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The schweinfurthins are a small set of diprenylated stilbenes isolated from an African plant. Schweinfurthins A, B, display significant and unique activity in the NCI's 60 cell line panel, and the breast cancer lines MCF7 and HS 578T were among the most sensitive. To study the mechanism of action and provide a reliable source, a chemical synthesis has been initiated. A cationic cyclization approach allowed the synthesis of 3-deoxyschweinfurthin B which is more slighlty cytotoxic than the natural products. This has led to the synthesis of several analogs for the elucidation of the essential pharmacophore of the schweinfurthin family. Our efforts to achieve an enantioselctive synthesis of the 3-deoxyschweinfurthin B core tricycle will be presented as well.

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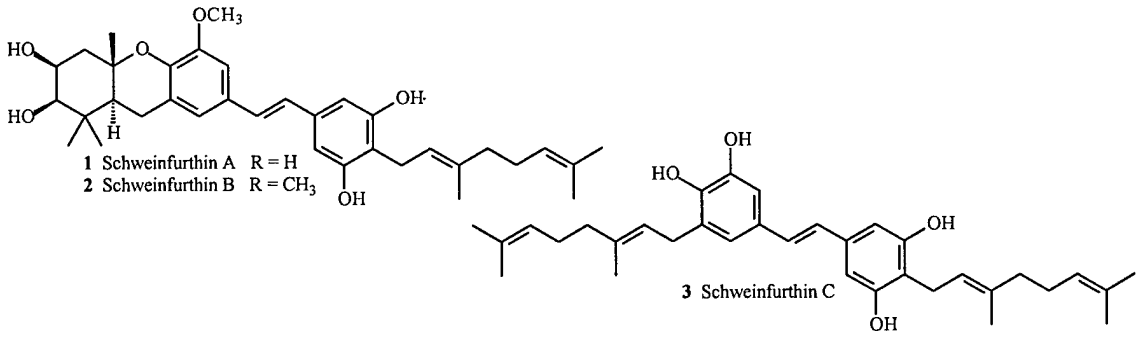
Introduction:

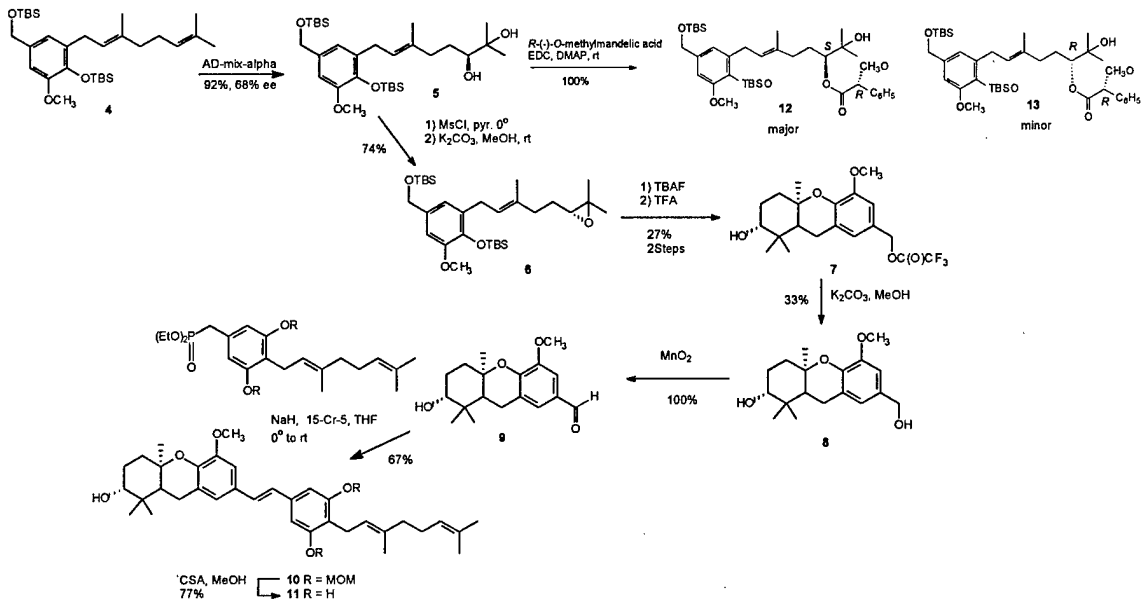
The schweinfurthins (**1–3**) are a small set of diprenylated stilbenes isolated from the African plant *Macaranga schweinfurthii* Pax. by Beutler *et al.* at the National Cancer Institute.[1,2] Schweinfurthins A (**1**), B (**2**), display significant activity in the NCI's 60-cell line anticancer assay with mean GI_{50}'s of less than 1.0 μM. Among the most sensitive cell lines were the breast cancer lines MCF7 and HS 578T. Inspection of the spectrum of activity shows no correlation with any currently used agents suggesting that these compounds may be acting at a previously unrecognized target or through a novel mechanism. The schweinfurthins have been isolated in low and varying amounts from the natural source, and their absolute stereochemistry has yet to be elucidated. For these reasons as well as their interesting biological activity, we have undertaken a total synthesis effort. An eventual asymmetric synthesis will allow assignment of the absolute stereochemistry and will provide a reliable source of schweinfurthins for further testing. Further chemical synthesis also allows access to analogs designed to probe the biological activity of these compounds.



