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Fluorescent schweinfurthin B and F analogs with anti-proliferative activity

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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. 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 analogs that could be readily visualized within cells. This report describes the synthesis of fluorescent analogs 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 analogs. The active compounds localize in peripheral puncta which may identify the site(s) 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 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 (Fig. 1).[4–6] Those that contain a hexahydroxanthene substructure, such as schweinfurthin 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 analogs. 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 analogs.[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

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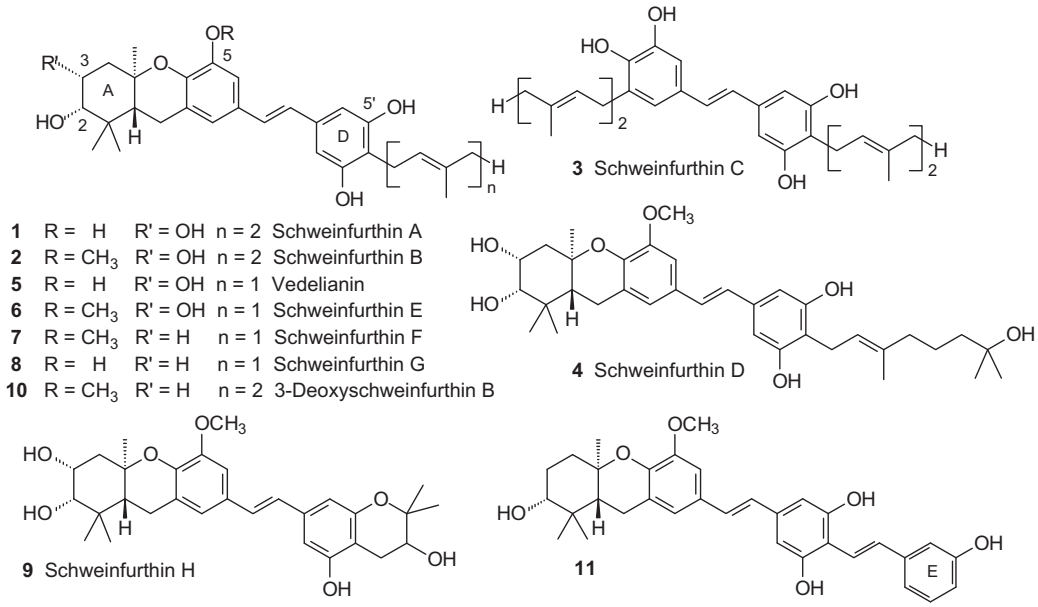


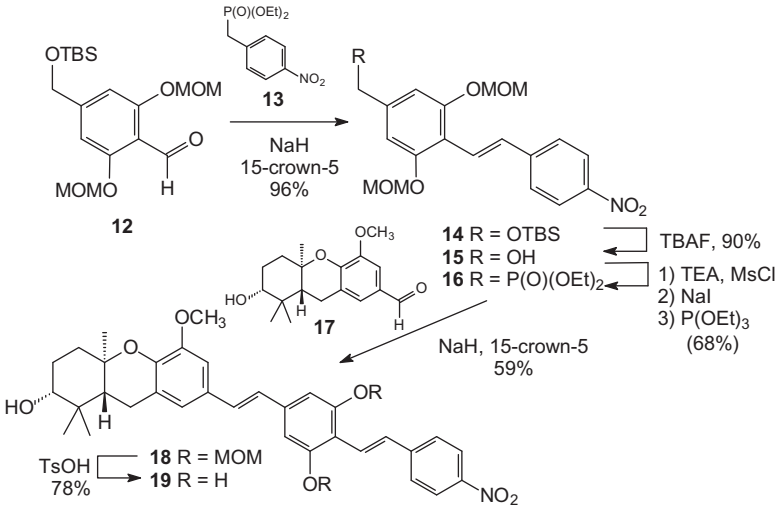
Figure 1. Structures of the natural schweinfurthins and selected analogs.

