Synthesis and Structure–Activity Relationships of Ring-Opened 17-Hydroxywortmannins: Potent Phosphoinositide 3-Kinase Inhibitors with Improved Properties and Anticancer Efficacy

Arie Zask,*[,†] Joshua Kaplan,[†] Lourdes Toral-Barza,[‡] Irwin Hollander,[‡] Mairead Young,[§] Mark Tischler,[§] Christine Gaydos,[‡] Michael Cinque,[‡] Judy Lucas,[‡] and Ker Yu[‡]

Discovery Medicinal Chemistry, Oncology Research, and Chemical Technologies, Wyeth Research, Pearl River, New York 10965

Received October 11, 2007

The phosphoinositide 3-kinase (PI3K) signaling pathway is frequently up-regulated in human cancer and is a promising target for the treatment of cancer. Wortmannin and its analogues are potent inhibitors of PI3K but suffer from inherent defects such as instability, insolubility, and toxicity. Opening of the reactive furan ring of 17-hydroxywortmannin with amines gives compounds with improved properties such as greater stability and aqueous solubility and a larger therapeutic index. Ring-opened analogues such as compound **13** containing basic amine groups have significantly increased PI3K inhibitory potency and greater efficacy in nude mouse xenograft assays.

The phosphoinositide 3-kinases (PI3K) signaling pathway is one of the most frequently mutated in human cancer, leading to amplification of signaling, and as such is a promising target for small-molecule inhibition, with promising therapeutic opportunities for the treatment of cancer.[1,2] The PI3Ks regulate cellular metabolism and growth by phosphorylation of the 3-position of phosphatidylinositol to generate phosphatidylinositol triphosphate that in turn couples the PI3Ks to downstream effectors.

The natural product wortmannin (**1**) and its analogue 17-hydroxywortmannin (**2**, Figure 1) are potent, nonselective inhibitors of PI3Ks that bind irreversibly to lysine-833 in the ATP binding pocket of PI3K via opening of the electrophilic furan ring at its C-20 position. The irreversible binding of wortmannin to PI3K γ has been proven by X-ray crystallography.[3] Wortmannin is a potent cytotoxic agent against human tumor cell lines in xenograft models in mice. The toxicity of wortmannin, as seen in its low therapeutic index, as well as its insolubility and aqueous instability, has hampered its development into a useful anticancer agent.[4,5] Recently, conjugates of **1** and **2** (e.g., **3**, PWT-458) with greatly improved properties were developed by the application of pegylation technology (Figure 1).[6,7]

Another approach to improving the properties of wortmannin (**1**) is through opening of the furan ring at C-20 with nucleophiles such as amines. Opening of the furan ring with dialkylamines was first reported in 1975 as a means of protecting the ring under the basic conditions necessary for deacetylation of the C-11 position.[8] Subsequently, it was shown that ring-opened wortmannin analogues retained PI3K inhibitory activity, although with much reduced potency.[9] Ring-opened wortmannin analogue **4** (PX-866), the product of furan ring opening of wortmannin with diallylamine,[10] was recently reported to be active in human tumor xenograft models in mice.[11] Mechanistically, the PI3K inhibitory activity of ring-opened compounds is consistent with the ability of the secondary amine to be displaced by primary amines (e.g., lysine-833 in PI3K).[9] We

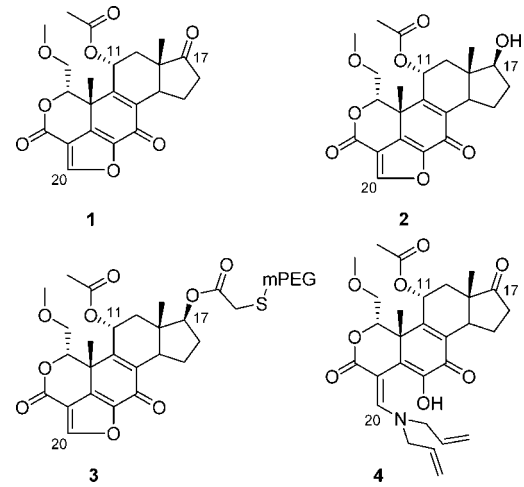


Figure 1. Wortmannin (**1**) and analogues.

predicted that the greater stability of ring-opened compounds would increase their half-lives under physiological conditions where reactive amine groups are present. The reduced reactivity of these analogues could lead to decreased toxicity as exemplified by a higher therapeutic index. Described herein is the synthesis and structure–activity relationships of ring-opened 17-hydroxywortmannin analogues with greatly improved aqueous solubility, in vivo stability, and therapeutic ratio relative to wortmannin.