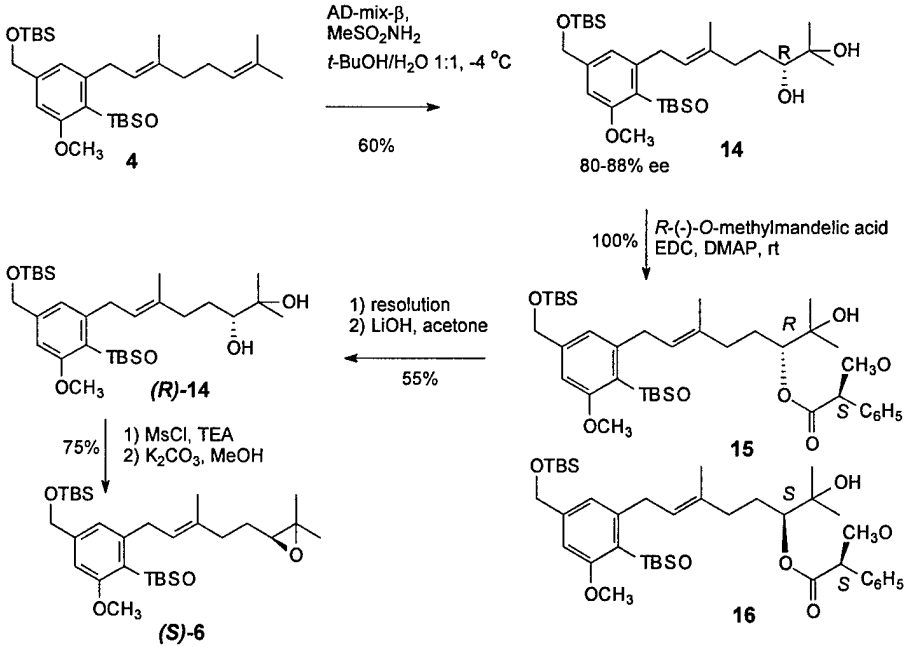
Body.

The last year has focused primarily on developing more detailed knowledge of the activity of the schweinfurthin family and on implementing a synthesis of an analog for use in determining the mechanism of action of these agents. In our last annual report we outlined an improved synthetic tactic that used an epoxide initiated cyclization to install one of the hydroxyl groups in the natural product leading to a synthesis of 3-deoxyschweinfurthin B **11** in enantioenriched form. This compound was sufficiently similar in structure to the natural schweinfurthins that we sent it to our collaborators at the National Cancer Institute for testing in their 60 cell-line assay. To our great delight this agent is slightly *more* active (0.22 μM) than the natural product schweinfiurthin B **2** (0.81 μM) and more importantly it shows a high degree of correlation to compound **2** in its pattern of activity. This suggests that it retains the same mechanism of action.

