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Biologically active biotin derivatives of schweinfurthin F

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ABSTRACT

As a prelude to efforts to identify schweinfurthin binding proteins, an ester conjugate and an amide conjugate of schweinfurthin F and biotin have been prepared by chemical synthesis. These compounds maintain activity in SF-295 cells comparable to the parent system, and display the lower potency in A549 cells that is a characteristic of the schweinfurthin pattern of activity.

The identification of a drug's molecular target is very desirable, both to elucidate the underlying biological mechanism(s) of action and to guide the rational design of more effective therapeutics.[1] A wide variety of techniques, including yeast three-hybrid systems,[2] phage display,[3] and protein microarrays[4] has been employed for the deconvolution of different small-molecule targets. Recently, affinity-based chromatography coupled with mass spectrometry (MS) has become increasingly popular due to the remarkable sensitivity and speed this technique offers.[1,5–7]

Identifying a molecular target using affinity-based chromatography typically requires the synthesis of an appropriate molecular probe amenable to immobilization on a solid support.[1] Biotinylation commonly has been used for the purification/identification of small molecule targets[8–14] (e.g., the identification of trapoxin as a histone deacetylase inhibitor).[15] This methodology requires derivatization of the molecule in question with biotin and subsequently relies on the extremely tight binding of biotin to streptavidin ($k_d = 10^{-15}$ M).[1]

One unique family of natural products with an as yet unknown molecular target is the schweinfurthins (e.g., schweinfurthin A, F, and 3-deoxyschweinfurthin B, **1**–**3**, Fig. 1). These compounds were isolated from the African plant *Macaranga schweinfurthii*,[16–20] and all known members of this family that possess a hexahydroxanthene system exhibit potent and differential antineoplastic activity in the National Cancer Institute's (NCI) 60-cell line anti-cancer screen. Of particular interest is the pattern of activity exhibited by the schweinfurthins toward specific cell lines in this large panel. A compound's pattern of activity in the NCI's 60-cell line assay can be analyzed by the COMPARE algorithm to determine whether it shows a significant correlation to any known agents in terms of mechanism of action.[21] Importantly, the pattern displayed in the 60-cell line screen by the schweinfurthins shows no correlation to any clinically used anti-neoplastic agent, indicating that this family of compounds probably acts via a novel mechanism or at a novel target. The COMPARE analysis of the schweinfurthins at the GI_{50} and TGI levels of response[20] does show significant correlations to several structurally unrelated natural products (Fig. 1), including the stellettins (e.g., stellettin A, **4**),[22–25] the cephalostatins (e.g., cephalostatin 1, **5**),[26–28] and OSW-1 (**6**).[29] Unfortunately, at this time none of these correlated natural products has a known molecular target or a defined mechanism of action. It has been determined that the schweinfurthin-sensitive cell lines possess no shared biochemical features and differ substantially in DNA repair phenotype, in vitro doubling time, and MDR status.[20] Discovery of the mechanism of action of the schweinfurthins may aid in similar determination of some or all of the correlated natural products, and in turn increase understanding of a new class of prospective chemotherapeutic agents.

In order to elucidate the basis of schweinfurthin toxicity, we have initiated an effort to identify the intracellular binding partner(s). Although numerous kinds of interacting molecules are possible, based on the schweinfurthins' reactivity, chemical structure, and overall pattern of cytotoxicity, we hypothesize that these compounds bind to protein(s). Herein we report chemical modifications of a potent natural schweinfurthin to yield biotinylated schweinfurthins that might be used to probe potential protein