Synthesis

Treatment of **2** with primary or secondary amines in methylene chloride or ethyl acetate gave the ring-opened analogues (Scheme 1). Similarly, 11-desacetyl 17-hydroxywortmannin[8] was treated with amines in dichloromethane to give the corresponding ring-opened analogues.

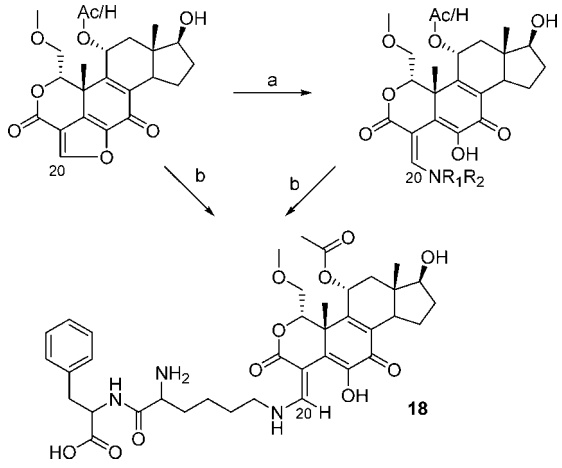
Results and Discussion

Preliminary libraries of ring-opened 17-hydroxywortmannin analogues were prepared with structurally diverse amines (Supporting Information Tables S1 and S2). This initial survey of the structure–activity relationships revealed that aliphatic monoamines gave analogues with PI3K IC_{50} values several

* To whom correspondence should be addressed. Phone: 845-602-2836. Fax: 845-602-2836. E-mail: zaska@wyeth.com.
† Discovery Medicinal Chemistry.
‡ Oncology Research.
§ Chemical Technologies.

10.1021/jm7012858 CCC: $40.75
Published on Web 02/13/2008

Scheme 1. Synthesis of Ring-Opened 17-Hydroxywortmannin Analogues[a]



[a] (a) HNR_1R_2, CH_2Cl_2 or EtOAc (R_1 and R_2 are as defined in the tables); (b) lysine−phenylalanine, pH 7.4.

orders of magnitude less potent than **2** (Table S1, Table 1), while much more potent inhibitors were obtained by reacting **2** with aliphatic diamines (Table S2, Table 2). The most potent inhibitors were formed from acyclic diamines and inhibited PI3K with IC_{50} values comparable to that of **2** (Table 2). To our knowledge, these are the most potent ring-opened wortmannin based inhibitors reported to date. Cyclic diamines typically gave less potent PI3K inhibitors than the acyclic diamines (Table S2). Although compound **4** is reported to have $IC_{50} = 0.5$ nM versus PI3K,[10,11] in our assays its potency ($IC_{50} = 88$ nM) is much lower, similar to that of other aliphatic ring-opened analogues in Table 1 and the literature.[5] Polar groups such as alcohols, esters, carboxylic acids, and nitriles had little effect on the potency of aliphatic ring-opened analogues (Table S1). Reaction of **2** with primary amines gave analogues (**7**, **10**, **14**) that were orders of magnitude less potent than those synthesized with the corresponding secondary amines (**5**, **9**, **13**, respectively).[9,12] This decrease in potency is consistent with the reported inability of primary amine analogues to revert to wortmannin[13] or to have their primary amine group displaced by amines.[9] The inaccessibility of these mechanistic pathways would preclude irreversible inhibition of PI3K through reaction with lysine-833. Ring-opened 17-hydroxywortmannin analogues, like **2**, were much weaker inhibitors of mTOR than PI3K. The X-ray structure of **11** shows the geometry of the olefin formed upon ring opening with a secondary amine (methylbutylamine) as E (Figure 2). This result confirms the olefin geometry of secondary amine ring-opened wortmannins previously proposed, based on heteronuclear NMR coupling constants.[9]

