Studies on the Mechanism of Phosphatidylinositol 3-Kinase Inhibition by Wortmannin and Related Analogs

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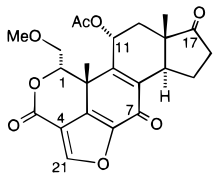
Wortmannin, a fungal metabolite, was identified as a potent inhibitor (IC_{50} = 4.2 nM) of phosphatidylinositol 3-kinase (PI 3-kinase). Due to the importance of PI 3-kinase in several intracellular signaling pathways, structure−activity studies on wortmannin analogs were performed in an effort to understand the structural requirements necessary for PI 3-kinase inhibition. Since wortmannin is an irreversible inhibitor of PI 3-kinase, it was postulated that covalent attachment at the electrophilic C-21 site was a possible mode of action for PI 3-kinase inhibition. We have prepared various wortmannin analogs which address the possibility of this mechanism. Of particular interest are compounds which affect the C-21 position of wortmannin either sterically or electronically. Our results support the conclusion that nucleophilic addition by the kinase onto the C-21 position of wortmannin is required for inhibition of PI 3-kinase by wortmannin analogs. Additionally, we have prepared several D-ring analogs of wortmannin, and their activities are reported herein. We conclude that the wortmannin D ring is an important recognition site since modifications have such a dramatic effect on inhibitor potency. Finally, the identification of 17β-hydroxywortmannin represents the first reported subnanomolar inhibitor of PI 3-kinase. These studies, along with *in vivo* antitumor experiments, suggest that the mechanism of PI 3-kinase inhibition correlates to the associated toxicity observed with wortmannin-based inhibitors of PI 3-kinase.

Introduction

The signaling pathways associated with cell growth and development are now recognized as potential new targets for therapeutic intervention in various proliferative diseases, such as cancer.[1] Research during the past decade has resulted in a greater understanding of oncogenes and their oncoproteins, giving us insight into the factors responsible for cellular signal transduction, especially as it applies to cell growth. One enzyme which has been implicated in growth factor signal transduction is phosphatidylinositol 3-kinase (PI 3-kinase).[2] This enzyme exists as a heterodimer of a 110 kDa catalytic subunit and a 85 kDa regulatory subunit.[3] PI 3-kinase, which selectively phosphorylates inositol lipids at the 3′ position, is an important enzyme in a number of cellular events which involve protein tyrosine kinases.[4] Recent studies show that PI 3-kinase is regulated by p21^{ras} *via* its association with the Ras effector site in a GTP-dependent manner.[5] Inhibitors of PI 3-kinase would be useful tools for the study of PI 3-kinase function and are potential therapeutic agents as well. Wortmannin (**1**) was recently identified as a potent and selective inhibitor of PI 3-kinase.[6] Structure−activity relationship (SAR) studies were initiated in an effort to better understand the structural requirements necessary for PI 3-kinase inhibition.

Kinetic analysis of the PI 3-kinase inhibition by wortmannin indicates that inhibition is irreversible.[6] We have postulated that the mechanism of PI 3-kinase inhibition by wortmannin occurs *via* covalent attachment of the kinase to the electrophilic C-21 position on the furan ring (Figure 1).[6] We felt that one strategy of probing the mechanism would be to make changes in the molecule (either steric or electronic), which may influence the electrophilicity of that center, and observe the effect on inhibitor potency. These studies, as well as *in vivo* evaluation of wortmannin-based inhibitors of PI 3-kinase, are presented here.



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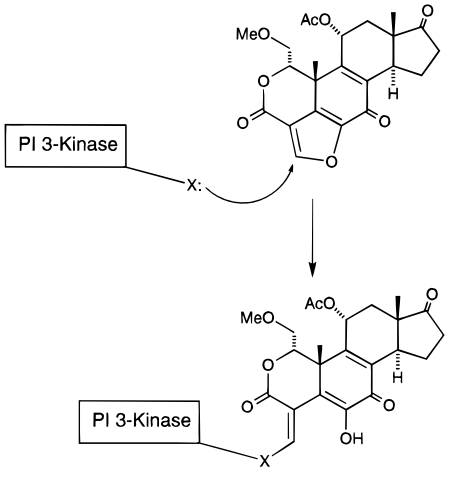

Figure 1.

Results

Furan Ring Modifications. The reaction of wortmannin with various amines and thiols has been shown to result in nucleophilic addition to the C-21 position followed by furan ring opening to give **2**−**4**.[7−9] Table 1

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1996.

