity of the natural products, and yet has properties that facilitate its visualization within cells.

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1. Introduction

Higher plants have proven to be an excellent source of novel compounds that are useful in the treatment of cancer.¹ Among the most notable successes in this area are the alkaloids vincristine and vinblastine isolated from *Catharanthus roseus*,² paclitaxel isolated from *Taxus brevifolia*,^{3,4} and camptothecin isolated from *Camptotheca acuminata*.⁵ As part of an ongoing National Cancer Institute (NCI) initiative to examine new plant extracts for potential chemotherapeutic agents, several closely related compounds named schweinfurthins have been discovered. After an extract from the Cameroonian plant *Macaranga schweinfurthii* displayed potent and cell type selective growth inhibition (GI) in the NCI's 60-cell line screen,⁶ the more active components of this extract were isolated, characterized, and named schweinfurthin A and B⁷ (**1** and **2**, Fig. 1). A less active analogue was named schweinfurthin C, and later studies yielded the closely related schweinfurthin D.⁸ Independent studies of *Macaranga alnifolia* later yielded schweinfurthins E–H.⁹

Further study of the schweinfurthins as potential cancer therapeutics is encouraged by the substantial differential activity seen among the cell lines of the NCI's 60-cell line screen. In addition, the unique pattern of activity can be considered a 'fingerprint' indicative of a possible novel mechanism of action.¹⁰ Finally, these agents demonstrated very potent antiproliferative activity against human-derived CNS cell lines such as SF-295, SF-539, and SNB-75

(GI₅₀'s of 11 nM, 10 nM, and 15 nM respectively, for schweinfurthin A). However, development of schweinfurthins as cancer therapeutics has been hindered by limited material due to difficulties in isolation of significant additional quantities of the active compounds from the original plant source. Therefore, we have undertaken the total synthesis of these natural products. While installation of the vicinal diol on the A-ring has proven to be elusive, analogues without the C-3 hydroxyl group have been prepared and shown to maintain the growth inhibition and differential activity exhibited by the natural schweinfurthin diols.^{6–9} Furthermore, compounds lacking the C-3 hydroxyl group were isolated during the more recent studies of *M. alnifolia*,⁹ and already have been synthesized as single enantiomers.¹¹

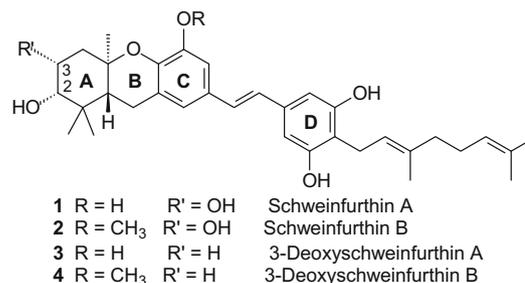


Figure 1. Structures of some natural (**1** and **2**) and synthetic (**3** and **4**) schweinfurthins.

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Two of the synthetic compounds, (*R,R,R*)-3-deoxyschweinfurthin A (**3**, 3dSA)¹² and B (**4**, 3dSB),¹³ have excellent potency with sub-micromolar mean GI₅₀'s, while maintaining differential activity across the NCI's 60-cell cancer screen. Since the inception of the synthetic program, our group has prepared three natural schweinfurthins and more than 40 analogues. These compounds have allowed us to explore some structure-function relationships within the family, particularly as they pertain to the D-ring.¹⁴ To date however, these analogues have not clarified the mechanism of action, which remains essentially unknown.

Development of schweinfurthins as cancer therapeutics might be accelerated by elucidation of their mechanism(s) of action. Fluorescence microscopy often has been used to determine the localization of drugs¹⁵ or proteins, and potential interaction with other cellular components.¹⁶ Synthesis of schweinfurthin analogues that incorporate fluorescence properties could advance our current understanding of schweinfurthin activity, as long as such compounds retain their anti-proliferative activity. Here, we present the synthesis and biological activity of a novel fluorescent schweinfurthin analogue.

During the synthesis of 3dSB, it was determined that many of these stilbenes were fluorescent under UV light. For example, 3dSB itself has an absorption maximum of 331 nm and an emission maximum of 391 nm (data not shown). The possibility that these properties might provide information on the sub-cellular localization of schweinfurthins immediately was recognized. After treatment with 3dSB, SF-295 cells (a human-derived glioblastoma multiforme cell line) were examined for potential UV fluorescence. However, 3dSB failed to generate a significant fluorescent signal above the autofluorescence of control cells (data not shown).