This result led to intensified efforts to develop an enantioselective pathway and to the concurrent development of analogs with modifications in the right-half for eventual use as probes of the mechanism of action. The absolute stereochemistry of the natural product is as yet undetermined and so AD-mix-α was chosen initially to install the diol. The mnemonic device outlined by Sharpless *et al.*[3] indicated the *S*-diol enantiomer was to be expected, but a more reliable instrumental method to establish the absolute stereochemistry was sought. Treatment of the diol **5** with the *R*-enantiomer of *O*-methylphenylacetic acid led to a quantitative yield of a mixture of diastereomers **12** and **13** which showed characteristic chemical shift differences expected based on Mosher-Trost models for ^{1}H-NMR assignment of absolute stereochemistry.[4] Specifically the vinyl hydrogen is shifted by 0.37 ppm upfield in the minor component as would be expected if this ester had the *R*-stereochemistry at the secondary alcohol center. This allowed assignment of the absolute stereochemistry of the major diol as *S*, as expected based on literature precedent.[3]



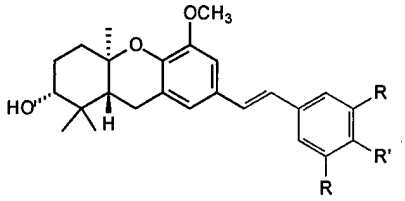
While preparation of the the Mosher-Trost esters allowed the absolute stereochemistry to be assigned these esters were also easily separable by column chromatography and thus allow for resolution and enatioselective synthesis of the 3-deoxyschweinfurthin B left-half tricycle. In order to acheive this objective the reaction of silyl ether **4** with AD-mix-β was utilized based on the slightly higher



enantiomeric excess afforded by this reagent. Thus the *R*-diol **14** could be esterified with *S*-*O*-methylphenylacetic acid to afford a mixture of diastereomeric esters **15** and **16** which were then subjected to resolution by chromatography. Treatment of the pure ester **15** with either lithium aluminum hydride or aqueous lithium hydroxide gives enantiopure diol ***(R)-14***. Formation of the mesylate and internal displacement then leads to the epoxide **(*S*)-6** as a single enantiomer. This then establishes a formal enantioselctive synthesis of the active analog 3-deoxyschweinfurthin B, and work towards an actual synthesis is ongoing.

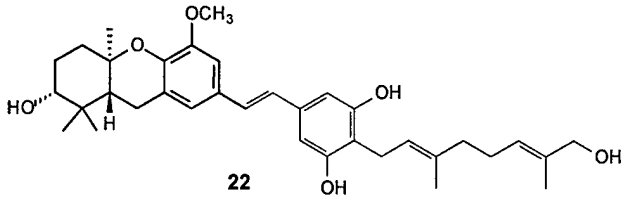
With these results in hand we set about exploring the nature of the right half moiety in an activity profile with hopes of finding a position for attachment of affinity reagents or

probes for yeast three-hybrid assays. We had some concerns about the stability of the right half resorcinol moiety and in this context synthesized the dimethoxy analog **17**, this molecule retained the differential activity of the schweinfurthins but showed a significant drop in overall cytotoxicity (see table). A series of such compounds was synthesized in an attempt to ascertain the extent of the importance of the free hydroxyls to activity. It became appearent from this data that the oxygen of the resorcinol moiety is indeed crucial to activity.



Compound	R	R'	Mean GI_{50} 60 cell-line assay	Differential activity
11	OH	Geranyl	0.2 μM	Yes
17	OCH_3	Geranyl	6.6 μM	Yes
18	F	Geranyl	43 μM	Yes
19	H	Geranyl	19 μM	No
20	H	H	16 μM	No
21	OH	H	7.8 μM	Yes

Thus compounds **19** and **20**, that lack any oxygenation in the left-half, fail to show any differential activity while still displaying some cytotoxicity. Interestingly the difluoro analog **18** retained a differential activity but showed greatly reduced overall cytotoxic behavior indicating some tolerance in the nature of the electron withdrawing group at these positions. Compound **21**, lacking the geranyl chain, retains differential activity and indeed is as active as the dimethoxy compound **17**. Overall these observations seemed to validate our original hypothesis that modification for mechanism of action studies be carried out at the terminus of the right-half geranyl chain. Gratifyingly, after synthesis of the terminal allylic alcohol **22,** testing showed this compound to be the most active of our 3-deoxyschweinfurthin analogs (mean GI_{50} 1.0 μM) and a good candidate for further modification along these lines.



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Key Accomplishments.

- The synthetic compound 3-deoxyschweinfurthin B is slightly *more* active than the natural product in enantioenriched form.
- The synthetic route has been expanded to establish an enantioselective synthesis of an advanced intermediate via an enantioselective oxidation and a chromatographic resolution.
- Several analogs of 3-deoxyschweinfurthin B bearing changes in the right-half resorcinol moiety have been synthesized and tested for bioactivity.
- An analog with suitably placed functionality to attach an affinity reagent or for yeast three hybrid assays has been synthesized and shown to retain significant and differential bioactivity.

