Published in final edited form as:

Bioorg Med Chem. 2010 September 15; 18(18): 6734–6741. doi:10.1016/j.bmc.2010.07.056.

Fluorescent schweinfurthin B and F analogues with anti-proliferative activity

Joseph J. Topczewski^a, Craig H. Kuder^b, Jeffrey D. Neighbors^a, Raymond J. Hohl^{b,c}, and David F. Wiemer^{a,b,*}

^aDepartment of Chemistry, University of Iowa, Iowa City, IA 52242-1294, USA

^bDepartment of Pharmacology, University of Iowa, Iowa City, IA 52242-1294, USA

^cDepartment of Internal Medicine, University of Iowa, Iowa City, IA 52242-1294, USA

Abstract

The natural tetracyclic schweinfurthins are potent and selective inhibitors of cell growth in the National Cancer Institute's 60-cell line screen. Most of the natural schweinfurthins display unique and potent activity in the 60 cell-line screen of the National Cancer Institute. At this time, the mechanism or cellular target that underlies this activity has not yet been identified, and efforts to illuminate the schweinfurthins' mode of action would benefit from development of potent fluorescent analogues that could be readily visualized within cells. This report describes the synthesis of fluorescent analogues of schweinfurthins B and F, and demonstrates that these compounds retain the potent and differentially toxic activities against select human cancer cells that are characteristic of the natural schweinfurthins. In addition, the synthesis of control compounds that maintain parallel fluorescent properties, but lack the potent activity of the natural schweinfurthin is described. Use of fluorescence microscopy shows differences between the localization of the active and relatively inactive schweinfurthin analogues. The active compounds localize in peripheral puncta which may identify the site (or sites) of activity.

1. Introduction

Natural products have been a significant source of drug leads in oncology.¹ Indeed several commonly used anti-cancer agents are themselves natural products or are the result of drug development programs based upon a natural product. In some instances, natural products have identified a novel target or mechanism of action that is useful in treating human cancer. One such agent is paclitaxel, which was found to stabilize micro-tubules causing cell cycle arrest just prior to mitosis.² From this perspective, the discovery of the schweinfurthins and their potent anti-cancer activity at the National Cancer Institute (NCI) may offer a similar opportunity for discovery of a novel drug target and/or mechanism of action.³ Nine compounds are now part of the natural family, including schweinfurthin A–H and vedelianin (Figure 1).^{4–6} Those that contain a hexahydroxanthene substructure, such as schweinfurthin

© 2010 Elsevier Science. All rights reserved

*Corresponding author. Tel: 319-335-1365; Fax: 319-335-1270; david-wiemer@uiowa.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary Data

General experimental procedures, the ¹H and ¹³C NMR spectra for compounds **14–16**, **18**, **19**, **21–27**, **29–32**, **34–37**, and a table of compound purity data, can be found here. This material is available free of charge via the Internet at:

A (**1**) and B (**2**), were found to have potent activity in the NCI 60 cell-line screen. Perhaps of even greater importance, the pattern of activity against the 60 cell lines indicated a potentially novel molecular target or mechanism of activity.^{7,8} Due to the scarcity of the natural schweinfurthins, we have had an ongoing program aimed at synthesis of natural schweinfurthins and preparation of analogues. This effort has culminated in the reported syntheses of schweinfurthin B (**2**),⁹ C (**3**),¹⁰ E (**6**),⁹ F (**7**),¹¹ and G (**8**),¹² as well as the lead compound 3-deoxyschweinfurthin B (3dSB, **10**) and more than 50 other analogues.¹⁴⁻¹⁹

Recently the bis-stilbene **11** was prepared as a fluorescent analog of 3dSB (**10**), with the hope that this probe would aid in elucidation of the mechanism of action of these natural products.¹⁶ Unfortunately, the fluorescence emission maximum of this compound (416 nm) displayed significant overlap with autofluorescence of the SF – 295 human-derived glioma cell line, and the compound demonstrated a rapid loss of fluorescence upon irradiation. To overcome these limitations and improve the fluorescent characteristics of potential probes, further synthetic efforts based on this core structure have been undertaken. Here the design, synthesis, and initial biological results of these studies will be reported, along with preparation of some related compounds with similar fluorescence properties and less potent biological activity to serve as controls.

2. Synthesis

While the stilbene **11** did show improved fluorescence properties over the parent 3dSB (**10**), identification of a related compound with an emission maximum further shifted to lower energy and more resistant to photo-bleaching would be attractive. Placement of an E-ring substituent on the para position would be expected to allow transmission of electronic effects throughout the stilbene system. Therefore, to increase the Stokes shift and secure an emission maximum further to the red, the *m*-phenol was replaced with a *p*-nitro group. This substitution was based upon the hypothesis that an effective electron withdrawing group across the bis-stilbene system would complement the electron donating C-ring ethers and improve the fluorescent properties of the compound.^{20, 21}

Synthesis of compound **19** began with the known aldehyde **12** (Scheme 1).¹⁶ Treatment with *p*-nitrobenzyl phosphonate²² (**13**) under Horner-Wadsworth-Emmons (HWE) conditions gave the protected stilbene **14**. After deprotection of the silyl ether **14** to the free alcohol **15**, a three-step procedure was used to convert the benzyl alcohol to the corresponding phosphonate **16** through the intermediate mesylate and iodide. An HWE condensation of phosphonate **16** with the known aldehyde **17**,¹³ proceeded smoothly to afford the bis-stilbene **18**. Hydrolysis of the methoxymethyl (MOM) acetals upon treatment with TsOH in methanol provided the target bis-stilbene **19**.

Unfortunately, compound **19** discolored quickly and undergoes decomposition upon storage. This tendency for decomposition has been observed with other schweinfurthin analogues, and had been attributed to the presence of the resorcinol substructure. Previous work has shown that at least one phenolic group was important to the differential activity of the schweinfurthins in the 60 cell-line screen.¹⁴ Several analogs with one resorcinol oxygen present as a methyl ether have been prepared, and this substitution has been shown to retain potent and specific activity in the schweinfurthin F series.¹¹ Therefore, preparation of a bis-stilbene with a D-ring mono methyl ether became attractive.

Synthesis of the desired mono methyl ether began with the known benzylic alcohol **20** (Scheme 2). Directed ortho metalation (DoM)²³ followed by treatment of the intermediate anion with DMF afforded the desired aldehyde **21** in modest yield. While this DoM reaction is lower yielding than the halogen-metal exchange approach used in our previous work,¹⁶

this strategy allows more facile access to the desired aldehyde. Compound **20** and its precursors have more favorable solubility properties than the brominated intermediates required for synthesis of aldehyde **12**, the DoM approach provides only starting material and product, and the DoM strategy can be performed without protecting the benzylic alcohol. Treatment of aldehyde **21** with phosphonate **13** and base gave the benzylic alcohol **22** in excellent yield. Application of the standard 3-step procedure for conversion of the alcohol to the phosphonate afforded compound **23**. Use of DMF as the solvent for the final Arbuzov reaction in this sequence greatly improves the yield.