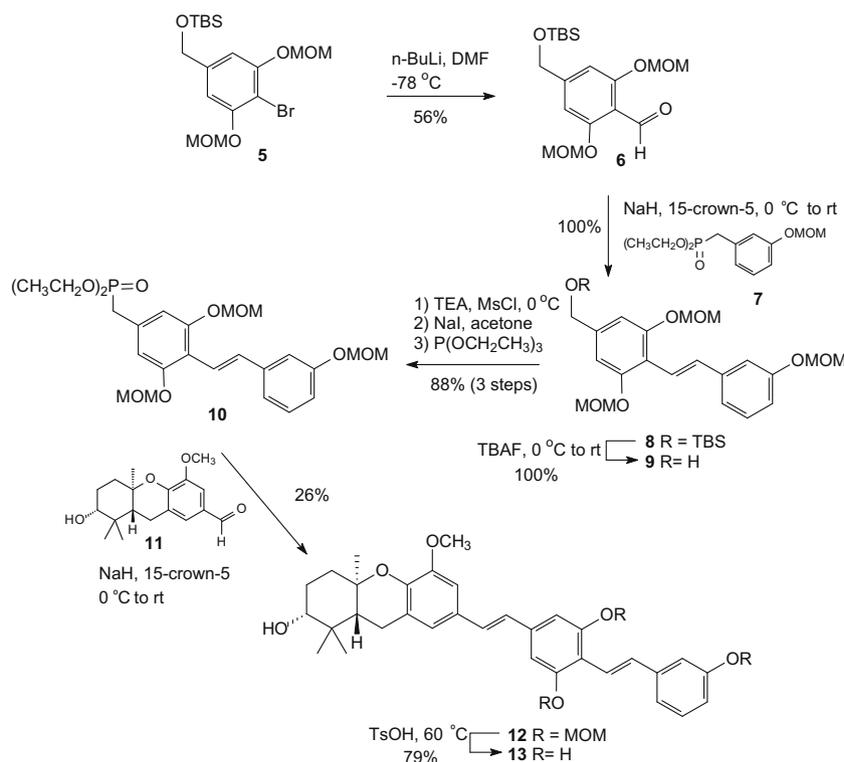
The limited utility of 3dSB as a fluorescent probe prompted a review of our prior structure-activity data with the hope of finding a position amenable to change and consistent with improved fluorescent characteristics. Previous work has demonstrated the importance of at least one free D-ring phenol for biological

activity.¹⁴ Consideration of the natural schweinfurthins indicates the *para* position of the D-ring as a point where the structure might be modified without loss of the desirable biological properties. For example, 3dSB bears a 10-carbon geranyl substituent at this position while schweinfurthin F carries only a 5-carbon prenyl substituent and both display comparable activity.¹¹ Therefore, a literature review was performed with an eye toward identification of accessible substitutions that might be made at this position to afford new analogues with improved fluorescence properties.

Substituted stilbenes are among the most studied fluorescent compounds.^{17,18} For example, Maddy et al. synthesized the fluorescent stilbene 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid in the mid 1960s.¹⁹ This compound then was shown to be an excellent fluorescent label for the plasma membrane of erythrocytes, and was subsequently shown to penetrate the blood-brain barrier.²⁰ More recently a bis-stilbene was demonstrated to penetrate mouse brain and fluorescently label myelin.²¹ The idea of attaching a second stilbene linkage at the *para* position of the D-ring also was attractive from a synthetic standpoint, because it fit well into our modular approach to the schweinfurthins. Therefore the novel bis-stilbene **13** was identified as the initial target.

2. Chemical synthesis

Synthesis of compound **13** began with the known aryl bromide **5** (Scheme 1).^{22,23} Halogen-metal exchange on bromide **5** presumably afforded a lithiated intermediate, which was subsequently allowed to react with anhydrous dimethylformamide to afford the new aldehyde **6** in acceptable yield. This aldehyde was treated with the known phosphonate **7**¹⁴ under modified Horner-Wadsworth-Emmons (HWE) conditions to give the protected stilbene **8**. Removal of the silyl protecting group under standard conditions gave the benzylic alcohol **9**. A three step procedure then was used to convert the alcohol **9** into the benzylic phosphonate **10**. Thus, treatment of the alcohol **9** with methanesulfonyl chloride and



Scheme 1. Synthesis of fluorescent stilbene **13**.

triethylamine affords the expected mesylate which can be smoothly transformed into the iodide upon reaction with NaI. Displacement of the iodide by the soft nucleophile triethyl phosphite gives the desired benzylic phosphonate **10** in high overall yield. A second HWE condensation with the nonracemic tricyclic aldehyde **11**¹³ gives the protected schweinfurthin analogue **12**. Final hydrolysis of the MOM protecting groups through treatment with camphorsulfonic acid or toluenesulfonic acid gives the desired bis-stilbene **13**. This compound was highly fluorescent with an absorbance maximum of 330 nm, an emission maximum of 416 nm and an extinction coefficient ϵ of 31,200 (Fig. 2).

3. Biological results and discussion

As noted above, the schweinfurthins display potent growth inhibition and differential activity in the NCI's 60-cell screen. In this assay, the SF-295 cell line is among the most sensitive to schweinfurthin-induced growth inhibition, while the human-derived lung adenocarcinoma A549 cells display only moderate growth inhibition upon parallel treatment. Due to this difference in sensitivity between SF-295 and A549 cells, these cell lines can be used to investigate the schweinfurthin-like activity of synthetic analogues such as compound **13**. Indeed, investigation of cytotoxicity with compound **13** demonstrates a significant toxicity in SF-295 cells (IC_{50} ~800 nM) while A549 cells are much less sensitive.