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]

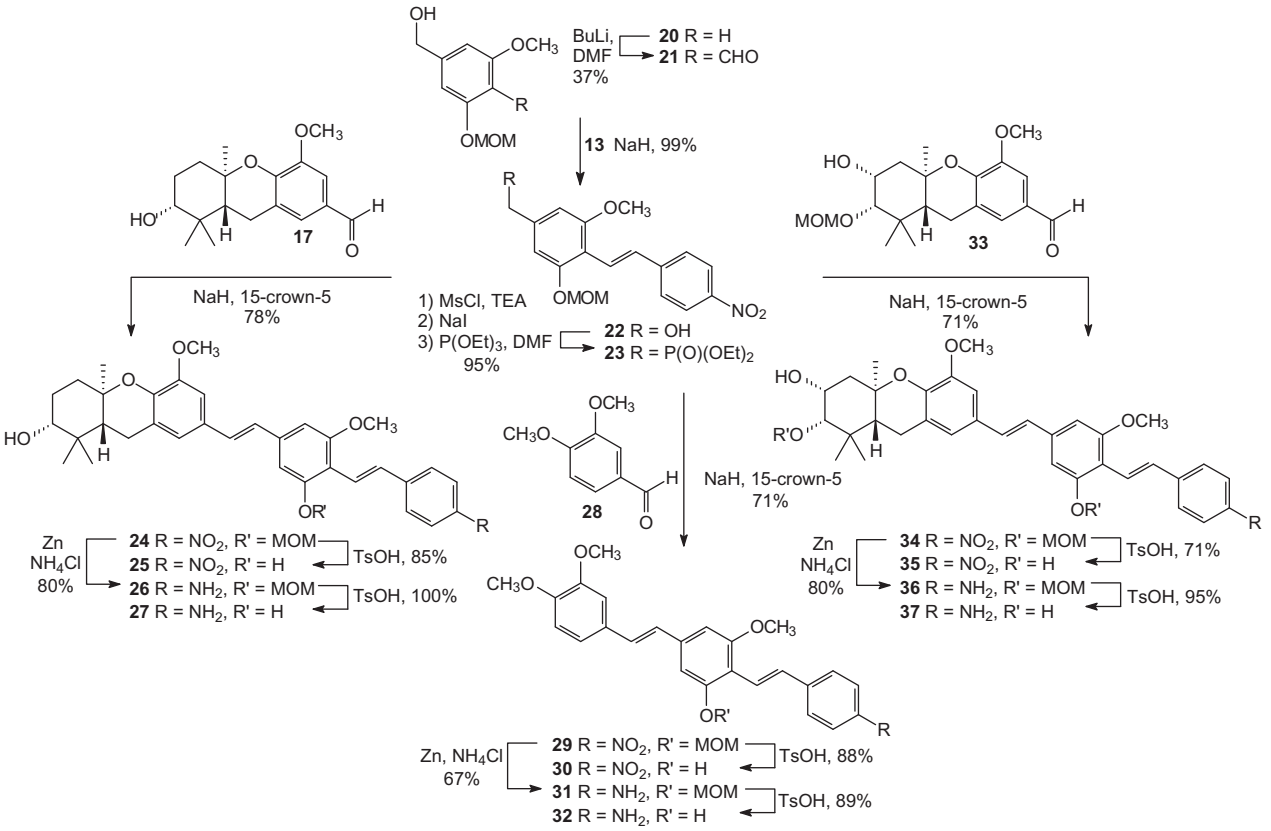
Synthesis of compound **19** began with the known aldehyde **12** (Scheme 1).[16] Treatment with *p*-nitrobenzyl phosphonate[22] (**13**) under Horner–Wadsworth–Emmons (HWE) conditions gave the protected stilbene **14**. After deprotection of the silyl ether **14** to the free alcohol **15**, a three-step 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**[9,13] 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 analogs, 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



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



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

three-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 analog 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 analog **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 compounds **11** and **19** addressed, it was possible to prepare further analogs 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 analogs 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 analog 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 analogs 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 Zn^0 in ammonium chloride[24] 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 >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 autofluroescence.[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 analogs, compounds **19**, **25**, **27**, **35**, and **37**, display potent effects on MTT activity in SF-295 cells after 48 h 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 ED_{50} values of 0.05, 0.1, and 0.04 μM, respectively. The amine bis-stilbene compounds **27** and **37** display similar ED_{50} values. As expected, compounds **30** and **32** lacking the hexahydroxanthene structure display greatly diminished activity in comparison to the other schweinfurthin analogs (Table 1). Consistent with the natural schweinfurthins, the fluorescent analogs (**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 μM.