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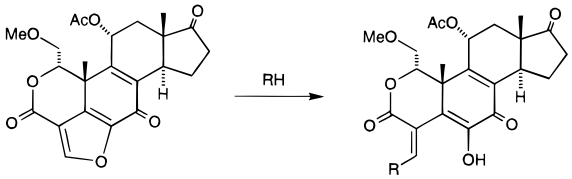
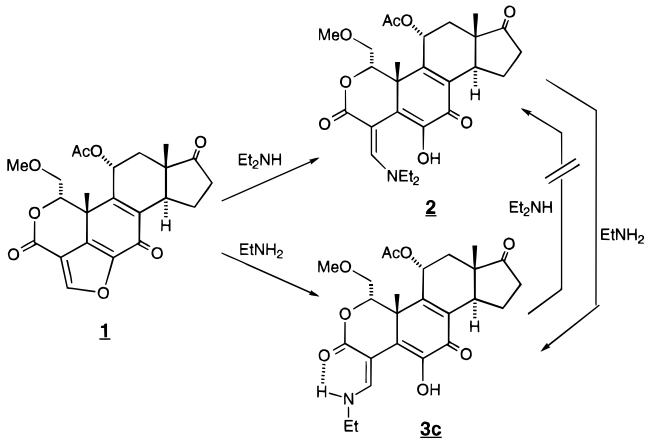
Table 1. PI 3-Kinase Activity of Wortmannin Nucleophilic Addition Products



1 **2-4**

entry	R	PI 3-kinase IC_{50} (nM)
2	Et_2N	80
3a	NH_2	≫500
3b	MeNH	≫500
3c	EtNH	≫500
3d	*n*-PrNH	≫500
4	*n*-BuS	52

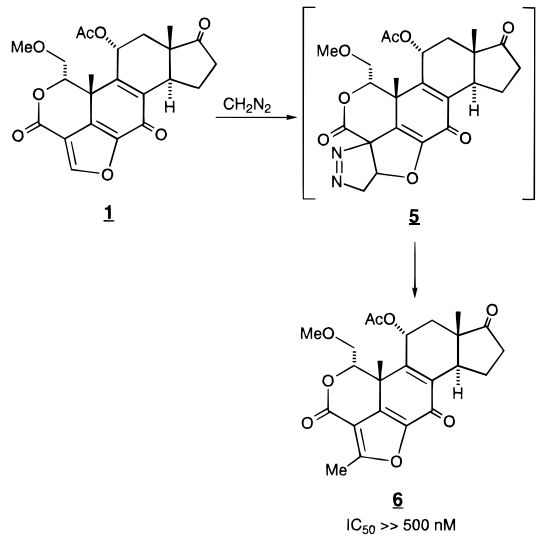
Scheme 1



shows the compounds which we prepared and their potency in PI 3-kinase inhibition assays.[10] It is interesting to note that **2** and **4** retain PI 3-kinase activity (albeit dramatically reduced), whereas **3a**−**e** show no inhibition up to 500 nM. We determined that the primary amine adducts **3** exist in a *Z* orientation with relation to the lactone carbonyl, while the secondary amine adduct **2** exists in the *E* configuration (Scheme 1).[11] It is clear that the *E* configuration could more closely mimic wortmannin in its ability to accept a nucleophile, while covalent attachment of the kinase to adducts such as **3** is precluded on steric grounds. In fact, when **2** was treated with excess ethylamine, efficient conversion to **3c** was observed. However, when **3c** was treated with diethylamine, no reaction was observed. This observation is consistent with our belief that nucleophilic addition to the inhibitor is required for inhibition of PI 3-kinase in these analogs.

To further test our hypothesis, we felt that direct substitution onto the C-21 position of wortmannin would provide a useful derivative which could more accurately test our steric hypothesis. When wortmannin was treated with diazomethane, 21-methylwortmannin (**6**) was produced in excellent yield (Scheme 2).[9] The structure of **6** was established by a single-crystal X-ray structure (Figure 2). It is believed that this compound is formed through an intermediate pyrrazoline, **5**, which undergoes loss of nitrogen to give the product. This compound showed no inhibition of PI 3-kinase up to 500 nM. Additionally, when **6** was treated with diethylamine, no reaction occurred. (It should be pointed out