In addition to their growth inhibitory effects, schweinfurthins induce drastic changes in cell morphology at time points beyond 24 h. In culture, SF-295 cells are large polygonal shaped cells with multiple outstretched processes (Fig. 3A). Treatment of SF-295 cells with schweinfurthin analogues such as 3dSB causes morphologic changes congruent with those induced by the natural products. These alterations are characterized by a decrease in cell area and a reduction in F-actin, which results in spindle-shaped cells with F-actin staining only at the periphery of the treated cells.

When SF-295 cells were treated for 24 h with compound **13** (500 nM) or 3dSB (500 nM), they exhibited morphologic characteristics somewhat similar to control cells (Fig. 3A). However at 48 h cells treated with 3dSB (500 nM) or compound **13** (500 nM) display altered morphology. These changes appear to differ from those induced by the Rho kinase (or ROCK) inhibitor Y-27632 (10 μ M),²⁴ which caused jagged F-actin at the periphery and long slender projections to form. Inhibition of ROCK disrupts RhoA signaling, which is critical in the maintenance of cell shape and motility.²⁵ In addition, ROCK inhibition induces changes readily detected at 24 h. At equivalent concentrations compound **13** displays similar activity to 3dSB in SF-295 cells, yet both compound **13** and 3dSB differ from the activity of Y-27632.

In comparison to the SF-295 cells, the schweinfurthins are much less active against the A549 cells. In control experiments, the A549 cells are rectangular and cover less surface area in

comparison to SF-295 cells (Fig. 3B). Treatment with 3dSB (500 nM) or compound **13** (500 nM) does not induce a morphologic change apparent at either 24 h or 48 h (Fig. 3B). The absence of change is consistent with previous studies which indicate that A549 cells are less sensitive to schweinfurthin treatment. Similar to the effects in SF-295 cells, Y-27632 (10 μ M) induces ruffled F-actin at the edges of the A549 cells (Fig. 3B). These findings further suggest that the structural changes introduced in compound **13** do not alter the differential activity observed with the schweinfurthins.

The bis-stilbene **13** represents the first compound that might further understanding of the schweinfurthin localization through fluorescence microscopy. Indeed, SF-295 cells treated with concentrations of compound **13** as low as 100 nM could be visualized at all time points tested (Fig. 4). Unlike previous experiments with other schweinfurthins, the fluorescence in treated cells was significantly greater than the background of control cells. As expected, treatment of SF-295 cells with increasing concentrations of compound **13** increased fluorescence intensity at all time points tested (Fig. 3). The localization of compound **13** within cells appears largely cytosolic with the most intense fluorescence in the perinuclear region. The fluorescent properties of compound **13** allow its visualization above the background autofluorescence in control cells.²⁶

4. Conclusions

In conclusion, these studies have led to the preparation of the first schweinfurthin analogue with fluorescence properties that allow its cellular localization to be tracked by microscopy. This compound maintains the activity of schweinfurthins in SF-295 cells, and results in characteristic changes in cell morphology over time. While the morphologic changes induced by schweinfurthins differ from those induced by the ROCK inhibitor Y-27632, these findings do not preclude the involvement of RhoA through other downstream mediators or other Rho family members such as Rac1 and Cdc 42, which are also crucial for proper cell structure and movement.²⁷ The localization of compound **13** appears to be cytosolic as well as perinuclear. Unfortunately, compound **13** undergoes rapid photobleaching and thus did not allow co-localization studies to determine its potential concentration in organelles. Nevertheless, incorporation of such a sizeable substituent at the para position of the D-ring suggests that other substituents might be placed at this site without significant loss of biological activity, and a series of fluorescent analogues can be readily envisioned. Other useful probes might include compounds linked to moieties such as biotin or polyethylene glycol to aid in the examination of mechanism.^{28–30} Such probes may allow determination of binding partners, signaling cascades, and/or localization. Studies in this vein are ongoing and will be reported in due course.

5. Experimental procedures and methods

5.1. General experimental conditions

Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use, CH_2Cl_2 was freshly distilled from CaH_2 , and DMF was dried over CaH_2 . All reactions in non-aqueous solvents were conducted in oven-dried glassware under a positive pressure of argon and with magnetic stirring. NMR spectra were recorded at 300 MHz for 1H , and 75 MHz for ^{13}C with $CDCl_3$ as solvent and $(CH_3)_4Si$ (1H) or $CDCl_3$ (^{13}C , 77.2 ppm) as internal standards unless otherwise noted. Silica gel (60 Å, 0.040–0.063 mm) was used for flash chromatography. Yields refer to pure compounds after chromatography. High resolution mass spectra were obtained at the University of Iowa Mass Spectrometry Facility.