Interestingly, inhibition of cell growth did not correlate directly with the potency of PI3K inhibition of these analogues (Tables 1 and 2). Ring-opened analogues of aliphatic monoamines were often more potent inhibitors of cell growth than **2**, despite having much higher PI3K IC_{50} values (Table 1). One explanation for this anomaly is the instability of **2** in culture systems due to covalent binding via its furan ring to amine groups in the serum proteins in the medium.[12,14,15] In contrast to **2**, ring-opened analogues are resistant to reaction with amines, thereby leading to longer exposure times of cells to these compounds. For example, treatment of **5** and **2** with the dipeptide lysine−phenylalanine at pH 7.4 led to compound **18** (Scheme 1) 14 times more slowly from **5** than from **2** ($T_{1/2}$ = 57 min vs 4 min, respectively).

Ring-opened analogues were also found to be much more stable in nude mouse plasma than **2** (Figure 3). Surprisingly, the expected mechanism of degradation by amine displacement at C-20 by plasma proteins did not take place to an appreciable extent. Instead, deacetylation of the C-11 group by esterases was the major metabolic pathway in plasma (e.g., **5** to **6** and **13** to **15**). Addition of the esterase inhibitor potassium fluoride suppressed the deacetylation reaction. Interestingly, analogue **8** with a bulky *tert*-butylethanolamine group was resistant to enzymatic deacetylation and showed relatively little degradation over 8 h. The loss of the 11-acetyl group led to ring-opened analogues (**6** and **15**) that were much less potent PI3K inhibitors.

The most potent inhibitor was the aliphatic diamine analogue **13** ($IC_{50} = 6.4$ nM). This analogue had weak activity in a panel of protein kinases but showed strong inhibition of PI3K isoforms α, β, and δ (Table 3). Consistent with **2** and the ring-opened analogues, **13** had diminished potency for the mammalian target of rapamycin (mTOR) with a 426-fold selectivity for PI3K α.

As with the wortmannins, the mode of action of these ring-opened analogues is through irreversible inhibition of the enzyme. Inhibition of the homologous enzyme mTOR, which also contains the reactive lysine-833 residue, showed time dependent inhibition consistent with irreversible binding (Figure 4). In contrast, the dual PI3K/mTOR inhibitor 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one (**19**, LY294002) that binds noncovalently showed no time dependence of inhibition of mTOR. Inhibitor wash-out assays in U87MG glioma cells also confirmed that these inhibitors irreversibly target PI3K.[16]

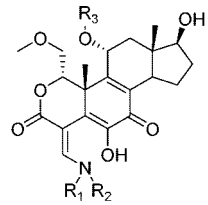
On the basis of its superior potency, **13** was further investigated in biological systems. Compound **13** inhibited the PI3K signaling cascade in the PTEN-negative U87MG glioma cells. It inhibited phospho-AKT (S473) and phospho-S6K (T389) phosphorylation at 30 ng/mL (Figure 5). Interestingly, the aliphatic amine analogue **8** was only 3-fold less potent. The enhanced cell activity of **8** is likely due to its superior stability in plasma (Figure 3).

Compound **13** showed potent inhibition of tumor growth (U87MG glioma) in the nude mouse xenograft model (Figure 6). The iv dosing was facilitated by the good aqueous solubility of **13** (>10 mg/mL). A minimum efficacious dose of <1.25 mg/kg with twice weekly dosing and 2.5 mg/kg with once weekly dosing was seen. Tumor suppression increased in a dose dependent fashion with 10 mg/kg once weekly giving the greatest inhibition of tumor growth, thus demonstrating a therapeutic index of at least 4. In contrast, similar studies with **2** showed a therapeutic index of 2.[6] As expected, the weaker PI3K inhibitor **8** was less potent in the U87MG xenograft model, requiring higher doses and/or more frequent dosing to achieve efficacy, with a minimum efficacious dose of 15 mg/kg once weekly, 7.5 mg/kg twice weekly, or 5 mg/kg 3× weekly (Figure 7).

The PK parameters of **13** were examined in the nude mouse following a single bolus iv administration at 5 mg/kg. Compound **13** exhibited moderate clearance (50 (mL/min)/kg), a low volume of distribution (0.7 L/kg), and a short half-life (0.5 h). A similar PK study conducted with **2** indicated that in contrast to **13**, levels of **2** are below the limits of detection upon iv dosing. As expected from the in vitro stability studies in nude mouse plasma, a proportionally high concentration of the 11-deacetylated metabolite **15** was seen in vivo. At longer time points (>1 h) the concentration of **15** surpassed that of **13**.