Scheme 2



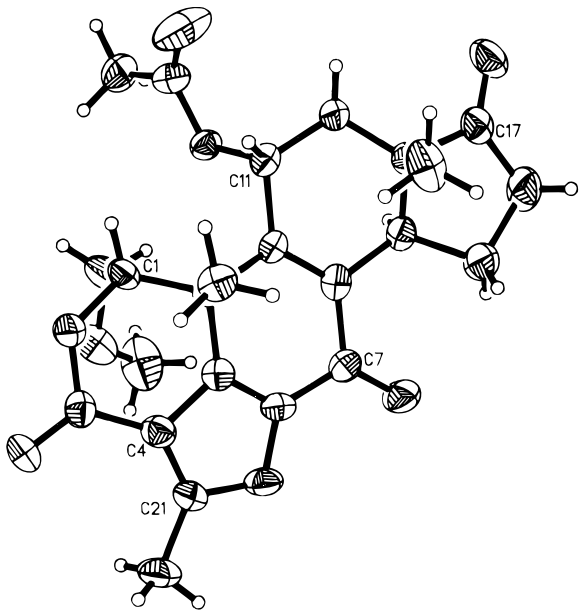
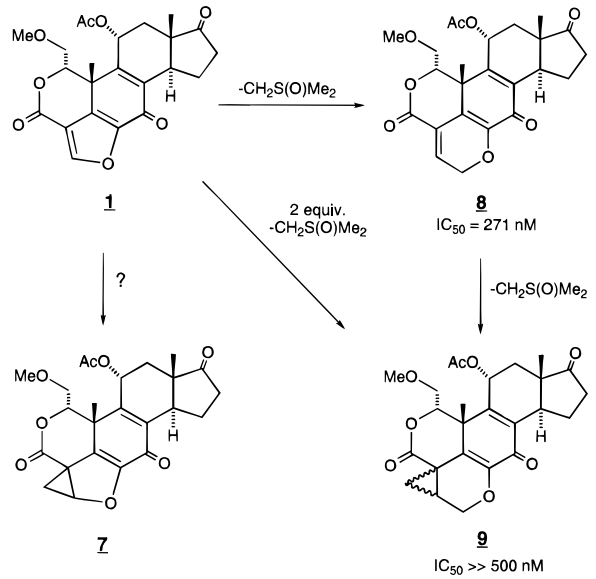


Figure 2. Ortep drawing of 21-methylwortmannin (**6**).

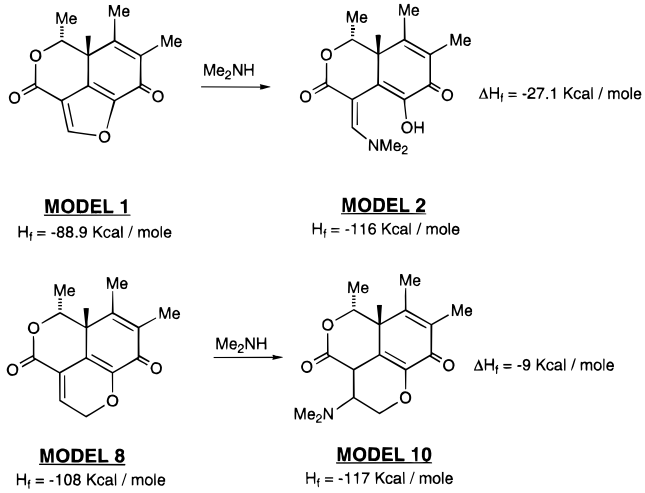
that the ring-opening reaction of wortmannin with diethylamine is instantaneous.) We feel that this is convincing evidence for our belief that PI 3-kinase forms a covalent attachment to wortmannin via the electrophilic C-21 position.

We were interested in preparing 4,21-cyclopropylwortmannin (**7**) *via* reaction of wortmannin with trimethylsulfoxoniumylide.[9] Although none of the desired product was observed, we did isolate an interesting ring expansion product, **8**, which could react with excess ylide to give the cyclopropyl derivative **9** as a mixture of diastereomers (Scheme 3).[9] The initial product **8** was a moderate inhibitor of PI 3-kinase (271 nM), while the cyclopropyl derivative **9** did not inhibit up to 500 nM. On the basis of our hypothesis, we were not surprised that **9** did not inhibit PI 3-kinase. However, we had expected **8** to be a more potent inhibitor. We can suggest two possible explanations for this loss of activity. First, the pyran ring in **8** is no longer planar like the furan ring in wortmannin. This slight perturbation in the three-dimensional space of that region may prevent efficient binding. An alternative explanation invokes a thermodynamic comparison of the nucleophilic