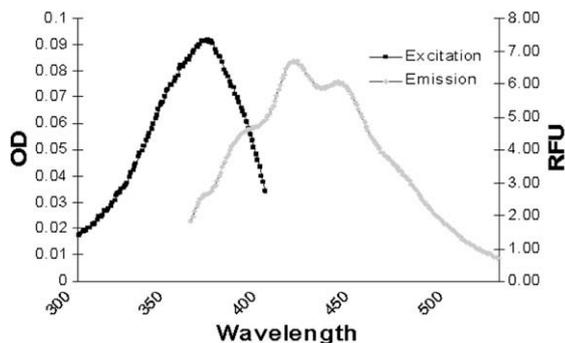


Figure 2. Absorption and emission spectra of compound **13**.

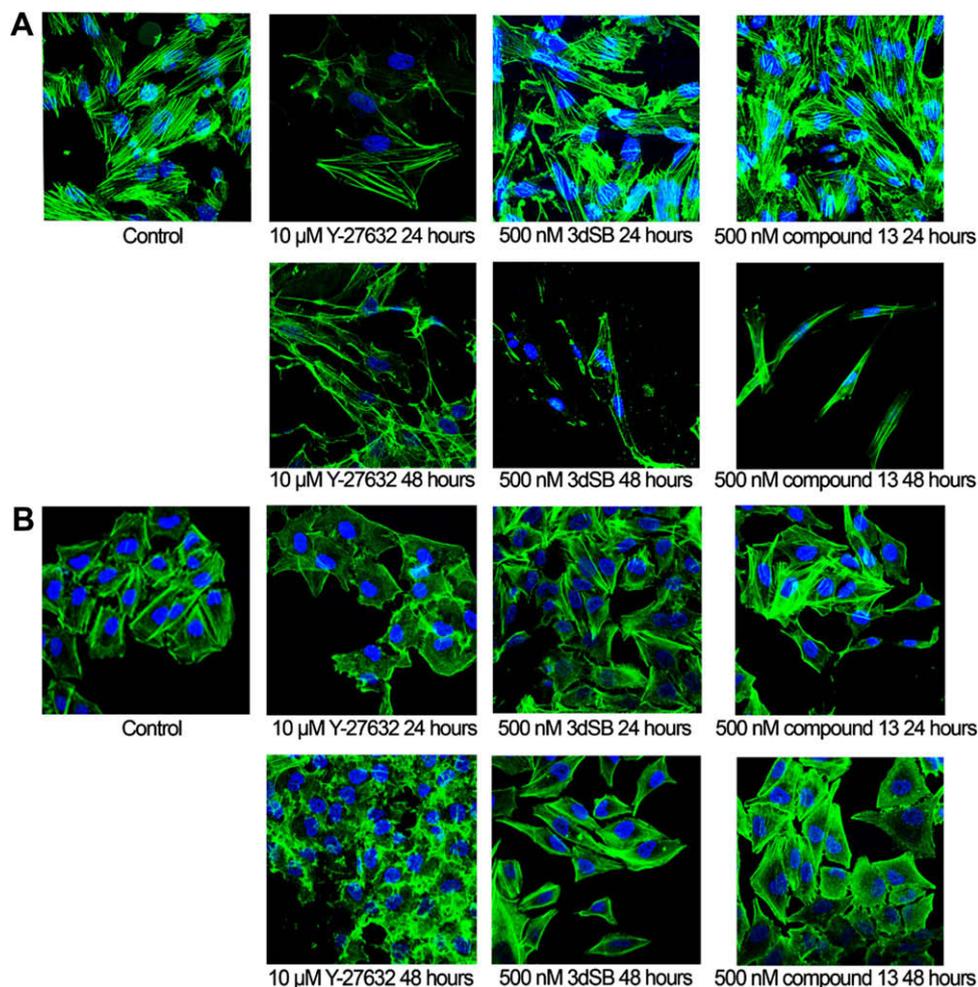


Figure 3. SF-295 (A) and A549 (B) cells treated with indicated concentrations and intervals of Y-27632, 3dSB, and compound **13** were stained with fluorescent phalloidin as an F-actin marker (green) and DAPI as nuclear stain (blue).

5.2. Aldehyde **6**

To a solution of the known bromide **5**^{22,23} (1.25 g, 3.0 mmol) in THF (10 mL) at -78°C was added *n*-BuLi (1.6 mL, 2.0 M in hexanes) dropwise over 2 min. After 30 min, DMF was added (0.5 mL, 6.5 mmol, containing some CaH_2 as a drying agent). The reaction was allowed to progress for an additional 3 h, and quenched by addition of saturated aq. NH_4Cl . The aqueous phase was extracted with EtOAc, and the combined organic phases were washed with brine, dried (MgSO_4), and concentrated in vacuo to afford a yellow oil. Final purification by column chromatography afforded aldehyde **6** (1.09 g, 99%) as a clear oil: $^1\text{H NMR}$ (CDCl_3) δ (10.5 s, 1H), 6.84 (s, 2H), 5.26 (s, 4H), 4.73 (s, 2H), 3.50 (s, 6H), 0.95 (s, 9H), 0.12 (s, 6H); $^{13}\text{C NMR}$ (CDCl_3) δ 188.9, 159.6 (2C), 151.0, 114.7, 105.6 (2C), 94.8 (2C), 64.5, 56.4 (2C), 25.8 (3C), 18.3, -5.4 (2C); HRMS (EI) calcd for $\text{C}_{18}\text{H}_{30}\text{O}_6\text{Si}$ (M+) 370.1812; found 370.1822.