Preparation of phosphonate **23** set the stage for synthesis of the *O*-methyl analogue of nitro-stilbene **19**. Condensation of phosphonate **23** with aldehyde **17** afforded the protected schweinfurthin **24** in good yield. Removal of the single MOM protecting group afforded the 5'-*O*-methyl analogue **25** in excellent yield. This compound displayed only slightly diminished activity relative to the parent resorcinol (*vide infra*) and, more importantly, demonstrated much improved stability.

With the key problems presented by compound **11** and **19** addressed, it was possible to prepare further analogues that could be utilized to probe the mechanism of schweinfurthin activity. The *p*-nitro stilbene **25** could, in principle, be used to identify the sub-cellular localization of the schweinfurthins. For such experiments to be truly informative however, a control compound with similar fluorescent properties but lacking the essential pharmacophore would be very helpful. Reduction of the nitro group would allow entrée to amine analogues which would vary the electronic properties of the bis-stilbenes and provide a site for further SAR studies. Rounding out the set of desired compounds would be the amine analogue of the control compound, along with the nitro- and amine-containing compounds based on the newly available schweinfurthin B A-ring diol system.⁹ These considerations led to identification of stilbene **35** as a target with potentially greater activity, to stilbenes **30** and **32** as relatively inactive but fluorescent controls, and to stilbenes **27** and **37** as amine-substituted targets.

The synthesis of these additional analogues was relatively straightforward. Condensation of the phosphonate **23** with the known aldehydes **28** and **33** gave the protected stilbenes **29** and **34**. Hydrolysis of the MOM acetal of each of the nitro compounds **29** and **34** afforded the targets **30** and **35** in good yields. Reduction of the nitro groups of compounds **24**, **29**, and **34** with Zn⁰ in ammonium chloride²⁴ afforded the desired amines **26**, **31**, and **36**. Deprotection of these compounds under standard conditions was uneventful, leading to compounds **27**, **32**, and **37**. This strategy provided the target compounds in excess of 95% chemical purity, with the exception of compound **37** (93%), and greater than 95% enantiomeric purity.

All of the target compounds exhibited significant fluorescence. The nitro compounds typically had an absorption maximum of about 430 nm and an emission maximum of ~575 nm, while the amines exhibited absorption maxima of 375 and typically had emission maxima ~495. Thus the nitro compounds **19**, **25**, **30**, and **35** displayed larger Stokes shifts (~150 nm) vis-a-vis the amines **27**, **32** and **37** (~120 nm). Of greater significance to the cellular localization studies, the nitro substituted compounds also demonstrate significantly red-shifted absorption and emission maxima compared to the previously synthesized bis-stilbene **11**. This shift in fluorescence emission allowed cellular studies on these compounds without interference from autofluorescence.²⁵ The fluorescence maxima of the amines is not shifted as greatly, but the utility of the nitro compounds may render this a moot point.

3. Biological Results and Discussion

The anti-cancer activities of many natural and synthetic schweinfurthins have been evaluated in the National Cancer Institute's 60 cell-line screen. Because there often is a long waiting time for this assay, we have described a two-cell line screen comprised of a sensitive (SF-295) and moderately insensitive (A549) cell line.¹⁷ The two cell-line assay measures schweinfurthin-like activity and is comparable to the discrimination yielded by the NCI 60 cell screen.¹⁷ The schweinfurthin analogues, compounds **19**, **25**, **27**, **35**, and **37**, display potent effects on MTT activity in SF-295 cells after 48 hours of treatment (Table 1). In this assay, SF-295 cells are especially sensitive to the nitro bis-stilbene compounds **19**, **25**, and **35**, which have ED₅₀ values of 0.05, 0.1, and 0.04 μ M, respectively. The amine bis-stilbene compounds **27** and **37** display similar ED₅₀ values. As expected, compounds **30** and **32** lacking the hexahydroxanthene structure display greatly diminished activity in comparison to the other schweinfurthin analogues (Table 1). Consistent with the natural schweinfurthins, the fluorescent analogues (**19**, **25**, **27**, **35**, and **37**) display decreased activity against the A549 cells in comparison to the SF-295 cells. The nitro bis-stilbenes are at least as potent in comparison to the amine bis-stilbenes. Control compounds **30** and **32** do not alter A549 MTT activity at concentrations as high as 10 μ M.

The fluorescent schweinfurthins may serve as probes for intracellular localization. Indeed, compound **25** displays peri-nuclear localization with distinct peripheral puncta, as demonstrated in Figure 2 (A-D). In comparison, compound **30**, which lacks schweinfurthin-like activity at concentrations tested, localizes extensively in the peri-nuclear nuclear region with minimal diffuse fluorescence in the periphery of the cell shown in Figure 2 (E-H). Based on the difference in activity and localization, the peripheral fluorescence displayed by compound **25** may identify the schweinfurthin site(s) of action. Although the active analogue is more dispersed to peripheral regions of the cell than is the less active control compound, it remains to be determined whether this identifies a binding target that is itself peripheral or a mislocalized perinuclear target.

4. Conclusions

The synthesis of a small set of fluorescent analogues based on the schweinfurthins has been accomplished. Data obtained from our two-cell line screen supports our prior observations that one of the phenol groups of the right half of the molecule can be replaced with a methyl ether without great loss of activity and with a contemporaneous increase in stability. In addition, compounds with the A-ring diol (**35** and **37**) are slightly more potent than the corresponding 3-deoxy compounds (**25** and **27**) in the SF-295 cells. Importantly, compounds **19**, **25**, **27**, **35**, and **37** retain the potency and differential activity displayed by the natural schweinfurthins. Due to these characteristics and their fluorescent properties, these compounds can be utilized as intracellular probes for sites of schweinfurthin activity. Microscopic examination of the fluorescence from compound **25** demonstrates that localization is both peri-nuclear and peripheral. The peripheral localization of **25** is unique when compared to the relatively inactive compound **30**; therefore, these sites may label the target of schweinfurthin activity. Based on puncta and dispersed nature of the observed fluorescence, compound **25** may localize in lysosomes or bind to protein(s) involved in trafficking. These possibilities will be investigated and described in due course.

5. Experimental Procedures and Methods

5.1 Protected Stilbene 14

A suspension of NaH (109 mg, 2.6 mmol, 60% in oil), and 15-crown-5 (1 drop) in THF was cooled to 0 °C. To this was added aldehyde **12** (245 mg, 0.66 mmol) and the known

phosphonate **13** (181 mg, 0.66 mmol) in THF (1.5 mL). After the mixture was allowed to stir for 45 min, 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 (2:1 hexanes:EtOAc) gave the stilbene **14** (197 mg, 61%) as a bright yellow oil: ¹H NMR δ 8.21–8.18 (m, 2H), 7.65 – 7.62 (m, 4H), 6.88 (s, 2H), 5.30 (s, 4H), 4.74 (s, 2H), 3.53 (s, 6H), 0.98 (s, 9H), 0.13 (s, 6H); ¹³C NMR δ 156.4 (2C), 146.1, 145.9, 143.8, 129.5, 126.4 (2C), 124.7, 123.9 (2C), 113.9, 105.6 (2C), 94.7 (2C), 64.5, 56.1 (2C), 25.7 (3C), 18.2, –5.4 (2C); HRMS (EI) calcd for C₂₅H₃₅NO₇Si (M⁺) 489.2183 found 489.2173.