Scheme 3



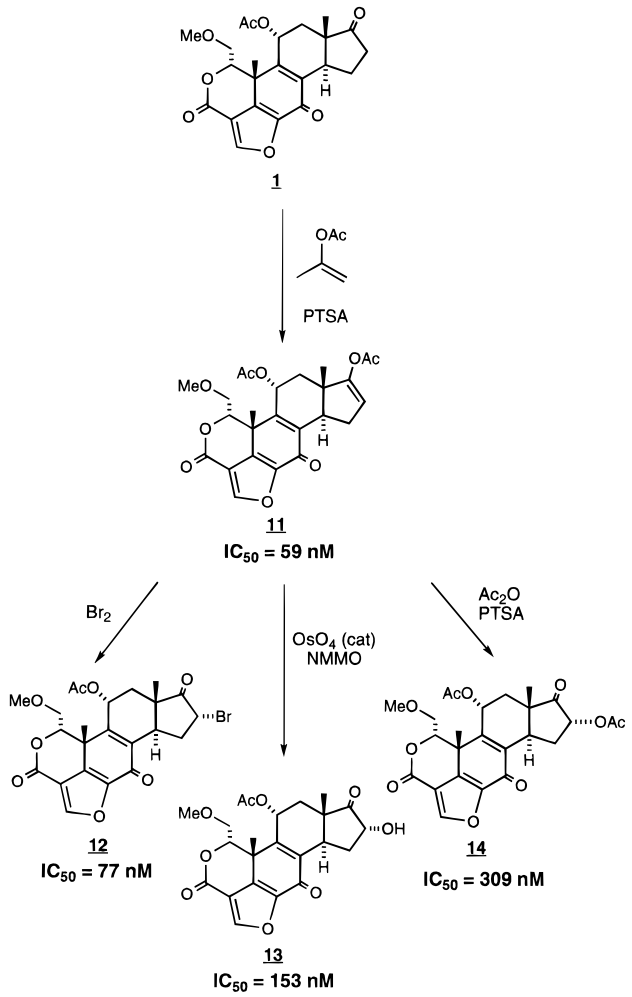
Scheme 4



addition products of **1** and **8**. For our calculations,[12] we used dimethylamine as the nucleophile for the presumed reaction of the inhibitor with the kinase. Additionally, we used a simplified inhibitor model (**model 1** and **model 8**) which deletes many of the constants between the two inhibitors, such as the C and D rings. The ring-opening reaction of **model 1** with dimethylamine produces **model 2**. This reaction has a $\Delta H_f = -27.1$ kcal/mol and is irreversible under these conditions. However, the reaction of **model 8** with dimethylamine produces **model 10**, which has a $\Delta H_f = -9.0$ kcal/mol and is reversible (Scheme 4). This difference could account for the dramatic difference in the activities of **1** vs **8**.

D-Ring Modifications. Although it is remote from the reactive furan ring, the D ring of wortmannin has proven to be very sensitive to structural modifications with relation to its PI 3-kinase activity. The enol acetate **11**, which showed about a 10-fold loss of activity relative to wortmannin ($IC_{50} = 59$ nM), was used as an intermediate in the preparation of several 16-substituted wortmannin derivatives. All of these derivatives (**12**−**14**) showed a sharp decrease in their ability to inhibit PI 3-kinase (Scheme 5). Interestingly, the enol acetate **11** was more active than any of the 16-

Scheme 5

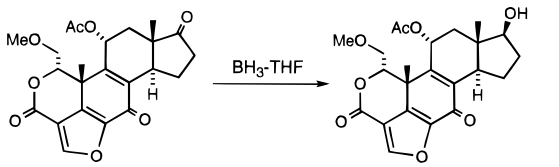


substituted inhibitors. We cannot draw many conclusions from this data since it is such a small set of compounds. However, this data suggests that the D ring of wortmannin is an important recognition element since even minor changes to the substitution pattern precludes efficient binding to PI 3-kinase. This is in contrast to our earlier studies on the C ring of wortmannin, which showed that changes in the C-ring substitution pattern had only a slight effect on inhibitor potency.[6,13] We feel that it is unlikely that a nonselective alkylating agent would show such dramatic SAR results so distant from the electrophilic site. The changes to the D ring shown here would certainly have a minimal effect on the electrophilicity of the C-21 position of wortmannin and its ability to form a covalent complex with a nucleophilic species.

One D-ring modification gave a dramatic increase in PI 3-kinase inhibitor potency. 17β-Hydroxywortmannin (**15**), prepared from the reduction of wortmannin with borane, showed a 10-fold increase in activity relative to wortmannin and pushed the activity into the subnanomolar range (Scheme 6).[13] With an IC_{50} of 0.50 nM, it is the most potent inhibitor of PI 3-kinase reported to date.