5.3. Stilbene **8**

A suspension of NaH (290 mg, 7.8 mmol, 60% in oil), and 15-crown-5 (1 drop, cat.) in THF (10 mL) was cooled to 0°C . To this was added a solution of aldehyde **6** (168 mg, 0.45 mmol) and phosphonate **7** (185 mg, 0.64 mmol) in THF (10 mL). The mixture was allowed to warm to rt and stirred a total of 10 h. Water was added dropwise, and the solution was extracted with EtOAc. The resulting

organic phase was washed with brine, dried (MgSO_4), and concentrated in vacuo. Final purification by column chromatography (6:1 to 2:1 hexanes/ethyl acetate) gave the stilbene **8** (228 mg, 100%) as a clear oil: $^1\text{H NMR}$ (CDCl_3) δ 7.61 (d, $J = 17$ Hz, 1H), 7.50 (d, $J = 17$ Hz, 1H), 7.38–7.29 (m, 3H), 7.03–7.00 (m, 1H), 6.93 (s, 2H), 5.34 (s, 4H), 5.30 (s, 2H), 4.80 (s, 2H), 3.60 (s, 6H), 3.59 (s, 3H), 1.05 (s, 9H), 0.02 (s, 6H); $^{13}\text{C NMR}$ (CDCl_3) δ 157.4, 156.1 (2C), 142.3, 140.7, 132.0, 129.4, 120.4, 120.1, 115.1, 114.6, 114.1, 106.0 (2C), 94.8 (2C), 94.3, 64.7, 56.1 (2C), 55.9, 25.8 (3C), 18.3, -5.3 (2C). Anal. Calcd for $\text{C}_{27}\text{H}_{40}\text{O}_7\text{Si}$: C, 64.30; H, 7.99. Found: C, 64.09; H, 8.02.

5.4. Benzylic alcohol **9**

Silyl ether **8** (227 mg, 0.45 mmol) was dissolved in THF (10 mL) and the solution was cooled to 0°C . To this solution was added TBAF (0.6 mL, 1.00 M in THF), after 5 min the ice bath was removed, and after 4 h the reaction was quenched by addition of sat. NH_4Cl . After extraction with ethyl acetate, the combined organic extracts were washed with water and brine, dried (MgSO_4), and concentrated in vacuo to give the desired benzylic alcohol **9** (176 mg, 100%) as a clear oil: $^1\text{H NMR}$ (CDCl_3) δ 7.52 (d, $J = 17$ Hz, 1H), 7.40 (d, $J = 17$ Hz, 1H), 7.29–7.19 (m, 3H), 6.94–6.91 (m, 1H), 6.82 (s, 2H), 5.24 (s, 4H), 5.21 (s, 2H), 4.62 (s, 2H), 3.49 (s, 9H), 2.51 (br s, 1H); $^{13}\text{C NMR}$ (CDCl_3) δ 157.4, 156.1 (2C), 141.9,