5.2 Benzylic alcohol 15

Silyl ether **14** (155 mg, 0.32 mmol) was dissolved in THF and the solution was cooled to 0 °C. To this solution was added TBAF (0.4 mL, 1.00 M in THF), and after 4 h the reaction was quenched by addition of NH₄Cl (sat.). After extraction with EtOAc, the combined organic extracts were washed with water and brine, dried (MgSO₄), and concentrated *in vacuo* to give the desired benzylic alcohol **15** (120 mg, 100%) as a clear oil: ¹H NMR ((CD₃)₂CO) δ 8.25–8.21 (m, 2H), 7.80 – 7.77 (m, 4H), 6.92 (s, 2H), 5.35 (s, 4H), 4.66 (d, *J* = 5.4 Hz, 2H), 4.43 (t, *J* = 5.4 Hz, 1H), 3.51 (s, 6H); ¹³C NMR ((CD₃)₂CO) δ 157.2 (2C), 146.9, 145.5, 130.0, 127.2 (2C), 125.3, 124.5 (2C), 114.4, 106.6 (2C), 100.5, 95.1 (2C), 64.1, 56.2 (2C); HRMS (EI) calcd for C₁₉H₂₁NO₇ (M⁺) 375.1318 found 375.1315.

5.3 Phosphonate 16

Methanesulfonyl chloride (0.04 mL, 0.52 mmol) was added to a solution of benzylic alcohol **15** (120 mg, 0.32 mmol) and Et₃N (0.2 mL, 1.4 mmol) in THF at 0 °C. The reaction mixture was allowed to warm to room temperature over 1 h, quenched by addition of H₂O, and extracted with EtOAc. The combined organic layers were washed with NH₄Cl (sat.) and brine, dried (MgSO₄), and concentrated *in vacuo*. The resulting residue and NaI (119 mg, 0.80 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 toluene (2 mL) the reaction mixture was heated at 110 °C for 24 h. After the solution was allowed to cool to rt, the excess phosphite was removed at high vacuum. The initial orange/red oil was purified by flash chromatography (1:2 hexanes:EtOAc to 100% EtOAc) to afford phosphonate **16** (99 mg, 88%) as an orange oil: ¹H NMR δ 8.21 – 8.18 (m, 2H), 7.64 – 7.61 (m, 4H), 6.81 – 6.80 (m, 2H), 5.28 (s, 4H), 4.07 (m, 4H), 3.52 (s, 6H), 3.13 (d, *J*_{HP} = 22 Hz, 2H), 1.29 (t, *J* = 7.0 Hz, 6H); ¹³C NMR δ 156.4 (d, *J*_{CP} = 3.9 Hz, 2C), 146.4, 145.8, 133.6 (d, *J*_{CP} = 9.2 Hz), 129.7, 126.6 (2C), 124.4, 124.0 (2C), 114.2 (d, *J*_{CP} = 3.6 Hz), 109.9 (d, *J*_{CP} = 6.2 Hz, 2C), 94.7 (2C), 62.2 (d, *J*_{CP} = 6.7 Hz, 2C), 56.4 (2C), 34.0 (d, *J*_{CP} = 138 Hz), 16.4 (d, *J*_{CP} = 6.3 Hz, 2C); ³¹P NMR δ 26.3; HRMS (EI) calcd for C₂₃H₃₀NO₉P (M⁺) 495.1658 found 495.1656.

5.4 Protected nitro stilbene 18

A suspension of NaH (60 mg, 1.5 mmol, 60% in oil), and 15-crown-5 (1 drop) in THF (5 mL) was cooled to 0 °C. To this mixture was added a solution of aldehyde **17** (17 mg, 0.06 mmol) and phosphonate **15** (28 mg, 0.06 mmol) in THF (1 mL). The resulting mixture was allowed to warm to rt and stirred a total of 6 hr. After water was added dropwise, 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:EtOAc) gave the stilbene **18** (23 mg, 59%) as an orange oil: ¹H NMR δ 8.21 (d, *J* = 8.3 Hz, 2H), 7.66 (s, 2H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.07 – 7.01 (m, 3H), 6.93 –

6.87 (m, 3H), 5.35 (s, 4H), 3.92 (s, 3H), 3.57 (s, 6H), 3.48 – 3.43 (m, 1H), 2.75 – 2.72 (m, 2H), 2.20 – 2.13 (m, 1H), 1.92 – 1.82 (m, 2H), 1.76 – 1.60 (m, 3H), 1.28 (s, 3H), 1.12 (s, 3H), 0.91 (s, 3H); ^{13}C NMR δ 156.8 (2C), 149.0, 146.3, 146.0, 143.0, 139.3, 129.9, 129.7, 128.5, 126.6 (2C), 125.7, 124.6, 124.0 (2C), 122.6, 121.0, 114.6, 106.9, 106.1 (2C), 94.8 (2C), 77.9, 77.2, 56.4 (2C), 56.0, 46.7, 38.4, 37.6, 28.1, 27.2, 23.1, 19.9, 14.3; HRMS (EI) calcd for $\text{C}_{37}\text{H}_{43}\text{NO}_9$ (M^+) 645.2938 found 645.2952.

5.5 Nitro stilbene 19

To a solution of protected stilbene **18** (20 mg, 0.03 mmol) in MeOH was added pTsOH (30 mg, 0.17 mmol), and the resulting solution was stirred at rt for 6 hr. The reaction was quenched by addition of sat. NaHCO_3 , extracted with EtOAc, and the organic phase was washed with brine and dried (MgSO_4). Concentration in vacuo, followed by final purification by column chromatography (2:1 to 1:1 hexanes:EtOAc) afforded the bis-stilbene **19** (12 mg, 78%) as a slightly yellow oil: UV (EtOH) λ_{max} (log ϵ) 426 (4.42); λ_{em} 593; ^1H NMR ($(\text{CD}_3)_2\text{CO}$) δ 9.06 (s, 2H), 7.05 – 6.78 (m, 6H), 3.78 (s, 3H), 3.40 – 3.35 (m, 1H), 2.71 – 2.68 (m, 2H), 1.82 – 1.58 (m, 6H), 1.18 (s, 3H), 1.09 (s, 3H), 0.87 (s, 3H); ^{13}C NMR ($(\text{CD}_3)_2\text{CO}$) δ 158.6 (2C), 150.0, 147.7, 146.7, 144.1, 140.2, 130.5, 129.5, 128.5, 127.2 (2C), 126.7, 126.3, 124.7 (2C), 123.6, 121.9, 111.7, 108.2, 105.9 (2C), 77.7, 77.5, 56.0, 55.4, 47.7, 39.0, 38.6, 27.8, 23.7, 20.3, 14.8; HRMS (EI) calcd for $\text{C}_{33}\text{H}_{35}\text{O}_7\text{N}$ (M^+) 557.2414 found 557.2422.