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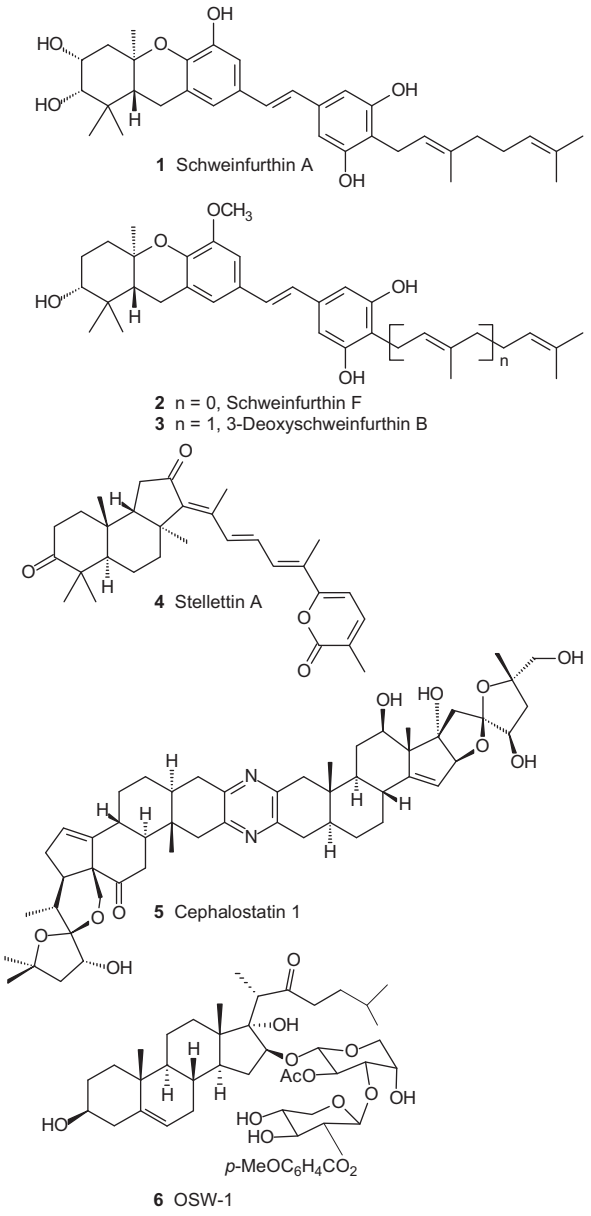


Figure 1. Some schweinfurthins (**1**–**3**), stellettin A (**4**), cephalostatin 1 (**5**), and OSW-1 (**6**).

interactions. For these analogues to have utility as probes for protein interactions, it would be at least desirable and perhaps essential for them to retain the characteristic schweinfurthin activity. Here we demonstrate that two specific biotinylated schweinfurthin analogues display schweinfurthin-like activity in a recently reported, two-cell line screen.[30]

To date, we have reported not only the total syntheses of several natural schweinfurthins,[31–36] but also the preparation of numerous synthetic analogues for structure–activity relationship (SAR) studies.[30,37,38] During the course of these studies, a schweinfurthin analogue with a terminal allylic alcohol[37] was synthesized and found to exhibit significant and differential cytotoxicity in the distinctive schweinfurthin pattern (mean GI_{50} = 1.0 μM in the 60-cell line assay), indicating that structural modifications at the distal end of the geranyl chain do not diminish significantly the biological activity. Conversely, the limited activity of schweinfurthin C, which possesses an undecorated geranyl left-half, suggests that the hexahydroxanthene core of the schweinfurthins (e.g., schweinfurthin A, **1**) is essential for activity, and that extensive modifications in this region would not be well-tolerated. Therefore we have pursued alterations to the D-ring geranyl tail to minimize interference in binding to potential protein targets. Finally, recent studies have demonstrated that methylation of one of the D-ring phenols provides additional stability to the oxidation-prone resorcinol moiety. These results suggested that analogues such as compound **7** (Scheme 1) may serve as probes, and the synthesis and bioassay of compounds bearing these design elements are reported here.

