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Structural analogues of schweinfurthin F: Probing the steric, electronic, and hydrophobic properties of the D-ring substructure

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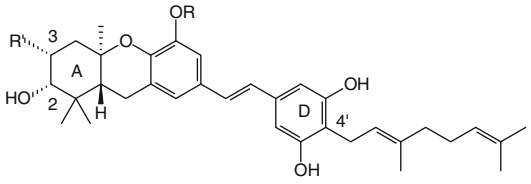
ABSTRACT

The natural tetracyclic schweinfurthins are potent and selective inhibitors of cell growth in the National Cancer Institute's 60-cell line screen. An interest in determination of their cellular or molecular target has inspired our efforts to prepare both the natural products and analogues. In this paper, chemical synthesis of analogues modified in different olefinic positions, and preliminary results from studies of their biological activity, are reported.

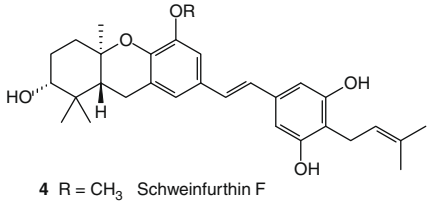
1. Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive form of central nervous system (CNS) cancer among adults. Unfortunately, patients undergoing the current standard of treatment have a median survival rate of only 15 months.[1] Despite numerous advances in the last several decades in understanding the molecular biology of GBM, only modest progress has been made in improving the prognosis of affected patients,[2] making new therapeutic alternatives vital. Fortunately, nature continues to be a rich source of compounds with chemotherapeutic potential.[3] As part of a continuing mission to obtain anticancer agents from natural sources, researchers at the National Cancer Institute (NCI) discovered a family of natural products known as the schweinfurthins which exhibit potent activity against human-derived CNS cancer cell lines. Schweinfurthins A and B (SA and SB, **1** and **2**, Fig. 1), along with the less active schweinfurthin C (SC), were isolated from the African plant *Macaranga schweinfurthii* and displayed potent and selective anti-proliferative activity in the NCI's 60-cell line screen (GI_{50} = 0.36 μM and 0.81 μM, respectively).[4] Shortly thereafter, schweinfurthin D was reported,[5] and the isolation of schweinfurthins E, F (**4**), G (**5**), and H by the Kingston group at Virginia Tech followed some years later.[6]

A program called COMPARE has been developed by the NCI to analyze similarities and differences among drug activity patterns,



1 R = H R' = OH Schweinfurthin A
2 R = CH_3 R' = OH Schweinfurthin B
3 R = CH_3 R' = H 3-deoxyschweinfurthin B



4 R = CH_3 Schweinfurthin F
5 R = H Schweinfurthin G

Figure 1. Selected natural (**1**–**2**, **4**–**5**) and synthetic (**3**) schweinfurthins.

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and these analyses can in turn provide insight into the mechanism of action of the drugs studied in the 60-cell cancer screen.[7] Importantly, the schweinfurthins show no correlation to any clinically used anti-neoplastic agent, indicating that this family of compounds may act via a novel mechanism or target.[8] Only three structurally unrelated families of natural products show any appreciable correlation to the schweinfurthins: the cephalostatins,[9] the stellettins,[10] and OSW-1.[11] Presumably, a determination of the molecular target of one of these compounds would aid in a similar determination with the others, thus increasing understanding of their biological activity.

Because of our ongoing interest in the synthesis of prenylated stilbenes and the unique activity of the schweinfurthins, we undertook a synthetic effort aimed at these compounds. This endeavor has led to the total synthesis of SC,[12] 3-deoxyschweinfurthin B (3dSB, **3**),[13] schweinfurthins F and G (**4** and **5**),[14,15] and, most recently, SB (**2**) and schweinfurthin E.[16] Furthermore, we have prepared a number of analogues to illuminate the pharmacophore(s) responsible for the schweinfurthins' differential activity.[17] First, based on the relative activity of SA or SB versus SC, it appears that the left-half hexahydroxanthene substructure is required for potent and selective activity. Second, replacing the phenolic groups of the right-half resorcinol structure with hydrogen or fluorine demonstrated that at least one of the phenolic hydroxyl groups is important for differential activity. Third, the effect of some D-ring substitutions has been examined. Comparison of the activity of schweinfurthin F (**4**), 3dSB (**3**), and a previously synthesized 3dSB analogue[17] (bearing prenyl, geranyl, and hydroxylated geranyl chains, respectively) with an analogue which lacks a prenyl chain, established that the absence of a prenyl chain led to greatly reduced activity. Finally, recent attempts at the NCI to obtain additional amounts of schweinfurthins from a natural source have resulted in the isolation of compounds tentatively identified as *cis*-stilbenes, raising questions about the importance of the central olefin stereochemistry.

Variations in activity observed through these past studies prompted investigation into the significance of a hydrophobic substituent on the D-ring and the stilbene olefin to the schweinfurthin pharmacophore. Specifically, we wished to explore whether attaching a simplified tail to the D-ring or altering the electronics of the stilbene moiety would be tolerated. We therefore targeted the synthesis of several new schweinfurthin analogues (Fig. 2). The preparation of these compounds and data on their biological activity are presented here.