5.6 Aldehyde 21

To a solution of the alcohol **20** (0.45 g, 2.3 mmol) in THF at 0 °C (194 mg, 43%): ^1H NMR δ 10.4 (s, 1H), 6.74 (s, 1H), 6.65 (s, 1H), 5.25 (s, 2H), 4.69 (s, 2H), 3.82 (s, 3H), 3.49 (s, 3H), 3.03 (br s, 1H); ^{13}C NMR δ 189.1, 162.0, 159.9, 150.4, 113.7, 104.7, 102.5, 94.6, 64.5, 56.5, 55.9. Anal. Calcd for $\text{C}_{11}\text{H}_{14}\text{O}_5$: C, 58.40; H, 6.24. Found: C, 58.69; H, 6.32. was added *n*-BuLi (2.8 mL, 2.0 M in hexanes) dropwise over 1 min. After 25 min, the solution was cooled to –30 °C and DMF (0.23 mL, 3.0 mmol, containing some CaH_2 as a drying agent) was added. The reaction was allowed to progress for an additional 1 hr, and then quenched by addition of NH_4Cl (sat.). After the aqueous phase was extracted with EtOAc, 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 (2:1 to 1:2 hexanes:EtOAc) afforded aldehyde **21** (192 mg, 37%) as a clear oil, along with a significant amount of recovered starting material

5.7 Benzyl Alcohol 22

To a solution of aldehyde **21** (128 mg, 0.57 mmol) in THF (12 mL) at rt was added 15-crown-5 (0.01 mL), diethyl 4-nitrobenzylphosphonate (**13**, 203 mg, 0.74 mmol), followed by NaH (220 mg, 5.5 mmol, 60% in oil), which resulted in the rapid appearance of a maroon color. After 8 min, the reaction was quenched by slow addition of water. The resulting solution was extracted with EtOAc, and the combined organic phases were washed with brine, dried (MgSO_4), and concentrated in vacuo. Final purification by column chromatography (6:4 hexanes/THF) afforded compound **22** (191 mg, 98%) as an orange solid: ^1H NMR δ 8.20 – 8.18 (m, 2H), 7.64 – 7.61 (m, 4H), 6.80 (s, 1H), 6.69 (s, 1H), 5.29 (s, 2H), 4.70 (s, 2H), 3.94 (s, 3H), 3.53 (s, 3H); ^{13}C NMR δ 159.2, 156.6, 145.9, 142.9, 129.8, 126.6 (2C), 124.5, 124.3, 124.0 (2C), 113.8, 105.5, 103.1, 94.8, 65.2, 56.4, 55.8; HRMS (EI) calcd for $\text{C}_{18}\text{H}_{19}\text{NO}_6$ (M^+) 345.1212 found 345.1216.

5.8 Phosphonate 23

To a solution of benzyl alcohol **22** (193 mg, 0.56 mmol) in THF at rt was added TEA (0.2 mL, 1.4 mmol) followed by MsCl (0.1 mL, 1.3 mmol). After 50 min, the reaction was

quenched by addition of water. The resulting solution was extracted with EtOAc, washed with brine, dried (MgSO₄), and concentrated in vacuo. This material was used immediately without further purification. The residue from the above step was dissolved in anhydrous acetone (8 mL) and NaI (210 mg, 1.4 mmol) was added at rt in the dark. After 1 h, the reaction was quenched by addition of water, the resulting solution was extracted with EtOAc, and was washed with brine. The organic phase was dried (MgSO₄) and concentrated in vacuo in the dark. This material was used immediately in the next step without further purification. The residue from the above step was dissolved in DMF (3 mL), P(OEt)₃ (0.4 mL, 2.3 mmol) was added, and the mixture was heated to 115 °C. After 17 h, an additional portion of P(OEt)₃ (0.4 mL, 2.3 mmol) was added. The reaction was allowed to cool to rt after an additional 3.5 h and concentrated in vacuo. Final purification by column chromatography (1% MeOH, 20% hexanes, 79% EtOAc) afforded the desired phosphonate **23** (245 mg, 95% over 3 steps) as a bright yellow oil: ¹H NMR δ 8.20 – 8.17 (m, 2H), 7.63 – 7.61 (m, 4H), 6.75 (s, 1H), 6.63 (s, 1H), 5.27 (s, 2H), 4.11 – 4.01 (m, 4H), 3.92 (s, 3H), 3.51 (s, 3H), 3.13 (d, *J*_{HP} = 22 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 6H); ¹³C NMR δ 158.8 (d, *J*_{CP} = 3.8 Hz), 156.4 (d, *J*_{CP} = 3.4 Hz), 146.3, 146.0, 133.5 (d, *J*_{CP} = 9.1 Hz), 129.7, 126.6 (2C), 124.5 (d, *J*_{CP} = 1.8 Hz), 124.0 (2C), 123.0, 109.1 (d, *J*_{CP} = 7.1 Hz), 106.5 (d, *J*_{CP} = 6.6 Hz), 94.9, 62.2, 62.1, 56.1 (d, *J*_{CP} = 34 Hz, 2C), 34.5 (d, *J*_{CP} = 138 Hz), 16.4 (d, *J*_{CP} = 6.0 Hz, 2C); ³¹P NMR δ 26.3; HRMS (EI) calcd for C₂₂H₂₈NO₈P (M⁺) 465.1553 found 465.1552.

5.9 Protected stilbene **24**

To a solution of aldehyde **17** (80 mg, 0.26 mmol) and phosphonate **23** (100 mg, 0.25 mmol) in THF at rt was added 15-crown-5 (0.01 mL) followed by NaH (63 mg, 1.6 mmol, 60% in oil), and after 75 min the reaction was quenched by addition of water. The resulting solution was extracted with EtOAc and the combined organic phases were washed with brine. After the organic phase was dried (MgSO₄) and concentrated in vacuo, final purification by column chromatography (1:1 hexanes/EtOAc) afforded recovered phosphonate **23** (12 mg, 12%) and compound **24** (94 mg, 71%) as an orange solid: ¹H NMR δ 8.22 – 8.18 (m, 2H), 7.67 – 7.63 (m, 4H), 7.09 – 7.04 (m, 1H), 6.97 – 6.90 (m, 4H), 6.78 – 6.74 (m, 1H), 5.36 (s, 2H), 4.00 (s, 3H), 3.92 (s, 3H), 3.58 (s, 3H), 3.48 – 3.44 (m, 1H), 2.78 – 2.73 (m, 2H), 2.19 – 2.13 (m, 1H), 1.92 – 1.82 (m, 2H), 1.73 – 1.54 (m, 3H), 1.28 (s, 3H), 1.13 (s, 3H), 0.91 (s, 3H); ¹³C NMR δ 159.1, 156.8, 149.0, 146.2, 146.1, 143.0, 139.1, 129.8, 129.4, 128.4, 126.5 (2C), 125.8, 124.6, 124.0 (2C), 122.7, 120.9, 113.8, 106.9, 105.4, 102.4, 94.8, 77.9, 77.2, 56.4, 56.0, 55.8, 46.7, 38.4, 37.6, 28.2, 27.3, 23.1, 19.8, 14.3; HRMS (EI) calcd for C₃₆H₄₁NO₈ (M⁺) 615.2832, found 615.2837.