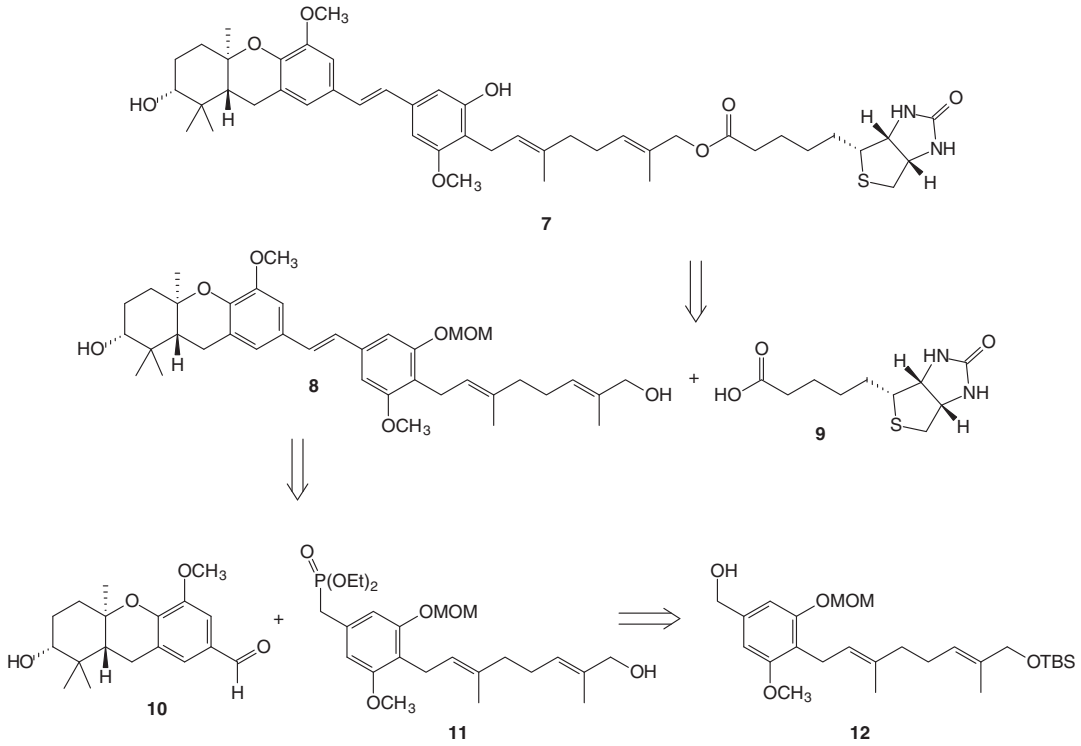
A retrosynthetic analysis of a biotin derivative (**7**) inspired by schweinfurthin F (**2**) and 3-deoxyschweinfurthin B (**3**) is detailed in Scheme 1. Late-stage esterification of D-biotin (**9**) and the schweinfurthin analogue **8** followed by removal of the phenolic protecting group would provide the indicated target. Disconnection of the stilbene olefin could afford the Horner–Wadsworth–Emmons coupling partners known aldehyde **10**[34] and phosphonate **11**. In turn phosphonate **11** could be obtained from the benzylic alcohol **12**, which could result from alkylation of the corresponding benzyl alcohol **13** under standard conditions for formation and alkylation of a dianion.[39]

Synthesis of arene **12** began with the known benzyl alcohol **13**[38] (Scheme 2). Regioselective formation of the lithiate, transmetallation to the cuprate, and subsequent alkylation with readily prepared bromide **14**[40,41] gave the C-alkylated product **12** in modest yield. Subsequent conversion to the phosphonate was accomplished via standard reaction conditions, and fluoride-mediated silyl group removal afforded phosphonate **11**. When the phosphonate **11** was allowed to react with the tricyclic aldehyde **10** in the presence of NaH and 15-crown-5, the protected stilbene **8** was obtained in good yield. A final EDC-mediated esterification of stilbene **8** and D-biotin (**9**) afforded the biotinylated analogue **15** in modest yield (48%), along with a substantial amount of recovered starting material (35%), but this reaction gave sufficient material to allow biological analysis. Attempted hydrolysis of the MOM acetal of compound **15** under various acidic conditions proved unsuccessful and resulted instead in cleavage of the allylic ester to return alcohol **8**. Chemical hydrolysis of the MOM groups would not be essential if compound **15** displays biological activity, but the facile hydrolysis of the allylic ester prompted concerns about its metabolic stability.