In summary, ring opening of 17-hydroxywortmannin (**2**) with secondary amines leads to analogues with improved properties such as aqueous solubility and stability toward amino acids and

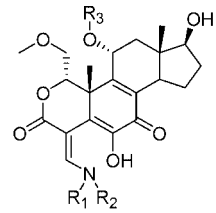
Table 1. Alkyl- and Cycloalkylamino Ring-Opened 17-Hydroxywortmannin Analogues



Cmpd.	R_1	R_2	R3	PI3K[a]	mTOR[a]	LNCap[a]
5	ethyl	ethyl	acetyl	0.382 +/- 0.028	14	0.160 +/- 0.012
6	ethyl	ethyl	H	7.85 +/- 1.40	>20	0.710 +/- 0.147
7	ethyl	H	acetyl	>10	>20	7.067 +/- 0.115
8	t-butyl	$-(CH_2)_2OH$	acetyl	1.110 +/- 0.137	28.6 +/- 0.8	0.128 +/- 0.052
9	t-butyl	Me	acetyl	0.440 +/- 0.172	6.5	0.189 +/- 0.055
10	t-butyl	H	acetyl	>10	>20	40.7 +/- 3.1
11	n-butyl	Me	acetyl	0.635 +/- 244	8.70 +/- 0.54	0.203 +/- 0.040
12	$-(CH_2)_4-$		acetyl	0.496 +/- 0.195	>20	0.257 +/- 0.095
2	17-hydroxywortmannin			0.0027 +/- 0.0009	0.193 +/- 0.059	1.464 +/- 0.645
4				0.088 +/- 0.013	3.1 +/- 0.14	0.573 +/- 0.064

[a] Average $IC_{50} \pm$ SEM (μM).

Table 2. Diaminoalkyl Ring-Opened 17-Hydroxywortmannin Analogues



Cmpd	R_1	R_2	R3	PI3K[a]	mTOR[a]	LNCap[a]
13	$-(CH_2)_3-N$	Me	Acetyl	0.0064 +/- 0.0018	2.92 +/- 1.85	0.389 +/- 0.175
14	$-(CH_2)_3-N$	H	Acetyl	>10	>20	2.02 +/- 0.36
15	$-(CH_2)_3-N$	Me	H	0.638 +/- 0.005	>20	2.34 +/- 0.34
16	$-(CH_2)_2-N$	t-butyl	Acetyl	0.0096 +/- 0.0025	0.816 +/- 0.476	0.919 +/- 0.261
17	$-(CH_2)_2-N$	$-CH_2Ph$	Acetyl	0.066 +/- 0.020	5.6 +/- 1.1	1.43 +/- 0.55

[a] Average $IC_{50} \pm$ SEM (μM).

plasma. Aliphatic diamines lead to analogues with enhanced PI3K inhibition potency relative to those with aliphatic monoamines. In our hands, **13** is the most potent ring-opened wortmannin based PI3K inhibitor reported to date with activity comparable to that of **2**. In human tumor xenograft assays in mice, **13** was a potent inhibitor of tumor growth with an improved therapeutic index over that of **2**. These findings show that ring-opened 17-hydroxywortmannin analogues have exciting potential as inhibitors of the PI3K pathway and therefore as anticancer agents.

Experimental Section

Materials and Instruments. Wortmannin was obtained from fermentation broths of the fungal culture ZIMV298 of the Wyeth microbial collection. All solvents were HPLC grade, and all other

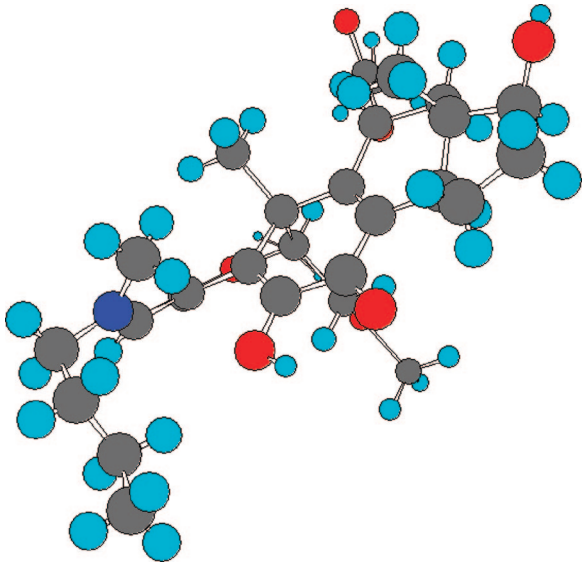


Figure 2. X-ray structure of **11**.