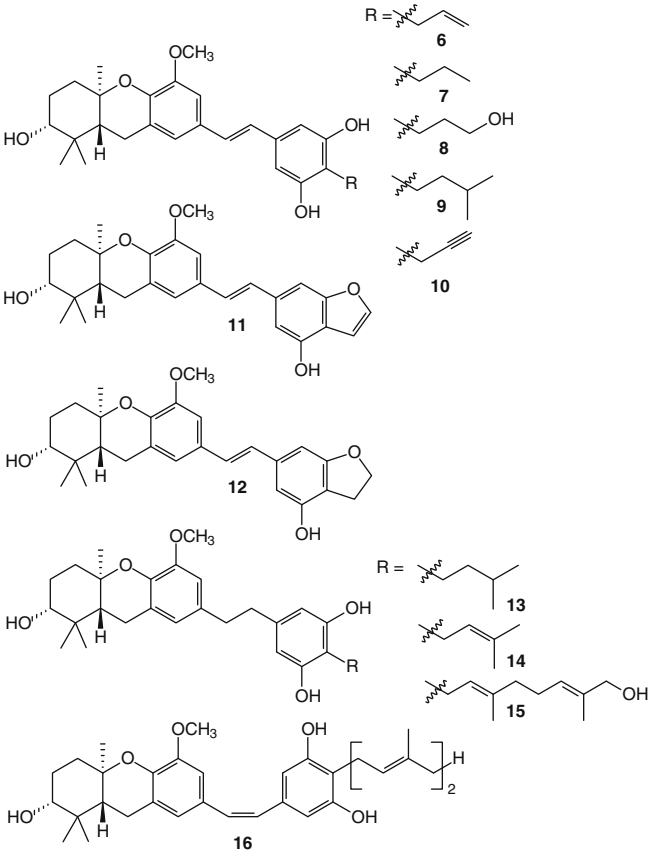


Figure 2. Olefin-modified schweinfurthin analogues.

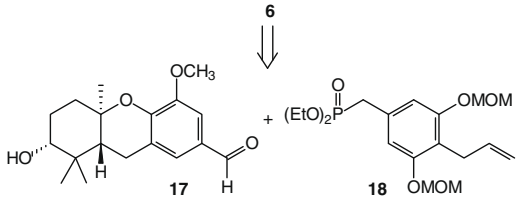


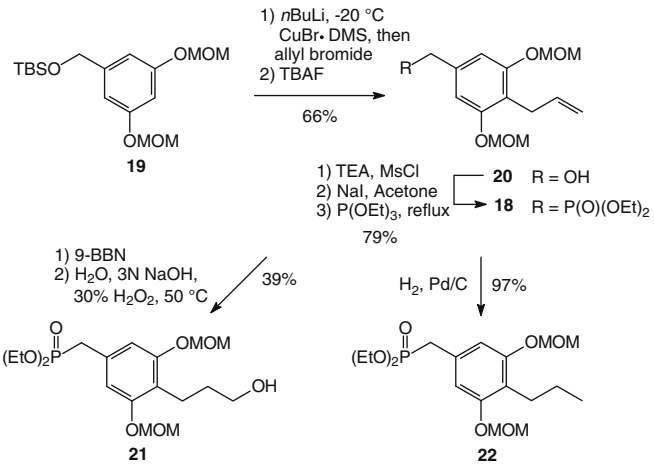
Figure 3. A representative retrosynthesis.

2. Synthesis

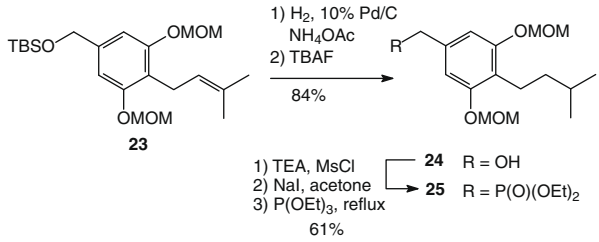
The total synthesis of SC established an early precedent for a highly convergent, stereoselective Horner–Wadsworth–Emmons condensation in formation of the central stilbene moiety,[12] a strategy then utilized in the synthesis of analogues such as 3dSB (**3**, Fig. 1). Because aldehyde **17** is known,[17] the first key intermediates for preparation of analogues **6**–**10** were the corresponding phosphonates as shown by the representative retrosynthesis depicted in Figure 3.

Benzyl alcohol **20** was prepared from the known arene **19**[18] by a directed *ortho* metallation/transmetallation/alkylation protocol[19] and subsequent removal of the silyl protecting group (Scheme 1). After formation of the mesylate and conversion to the iodide, Arbuzov reaction with triethyl phosphite provided phosphonate **18**. Hydroboration/oxidation of compound **18** with 9-BBN and H_2O_2 gave hydroxylated phosphonate **21**, while palladium-catalyzed hydrogenation of phosphonate **18** produced the *n*-propyl phosphonate **22**.

The preparation of isopentyl phosphonate **25** began with known prenylated arene **23**[20] (Scheme 2). Hydrogenation of alkene **23**



Scheme 1. Synthesis of phosphonates **18**, **21**, and **22**.



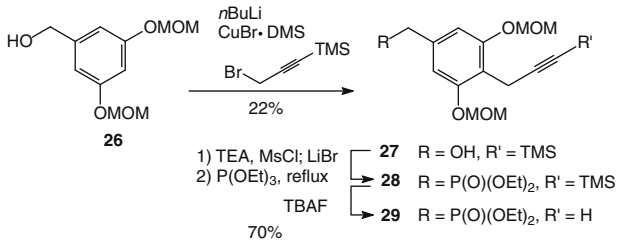
Scheme 2. Synthesis of phosphonate **25**.

over Pd/C in the presence of NH_4OAc[21] followed by silyl deprotection provided alkane **24**, which was then converted to phosphonate **25** via standard conditions.

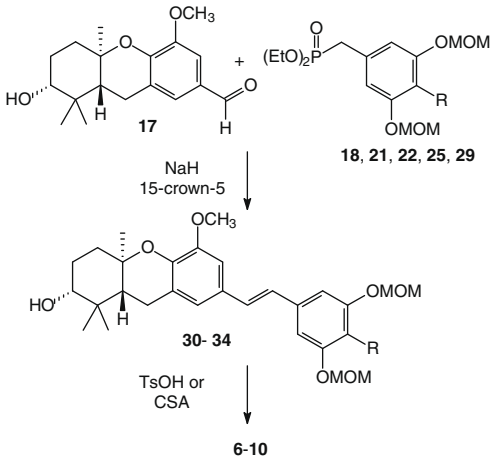
Alkynyl phosphonate **29** was obtained via alkylation of known benzyl alcohol **26**[18] with 3-(trimethylsilyl)propargyl bromide, conversion to the phosphonate, and removal of the TMS group (Scheme 3).