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 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 analog 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 peri-nuclear target.

4. Conclusions

The synthesis of a small set of fluorescent analogs 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 ($MgSO_4$), 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: ^{1}H 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); ^{13}C 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 $C_{25}H_{35}NO_7Si$ (M^+) 489.2183, found 489.2173.

Table 1
The ED_{50} values for schweinfurthin analogs **19**, **25**, **27**, **30**, **32**, **35**, and **37** in the SF-295 and A549 cells at 48 h, 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

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 NH_4Cl (satd). After extraction with EtOAc, the combined organic extracts were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo to give the desired benzylic alcohol **15** (120 mg, 100%) as a clear oil: ^{1}H NMR ($(CD_3)_2CO$) 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); ^{13}C NMR ($(CD_3)_2CO$) 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 $C_{19}H_{21}NO_7$ (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 Et_3N (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 H_2O, and extracted with EtOAc. The combined organic layers were washed with NH_4Cl (satd) and brine, dried ($MgSO_4$), 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 $Na_2S_2O_3$ until the color faded, it was washed with brine, dried ($MgSO_4$), 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: ^{1}H 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); ^{13}C 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); ^{31}P NMR δ 26.3; HRMS (EI) calcd for $C_{23}H_{30}NO_9P$ (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 h. After

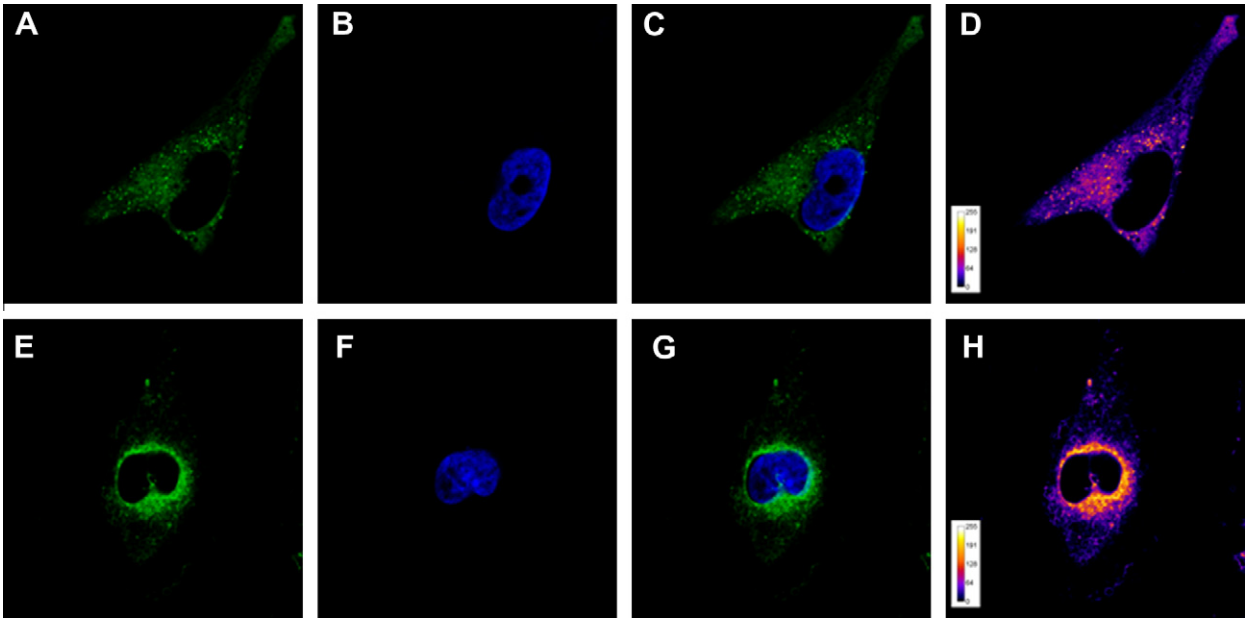


Figure 2. The intracellular localization of compounds **25** and **30**. SF-295 cells were treated with compound **25** (500 nM) (A–D) or compound **30** (500 nM) (E–H) for 24 h. Images depict intracellular localization of compound **25** (A) or compound **30** (E) alone, nuclear labeling of treated 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).

water was added dropwise, 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 (3:1 to 1:1 hexanes/EtOAc) gave the stilbene **18** (23 mg, 59%) as an orange oil: ^{1}H 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 $C_{37}H_{43}NO_9$ (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 *p*TsOH (30 mg, 0.17 mmol), and the resulting solution was stirred at rt for 6 h. The reaction was quenched by addition of satd $NaHCO_3$, 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; ^{1}H NMR ($(CD_3)_2CO$) δ 9.06 (s, 2H), 8.20 (d, J = 8.7 Hz, 2H), 7.88 (s, 2H), 7.40 (d, J = 8.7 Hz, 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 ($(CD_3)_2CO$) 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 $C_{33}H_{35}O_7N$ (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%): ^{1}H 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 $C_{11}H_{14}O_5$: 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 h, and then quenched by addition of NH_4Cl (satd). 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: ^{1}H 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 $C_{18}H_{19}NO_6$ (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 ($MgSO_4$), 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 ($MgSO_4$) 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 three steps) as a bright yellow oil: ^{1}H 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); ^{13}C 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); ^{31}P NMR δ 26.3; HRMS (EI) calcd for $C_{22}H_{28}NO_8P$ (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 ($MgSO_4$) 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: ^{1}H 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); ^{13}C 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 $C_{36}H_{41}NO_8$ (M^+) 615.2832, found 615.2837.