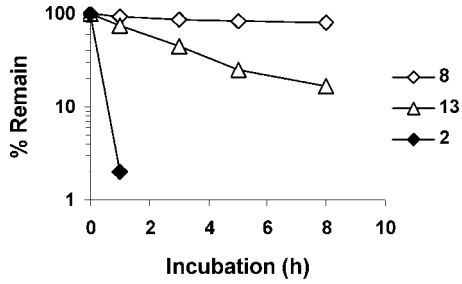


Figure 3. Stability of **8** and **13** in nude mouse plasma.

Table 3. IC_{50} Values (μM) of **13** and **8** in a Panel of Protein and Lipid Kinases

kinase	**13**	**8**
PI3K α	0.0064	1.110
PI3K β	0.013	
PI3K γ	0.008	
PI3K δ	0.011	
AKT1	>30	>30
BTK	>10	>10
CDK4	>50	>50
EGFR	>10	>10
GSK3	>10	>10
IGFR	>10	>10
IKKβ	>10	>10
KDR	>10	>10
LCK	>30	>30
LYN	>30	>30
MEK1	>10	>10
MK2	>200	>200
mTOR	2.92	28.6
PDK1	>200	>200
S6K1	>30	>30
Tpl2	>40	>40

chemicals were analytical reagents or equivalent. A BUCHI rotary evaporation system (RE 260 and R 124) was from Buchi (Flawil, Switzerland). ^{1}H NMR spectra were recorded on a 400 MHz NMR spectrophotometer using DMSO-d_6 or $CDCl_3$ neutralized with alumina. Electrospray (ES) mass spectra were recorded on a Micromass Platform spectrometer. High-resolution mass spectra were recorded on a Finnigen MAT-90 spectrometer.

Biology. Inhibition of PI3K enzyme activity is measured in a microtiter plate-based fluorescence polarization (FP) assay adapted from Echelon's K-1100 PI3K FP assay kit protocol with PI3K enzyme purchased from Upstate Biotech, PIP2, and TAMRA-labeled detector

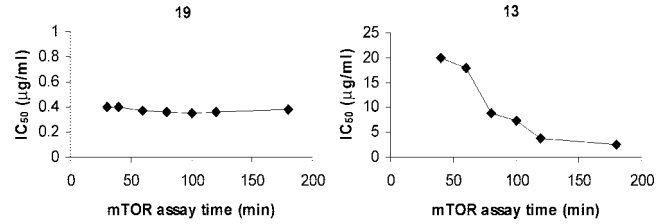


Figure 4. Time dependent inhibition of mTOR by **13**. Compounds **19** and **13** were assayed in the mTOR in vitro enzyme assay for various indicated times. For each assay time, the IC_{50} value was determined and plotted.

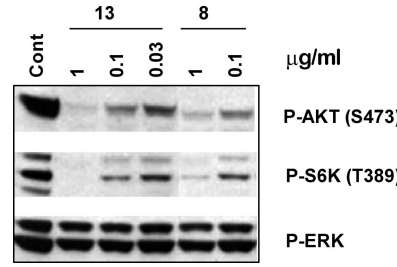


Figure 5. Inhibition of PI3K signaling in PTEN-negative U87MG cells. U87MG cells were plated in six-well culture plates for 24 h and treated with DMSO or the indicated doses of **13** or **8** in growth media for 6 h. Protein lysates were prepared and immunoblotted for phospho-AKT, phospho-S6K1, and phospho-ERK.