With several of the necessary phosphonates in hand, the Horner–Wadsworth–Emmons condensations were effected in the presence of NaH and 15-crown-5 (Scheme 4). In all cases, only the *trans*-stilbene product was observed. Subsequent hydrolysis of the MOM protecting groups was carried out in the presence of TsOH or CSA to provide analogues **6**–**10** (Table 1).

To examine the impact of a benzofuran or dihydrobenzofuran system as an E-ring, the key intermediate **36** was prepared from the known benzofuran core **35**[22] through MOM-protection and reduction as shown in Scheme 5. Hydrogenation of compound **36** occurred smoothly upon treatment with hydrogen and Pd/C, to afford the reduced compound **37**. Conversion to the phosphonate **38** took place under standard conditions, and the HWE condensation with aldehyde **17** occurred smoothly as well (Scheme 5). Final



Scheme 3. Synthesis of phosphonate **29**.



Scheme 4. Synthesis of analogues **6**–**10**.

deprotection gave the desired analogue **12**. When difficulty was encountered preparing the phosphonate analogue of the benzofuran alcohol **36**, a reversed HWE strategy was explored, based upon synthesis of the corresponding right-half aldehyde **40** through oxidation of alcohol **36**. Condensation of aldehyde **40** with the known left-half phosphonate **41**[15] gave the expected *trans*-stilbene **42**, and deprotection provided the desired benzofuran analogue **11.**

To gauge the significance of the *trans*-stilbene olefin, other analogues were prepared where this moiety was replaced by a simple alkyl chain or isomerized to the corresponding cis stilbene. For example, hydrogenation of the known stilbene **43**[14] over Pd/C gave the fully saturated analogue **44**, and MOM hydrolysis provided the desired analogue **13** (Scheme 6). Alternatively, selective reduction of the stilbene moiety in the presence of the isoprenoid olefins[23] was accomplished via reaction of compounds **43** and **45** with Mg and NH_4Cl in methanol. Standard deprotection of the products **46** and **47** provided analogues **14** and **15**. Finally, the last of our targeted analogues, the *cis*-stilbene **16**, was prepared by irradiation of 3dSB (**3**) to promote olefin isomerization, followed by chromatography to isolate the desired cis isomer.

3. Biological results and discussion

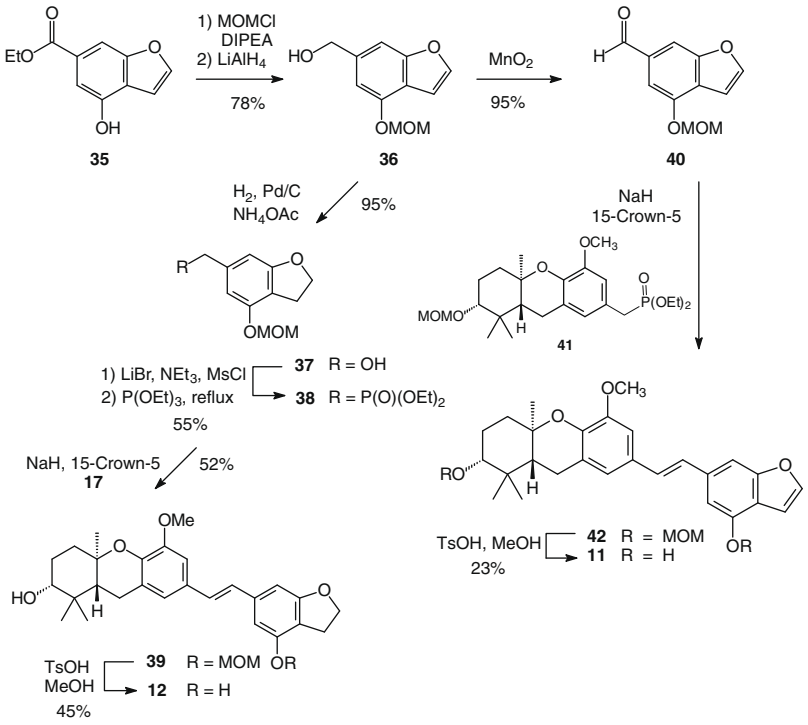
As presented above, the schweinfurthins exhibit potent and differential cytotoxicity in the NCI's 60-cell cancer screen. Of the cell lines tested in the NCI assay, the human-derived glioblastoma cell line SF-295 is one of the most sensitive to the growth inhibitory effects of the schweinfurthins, while the human-derived lung adenocarcinoma cell line A549 displays only moderate sensitivity to the schweinfurthins. Based on this difference in antiproliferative activities, a two-cell line screen was developed to determine whether synthetic analogues of the schweinfurthins exhibit the same basic pattern of cytotoxicity as the natural compounds. Within this testing scheme, all of the prepared compounds display schweinfurthin-like activity, but the observed potencies vary greatly.

One subset of these analogues can be viewed as the group with small alkyl substituents on the D-ring, compounds **6**–**10**. All five of these compounds showed anti-proliferative effects in the low or sub-micromolar range when tested against SF-295 cells (Table 2), and substantially less activity when tested in the A549 cells. The most potent compound in this set was the isopentyl compound **9**, indicating that the presence of an olefin at this position is unnecessary for activity in the SF-295 cell assay, and this compound showed approximately 10-fold lower potency against the A549 cells. Comparison of the Clog *P* values of compounds **7**, **8**, and **9** reveals a tentative correlation between hydrophobicity and cytotoxic activity. The hydroxyl moiety in analogue **8** leads to a decrease in activity in the sensitive cell line, while the presence of hydrophobic methyl groups in analogue **9** appears to contribute to slightly increased anti-proliferative activity.

