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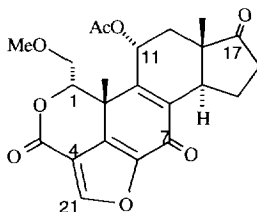
## SYNTHETIC STUDIES ON THE FURAN RING OF WORTMANNIN

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**Abstract:** Synthetic studies on the fungal metabolite, wortmannin, were undertaken in an effort to gain insight into the structure activity relationships of wortmannin analogs on phosphatidylinositol-3'-kinase (PI-3-kinase). Our work has focused on the chemistry of the previously unexplored furan ring and has uncovered several interesting and novel chemical transformations, which are described herein.

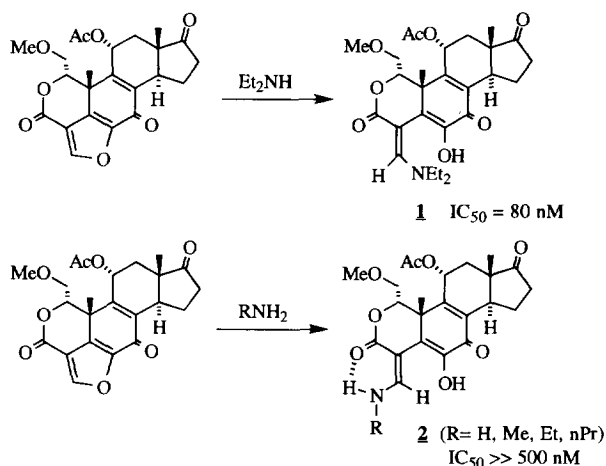
In the early 1970's, the structure of the fungal metabolite, wortmannin, was elucidated<sup>1</sup> and researchers at Sandoz reported on its potent antiinflammatory activity.<sup>2</sup> Structure activity relationship (SAR) studies were performed with the goal of reducing the toxicity observed in wortmannin while retaining its antiinflammatory activity. Unfortunately, development of wortmannin or wortmannin analogs as antiinflammatory agents was precluded due to this toxicity. A series of papers were published which outlined the synthetic efforts of the Sandoz group.<sup>3</sup> Some very elegant chemistry was developed which allowed for functionalization of the steroid D ring. Additionally, it was shown that the A ring could be opened under conditions which left much of the rest of the molecule intact. However, limited manipulation of the furan ring was reported.



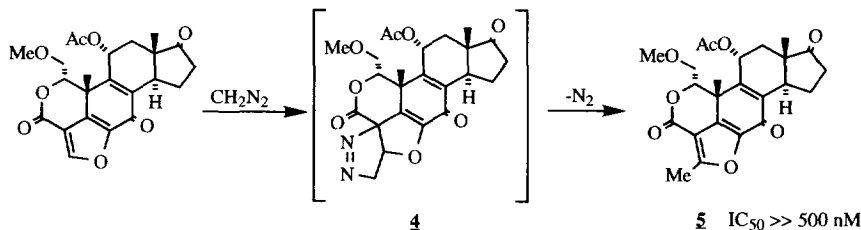
**Wortmannin** (IC<sub>50</sub> = 4.2 nM)

The chemistry of wortmannin apparently lay dormant for about 15 years until Broka and Ruhland reported on the total synthesis of a simplified analog of wortmannin in 1992.<sup>4</sup> Then in 1994, it was reported that wortmannin was a potent (4.2 nM) and selective inhibitor of phosphatidylinositol-3'-kinase (PI-3-kinase).<sup>5</sup> PI-3-kinase has been identified as an important enzyme in a number of growth factor signalling pathways and is a potential target for intervention in various proliferative diseases, such as cancer.<sup>6</sup> We were interested in the chemistry of wortmannin for the purpose of developing an SAR for PI-3-kinase inhibition. We were particularly interested in making changes to the furan portion of the molecule since we felt that the highly electrophilic C-21 position of wortmannin might be responsible for many of its biological activities.

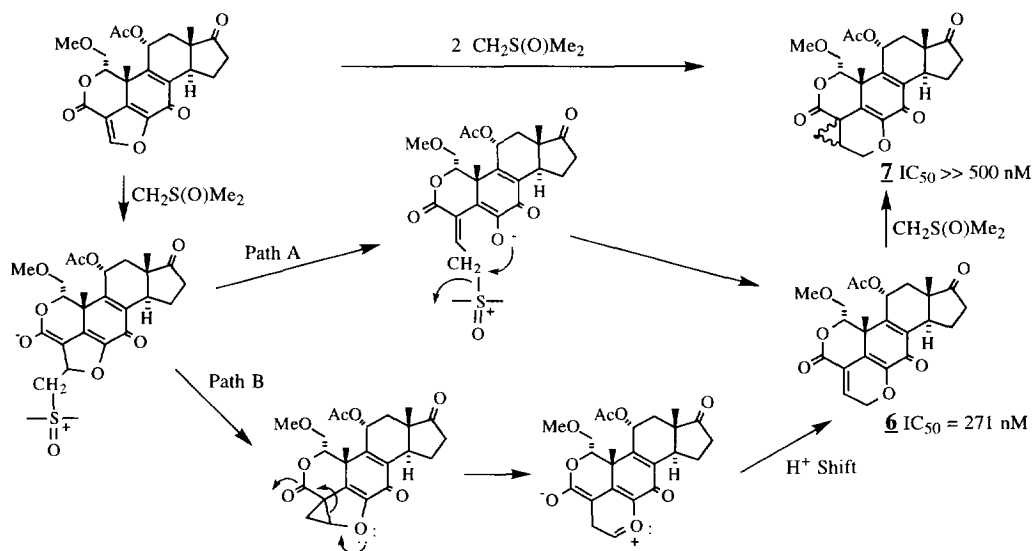
The C-21 position of wortmannin is highly susceptible to nucleophilic attack. Although it has been shown that secondary amines react readily with wortmannin to give the ring-opened derivatives **1**,<sup>3</sup> the configuration of the double bond has never been reported. When we observed strikingly different inhibition activities between the secondary amine adducts **1** and the primary amine adducts **2** we decided to probe this issue.<sup>5</sup> Upon examining the heteronuclear coupling constants between the olefinic protons and the lactone carbonyl carbon, we were able to determine that the double bond configurations were indeed different in the two systems.<sup>7</sup> We believe that the *Z* relationship observed in **2** is preferred due to an intramolecular hydrogen bond which is not possible for adduct **1**.