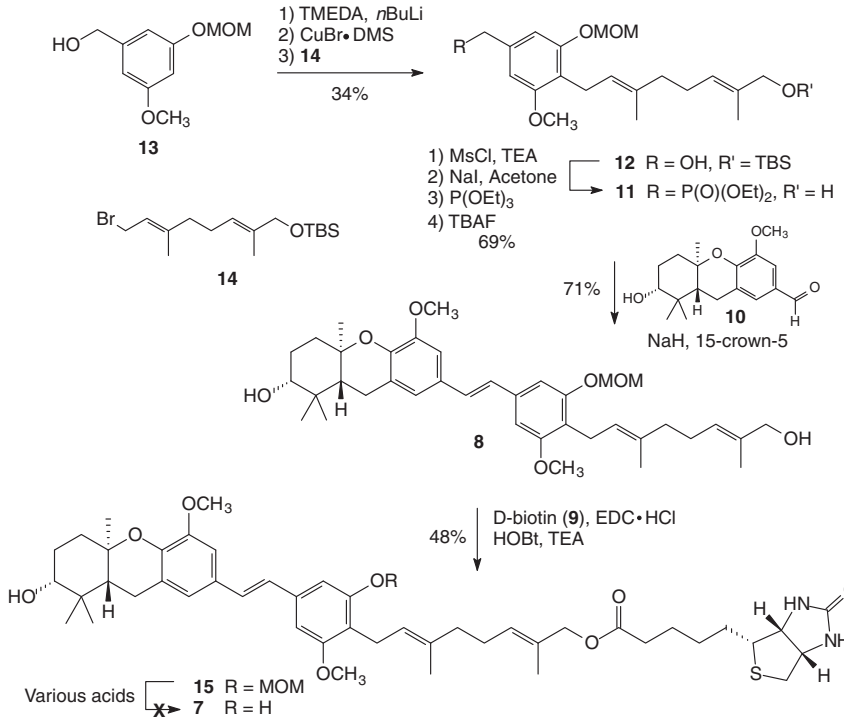
In an effort to increase the stability of the allylic linkage,[42–45] a more stable amide group was envisioned (Scheme 3). Treatment of analogue **8** with phthalimide under standard Mitsunobu conditions[46] gave the desired compound **16**. Treatment of phthalimide **16** with hydrazine hydrate followed by EDC-mediated condensation with D-biotin (**9**) afforded the desired biotinylated schweinfurthin **17**. After preparation of this compound, both the biotin amide **17** and the ester **15** were advanced to assays to gauge their biological activity.

As described above, maintaining schweinfurthin-like activity may be essential to the utility of these biotinylated compounds. To address whether these analogues possess schweinfurthin-like activity, compounds **15** and **17** were tested in a relevant two-cell line screen.[30] This screen measures the cytotoxicity of schweinfurthin analogues in the human-derived glioblastoma multiforme cell line SF-295, which is highly sensitive to schweinfurthin treatment, and the human-derived lung adenocarcinoma cell line A549, which is relatively insensitive. Results from these assays are indicative of overall schweinfurthin-like activity, as demonstrated in a recent report.[30]

Biotinylated analogues **15** and **17** were tested against SF-295 and A549 cell lines under standard conditions (Fig. 2). Both compounds exhibited activity against SF-295 cells (EC_{50} = 2.7 and 1.1 μM, respectively). Although this activity is somewhat decreased in comparison to the schweinfurthin analogue 3-deoxyschweinfurthin B (**3**, EC_{50} = 0.5 μM in this assay), these results