Compared to the first set of compounds, the two heterocyclic compounds, benzofuran **11** and its dihydro analogue **12,** showed somewhat less potency against the SF-295 cell line, although dihydrofuran **12** was more active than its benzofuran counterpart. This result is in agreement with findings observed in a similar study.[24]

Assays on the final compounds suggest that reduction of the *trans*-stillbene olefin diminishes activity (e.g., **13–15** vs **9**). Isomerization of 3dSB (**3**, EC_{50}'s of 0.5 and 6.4 μM, respectively, in this assay) to the cis olefin **16** has an even greater negative impact. This outcome is interesting given the varied potencies observed in cis and trans analogues of medicinally important stilbenes such as resveratrol[25] and combretastatin.[26]

Of the 11 compounds tested in the two-cell assay, compound **9** demonstrated the greatest potency against SF-295 cells, along with a 10-fold difference in activity in the two-cell assays. When this

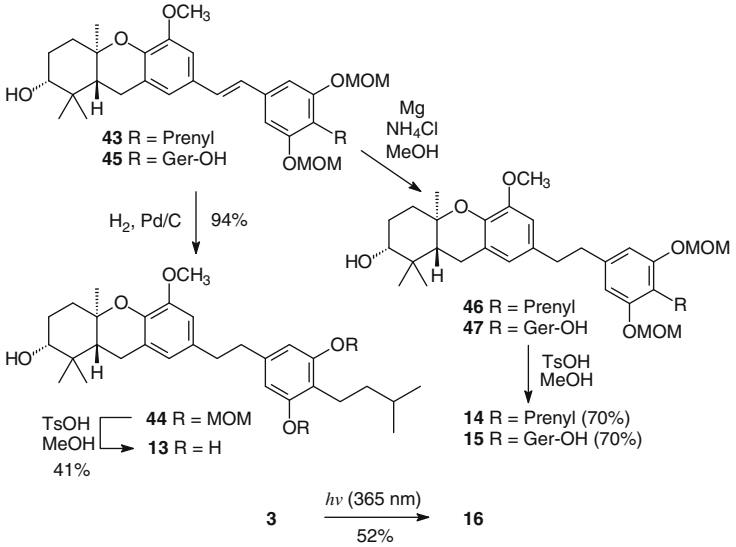


Scheme 5. Synthesis of analogues **11** and **12**.

Table 1
HWE condensations and hydrolysis reactions

Phosphonate	R	Stilbene	Yield (%)	Target	Yield (%) (TsOH)
18	Allyl	30	70	**6**	67
22	*n*-Propyl	31	75	**7**	73
21	3-Propanol	32	65	**8**	74
25	Isopentyl	33	—	**9**	73 (CSA) (two steps)
29	Propynyl	34	—	**10**	26 (two steps)

compound was tested in the 60-cell line assay at the NCI, it also showed significant potency. In this assay, the average GI_{50} across the 60 cell lines was 0.29 μM, and the GI_{50} in the SF-295 cell line was ~33 nM, making this one of the most potent schweinfurthin analogues to date. Its potency in the 60-cell line screen exceeds that of several of the natural products (e.g., SA and SB), which will encourage additional efforts to improve the activity of schweinfurthin analogues. Furthermore, the GI_{50} values varied over the 60-cell lines examined by more than three orders of magnitude (cf. Supplementary data). This large range is indicative of selective toxicity. Conversely, compound **15** proved to be one of the least active compounds tested in the two-cell assay. When this compound was tested in the NCI's 60-cell line assay, its average GI_{50} was 4.9 μM, suggesting a weakly toxic compound, and it exhibited virtually no differential activity across the 60 cell lines (cf. Supplementary data). Taken together with the results obtained from testing com-



Scheme 6. Synthesis of analogues **13**–**16**.

Table 2
Activity of synthetic schweinfurthins in a two-cell screen

Compound	*Clog P*	SF-295 EC_{50} (μM)	A549 EC_{50} (μM)
6	5.75	1.7	>10
7	6.05	0.9	>10
8	4.62	2.5	>10
9	6.79	0.4	4.2
10	5.16	1.3	>10
11	5.11	4.8	>10
12	4.84	2.9	>10
13	6.98	2.8	>10
14	6.58	2.9	>10
15	6.95	>10	>10
16	8.04	>10	>10

pound **9**, this suggests that the two-cell line assay is an effective means for rapidly screening analogues.

4. Conclusions

In conclusion, these studies have led to the preparation of a set of eleven new schweinfurthin analogues with variations in the nature of the stilbene olefin and the substituent at C-4′ of the D-ring. The paucity of functionality in the side chain of the most potent compound, the isopentyl analogue **9**, may suggest that increasing hydrophobicity is more important than interaction with a specific functional group. Given this perspective, the activity observed in the heterocyclic compounds **11** and **12** encourages exploration of other heterocyclic systems, especially if they can be prepared with an additional alkyl substituent in the E-ring. Finally, either reduction of the stilbene olefin (e.g., **13**–**15**) or isomerization from the trans stereochemistry to the cis (**16**) diminishes activity in the SF-295 cell line.

The two-cell assay for screening synthetic analogues has proven quite effective in quickly identifying potent and selective compounds. In this study, all eleven new schweinfurthin analogues were pre-screened in the two-cell assay. After the most potent compound of the set was identified, confirmation of this analogue's activity was obtained via the NCI's 60-cell line assay. As a proof of concept, one of the least active of the analogues in the two-cell assay also was tested in the 60-cell assay and displayed both reduced cytotoxicity and lessened differential activity. Thus it appears reasonable to use this facile screening process for more efficient testing of future synthetic analogues.

At this time, the mode of action and/or molecular target of the schweinfurthins remain unknown. Given the preservation of activity despite variations in the alkyl chains at C-4′, this position appears to be a reasonable site for preparation of biotinylated derivatives. Preparation of such compounds is underway and will be reported in due course.