5.10 Nitro schweinfurthin **25**

To a solution of compound **24** (16 mg, 0.03 mmol), in CH₃OH (2.5 mL) and EtOAc (0.5 mL) was added TsOH·H₂O (32 mg, 0.16 mmol). After 24 h, the reaction was quenched by addition of NaHCO₃ and concentrated in vacuo. The resulting solution was extracted with EtOAc, and the combined organic phase was washed with brine, dried (MgSO₄), and concentrated in vacuo. Final purification by column chromatography (1:1 hexanes/EtOAc) afforded compound **25** (13 mg, 85%) as a dark orange solid: UV (EtOH) λ_{max} (log ε) 429 (4.25); λ_{em} 557; ¹H NMR δ 8.20 (d, *J* = 8.8 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 16.8 Hz, 1H), 7.54 (d, *J* = 16.8 Hz, 1H), 7.01 (d, *J* = 16.0 Hz, 1H), 6.89 (s, 1H), 6.88 (s, 1H), 6.85 (d, *J* = 16.0 Hz, 1H), 6.65 (s, 1H), 6.63 (s, 1H), 5.50 (br d, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.46 – 3.44 (m, 1H), 2.75 – 2.72 (m, 2H), 2.17 – 2.14 (m, 2H), 1.90 – 1.69 (m, 4H), 1.27 (s, 3H), 1.11 (s, 3H), 0.90 (s, 3H); ¹³C NMR δ 159.2, 155.0, 149.0, 146.3, 145.5, 143.1, 139.1, 129.9, 129.2, 128.4, 126.6 (2C), 125.4, 124.8, 124.0 (2C), 122.7, 120.9, 111.8, 106.9, 106.5, 101.5, 78.0, 77.7, 56.0, 55.8, 46.7, 38.4, 37.6, 28.3, 27.3, 23.1, 19.9, 14.3; HRMS (ESI) *m/z* calcd for C₃₄H₃₇NO₇ (M⁺) 571.2570, found 571.2567.

5.11 Protected amine 26

To a solution of compound **24** (33 mg, 0.05 mmol) in acetone (3 mL) was added NH_4Cl (sat., 1 mL) followed by Zn^0 dust (67 mg, 1.0 mmol) and the mixture was heated to reflux. After 70 min, the solution was allowed to cool and decanted into a separatory funnel. The resulting solution was extracted with EtOAc, and the combined organic phases were washed with brine, dried (MgSO_4), and concentrated in vacuo. Final purification by column chromatography (4:6 hexanes/EtOAc to 99:1 EtOAc/MeOH) afforded amine **26** (25 mg, 80%) as an orange oil: ^1H NMR δ 7.51 (d, J = 16.4 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 16.4 Hz, 1H), 7.01 (d, J = 16.4 Hz, 1H), 6.92 – 6.88 (m, 4H), 6.76 (d, J = 0.8 Hz, 1H), 6.67 (d, J = 8.0 Hz, 2H), 5.30 (s, 2H), 3.94 (s, 3H), 3.90 (s, 3H), 3.54 (s, 3H), 3.42 (dd, J = 11.8, 4.0 Hz, 1H), 2.72 (m, 2H), 2.16 – 2.11 (m, 1H), 1.88 – 1.81 (m, 3H), 1.73 – 1.60 (m, 2H), 1.25 (s, 3H), 1.10 (s, 3H), 0.89 (s, 3H); ^{13}C NMR δ 158.4, 156.0, 148.9, 145.6, 142.6, 136.8, 132.4, 129.9, 128.7, 128.5, 127.5 (2C), 126.2, 122.6, 120.6, 116.3, 115.7, 115.1 (2C), 106.7, 105.9, 102.7, 94.9, 77.9, 77.0, 56.2, 55.9, 55.7, 46.7, 38.3, 37.6, 28.2, 27.3, 23.1, 19.8, 14.3; HRMS (ESI) m/z calcd for $\text{C}_{36}\text{H}_{43}\text{NO}_6$ (M^+) 585.3090, found 585.3088.

5.12 Amine 27

To a solution of amine **26** (22 mg, 0.036 mmol), in CH_3OH (2 mL) and EtOAc (0.5 mL) was added $\text{TsOH}\cdot\text{H}_2\text{O}$ (43 mg, 0.13 mmol). After 23 h, the reaction was quenched by addition of NaHCO_3 and concentrated in vacuo. The resulting solution was extracted with EtOAc, and the combined organic phases were washed with brine. The organic phase was dried (MgSO_4) and concentrated in vacuo. This afforded schweinfurthin **26** (20 mg, 100%) without further purification as a dark orange solid: UV (EtOH) λ_{max} (log ϵ) 377 (4.53); λ_{em} 493; ^1H NMR δ 7.33 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 16.8 Hz, 1H), 7.12 (d, J = 16.8 Hz, 1H), 6.95 (d, J = 16.8 Hz, 1H), 6.87 – 6.81 (m, 3H), 6.67 – 6.65 (m, 3H), 6.59 (s, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.41 (dd, J = 11.8, 3.4 Hz, 1H), 2.71 (m, 2H), 2.14 – 2.10 (m, 1H), 1.87 – 1.83 (m, 2H), 1.71 – 1.58 (m, 2H), 1.24 (s, 3H), 1.09 (s, 3H), 0.87 (s, 3H); ^{13}C NMR δ 159.5, 155.6, 150.0, 147.0, 143.8, 138.4, 133.3, 130.1, 129.9, 129.8, 128.7 (2C), 127.3, 123.8, 121.8, 117.9, 116.3 (2C), 114.4, 108.1, 107.7, 102.2, 79.0, 78.2, 57.1, 56.8, 47.8, 39.4, 38.7, 29.3, 28.4, 24.2, 20.9, 15.3; HRMS (ESI) m/z calcd for $\text{C}_{34}\text{H}_{39}\text{NO}_5$ (M^+) 541.2828, found 541.2835.

5.13 Stilbene 29

To a solution of 3,4-dimethoxybenzaldehyde (**28**, 50 mg, 0.26 mmol) and phosphonate **23** (100 mg, 0.25 mmol) in THF (4 mL) at rt was added 15-crown-5 (0.01 mL) followed by NaH (57 mg, 1.4 mmol, 60% in oil), and after 65 min the reaction was quenched by addition of water. The resulting solution was extracted with EtOAc, and the combined organic phases were washed with brine. The organic phase was dried (MgSO_4) and concentrated in vacuo. Final purification by column chromatography (3:1 hexanes/EtOAc to EtOAc) afforded recovered phosphonate **23** (27 mg, 27%) and compound **29** (94 mg, 71%) as an orange solid: ^1H NMR δ 8.19 (d, J = 8.8 Hz, 2H), 7.66 (s, 2H), 7.63 (d, J = 8.8 Hz, 2H), 7.11 – 7.07 (m, 3H), 6.96 (s, 1H), 6.96 (s, 1H), 6.87 (d, J = 8.0 Hz, 1H), 6.78 (s, 1H), 5.34 (s, 2H), 3.99 (s, 3H), 3.96 (s, 3H), 3.91 (s, 3H), 3.56 (s, 3H); ^{13}C NMR δ 159.1, 156.8, 149.1, 149.0, 146.1, 146.1, 138.9, 129.9, 129.5, 129.5, 126.6 (2C), 126.3, 124.6, 124.0 (2C), 120.2, 113.9, 111.1, 108.5, 105.4, 102.4, 94.8, 56.4, 55.9, 55.8, 55.7; HRMS (ESI) m/z calcd for $\text{C}_{27}\text{H}_{27}\text{NO}_7$ (M^+) 477.1788, found 477.1771.