Cytotoxicity and Antitumor Activity of Wortmannin. Wortmannin has been shown to inhibit PI 3-kinase in PDGF stimulated v-*sis* NIH 3T3 cells.[6] Additionally, it has been reported that wortmannin displays cytotoxicity in several human tumor cell lines, with IC_{50}'s in the 0.3−0.9 μg/mL range for GC3 colon

Scheme 6



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17-β-Hydroxywortmannin 15
IC_{50} = 0.5 nM

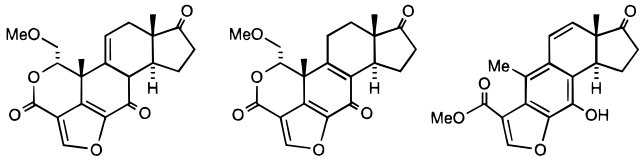
Table 2. *In Vivo* Evaluation of Wortmannin

tumor line	dose (mg/kg)[a]	inhibition[b]
6C3HED lymphosarcoma	0.75	−
	1.50	toxic
B-16 melanoma	0.75	−
	1.50	toxic
BXPC-3 pancreatic	0.75	+
	1.50	toxic
C-26 colon	0.75	−
	1.50	toxic
C3H mammary	1.25	+
	2.50	toxic
CX-1 colon	0.75	−
	1.50	toxic
GC3 colon	0.38	toxic
	0.75	toxic
HC1 colon	0.75	−
	1.50	toxic
IGROV1 ovarian	0.75	−
	1.50	toxic
Lewis lung	0.75	−
	1.50	toxic
LX-1 lung	0.38	−
	0.75	toxic
M-5076 ovarian	0.75	−
	1.50	toxic
MX-1 mammary	0.75	−
	1.50	toxic
PACA-2 pancreatic	0.75	−
	1.50	toxic
PANC-1 pancreatic	0.75	−
	1.50	toxic
VRC5 colon	0.75	−
	1.50	toxic
X-5563 plasma cell myeloma	0.75	−
	1.50	toxic

[a] Wortmannin was given po daily ×10 (except for the 6C3HED lymphosarcoma tumor in which wortmannin was given po daily ×8). [b] Antitumor activity (inhibition of tumor growth): −, <60% inhibition; +, 60−79%; ++, 80−94%; +++, 95−100%; toxic, >25% lethality.

carcinoma, IGROV1 ovarian carcinoma, and CCRF-CEM leukemia.[14] We have evaluated wortmannin in 16 different *in vivo* tumor models using daily po dosing in an effort to find an appropriate model for antitumor evaluation of wortmannin and related analogs. Our results are shown in Table 2. The narrow therapeutic index has made *in vivo* evaluation difficult for wortmannin and related analogs, but the C3H mammary adenocarcinoma cell line was chosen to complete a more thorough examination of issues such as dosage and schedule dependency. Other routes (iv, sc, and ip) and dosing schedules (q2d×5 and b.i.d. × 10) were investigated in the C3H mammary tumor, but the antitumor activity was not significantly different than seen for daily ×10 po dosing.

We have evaluated some additional wortmannin analogs in the C3H mammary antitumor model, and the results are shown in Table 3. In addition to **2** and **15**, antitumor activity was evaluated for three previously



16 17 18

reported wortmannin analogs, **16**−**18**.[6] Included in Table 3 is the *in vitro* PI 3-kinase activity of these analogs.

Table 3. Antitumor Activity of Wortmannin Analogs in C3H Mammary Model

compd	PI 3-kinase IC_{50} (nM)	dose (mg/kg)[a]	inhibition[b]
1	4.2	1.25	+
		2.50	toxic
2	80	5.0	+
		10.0	toxic
15	0.50	0.5	−
		1.0	toxic
16	54	8.0	+
		16	toxic
17	17	2.0	−
		4.0	toxic
18	4600	16	−

[a,b] See Table 2 footnotes.

Discussion and Conclusions

One of the goals of our PI 3-kinase inhibitor program was to further our understanding of the relationship between PI 3-kinase activity and toxicity in our animal models. The data in Table 3 show a relationship between *in vitro* inhibitor potency and antitumor activity for the compounds which displayed a sufficient therapeutic index. A relationship also exists between *in vitro* potency and toxicity. The most potent PI 3-kinase inhibitors show toxicity at the lowest doses. Additionally, the lactone ring-opened compound **18**, which was about 1000 times less potent than wortmannin *in vitro*, showed no activity and no toxicity in the C3H mammary tumor model up to 16 mg/kg. Table 3 clearly shows that all of the potent inhibitors (**1**, **2**, **16**, and **17**) had a tolerable dose at one-half the toxic dose. Compounds **1**, **2**, and **16** showed antitumor effects at this dose. These data correlate well with the *in vitro* potency. An additional interesting observation is the fact that 21-methylwortmannin (**6**), which differs from wortmannin only by the replacement of a hydrogen with a methyl group, showed no toxicity in our C3H mammary mouse lethality model up to 100 mg/kg (po, daily ×5), while wortmannin shows acute toxicity and death at 3 mg/kg (po, daily ×4). Since 21-methylwortmannin (**6**) does not inhibit PI 3-kinase (up to 500 nM) but is so structurally similar to wortmannin, we feel that the toxicity of wortmannin and related analogs is related to the mechanism for PI 3-kinase inhibition. Additionally, we have shown that this activity seems to be associated with the ability of the inhibitor to bind covalently to the kinase at the electrophilic C-21 position.