5. Experimental procedures and methods

5.1. General experimental conditions

Tetrahydrofuran was freshly distilled from sodium/benzophenone. Methylene chloride and triethylamine were distilled from calcium hydride prior to use. Solutions of butyl lithiums were purchased from a commercial source and titrated with diphenyl acetic acid prior to use. All other reagents and solvents were purchased from commercial sources and used without further purification. All reactions in nonaqueous solvents were conducted in flame-dried glassware under a positive pressure of argon and with magnetic stirring. NMR spectra were obtained at 300 MHz for ^{1}H, and 75 MHz for ^{13}C with $CDCl_3$ as solvent, and $(CH_3)_4Si$ (^{1}H, 0.00 ppm) or $CDCl_3$ (^{13}C, 77.0 ppm) as internal standards unless otherwise noted. The ^{31}P chemical shifts were reported in ppm relative to 85% H_3PO_4 (external standard). High resolution mass spectra were obtained at the University of Iowa Mass Spectrometry Facility, and elemental analyses were obtained at Atlantic Microlabs, Inc. Silica gel (60 Å, 0.040–0.063 mm) was used for flash chromatography. Most of the analogues were prepared using left-half aldehyde **17** with an ee of 90%, as determined by HPLC.

5.2. Benzyl alcohol 20

To a solution of known silyl protected benzyl alcohol **19**[18] (634 mg, 1.9 mmol) in THF (12 mL) at −20 °C was added *n*-BuLi (0.9 mL, 2.4 M in hexane, 2.2 mmol), and the reaction was allowed to warm to 0 °C over 1 h. The solution was then cooled to −20 °C, CuBr·DMS (380 mg, 1.9 mmol) was added in one portion, and the resulting solution was allowed to stir for 1 h. To the solution was added allyl bromide (0.2 mL, 2.1 mmol) and the resulting solution was allowed to stir for 2 h. After the reaction was quenched by addition of NH_4Cl (satd), the aqueous layer was extracted with EtOAc, dried ($MgSO_4$), and concentrated in vacuo. The resulting oil was dissolved in THF (10 mL) at rt, TBAF (1.5 mL, 1.0 M in THF, 1.5 mmol) was added, and the reaction was allowed to stir for 2 h. Once TLC analysis indicated complete consumption of the starting material, the reaction was quenched by addition of H_2O and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting oil was purified by column chromatography (60% EtOAc/hexanes) to afford compound **20** (329 mg, 66%) as a colorless oil: ^{1}H NMR δ 6.77 (s, 2H), 5.97–5.91 (m, 1H), 5.17 (s, 4H), 5.00–4.91 (m, 2H), 4.59 (s, 2H), 3.45 (s, 6H), 3.40–3.37 (m, 2H); ^{13}C NMR δ 155.7 (2C), 140.6, 136.6, 117.3, 114.2, 106.3 (2C), 94.3 (2C), 65.2, 56.0 (2C), 27.5; HRMS (EI$^+$) calcd for $C_{14}H_{20}O_5$ [M$^+$] 268.1311; found 268.1309.

5.3. Phosphonate 18

Methanesulfonyl chloride (0.1 mL, 1.6 mmol) was added dropwise to a solution of alcohol **20** (329 mg, 1.2 mmol) and Et_3N (0.3 mL, 1.8 mmol) in THF (7 mL) at 0 °C, and the solution was allowed to stir for 1 h. The resulting precipitate was dissolved by addition of H_2O, and the aqueous layer was extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was dissolved in acetone (10 mL), and NaI (670 mg, 4.5 mmol) was added in one portion. After the mixture was allowed to stir for 24 h, it was quenched by addition of H_2O and extracted with EtOAc. The combined extracts were washed with $Na_2S_2O_3$ (satd) until the color had disappeared, dried ($MgSO_4$), and concentrated in vacuo. The resulting yellow oil was added to a solution of triethyl phosphite (0.2 mL, 1.4 mmol) in THF (3 mL), and the solution was heated at reflux overnight. After concentration in vacuo, the resulting oil was purified by column chromatography (2% MeOH/$CHCl_3$) to yield phosphonate **18** (375 mg, 79%) as a pale oil: ^{1}H NMR δ 6.70 (d, J_{PH} = 2.5 Hz, 2H), 6.01–5.86 (m, 1H), 5.18 (s, 4H), 5.00–4.90 (m, 2H), 4.09–3.99 (m, 4H), 3.45 (s, 6H), 3.44–3.40 (m, 2H), 3.08 (d, J_{PH} = 21.5 Hz, 2H), 1.38–1.32 (m, 6H); ^{13}C NMR δ 155.6 (d, J_{CP} = 2.6 Hz, 2C), 142.4, 136.7 (d, J_{CP} = 1.8 Hz), 116.8, 114.1, 109.5 (d, J_{CP} = 6.8 Hz, 2C), 94.4 (2C), 62.0 (d, J_{CP} = 6.6 Hz, 2C), 56.0 (2C), 33.0 (d, J_{CP} = 137.0 Hz), 27.5, 16.4 (d, J_{CP} = 7.2 Hz, 2C); ^{31}P NMR δ 26.9; HRMS (EI$^+$) calcd for $C_{18}H_{29}O_7P$ [M$^+$] 388.1651; found 388.1655.

5.4. Phosphonate 21

To a solution of phosphonate **18** (101 mg, 0.3 mmol) in THF (1 mL) at 0 °C was added 9-BBN (2.0 mL, 0.5 M in THF, 1.0 mmol), and the solution was allowed to warm to rt and stirred overnight.

To the solution was added H_2O (0.1 mL), 2 N NaOH (0.8 mL), and 30% H_2O_2 (0.4 mL), and the reaction was heated at 50 °C for 2 h, then allowed to cool to rt and stirred for 2 days. After the resulting solution was extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated in vacuo, the remaining oil was purified by flash column chromatography (2% MeOH/$CHCl_3$) to afford compound **21** (41 mg, 39%) as a colorless oil: ^{1}H NMR δ 6.66 (s, 2H), 5.11 (s, 4H), 3.99–3.94 (m, 4H), 3.47 (t, J = 6.0 Hz, 2H), 3.40 (s, 6H), 3.01 (d, J_{PH} = 21.4 Hz, 2H), 2.70 (t, J = 7.1 Hz, 2H), 2.10 (br s, 1H), 1.76–1.68 (m, 2H), 1.20 (t, J_{PH} = 5.9 Hz, 6H); ^{13}C NMR δ 155.8 (d, J_{CP} = 3.4 Hz, 2C), 130.7 (d, J_{CP} = 8.9 Hz), 118.1, 109.5 (d, J_{CP} = 6.7 Hz, 2C), 94.6 (2C), 62.0 (d, J_{CP} = 6.8 Hz, 2C), 61.6, 56.1 (2C), 33.8 (d, J_{CP} = 138.2 Hz), 31.7, 18.8, 16.3 (d, J_{CP} = 6.1 Hz, 2C); ^{31}P NMR δ 26.7; HRMS (EI$^+$) calcd for $C_{18}H_{31}O_8P$ [M$^+$] 406.1757; found 406.1761.