5.14 Compound 30

To a solution of compound **29** (20 mg, 0.042 mmol), in CH_3OH (4 mL) and EtOAc (2 mL) was added $\text{TsOH}\cdot\text{H}_2\text{O}$ (54 mg, 0.28 mmol) and the solution was stirred at rt. After 18.5 h,

the reaction was quenched by addition of NaHCO_3 and concentrated in vacuo. The resulting solution was extracted with EtOAc, and the combined organic phases were washed with brine. After the organic phase was dried (MgSO_4) and concentrated in vacuo, final purification by column chromatography (6:4 hexanes/EtOAc to 4:6 hexanes/EtOAc) afforded compound **30** (16 mg, 88%) as a dark orange solid: UV (EtOH) λ_{max} (log ϵ) 431 (4.39); λ_{em} 579; ^1H NMR ($(\text{CD}_3)_2\text{CO}$) δ 8.22 (d, $J = 9.2$ Hz, 2H), 7.81 (s, 2H), 7.76 (d, $J = 9.2$ Hz, 2H), 7.25 (d, $J = 2.0$ Hz, 1H), 7.19 (d, $J = 16.0$ Hz, 1H), 7.00 (dd, $J = 8.4, 2.0$ Hz, 1H), 7.04 (d, $J = 16.4$ Hz, 1H), 6.95 (d, $J = 8.4$ Hz, 1H), 6.85 (d, $J = 1.2$ Hz, 1H), 6.81 (d, $J = 1.2$ Hz, 1H), 4.00 (s, 3H), 3.87 (s, 3H), 3.83 (3H); ^{13}C NMR ($(\text{CD}_3)_2\text{CO}$) δ 160.5, 158.1, 150.7, 150.6, 147.4, 147.1, 140.3, 131.2, 130.3, 129.3, 127.4 (2C), 127.1, 126.0, 124.8 (2C), 121.1, 112.7, 110.4, 107.6, 104.5, 101.5, 56.2, 56.1, 56.1; HRMS (ESI) m/z calcd for $\text{C}_{25}\text{H}_{23}\text{NO}_6$ (M^+) 433.1525, found 433.1522.

5.15 Compound 31

According to the procedure described for compound **26**, compound **29** (20 mg, 0.04 mmol), NH_4Cl (sat., 1 mL), and Zn^0 dust (30 mg, 0.46 mmol) gave after final purification by column chromatography (4:6 hexanes/EtOAc) amine **31** (12 mg, 67%) as orange solid: ^1H NMR δ 7.53 (d, $J = 16.8$ Hz, 1H), 7.37 (d, $J = 8.8$ Hz, 2H), 7.28 (d, $J = 16.8$ Hz, 1H), 7.09 (d, $J = 2.0$ Hz, 1H), 7.06 (dd, $J = 8.4, 2.0$ Hz, 1H), 7.04 (d, $J = 16.4$ Hz, 1H), 6.94 (d, $J = 1.2$ Hz, 1H), 6.93 (d, $J = 16.4$ Hz, 1H), 6.87 (d, $J = 8.4$ Hz, 1H), 6.77 (d, $J = 0.8$ Hz, 1H), 6.68 (d, $J = 8.4$ Hz, 2H), 5.31 (s, 2H), 3.96 (s, 3H), 3.95 (s, 3H), 3.91 (s, 3H), 3.55 (s, 3H); ^{13}C NMR δ 158.4, 156.0, 149.0, 148.9, 145.7, 136.6, 132.5, 130.3, 129.9, 128.3, 127.6 (2C), 126.8, 119.9, 116.3, 115.8, 115.2 (2C), 111.1, 108.5, 106.0, 102.7, 94.9, 56.3, 55.9, 55.8, 55.7; HRMS (ESI) m/z calcd for $\text{C}_{27}\text{H}_{29}\text{NO}_5$ (M^+) 447.0246, found 447.2051.

5.16 Compound 32

According to the procedure described for compound **30**, compound **31** (10 mg, 0.02 mmol), in CH_3OH and EtOAc was treated with $\text{TsOH} \cdot \text{H}_2\text{O}$ (60 mg, 0.31 mmol), and after final purification by column chromatography (4:6 hexanes/EtOAc) afforded compound **32** (8 mg, 89%) as a dark orange solid: UV (EtOH) λ_{max} (log ϵ) 377 (4.63); λ_{em} 512; ^1H NMR ($(\text{CD}_3)_2\text{CO}$) δ 7.56 (d, $J = 16.8$ Hz, 1H), 7.29 – 7.22 (m, 3H), 7.10 – 6.92 (m, 6H), 6.76 (d, $J = 2.8$ Hz, 1H), 6.65 (d, $J = 8.4$ Hz, 2H), 4.69 (brd, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.81 (s, 3H); ^{13}C NMR ($(\text{CD}_3)_2\text{CO}$) δ 159.6, 156.9, 150.6, 150.4, 148.7, 137.6, 132.8, 131.5, 129.3, 128.9, 128.0 (2C), 127.5, 120.8, 116.4, 115.3 (2C), 112.8, 110.3, 107.7, 104.5, 101.6, 56.1, 56.1, 56.0; HRMS (ESI) m/z calcd for $\text{C}_{25}\text{H}_{25}\text{NO}_4$ (M^+) 403.1784, found 403.1779.

5.17 Compound 34

To a solution of aldehyde **33** (25 mg, 0.07 mmol) and phosphonate **23** (50 mg, 0.11 mmol) in THF (4 mL) at rt was added 15-crown-5 (0.01 mL) followed by NaH (51 mg, 1.3 mmol, 60% in oil). After 35 min, the reaction was quenched by addition of water. The resulting solution was extracted with EtOAc, washed with brine, dried (MgSO_4) and concentrated in vacuo. Final purification by column chromatography (4:6 hexanes/EtOAc) afforded compound **34** (36 mg, 78%) as an orange solid: ^1H NMR δ 8.19 (d, $J = 8.8$ Hz, 2H), 7.66 – 7.62 (m, 4H), 7.06 (d, $J = 16.4$ Hz, 1H), 6.96 (s, 1H), 6.95 – 6.90 (m, 3H), 6.77 (s, 1H), 5.35 (s, 2H), 4.83 (d, $J = 6.8$ Hz, 1H), 4.73 (d, $J = 6.8$ Hz, 1H), 4.32 (ddd, $J = 3.2, 3.2, 3.2$ Hz, 1H), 3.99 (s, 3H), 3.92 (s, 3H), 3.57 (s, 3H), 3.47 (s, 3H), 3.28 (d, $J = 3.6$ Hz, 1H), 2.81 – 2.75 (m, 2H), 2.57 (dd, $J = 14.2, 3.0$ Hz, 1H), 2.34 (br d, 1H), 1.98 (dd, $J = 14.0, 2.8$ Hz, 1H), 1.80 (dd, $J = 12.5, 5.2$ Hz, 1H), 1.49 (s, 3H), 1.12 (s, 3H), 1.10 (s, 3H); ^{13}C NMR δ 159.1, 156.8, 149.0, 146.1, 146.1, 142.6, 139.1, 129.8, 129.3, 128.4, 126.5 (2C), 125.8, 124.6, 124.0 (2C), 122.8, 120.8, 113.7, 106.8, 105.3, 102.4, 96.8, 94.8, 84.7, 76.6, 68.6,

56.3, 56.1, 55.9, 55.7, 47.0, 42.2, 37.8, 28.7, 22.9, 21.5, 16.6; HRMS (ESI) m/z calcd for $C_{38}H_{45}NO_{10}$ (M^+) 675.3043, found 675.3040.