5.10. Nitro schweinfurthin 25

To a solution of compound **24** (16 mg, 0.03 mmol) in CH_3OH (2.5 mL) and EtOAc (0.5 mL) was added $TsOH{\cdot}H_2O$ (32 mg, 0.16 mmol). After 24 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 phase was washed with brine, dried ($MgSO_4$), 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; ^{1}H 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); ^{13}C 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 $C_{34}H_{37}NO_7$ (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 (satd, 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: ^{1}H 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 $C_{36}H_{43}NO_6$ (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 $TsOH{\cdot}H_2O$ (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; ^{1}H 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 $C_{34}H_{39}NO_5$ (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: ^{1}H 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 $C_{27}H_{27}NO_7$ (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 $TsOH{\cdot}H_2O$ (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; ^{1}H NMR ($(CD_3)_2CO$) 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 ($(CD_3)_2CO$) 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 $C_{25}H_{23}NO_6$ (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 (satd, 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: ^{1}H 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 $C_{27}H_{29}NO_5$ (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 $TsOH{\cdot}H_2O$ (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; ^{1}H NMR ($(CD_3)_2CO$) 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 (br d, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.81 (s, 3H); ^{13}C NMR ($(CD_3)_2CO$) 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 $C_{25}H_{25}NO_4$ (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: ^{1}H 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: ^{1}H 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 (satd, 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: ^{1}H 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; ^{1}H 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 varying concentrations of compounds **19**, **25**, **27**, **32**, **35**, or **37**. After 44 h 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 h 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 (ED_{50}) concentrations were determined using Calcusyn 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 h. At the conclusion of the treatment interval, cells were washed three times in PBS and fixed in 4% formaldehyde for 15 min. 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.

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Supplementary data

General experimental procedures, the ^{1}H and ^{13}C NMR spectra for compounds **14–16**, **18**, **19**, **21–27**, **29–32**, **34–37**, and a table of compound purity data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.056.

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