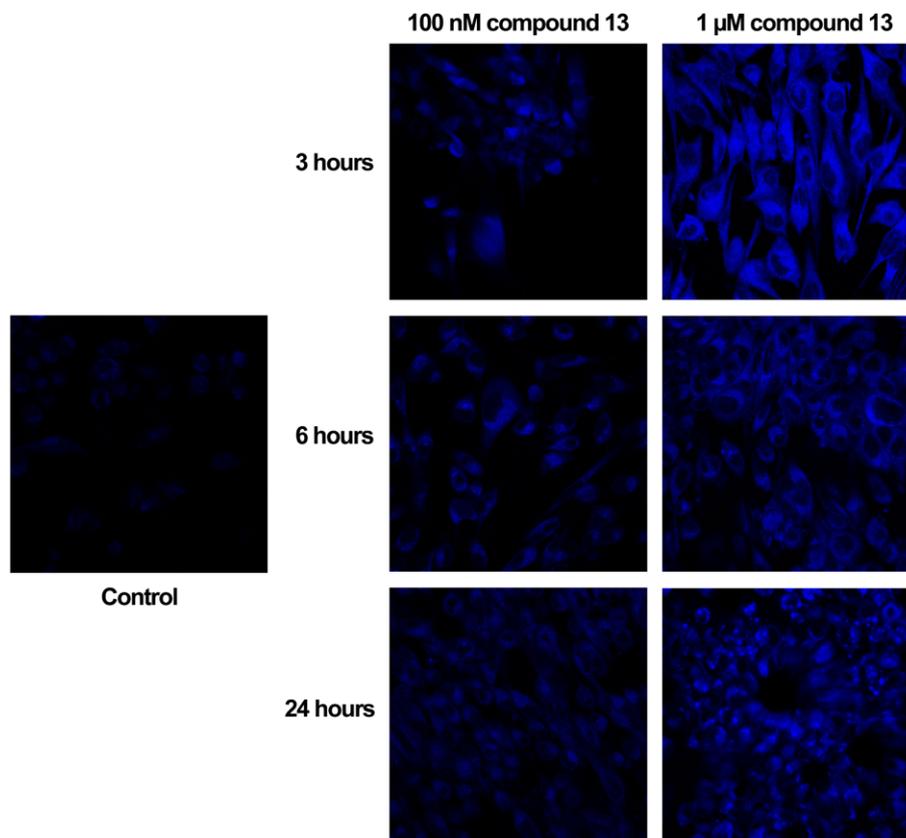


Figure 4. Fluorescence of compound **13** in SF-295 cells at indicated concentrations and time points.

140.5, 132.3, 129.4, 120.1, 120.1, 115.7, 114.7, 114.1, 106.7 (2C), 94.6 (2C), 94.3, 64.9, 56.2 (2C), 55.9.

5.5. Phosphonate **10**

Methanesulfonyl chloride (0.15 mL, 1.9 mmol) was added to a solution of benzylic alcohol **9** (176 mg, 0.45 mmol) and Et₃N (0.2 mL, 1.4 mmol) in CH₂Cl₂ (10 mL). The reaction mixture was allowed to warm to room temperature over 1 h, quenched by addition of H₂O, and extracted with CH₂Cl₂. The combined organic layers were washed with NH₄Cl (sat), brine, dried (MgSO₄), and concentrated in vacuo. The resulting residue and NaI (150 mg, 1.0 mmol) were stirred in acetone (10 mL) for 8 h. The reaction mixture was concentrated in vacuo to afford a red solid, which was dissolved in EtOAc. After the resulting yellow solution was washed with Na₂S₂O₃ until the color faded, it was washed with brine, dried (MgSO₄) and concentrated in vacuo. The resulting yellow oil was added to triethyl phosphite (2 mL) and the reaction mixture was heated at 80 °C for 24 h. After the solution was allowed to cool to rt, the excess phosphite was removed at high vacuum. The initial yellow oil was purified by flash chromatography (1:2 hexanes/ethyl acetate to 100% EtOAc) to afford phosphonate **10** (201 mg, 88%) as a clear oil: ¹H NMR (CDCl₃) δ 7.53 (d, *J* = 16.7 Hz, 1H), 7.40 (d, *J* = 16.7 Hz, 1H), 7.28–7.21 (m, 3H), 6.97–6.94 (m, 1H), 6.80–6.79 (m, 2H), 5.26 (s, 4H), 5.23 (s, 2H), 4.12–4.02 (m, 4H), 3.52 (s, 9H), 3.13 (d, *J*_{HP} = 21.8 Hz, 2H), 1.30 (t, *J* = 7.0 Hz, 6H); ¹³C NMR (CDCl₃) δ 157.4, 155.9 (d, *J*_{CP} = 3.7 Hz, 2C), 140.6, 132.3 (d, *J*_{CP} = 1.6 Hz), 132.0 (d, *J*_{CP} = 8.0 Hz), 129.4, 120.1, 120.1, 115.3 (d, *J*_{CP} = 4.1 Hz), 114.7, 114.1, 110.2 (d, *J*_{CP} = 6.7 Hz, 2C), 94.8 (2C), 94.4, 62.1 (d, *J*_{CP} = 6.8 Hz, 2C), 56.2 (2C), 55.9, 33.5 (d, *J*_{CP} = 138 Hz), 16.3 (d,

*J*_{CP} = 6.0 Hz, 2C), HRMS (EI) calcd for C₂₅H₃₅O₉P (M⁺) 510.2019; found 510.2018.