In summary, we have evaluated wortmannin analogs and studied the relationship between PI 3-kinase inhibitor potency and the ability of these analogs to react with nucleophiles at the C-21 position. Our data suggest that nucleophilic addition of the kinase to the electrophilic C-21 position of wortmannin and related

analogs is required for inhibitor potency and antitumor activity. Unfortunately, this mechanism appears to be linked to the observed toxicity.

Experimental Section

Compounds **2** and **11**−**18** are not new and were prepared according to the literature procedures outlined in ref 7.

General Procedure for the Reaction of Wortmannin with Primary and Secondary Amines. Wortmannin (1.00 g, 2.33 mmol) was dissolved in 40 mL of methylene chloride and stirred at 25 °C as 2.56 mmol of the amine was added dropwise. The reaction mixture immediately turned dark orange and was concentrated *in vacuo*. The resulting orange amorphous solid was recrystallized from 50% ethyl acetate−isooctane to give yellow-orange crystalline solids, whose structures were assigned on the basis of their spectroscopic and analytical data. This procedure was used for the preparation of diethylamino adduct **2**, methylamino adduct **3b**, ethylamino adduct **3c**, and *n*-propylamino adduct **3d**. Each of these compounds gave acceptable spectroscopic and analytical data (^{1}H NMR, IR, MS, elemental analysis). The spectroscopic data was nearly identical with that of **3a** in all of these cases.

Amino Adduct 3a. A solution of 25 mg (0.058 mmol) of wortmannin, 9.0 mg (0.116 mmol) of ammonium acetate, and 16 mg (0.116 mmol) of anhydrous powdered potassium carbonate in 2 mL of 50% THF−water was stirred at 25 °C for 30 min. The reaction mixture was poured into 10 mL of methylene chloride and the organic phase washed once with water, dried over sodium sulfate, and concentrated *in vacuo*. The resulting solid was recrystallized from 50% ethyl acetate−isooctane to give 21 mg (81%) of a yellow solid (mp 140−143 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (s, 3H), 1.55 (s, 3H), 1.88 (dd, 1H, J = 15.1, 3.3 Hz), 2.03 (s, 3H), 2.20−2.40 (m, 3H), 2.55 (m, 1H), 2.82−2.98 (m, 2H), 3.19 (m, 2H), 3.26 (s, 3H), 4.34 (dd, 1H, J = 7.2, 1.2 Hz), 5.80 (bs, 1H), 5.99 (dd, 1H, J = 7.9, 3.2 Hz), 7.14 (s, 1H), 8.61 (dd, 1H, J = 15.1, 8.3 Hz), 9.36 (m, 1H); IR (KBr) 1222, 1580, 1627, 1642, 1687, 1743, 3367 cm^{-1}; MS (FAB) 446 ($M^+ + 1$). Anal. ($C_{23}H_{27}NO_8$) C, H, N.

Butanethiol adduct 4. To a solution of wortmannin (200 mg, 0.47 mmol) in methylene chloride (2 mL) were added *n*-butanethiol (0.08 mL, 0.75 mmol) and 1 drop of triethylamine. After the mixture had stirred overnight under nitrogen, another drop of triethylamine was added and the reaction mixture stirred for an additional 1 h. The volatiles were removed *in vacuo*, and the residue was chromatographed by radial chromatography (silica gel, 1:1 EtOAc/hexanes) to give 186 mg (77%) of product as a bright yellow-orange solid (mp 88−90 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (s, 3H), 1.55 (s, 3H), 1.88 (dd, 1H, J = 15.1, 3.3 Hz), 2.03 (s, 3H), 2.20−2.40 (m, 3H), 2.55 (m, 1H), 2.82−2.98 (m, 2H), 3.19 (m, 2H), 3.26 (s, 3H), 4.34 (dd, 1H, J = 7.2, 1.2 Hz), 5.80 (bs, 1H), 5.99 (dd, 1H, J = 7.9, 3.2 Hz), 7.14 (s, 1H), 8.61 (dd, 1H, J = 15.1, 8.3 Hz), 9.36 (m, 1H); IR ($CHCl_3$) 1197, 1317, 1421, 1625, 1743, 2976 cm^{-1}; MS (FD) m/e 518 (M^+). Anal. ($C_{27}H_{34}O_8S$) C, H.