5.18 Compound 35

According to the procedure for compound **30**, compound **34** (16 mg, 0.024 mmol), in CH_3OH and EtOAc was treated with $TsOH \cdot H_2O$ (60 mg, 0.32 mmol) and after final purification by column chromatography (3:7 hexanes/EtOAc) afforded schweinfurthin **35** (10 mg, 71%) as a dark orange wax: 1H NMR δ 8.20 (d, $J = 8.8$ Hz, 2H), 7.67 – 7.61 (m, 3H), 7.55 (d, $J = 16.4$ Hz, 1H), 7.01 (d, $J = 16.0$ Hz, 1H), 6.89 (s, 1H), 6.88 (s, 1H), 6.85 (d, $J = 16.0$ Hz, 1H), 6.64 (s, 2H), 4.26 (ddd, $J = 3.2, 3.2, 3.2$ Hz, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.39 (d, $J = 3.6$ Hz, 1H), 2.82 – 2.76 (m, 2H), 2.52 (dd, $J = 14.4, 2.8$ Hz, 1H), 2.30 (dd, $J = 14.4, 3.2$ Hz, 1H), 1.79 (dd, $J = 12.8, 5.2$ Hz, 1H), 1.47 (s, 3H), 1.13, (s, 3H), 1.09 (s, 3H); ^{13}C NMR δ 159.2, 155.3, 149.0, 146.2, 145.7, 142.6, 139.1, 129.8, 129.2, 128.4, 126.6 (2C), 125.5, 124.8, 124.0 (2C), 122.9, 120.9, 111.9, 107.0, 106.5, 101.4, 77.5, 76.8, 70.7, 56.0, 55.8, 46.8, 43.3, 38.0, 28.9, 23.0, 21.6, 16.0; HRMS (ESI) m/z calcd for $C_{34}H_{37}NO_8$ (M^+) 587.2519, found 587.2518.

5.19 Amine 36

According to the procedure described for compound **26**, compound **35** (16 mg, 0.024 mmol), NH_4Cl (sat., 1 mL) and Zn^0 dust (26 mg, 0.4 mmol) gave after final purification by column chromatography (4:6 hexanes/EtOAc to 99:1 EtOAc/MeOH) amine **36** (12 mg, 80%) as an orange oil: 1H NMR δ 7.52 (d, $J = 16.8$ Hz, 1H), 7.37 (d, $J = 8.4$ Hz, 2H), 7.28 (d, $J = 16.0$ Hz, 1H), 7.01 (d, $J = 16.0$ Hz, 1H), 6.93 – 6.88 (m, 4H), 6.76 (s, 1H), 6.70 (d, 8.0 Hz, 2H), 5.30 (s, 2H), 4.84 (d, $J = 6.8$ Hz, 1H), 4.73 (d, $J = 6.8$ Hz, 1H), 4.32 (ddd, $J = 2.8, 2.8, 2.8$ Hz, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.55 (s, 3H), 3.47 (s, 3H), 3.28 (d, $J = 2.8$ Hz, 1H), 2.84 – 2.71 (m, 2H), 2.57 (dd, $J = 13.6, 2.4$ Hz, 1H), 1.98 (dd, $J = 11.2, 2.6$ Hz, 1H), 1.80 (dd, $J = 12.6, 5.0$ Hz, 1H), 1.49 (s, 3H), 1.12 (s, 3H), 1.10 (s, 3H); ^{13}C NMR δ 158.5, 156.1, 149.1, 145.2, 142.5, 136.9, 132.4, 130.4, 128.8, 128.7, 127.6 (2C), 126.3, 122.8, 120.6, 116.6, 115.8, 115.5 (2C), 107.0, 106.0, 102.8, 96.9, 95.0, 84.9, 76.5, 68.7, 56.3, 56.1, 56.0, 55.8, 47.2, 42.3, 37.9, 28.8, 23.0, 21.6, 16.7; HRMS (ESI) m/z calcd for $C_{38}H_{47}NO_8$ (M^+) 645.3302, found 645.3312.

5.20 Amine 37

According to the procedure for compound **30**, compound **36** (11 mg, 0.017 mmol), in CH_3OH was treated with $TsOH \cdot H_2O$ (25 mg, 0.13 mmol), and gave after final purification by column chromatography (EtOAc) schweinfurthin **37** (9 mg, 95%) as a dark orange wax: UV (EtOH) λ_{max} (log ϵ) 375 (4.63); λ_{em} 481; 1H NMR (CD_3OD) δ 7.50 (d, $J = 16.4$ Hz, 1H), 7.25 (d, $J = 8.4$ Hz, 2H), 7.24 (d, $J = 16.4$ Hz, 1H), 7.01 – 6.88 (m, 4H), 6.69 (d, $J = 8.4$ Hz, 2H), 6.66 (d, $J = 1.2$ Hz, 1H), 6.64 (s, 1H), 4.14 (ddd, $J = 3.6, 3.6, 3.6$ Hz, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.30 (obscured by solvent, 1H), 2.78 – 2.76 (m, 2H), 2.35 (dd, $J = 13.8, 3.0$ Hz, 1H), 1.92 (dd, $J = 13.4, 3.0$ Hz, 1H), 1.73 (dd, $J = 11.8, 6.2$ Hz, 1H), 1.41 (s, 3H), 1.10 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (CD_3OD) δ 160.1, 157.4, 150.3, 147.9, 143.6, 138.3, 131.3, 130.6, 129.5, 128.1 (2C), 127.4, 124.4, 122.0, 117.5, 116.7 (2C), 115.8, 114.6, 108.5, 107.5, 101.9, 78.8, 78.1, 71.8, 56.5, 56.2, 48 (obscured by solvent), 44.8, 39.2, 29.4, 24.0, 22.0, 16.6; HRMS (ESI) m/z calcd for $C_{34}H_{39}NO_6$ (M^+) 557.2777, found 557.2784.

5.21 Cell culture

The SF-295 and A549 cell lines were purchased from the NCI and ATCC, respectively. These cell lines were maintained in RPMI 1640 (SF-295) or F-12 media (A549), each supplemented with 10% FBS, amphotericin B, penicillin/streptomycin, and L-glutamine. Cells were cultured in the presence of 5% CO_2 at 37° C.