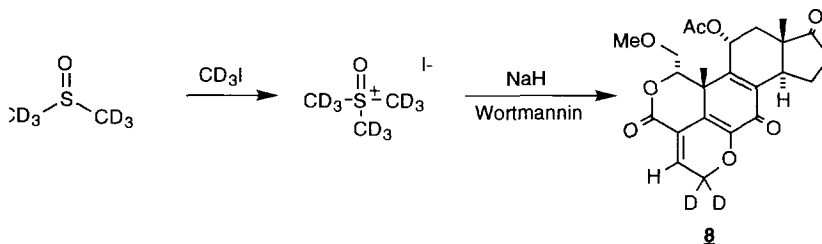
In an effort to further explore the chemistry of the furan ring and specifically the C-21 position, we treated wortmannin with diazomethane. The product of this reaction was not the expected cyclopropane, or even the pyrazoline **4**, but 21-methylwortmannin **5**.<sup>8</sup> Although we were unable to detect the pyrazoline **4**, we believe it to be an intermediate and loses nitrogen to form **5**. It should be noted that simple addition of a C-21 methyl group to wortmannin destroys PI-3-kinase activity.



A unique ring expansion product **6** was observed when wortmannin was treated with trimethylsulfoxonium ylide.<sup>9</sup> The cyclopropyl adduct **7** could be formed, as a 2:1 mixture of diastereomers, when wortmannin was treated with an excess of ylide or when **6** was directly treated with the ylide. While **6** retained moderate PI-3-kinase inhibitor activity, **7** was inactive up to 500 nM. We envisioned two possible mechanisms for the ring expansion reaction (Path A vs. Path B) and set out to determine which mechanism was operative since we were unable to detect any of the intermediates in this transformation. We reasoned that if the fully deuterated ylide was used in the reaction, the two potential mechanisms could be distinguished by the location of the deuterium atoms in the ring expansion product. We prepared the fully deuterated ylide



precursor in the usual manner by simply treating  $\text{d}^6$ -DMSO with an excess of  $\text{CD}_3\text{I}$  at reflux<sup>9</sup> and collected the solid sulfoxonium iodide. When the ylide was prepared and treated with wortmannin, the product **8** was formed exclusively.<sup>10</sup> This experiment indicates that Path A is preferred for the transformation.



The chemistry reported here allowed us to learn a great deal about the structural requirements for PI-3-kinase inhibition in wortmannin analogs. As we suspected, the electrophilic C-21 position in the furan ring proved to be an important site in the molecule. We found that changes at that position, such as the ones reported here, have a dramatic effect on PI-3-kinase inhibitor potency. A more comprehensive description of our PI-3-kinase SAR will be reported in a separate paper to appear shortly.

**Acknowledgement:** We thank Laverne Boek, Patrick Baker and Stephen Larson and their coworkers for the microbial production and chemical isolation of the wortmannin used for this work. We acknowledge Professor Garth Powis (University of Arizona) and his coworkers for their contributions to our PI-3-kinase inhibitor program. We would like to thank the Physical Chemistry Department at Eli Lilly and Company for their help in the characterization of compounds. Finally, we would like to thank Jennifer Olkowski for her timely solution of the X-Ray crystal structure of 21-methylwortmannin **5**.

All new compounds were characterized on the basis of their H-NMR,  $^{13}\text{C}$ -NMR, IR, Mass Spec. and Elemental Analysis data.

#### References and Notes

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2. Wiesinger, D.; Gubler, H. U.; Haeffliger, W.; Hauser, D. *Experientia* **1974**, 135.
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4. Broka, C. A.; Ruhland, B. *J. Org. Chem.* **1992**, 57, 4888.
5. Our initial findings and a detailed description of our assay conditions can be found in the following reference. Powis, G.; Bonjouklian, R.; Berggren, M. M.; Gallegos, A.; Abraham, R.; Ashendel, C.; Zalkow, L.; Matter, W. F.; Dodge, J.; Grindey, G.; Vlahos, C. J. *Cancer Research* **1994**, **54**, 2419.
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7. The heteronuclear coupling between the A ring carbonyl and the olefinic proton is dependent upon the stereochemistry with a value of 7 Hz or greater signifying an *E* configuration (between the C and H). Less than 7 Hz is indicative of a *Z* orientation. The coupling was measured using a gated  $^{13}\text{C}$  spectrum or a spectrum in which the proton was decoupled using low power. The following coupling constants were observed. **1**:  $J_{\text{CH}} = 4.5$  Hz. **2**:  $J_{\text{CH}} = 9.8$  Hz. This data confirms our assignments.
8. The structure of 21-methylwortmannin **5** was proven by a single crystal X-ray structure.
9. Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, 87, 1353.
10. The ABX pattern observed for the pyran ring protons in the H-NMR of **6** is first order and clear. In the H-NMR of the deuterated compound **8**, the AB protons are absent and the olefinic X proton has collapsed to a singlet.

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