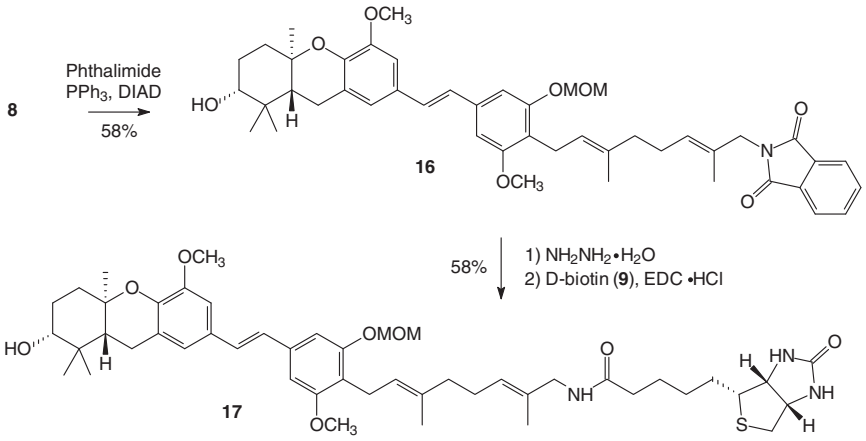


Scheme 1. Retrosynthesis of the biotin–schweinfurthin conjugate **7**.



Scheme 2. Attempted synthesis of allylic ester **7**.

indicate that biotinylation of these compounds does not substantially diminish activity. As with the natural schweinfurthins, analogues **15** and **17** also are relatively inactive against the A549 cells (EC_{50} >10 and 5.0 μM, respectively), as is 3-deoxyschweinfurthin B (EC_{50} 4.9 μM). Taken together, these data suggest overall schweinfurthin-like activity and indicate that attachment of biotin



Scheme 3. Preparation of amide **17**.

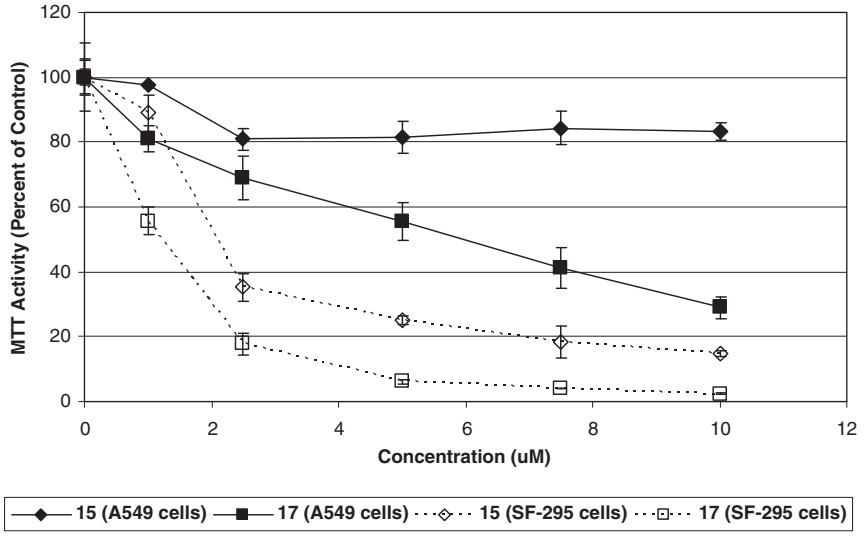


Figure 2. Concentration–response curves of compounds **15** and **17** in SF-295 and A549 cells. Values shown are the mean of quadruplicate treatments ± the standard deviation.

at this position does not affect significantly the interaction between the compounds and their presumed target.

In conclusion, two biotinylated schweinfurthin analogues have been prepared. After ester analogue **15** proved to be acid-labile, the amide analogue **17** was prepared in order to increase the chemical/metabolic stability of the biotin linkage. Both analogues exhibit schweinfurthin-like activity in SF-295 and A549 cancer cell lines, indicating that biotinylation at the right-half of the schweinfurthins does not significantly affect cytotoxicity. Pull-down experiments utilizing these probes are currently underway, and the results of these studies will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.143.

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