21-Methylwortmannin (6). Diazomethane (10 mmol) was prepared according to the procedure of De Boer and Backer.[15] The solution of diazomethane (about 30 mL) was transferred to a fire-polished, 250 mL Ehrlenmeyer flask. To this solution was added 500 mg (1.17 mmol) of wortmannin in 50 mL of THF. The top of the flask was covered with parafilm, and the solution was stirred at 25 °C and monitored by TLC. The reaction required 3 days to go to completion, after which the mixture was concentrated *in vacuo* and recrystallized from 50% ethyl acetate−isooctane to give 420 mg (81%) of a yellow crystalline solid (mp 178−179 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.57 (m, 1H), 1.70 (s, 3H), 2.02 (m, 1H), 2.13 (s, 3H), 2.23 (m, 1H), 2.56−2.62 (m, 2H), 2.73 (s, 3H), 2.86 (ddd, 1H, J = 12.6, 5.8, 2.8 Hz), 2.99 (dd, 1H, J = 11.1 and 7.5 Hz), 3.16 (m, 1H), 3.21 (s, 3H), 3.43 (dd, 1H, J = 11.1, 1.5 Hz), 4.71 (dd, 1H, J = 7.4, 1.8 Hz), 6.13 (dt, 1H, J = 7.7, 2.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.9, 14.5, 21.0, 22.9, 26.5, 35.7, 36.2, 40.7, 44.1, 49.2, 59.4, 70.1, 72.9, 88.5, 109.3, 140.3, 142.8, 144.2, 148.9, 158.6, 164.8, 169.5, 172.1, 216.2; IR (KBr) 1221, 1582, 1653, 1680, 1740, 2920 cm^{-1}; MS (FD) m/e 443 ($M^+ + 1$).

Wortmannin Pyran 8. Trimethylsulfoxonium iodide (321 mg, 1.46 mmol) was suspended in 4 mL of DMSO and stirred at 25 °C as 58 mg (1.46 mmol) of sodium hydride (60% in mineral oil) was added. The formation of the ylide was complete in about 30 min, as evidenced by the cessation of hydrogen evolution and the formation of a clear solution. To this solution was added 500 mg (1.17 mmol) of wortmannin in 2 mL of DMSO. The dark reaction mixture was stirred for an additional 30 min at 25 °C and the reaction quenched by pouring the mixture into 20 mL of water. The mixture was extracted with ethyl acetate (2 × 30 mL), and the combined organic extracts were washed once with brine. The resultant orange solution was dried and concentrated *in vacuo* to give a brown oil. A crude ^{1}H NMR indicated that the reaction mixture contained about 25% wortmannin, 50% pyran **8**, and 25% cyclopropylpyran **9**. Column chromatography on silica gel using 50% ethyl acetate−hexane as the eluent provided pure pyran **8**, which was recrystallized using 50% ethyl acetate−isooctane to give 182 mg (35%) of a white crystalline solid (mp 130−131 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.57 (s, 3H), 1.72 (dd, 1H, J = 14.2, 5.7 Hz), 2.02 (m, 1H), 2.08 (s, 3H), 2.25 (m, 1H), 2.48 (dd, 1H, J = 13.9, 7.6 Hz), 2.59 (m, 1H), 2.86−3.04 (m, 2H), 3.18 (dd, 1H, J = 11.1, 6.4 Hz), 3.24 (s, 3H), 3.46 (dd, 1H, J = 11.1, 2.2 Hz), 4.61 (dd, 1H, J = 6.3, 2.2 Hz), 4.74 (dd, 1H, J = 16.1, 3.3 Hz), 5.14 (dd, 1H, J = 16.1, 5.5 Hz), 6.02 (m, 1H), 6.98 (dd, 1H, J = 5.5, 3.3 Hz); IR ($CHCl_3$) 1236, 1653, 1743, 2936 cm^{-1}; MS (FD) m/e 442 (M^+). Anal. ($C_{24}H_{26}O_8$) C, H.