Reportable Outcomes.

Abstract: Neighbors, Jeffrey D.; Salnikova, M.; Wiemer, David F. Synthetic studies towards the schweinfurthin family of cytotoxins. Abstracts of Papers, 227th ACS National Meeting, Anaheim, CA, United States, March 28 – April 1, 2004. (see abstract)

Abstract: Salnikova, Maya S.; Neighbors, Jeffrey D.; Wiemer, David F. Studies directed towards synthesis of schweinfurthin B analogs. Abstracts, 38th Midwest Regional Meeting of the American Chemical Society, Columbia, MO, United States, November 5-7 (2003)

Patent: Wiemer, David F.; Neighbors, Jeffrey D. ANALOGUES OF THE SCHWEINFURTHINS AS POTENTIAL ANTI-CANCER AGENTS (submitted)

Conclusions.

The finding of higher activity in the synthetic analog 3-deoxyshcweinfurthin B has changed the focus of our effort. The significant activity evident in this left-half modification has allowed us to make a series of analogs to ascertain the nature of the right-half functionality within the essential pharmacophore of this series of potential anti-cancer agents. Several analogs showing highly correlated activity in the NIH 60 cell-line panel have been synthesized. The ultimate result of this effort is an active compound (**22**) with suitable functionality for further modification to give affinity reagents, or for attachment to probes for three-hybrid assays. We are currently exploring the chemistry to establish such reagents.

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4. a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973,** *95*, 512–519. b) Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M.; Baldwin, J. J.; Christy, M. E.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.* **1986**, *51*, 2370–2374. c) For application to an extended alicyclic system see for example: Gallinis, D. L.; Wiemer, D. F. *J. Org. Chem.* **1993**, *58*, 7804–7807.

Appendices.

1. *Abstract of:* Neighbors, Jeffrey D.; Salnikova, M.; Wiemer, David F. Synthetic studies towards the schweinfurthin family of cytotoxins. Abstracts of Papers, 227th ACS National Meeting, Anaheim, CA, United States, March 28 – April 1, 2004.

2. *Abstract of:* Salnikova, Maya S.; Neighbors, Jeffrey D.; Wiemer, David F. Studies directed towards synthesis of schweinfurthin B analogs. Abstracts, 38th Midwest Regional Meeting of the American Chemical Society, Columbia, MO, United States, November 5-7 (2003)

Appendix

Synthetic studies toward the schweinfurthin family. Neighbors, Jeffrey D.; Salnikova, Maya S.; Wiemer, David F. Department of Chemistry, University of Iowa, Iowa City, IA, USA. Abstracts of Papers, 227th ACS National Meeting, Anaheim, CA, United States, March 28-April 1, 2004 (2004)

Abstract

The schweinfurthins are a small set of prenylated stilbenes isolated from Macaranga schweinfurthii, and three of the four known compds. display significant anticancer activity. These three have a common "left-half" tricycle exemplified in the structures of schweinfurthins A (1) and B (2). To prep. these natural products, we envision a late stage introduction of the stilbene olefin via HWE condensation and thus require a "left half" tricyclic aldehyde to couple with our previously synthesized "right-half" phosphonate. It has been established that a 3-deoxyschweinfurthin "left half" can be synthesized via an epoxide initiated cascaded cyclization. Our ongoing efforts to install the diol moiety, as well as efforts culminating in the synthesis of schweinfurthin analogs will be discussed.

Studies directed towards synthesis of schweinfurthin B analogs. Salnikova, Maya S.; Neighbors, Jeffrey D.; Wiemer, David F. Chemistry, University of Iowa, Iowa City, IA, USA. Abstracts, 38th Midwest Regional Meeting of the American Chemical Society, Columbia, MO, United States, November 5-7 (2003).

Abstract

Schweinfurthin B, a natural product from Macaranga schweinfurthii, exhibits an unusual cytotoxicity profile in the NCI's 60-cell line panel with a mean GI50 of 0.81 μM. We have undertaken a synthetic effort aimed at schweinfurthin B and related compds. Our synthetic strategy is based on a convergent approach, where two subunits (a tricyclic "left half" and a resorcinol "right half") are joined in formation of the central stilbene bond. This strategy should allow access to various analogs of the natural compd. for bioactivity and mechanism of action studies. Synthesis of modified "right halves" with various substituents and their influence on bioactivity will be presented in this poster.