5.6. Protected bis-stilbene **12**

A suspension of NaH (80 mg, 20 mmol, 60% in oil), and 15-crown-5 (1 drop, cat.) in THF (5 mL) was cooled to 0 °C. To this mixture was added a solution of aldehyde **11** (60 mg, 0.20 mmol) and phosphonate **10** (95 mg, 0.19 mmol) in THF (5 mL). The resulting mixture was allowed to warm to rt and stirred a total of 30 h. Water was added dropwise, and the solution was extracted with EtOAc. The resulting organic phase was washed with brine, dried (MgSO₄), and concentrated in vacuo. Final purification by column chromatography (3:1 to 1:1 hexanes/ethyl acetate) gave the stilbene **12** (71 mg, 56%) as a green waxy solid oil: ¹H NMR (CDCl₃) δ 7.55 (d, *J* = 16.6 Hz, 1H), 7.43 (d, *J* = 16.6 Hz, 1H), 7.32–7.20 (m, 4H), 7.04–6.87 (m, 6H), 5.32 (s, 4H), 5.23 (s, 2H), 3.91 (s, 3H), 3.55 (s, 6H), 3.52 (s, 3H), 3.47–3.42 (m, 1H), 2.74–2.71 (m, 2H), 2.20–2.12 (m, 1H), 1.90–1.47 (m, 5H), 1.27 (s, 3H), 1.14 (s, 3H), 0.90 (s, 3H); ¹³C NMR (CDCl₃) δ 157.4, 156.3 (2C), 148.9, 142.7, 140.7, 137.8, 132.1, 129.5, 129.1, 128.6, 125.9, 122.6, 120.7, 120.3, 120.1, 125.7, 114.7, 114.2, 106.7, 106.4 (2C), 94.8 (2C), 94.4, 77.9, 77.1, 60.3, 56.3 (2C), 56.0, 46.7, 38.3, 37.6, 28.2, 27.3, 23.1, 19.8, 14.1; HRMS (EI) calcd for C₃₉H₄₈O₉ (M⁺) 660.3298, found 660.3301.

5.7. Bis-stilbene **13**

To a solution of stilbene **12** (16 mg, 0.024 mmol) in MeOH (5 mL) was added p-toluenesulfonic acid (3 mg, 0.014 mmol). The resulting solution was heated 60 °C for 5 h. The reaction was

quenched by addition of sat. NaHCO₃, extracted with ethyl acetate, and the organic phase was washed with brine and dried (MgSO₄). Concentration in vacuo, followed by final purification by column chromatography (2:1 to 1:1 hexanes/ethyl acetate) afforded the bis-stilbene **13** (10 mg, 79%) as a slightly yellow oil: ¹H NMR ((CD₃)₂CO) δ 7.19 d, *J* = 17 Hz, 1H), 7.55 (d, *J* = 17 Hz, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.04–6.98 (m, 4H), 6.95 (d, *J* = 6.9 Hz, 2H), 6.90–6.88 (m, 1H), 6.73–6.70 (m, 2H), 3.82 (s, 3H), 3.43–3.38 (m, 1H), 2.76–2.73 (m, 2H), 1.85–1.63 (m, 6H), 1.23 (s, 3H), 1.13 (s, 3H), 0.90 (s, 3H); ¹³C NMR ((CD₃)₂CO) δ 158.5, 157.6 (2C), 150.2, 144.1, 142.1, 138.6, 131.5, 130.4, 129.7, 129.6, 126.5, 123.7, 121.8, 121.5, 118.7, 114.7, 113.0, 112.3, 108.3 (2C), 77.7, 77.6, 56.1, 47.2, 39.2, 38.7, 29.2, 27.9, 23.8, 20.4, 14.9; HRMS (EI) calcd for C₃₃H₃₆O₆ (M⁺) 528.2512; found 528.2523.

5.8. Cell culture

The SF-295 cells were maintained in RPMI 1640 media supplemented with 10% bovine fetal calf serum and penstreptomycin. The A-549 cells were maintained in F-12 media with 10% fetal calf serum and penstreptomycin. Both cell lines were incubated at 37 °C and 5% CO₂.

5.9. Cytotoxicity

SF-295 and A549 cells were plated in 24-well plates. Upon 65% confluency, cells were treated with 3dSB, compound **9** or Y-27632. To measure cytotoxicity, the MTT assay was performed. Briefly, thiazolyl blue tetrazolium bromide (Sigma, St. Louis, MO, USA) was added to all wells 44 h after compound treatment. At 48 h solubilization solution (HCl, triton X-100, Isopropyl alcohol) was added to each well and incubated overnight with gentle agitation at 37 °C. To quantify toxicity, the absorbance of each well at 540 nm and 650 nm (reference λ) was measured spectrophotometrically.

5.10. Cellular morphology

Cells were plated on sterilized coverslips in 6-well plates and allowed to reach 65% confluency before treatment with the indicated compounds. Rock inhibitor Y-27632 was purchased from Calbiochem San Diego, CA. At the conclusion of the treatment interval, cells were rinsed and fixed with 3.7% formaldehyde for 15 min. After subsequent rinses in PBS, cells were permeabilized with 0.2% triton X-100 for 4 min and rinsed in PBS. Cells were then incubated with 1% BSA for 30 min before incubation with phalloidin stain (Molecular Probes, Eugene, OR) for 20 min. At the completion of the staining interval, cells were rinsed and mounted using Vectashield mounting media containing DAPI (Vector laboratories, Inc., Burlingame, CA). Slides were imaged using a Bio-Rad Multi-photon microscope and processed using Image-J software.

5.11. Fluorescence microscopy

SF-295 cells were plated on sterilized coverslips in 6-well plates and allowed to reach 65% confluency and then treated for indicated intervals. At the conclusion of the treatment

interval cells were washed three times in complete media to remove residual compound. Immediately following rinsing, cells were mounted onto microscope slides and imaged using a Bio-Rad Multi-photon microscope at the University of Iowa Central Microscopy facility. Images were further processed using Image-J software.