5.5. Phosphonate 22

To a solution of phosphonate **18** (69 mg, 0.2 mmol) in MeOH (3 mL) was added 10% Pd–C (67 mg, cat.) and an excess of H_2, and the mixture was agitated overnight. Following filtration through Celite, the resulting solution was concentrated in vacuo to afford compound **22** (67 mg, 97%) as a colorless oil: ^{1}H NMR δ 6.63 (s, 2H), 5.10 (s, 4H), 3.99–3.94 (m, 4H), 3.39 (s, 6H), 3.02 (d, J_{PH} = 21.6 Hz, 2H), 2.54 (t, J = 7.5 Hz, 2H), 1.48–1.40 (m, 2H), 1.19 (t, J_{PH} = 6.9 Hz, 6H), 0.86 (t, J = 7.2 Hz, 3H); ^{13}C NMR δ 155.7 (d, J_{CP} = 3.3 Hz, 2C), 129.9 (d, J_{CP} = 9.2 Hz), 119.5 (d, J_{CP} = 4.2 Hz), 109.2 (d, J_{CP} = 6.7 Hz, 2C), 94.3 (2C), 62.0 (d, J_{CP} = 6.7 Hz, 2C), 55.8 (2C), 33.7 (d, J_{CP} = 137.7 Hz), 25.1, 22.6 (d, J_{CP} = 2.4 Hz), 16.2 (d, J_{CP} = 6.1 Hz, 2C), 14.1; ^{31}P NMR δ 27.0; HRMS (EI$^+$) calcd for $C_{18}H_{31}O_7P$ [M$^+$] 390.1807; found 390.1811.

5.6. Benzyl alcohol 27

To a solution of benzyl alcohol **26** (203 mg, 0.9 mmol) in THF (10 mL) at −20 °C was slowly added *n*-BuLi (0.8 mL, 2.2 M in hexane, 1.8 mmol), and the reaction was allowed to warm to 0 °C over 1 h. The solution was then cooled to −20 °C, CuBr·DMS (390 mg, 1.9 mmol) was added in one portion, and the resulting solution was allowed to stir for 1 h. To this solution was added 3-(trimethylsilyl)propargyl bromide (0.2 mL, 1.2 mmol), and the resulting solution was allowed to warm to rt and stirred for 2 h. After the reaction was quenched by addition of NH_4Cl (satd), extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated in vacuo, final purification by column chromatography (35% EtOAc/hexanes) provided alcohol **27** (66 mg, 22%) as a colorless oil: ^{1}H NMR δ 6.74 (s, 2H), 5.18 (s, 4H), 4.54 (s, 2H), 3.59 (s, 2H), 3.45 (s, 6H), 2.99 (br s, 1H), 0.06 (s, 9H); ^{13}C NMR δ 155.2 (2C), 141.4, 114.4, 106.3 (2C), 105.5, 94.0 (2C), 82.0, 64.7, 55.8 (2C), 14.1, −0.1 (3C); HRMS (EI$^+$) calcd for $C_{17}H_{26}O_5Si$ [M$^+$] 338.1550; found 338.1548.

5.7. Stilbene 30

To a mixture of NaH (28 mg, 0.8 mmol) and 15-crown-5 (1 drop, cat.) in THF (5 mL) at 0 °C was added a solution of phosphonate **18** (38 mg, 0.1 mmol) and aldehyde **17** (29 mg, 0.1 mmol) in THF (1 mL), and the reaction was allowed to stir for 1 h at 0 °C. The reaction mixture was then quenched via dropwise addition of H_2O, extracted with ether, washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting oil was purified by flash column chromatography (45% EtOAc/hexanes) to afford stilbene **30** (37 mg, 70%) as a colorless oil: ^{1}H NMR δ 6.91–6.79 (m, 6H), 5.90–5.85 (m, 1H), 5.16 (s, 4H), 4.95–4.85 (m, 2H), 3.82 (s, 3H), 3.42 (s, 6H), 3.38–3.37 (m, 3H), 2.65–2.56 (m, 2H), 2.07–2.02 (m, 1H), 1.81–1.52 (m, 4H), 1.18 (s, 3H), 1.03 (s, 3H), 0.81 (s, 3H); ^{13}C NMR δ 155.9 (2C), 148.9, 142.6, 137.1, 136.6, 128.8, 128.4, 126.3, 122.6, 120.6, 117.5, 114.2, 106.8, 105.9 (2C), 94.4 (2C), 77.9, 77.1, 56.0 (2C), 55.9, 46.7, 38.4, 37.7, 29.2, 28.3, 27.7, 27.3, 23.1, 19.9; HRMS (EI$^+$) calcd for $C_{32}H_{42}O_7$ [M$^+$] 538.2931; found 538.2930.