Wortmannin Cyclopropylpyran 9. Using the above procedure, **9** can be formed exclusively by simply using 2 equiv of trimethylsulfoxoniumylide and sodium hydride. The white crystalline product **9** (mp 122−125 °C) was isolted in 72% yield as a 2:1 mixture of diastereomers (which were not separated). Major isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (s, 3H), 2.70 (dd, 1H, J = 9.1, 6.5 Hz), 2.79 (dd, 1H, J = 9.1, 3.6 Hz), 2.05 (dd, 1H, J = 6.5, 3.6 Hz), 2.10 (s, 3H), 2.22 (m, 2H), 2.51 (m, 2H), 2.78−2.97 (m, 2H), 3.00 (dd, 1H, J = 8.6, 6.3 Hz), 3.33 (s, 3H), 3.48−3.75 (m, 3H), 4.53 (d, 1H, J = 11.0 Hz), 4.59 (dd, 1H, J = 7.8, 1.8 Hz), 5.97 (m, 1H); IR ($CHCl_3$) 1146, 1653, 1744, 3019 cm^{-1}; MS (FAB) m/e 457 ($M^+ + 1$). Anal. ($C_{25}H_{28}O_8$), C, H.

***In Vitro* Evaluation of PI 3-Kinase Inhibitors.** The PI 3-kinase inhibition assay has been described previously.[6]

***In Vivo* Inhibition of Tumor Growth.** The antitumor activity of wortmannin and its analogs was evaluated in a series of solid murine[16] and human xenograft[17] tumor lines. Tumors were carried by serial passage with subcutaneous implants of tumor fragments *via* trochar in the axillary region. Dosing was initiated 1 day after implant for the murine tumors (6C3HED lymphosarcoma, B-16 melanoma, C-26 colon, C3H mammary, Lewis lung, and X-5563 plasma cell myeloma) except for the M-5076 ovarian for which dosing was initiated 5 days after implant. For the human xenograft tumors (BXPC-3 pancreatic, CX-1 colon, GC3 colon, LX-1 lung, PACA-2 pancreatic, and PANC-1 pancreatic), dosing was initiated 7 days after the implant except for the HC1 colon and IGROV1 ovarian for which dosing was initiated 14 days after tumor implant. Wortmannin and its analogs were dosed as a suspension in 2.5% GAF emulphor EL-620 (Warren-Graham, Cockeysville, MD) in 0.9% NaCl except for **2** which was dosed in water. Tumor weights were calculated from measurements of the width and length of tumors using electronic calipers interfaced to a microcomputer using the following formula:[18]

$$\text{tumor weight in mg} = [\text{tumor length in mm} \times (\text{tumor width in mm})^2] \times 0.5$$

Tumor measurements were taken on the day after the last dose for murine tumors and 5 days after the last dose for the human xenograft tumors. Percentage inhibition of tumor growth was calculated as:

percent inhibition of tumor growth =

$$100 \times (1 - \frac{\text{average tumor weight of treated group}}{\text{average tumor weight of control group}})$$

Groups consisted of 10 mice that were randomized from those implanted. A group was considered toxic if lethality was >25%.

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Supporting Information Available: X-ray data for 21-methylwortmannin (**6**) (8 pages). Ordering information is given on any current masthead page.

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(8) Haefliger and co-workers have shown that the furan ring can be opened with diethylamine to give **2** (see ref 7). Analysis of the olefin geometry (*E* vs *Z*) was not reported.

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(10) Our PI 3-kinase assay, which utilizes purified enzyme from bovine brain has been described in detail in ref 6.

(11) 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}C NMR spectrum or a spectrum in which the proton was decoupled using low power. The following coupling constants were observed. **2**: $J_{CH} = 4.5$ Hz. **3c**: $J_{CH} = 9.8$ Hz. These data confirm our assignments. Additionally, an intramolecular hydrogen bond exists between the lactone carbonyl (A ring) and the N-H hydrogen. This effect results in a downfield shift in the N-H proton signal in the ^1H NMR and was observed for all of the primary amine adducts (**3a**−**d**). This effect has been previously addressed (see ref 9).

(12) Molecular modeling calculations were performed using the program Spartan (version 3.1.1; Wavefunction, Inc., 18401 Von Karmen, Suite 370, Irvine, CA 92715) running on an SGI IRIS Indigo workstation. *In vacuo* heats of formation were obtained with the semiemperical AM1 Hamiltonian; geometries were optimized until the SCF energy converged such that the difference between two consecutive cycles was $<1.0 \times 10^{-5}$. It should be pointed out that wortmannin reacts with dimethylamine to give the product **model 2**, analogous to the reaction with diethylamine. We have chosen to use dimethylamine to simplify our thermodynamic calculations.

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