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References and notes

- Cragg, G. M.; Newman, D. J. *J. Ethnopharm.* **2005**, *100*, 72.
- Noble, R. L.; Beer, C. T.; Cutts, J. H. *Biochem. Pharmacol.* **1958**, *1*, 347.
- Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
- Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665.
- Hsiang, Y. H.; Hertzberg, R.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873.
- Beutler, J. A.; Jato, J. G.; Cragg, G.; Wiemer, D. F.; Neighbors, J. D.; Salnikova, M. S.; Hollingshead, M.; Scudiero, D. A.; McCloud, T. G. In *Medicinal and Aromatic Plants*; Bogers, R. J., Ed.; Springer, 2005; pp 301–309.
- Beutler, J. A.; Shoemaker, R. H.; Johnson, T.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 1509.
- Beutler, J. A.; Jato, J.; Cragg, G. M.; Boyd, M. R. *Nat. Prod. Lett.* **2000**, *14*, 399.
- Yoder, B. J.; Cao, S. G.; Norris, A.; Miller, J. S.; Ratovoson, F.; Razafitsalama, J.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. *J. Nat. Prod.* **2007**, *70*, 342.
- Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088.
- Mente, N. R.; Wiemer, A. J.; Neighbors, J. D.; Beutler, J. A.; Hohl, R. J.; Wiemer, D. F. *Biorg. Med. Chem. Lett.* **2007**, *17*, 911.
- Mente, N. R.; Neighbors, J. D.; Wiemer, D. F. *J. Org. Chem.* **2008**, *73*, 7963.
- Neighbors, J. D.; Beutler, J. A.; Wiemer, D. F. *J. Org. Chem.* **2005**, *70*, 925.
- Neighbors, J. D.; Salnikova, M. S.; Beutler, J. A.; Wiemer, D. F. *Biorg. Med. Chem.* **2006**, *14*, 1771.
- Kashemirov, B. A.; Bala, J. L. F.; Chen, X.; Ebetino, F. H.; Xia, Z.; Russell, R. G. G.; Coxon, F. P.; Roelofs, A. J.; Rogers, M. J.; McKenna, C. E. *Bioconjugate Chem.* **2008**, *19*, 2308.
- Day, R. N. *Mol. Cell. Endocrinol.* **2005**, *230*, 1.
- Papper, V.; Likhtenshtein, G. I. *J. Photochem. Photobiol., A* **2001**, *140*, 39.
- Mazzucato, U.; Momicchioli, F. *Chem. Rev.* **1991**, *91*, 1679.
- Maddy, A. H. *Biochim. Biophys. Acta* **1964**, *88*, 390.
- Schmued, L. C.; Fallon, J. H. *Brain Res.* **1985**, *346*, 124.
- Wu, C. Y.; Tian, D. H.; Feng, Y.; Polak, P.; Wei, J. J.; Sharp, A.; Stankoff, B.; Lubetzki, C.; Zalc, B.; Mufson, E. J.; Gould, R. M.; Feinstein, D. L.; Wang, Y. M. *J. Histochem. Cytochem.* **2006**, *54*, 997.
- Furstner, A.; Stelzer, F.; Rumbo, A.; Krause, H. *Chem. Eur. J.* **2002**, *8*, 1856.
- Wang, Q. L.; Huang, Q. G.; Chen, B.; Lu, J. P.; Wang, H.; She, X. G.; Pan, X. F. *Angew. Chem., Int. Ed.* **2006**, *45*, 3651.
- Uehata, M.; Ishizaki, T.; Satoh, H.; Ono, T.; Kawahara, T.; Morishita, T.; Tamakawa, H.; Yamagami, K.; Inui, J.; Maekawa, M.; Narumiya, S. *Nature* **1997**, *389*, 990.
- Ridley, A. J. *Trends Cell Biol.* **2006**, *16*, 522.
- Schneckenburger, H.; Wagner, M.; Weber, P.; Strauss, W. S. L.; Sailer, R. *J. Fluoresc.* **2004**, *14*, 649.
- Heasman, S. J.; Ridley, A. J. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 690.
- Bach, S.; Knockaert, M.; Reinhardt, J.; Lozach, O.; Schmitt, S.; Baratte, B.; Koken, M.; Coburn, S. P.; Tang, L.; Jiang, T.; Liang, D.-C.; Galons, H.; Dierick, J.-F.; Pinna, L. A.; Meggio, F.; Totzke, F.; Schachtele, C.; Lerman, A. S.; Carnero, A.; Wan, Y.; Gray, N.; Meijer, L. *J. Biol. Chem.* **2005**, *280*, 31208.
- McPherson, M.; Yang, Y.; Hammond, P. W.; Kreider, B. L. *Chem. Biol.* **2002**, *9*, 691.
- Sato, S.; Kwon, Y.; Kamisuki, S.; Srivastava, N.; Mao, Q. A.; Kawazoe, Y.; Uesugi, M. *J. Am. Chem. Soc.* **2007**, *129*, 873.