5.8. Analogue 6

To a solution of stilbene **30** (37 mg, 0.07 mmol) in MeOH (2 mL) at rt was added TsOH (60 mg, 0.3 mmol) and the solution was allowed to stir for 18 h. The reaction was quenched by addition of $NaHCO_3$ (satd) and extracted with EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), concentrated in vacuo, and purified by flash column chromatography (50% EtOAc/hexanes) to afford analogue **6** (21 mg, 67%) as a yellow oil: ^{1}H NMR δ 6.83–6.65 (m, 4H), 6.49 (s, 2H), 5.99–5.88 (m, 1H), 5.14–5.03 (m, 2H), 3.79 (s, 3H), 3.41–3.33 (m, 3H), 2.65–2.62 (m, 2H), 2.07–2.02 (m, 1H), 1.82–1.51 (m, 4H), 1.14 (s, 3H), 1.02 (s, 3H), 0.89 (s, 3H); ^{13}C NMR δ 155.3 (2C), 148.8, 142.7, 137.4, 136.1, 128.8, 128.6, 125.8, 122.7, 120.7, 115.8, 111.4, 107.0, 106.1 (2C), 78.1, 77.2, 56.0, 46.7, 38.4, 37.6, 29.2, 28.2, 27.6, 27.3, 23.1, 19.8; HRMS (EI$^+$) calcd for $C_{28}H_{34}O_5$ [M$^+$] 450.2406; found 450.2408.

5.9. Benzyl alcohol 36

To a solution of known ester **35**[22] (202 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added DIPEA (0.4 mL, 2.3 mmol). After stirring for 30 min, MOMCl (0.2 mL, 2.6 mmol) was added. The reaction was allowed to warm to rt, stirred for 12 h, then quenched by addition of H_2O and extracted with EtOAc. The organic layers were combined and washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. To a solution of the crude ester dissolved in THF (20 mL) at 0 °C was added $LiAlH_4$ (276 mg, 7.3 mmol), and the reaction was allowed to warm to rt and stirred for 4 h. The reaction was quenched by the addition of H_2O and extracted with EtOAc. The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to afford alcohol **36** (155 mg, 78% over two steps) as an off-white solid: ^{1}H NMR δ 7.54 (d, J = 2.2 Hz, 1H), 7.20 (m, 1H), 6.91 (m, 1H), 6.85 (dd, J = 2.2, 1.0 Hz, 1H), 5.30 (s, 2H), 4.74 (s, 2H), 3.52 (s, 3H), 1.97 (br s, 1H); ^{13}C NMR δ 156.4, 150.9, 144.1, 138.7, 117.8, 106.3, 104.1, 103.9, 94.7, 65.6, 56.2; HRMS (EI$^+$) calcd for $C_{11}H_{12}O_4$ [M$^+$] 208.0736; found 208.0733.

5.10. Benzyl alcohol 37

To a solution of benzofuran **36** (387 mg, 1.9 mmol) in MeOH (2 mL) at rt was added NH_4OAc (72 mg, 0.9 mmol), 10% Pd–C (40 mg, cat.), and excess H_2, and the resulting mixture was agitated for 12 h. The reaction mixture then was filtered through Celite, washed with EtOAc, and concentrated in vacuo to afford compound **37** (373 mg, 95%) as a light yellow oil: ^{1}H NMR δ 6.57 (s, 1H), 6.45 (s, 1H), 5.15 (s, 2H), 4.56 (t, J = 8.7 Hz, 2H), 4.53 (s, 2H), 3.45 (s, 3H), 3.20 (br s, 1H), 3.13 (t, J = 8.7 Hz, 2H); ^{13}C NMR δ 161.5, 153.8, 142.9, 114.1, 105.1, 102.0, 94.1, 71.6, 65.0, 55.9, 27.0; HRMS (EI$^+$) calcd for $C_{11}H_{14}O_4$ [M$^+$] 210.0892; found 210.0895.

5.11. Phosphonate 38

To a solution of alcohol **37** (187 mg, 0.9 mmol) in THF (10 mL) was added NEt_3 (0.5 mL, 3.6 mmol) and LiBr (460 mg, 5.3 mmol), and the reaction was cooled to 0 °C. After 10 min, MsCl (0.2 mL, 2.2 mmol) was added slowly and the mixture was allowed to stir for 1 h. Additional LiBr (200 mg, 2.3 mmol) was added and the reaction mixture was allowed to stir for 45 min, then poured into Et_2O. The reaction mixture was quenched by addition of NH_4Cl (satd) and extracted with Et_2O. After the combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo, the resulting oil was dissolved in $P(OEt)_3$ (2 mL) and

heated at reflux overnight. The reaction was then allowed to cool to rt, poured into Et_2O, quenched by addition of H_2O, and extracted with Et_2O. After the combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo, final purification by flash column chromatography (2–3% MeOH/Et_2O) afforded phosphonate **38** (160 mg, 55%) as a light yellow solid: ^{1}H NMR δ 6.53 (m, 1H), 6.44 (m, 1H), 5.17 (s, 2H), 4.57 (t, J = 8.7 Hz, 2H), 4.04 (qd, J = 7.8, 7.1 Hz, 4H), 3.47 (s, 3H), 3.14 (dt, J = 8.8, 3.3 Hz, 2H), 3.07 (d, J_{PH} = 21.4 Hz, 2H), 1.28 (t, J = 7.0 Hz, 6H); ^{13}C NMR δ 161.5 (d, J_{CP} = 3.0 Hz), 153.7 (d, J_{CP} = 3.2 Hz), 132.7 (d, J_{CP} = 9.2 Hz), 113.7 (d, J_{CP} = 3.7 Hz), 108.2 (d, J_{CP} = 6.7 Hz), 105.0 (d, J_{CP} = 6.7 Hz), 94.3, 71.6, 61.9 (d, J_{CP} = 6.9 Hz, 2C), 55.9, 33.8 (d, J_{CP} = 138.1 Hz), 27.1 (d, J_{CP} = 1.0 Hz), 16.2 (d, J_{CP} = 6.2 Hz, 2C); ^{13}P NMR δ 26.2; HRMS (EI$^+$) calcd for $C_{15}H_{23}O_6P$ [M$^+$] 330.1232; found 330.1224.