5.22 Cytotoxicity Assay

Either SF-295 or A549 cells were incubated with indicated concentrations of compounds **19**, **25**, **27**, **32**, **35**, or **37**. After 44 hours of exposure, media was aspirated and replaced with either RPMI 1640 lacking phenol Red (SF-295) or F-12 (A549) media containing MTT salt (Calbiochem, San Diego, CA). At the conclusion of the 48 hour interval, cells were solubilized in stop solution (10% 1 N HCl, 10% triton X-100, and isopropyl alcohol) overnight at room temperature. The absorption of the resulting solution was measured at 540 nm and 650 nm. The effective dose 50 concentrations were determined using Calcsyn software (Biosoft, Cambridge, UK).

5.23 Fluorescence microscopy

The SF-295 cells were plated on 22 × 22 mm coverslips. After reaching 45% confluency, cells were treated with indicated concentrations of compound **25** or **30** for 24 hours. At the conclusion of the treatment interval, cells were washed three times in PBS and fixed in 4% formaldehyde for 15 minutes. Coverslips were then washed in PBS three times and mounted in Vectashield containing DAPI. Compound and DAPI fluorescence was visualized using a Bio-Rad Multi-photon microscope. Images were processed with ImageJ. Images demonstrating brightness distribution were created with the fire look up table.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the University of Iowa for a Shriner Fellowship (JJT). Financial support from the Roy J. Carver Charitable Trust and the National Cancer Institute (R41CA126020 via Terpenoid Therapeutics, Inc.) is gratefully acknowledged. The content is solely the responsibility of the authors, and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

References

1. Newman DJ, Cragg GM. *J. Nat. Prod.* 2007; 70:461–477. [PubMed: 17309302]
2. Kingston DGI. *J. Nat. Prod.* 2000; 63:726–734. [PubMed: 10843603]
3. Beutler JA, Shoemaker RH, Johnson T, Boyd MR. *J. Nat. Prod.* 1998; 61:1509–1512. [PubMed: 9868152]
4. Beutler JA, Jato J, Cragg GM, Boyd MR. *Nat. Prod. Lett.* 2000; 14:399–404.
5. Yoder BJ, Cao S, Norris A, Miller JS, Ratovoson F, Razafitsalama J, Andriantsiferana R, Rasamison VE, Kingston DGI. *J. Nat. Prod.* 2007; 70:342–346. [PubMed: 17326683]
6. Thoison O, Hnawia E, Gueritte-Voegelein F, Sevenet T. *Phytochemistry.* 1992; 31:1439–1442.
7. Paull KD, Shoemaker RH, Hodes L, Monks A, Scudiero DA, Rubinstein L, Plowman J, Boyd MR. *J. Nat. Cancer Inst.* 1989; 81:1088–1092. [PubMed: 2738938]
8. a) Deng JZ, Newman DJ, Hecht SM. *J. Nat. Prod.* 2000; 63:1269–1272. [PubMed: 11000034] b) Zhou BN, Hoch JM, Johnson RK, Mattern MR, Eng WK, Ma J, Hecht SM, Newman DJ, Kingston DGI. *J. Nat. Prod.* 2000; 63:1273–1276. [PubMed: 11000035]
9. Topczewski JJ, Neighbors JD, Wiemer DF. *J. Org. Chem.* 2009; 74:6965–6972. [PubMed: 19697910]
10. Treadwell EM, Cermak SC, Wiemer DF. *J. Org. Chem.* 1999; 64:8718–8723.
11. Mente NR, Wiemer AJ, Neighbors JD, Beutler JA, Hohl RJ, Wiemer DF. *Biorg. Med. Chem. Lett.* 2007; 17:911–915.
12. Mente NR, Neighbors JD, Wiemer DF. *J. Org. Chem.* 2008; 73:7963–7970. [PubMed: 18795788]
13. a) Neighbors JD, Beutler JA, Wiemer DF. *J. Org. Chem.* 2005; 70:925–931. [PubMed: 15675850] b) Wiemer, DF.; Neighbors, JD.; Beutler, JA. *PCT Int. Appl. Preparation of Schweinfurthin*

- analogues. 2005. CODEN: PIXXD2 WO 2005092878 A2 20051006 CAN 143:347317 AN 2005:1075787
14. Neighbors JD, Salnikova MS, Beutler JA, Wiemer DF. *Biorg. Med. Chem.* 2006; 14:1771–1784.
 15. Neighbors JD, Mente NR, Boss KD, Zehnder DW II, Wiemer DF. *Tetrahedron Lett.* 2008; 49:516–519.
 16. Neighbors JD, Kuder CH, Hohl RJ, Wiemer DF. *Biorg. Med. Chem.* 2009; 17:4718–4723.
 17. Ulrich NC, Kodet JG, Mente NR, Kuder CH, Beutler JA, Hohl RJ, Wiemer DF. *Bioorg. Med. Chem.* 2010; 18:1676–1683. [PubMed: 20116262]
 18. Neighbors JD, Topczewski JJ, Wiemer DF. *Tetrahedron Lett.* 2009; 50:3881–3884.
 19. Wiemer, David F, Neighbors, Jeffrey D, Hohl, Raymond J, Kuder Craig. Schweinfurthin analogs. *PCT Int. Appl.* 2009 56 pp. CODEN: PIXXD2 WO 2009158516 A1 20091230 CAN 152:119838 AN 2009:1618103.
 20. Meier H. *Angew. Chem. Int. Ed. Eng.* 2005; 44:2482–2506.
 21. Gompper R, Wagner HU. *Angew. Chem. Int. Ed. Eng.* 1988; 27:1437–1455.
 22. a) Kagan F, Birkenmeyer RD, Strube RE. *J. Am. Chem. Soc.* 1959; 81:3026–3031. b) Wadsworth DH, Schupp OE III, Seus EJ, Ford JA Jr. *J. Org. Chem.* 1965; 30:680–685.
 23. Neighbors JD, Salnikova MS, Wiemer DF. *Tetrahedron Lett.* 2005; 46:1321–1324.
 24. Miljanic S, Frkanec L, Meic Z, Zinic M. *Langmuir.* 2005; 21:2754–2760. [PubMed: 15779945]
 25. Schneckenburger H, Wagner M, Weber P, Strauss WSL, Sailer R. *J. Fluoresc.* 2004; 14:649–654. [PubMed: 15617271]

Figure 1.
Structures of the natural schweinfurthins and selected analogues.

Figure 2.

The intracellular localization of compound **25** and **30**. SF-295 cells were treated with compound **25** (500 nM) (A-D) or compound **30** (500 nM) (E-H) for 24 hours. Images depict intracellular localization of compound **25** (A) or compound **30** (E) alone, nuclear labeling of treated cell with DAPI (B and F), merged images of DAPI with compound **25** (C) or compound **30** (G), or fluorescence brightness distribution images of compound **25** (D) or **30** (H).

Scheme 1.
Synthesis of the *bis*-stilbene **19**.

Scheme 2.
Preparation of *p*-nitro and *p*-amino *bis*-stilbenes.

Table 1

The ED₅₀ values for schweinfurthin analogues **19**, **25**, **27**, **30**, **32**, **35**, and **37** in the SF-295 and A549 cells at 48 hours, as measured by the MTT assay.

Compound	ED ₅₀ Values (μM)	
	SF-295	A549
19	0.05	0.8
25	0.1	2.7
27	0.4	3.0
30	>1	>10
32	>1	>10
35	0.04	0.7
37	0.02	3.2