5.12. Aldehyde 40

Alcohol **36** (121 mg, 0.6 mmol) was dissolved in CH_2Cl_2 (15 mL), and MnO_2 (980 mg, 11.3 mmol) was added. The reaction was allowed to stir for 5 h at rt, and then filtered through Celite and the pad was washed with CH_2Cl_2. The solvent was removed in vacuo to yield aldehyde **40** (114 mg, 95%) as a white solid: ^{1}H NMR δ 10.0 (s, 1H), 7.75 (d, J = 2.2 Hz, 1H), 7.70 (m, 1H), 7.45 (d, J = 1.1 Hz, 1H), 6.96 (dd, J = 2.2, 1.0 Hz, 1H), 5.37 (s, 2H), 3.54 (s, 3H); ^{13}C NMR δ 191.5, 155.8, 151.3, 147.4, 134.3, 124.4, 108.9, 106.5, 104.7, 94.8, 56.4. Anal. Calcd for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89. Found: C, 63.69; H, 4.95.

5.13. Analogue 14

To a solution of known stilbene **43**[14] (23 mg, 0.04 mmol) in MeOH (2 mL) at rt was added freshly ground Mg (50 mg, 0.9 mmol) and NH_4Cl (50 mg, 0.9 mmol). The reaction was allowed to stir until all the Mg had dissolved. Once TLC analysis indicated the disappearance of starting material, the reaction mixture was poured into NH_4Cl (satd), and EtOAc was added. The aqueous layer was acidified with 1 M HCl until the precipitate dissolved, and then was extracted with EtOAc. The combined organics extracts were washed with $NaHCO_3$ (satd) and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. To a solution of the resulting oil in MeOH (3 mL) was added TsOH (20 mg, 0.1 mmol), the reaction was allowed to stir for 2 days, and then was quenched by addition of $NaHCO_3$ (satd) and extracted with EtOAc. After the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo, final purification by flash column chromatography (40% EtOAc/hexanes) afforded analogue **14** (14 mg, 70% over two steps) as a yellow oil: ^{1}H NMR δ 6.53 (d, J = 1.6 Hz, 1H), 6.49 (d, J = 1.6 Hz, 1H), 6.26 (s, 2H), 5.28–5.24 (m, 1H), 3.79 (s, 3H), 3.45–3.38 (m, 3H), 2.80–2.72 (m, 4H), 2.69–2.66 (m, 2H), 2.13–2.09 (m, 1H), 1.89–1.58 (m, 5H), 1.82 (s, 3H), 1.75 (d, J = 0.9 Hz, 3H), 1.23 (s, 3H), 1.09 (s, 3H), 0.87 (s, 3H); ^{13}C NMR δ 154.8 (2C), 148.4, 141.7, 140.7, 135.1, 132.8, 122.4, 121.8, 121.0, 110.9, 109.7, 108.3 (2C), 78.1, 56.0, 46.8, 38.4, 37.7, 37.7, 37.2, 28.3, 27.4, 25.8, 23.1, 22.3, 19.8, 17.9, 14.3; HRMS (EI$^+$) calcd for $C_{30}H_{40}O_5$ [M$^+$] 480.2876; found 480.2885.

5.14. Analogue 16

A solution of 3dsB[13] (**3**, 19 mg, 0.03 mmol) in EtOH (2 mL) was stirred and irradiated with 365 nm UV-light. After 6 h the solvent was removed in vacuo and purification by flash column chromatography (33% EtOAC/hexanes) afforded some recovered starting material (5 mg, 27%) along with *cis*-stilbene **16** (10 mg, 52%) as a straw-colored oil: ^{1}H NMR (CD_3OD) δ 6.70 (s, 1H), 6.62 (s, 1H), 6.36 (d, J = 12.3 Hz, 1H), 6.32 (d, J = 12.3 Hz, 1H), 6.27 (s, 2H), 5.23 (t, J = 6.9 Hz, 1H), 5.08 (t, J = 6.9 Hz, 1H), 3.49 (s, 3H), 3.36–3.30 (m, 1H), 3.28 (d, J = 7.2 Hz, 2H), 2.64 (s, 1H), 2.61 (s, 1H), 2.07–1.92 (m, 6H), 1.83–1.77 (m, 1H), 1.75 (s, 3H), 1.73–1.66 (m, 2H), 1.64 (s, 3H), 1.58 (s, 3H), 1.18 (s, 3H), 1.06 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD) δ 157.1 (2C), 149.0, 143.0, 137.7, 134.8, 132.0, 130.5, 130.0, 129.7, 125.5, 124.6, 124.4, 123.4, 115.4, 111.3, 108.0 (2C), 78.7, 78.1, 56.0, 48.5, 41.1, 39.5, 38.9, 29.0, 27.9, 27.8, 25.9, 24.0, 23.1, 20.2, 17.7, 16.3, 14.8; HRMS (EI$^+$) calcd for $C_{35}H_{46}O_5$ [M$^+$] 546.3345; found 546.3351.

5.15. Cell culture

Cells were cultured in RPMI 1640 (SF-295) or F-12 (A549) media containing 10% fetal bovine serum, penicillin–streptomycin, amphotericin B, and L-glutamine at 37 °C in the presence of 5% CO_2.

5.16. Cytotoxicity assay

SF-295 and A549 cells were grown to 60% confluency before treatment with indicated compound concentrations. After 44 h, the media was aspirated and changed to RPMI 1640 without phenol red (SF-295) or F-12 (A549) with 0.06 mg/mL MTT salt (Calbiochem, San Diego, CA, USA). MTT stop solution (10% 1 N HCl, 10% triton X-100, and isopropyl alcohol) was added after 4 h of incubation with MTT containing media. Plates were wrapped and incubated overnight at 37 °C with gentle agitation. The optical density of each condition was determined spectrophotometrically at 540 nm and 650 nm. EC_{50} values were calculated using Calcusyn (Biosoft, Cambridge, UK).

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Supplementary data

Supplementary data (spectroscopic data and experimental conditions for preparation of compounds **7**–**13**, **15**, **24**, **25**, **29**, **31**, **32**, **39**, and **44** are provided, along with the two-cell data for compounds **6**–**16** and the 60-cell data for compounds **9** and **15**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.12.063.

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