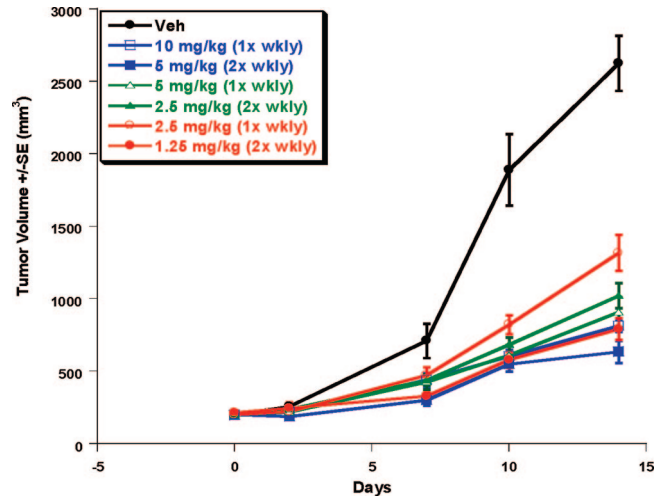


Figure 6. Tumor efficacy of **13** in the xenograft glioma model. U87MG glioma were grown in the nude mice as described.[6] The tumors were staged on day 0. Compound **13** was formulated in PBS and administrated intravenously (iv) at the indicated dosages and schedules for 2 weeks. Tumor growth was monitored twice a week. Tumor volume was determined as described.[6]

(Echelon) and GST-GRP1 (expressed and purified from *E. coli*). The reaction was carried out in 20 μL of reaction buffer (20 mM Hepes, pH 7.5, 2 mM $MgCl_2$, 0.05% CHAPS, and 0.01% βME) containing 20 μM PIP2, 25 μM ATP with vehicle control or inhibitors. The assay was incubated for 15–30 min at room temperature and stopped with 20 μL of stop/detection buffer (10 nM probe and 40 nM GST-GRP). The plates were incubated for 1–2 h, and FP was measured in a Perkin-Elmer Envision plate reader with TAMRA- FP filters. The mTOR kinase assay was carried out as described previously.[17] In vitro cell growth assays[18] and in vivo efficacy studies[6] were carried out as described previously.

(1*E*,4*S*,4a*R*,5*R*,6a*S*,7*S*)-1-{[[3-(Dimethylamino)propyl](methyl)amino]methylene}-7,11-dihydroxy-4-(methoxymethyl)-4a,6a-dimethyl-2,10-dioxo-1,2,4,4a,5,6,6a,7,8,9,9a,10-dodecahydroindeno[4,5-*h*]isochromen-5-yl Acetate (13). To a slurry of **2** (15.3 g) and EtOAc (107.7 mL) cooled in an ice bath was added *N*,*N*,*N*′-

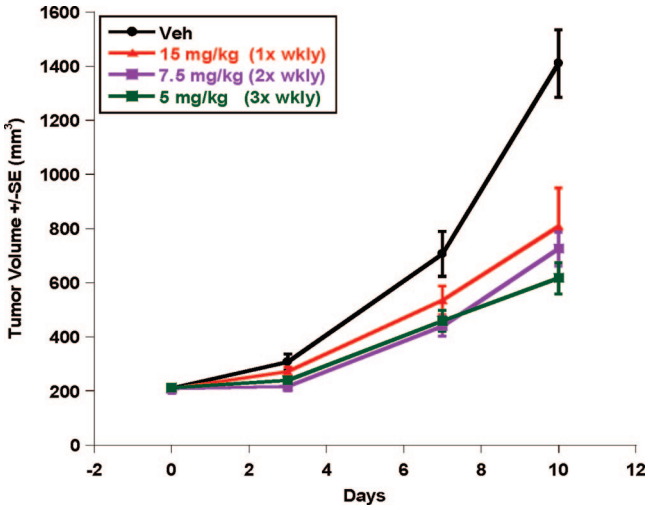


Figure 7. Tumor efficacy of **8** in the xenograft glioma model. U87MG glioma were grown in the nude mice as described.[6] The tumors were staged on day 0. Compound **8** was formulated in PBS and administrated intravenously (iv) at the indicated dosages and schedules for 2 weeks. Tumor growth was monitored twice a week. Tumor volume was determined as described.[6]

trimethyl-1,3-propanediamine (5.2 mL) dropwise over a period of 12 min. The ice bath was removed, and the resulting clear, orange solution was stirred at room temperature. After 48 min heptane was slowly added, and seed crystals of **13** were added. The resulting yellow slurry was stirred at room temperature overnight. This material was combined with slurry from a reaction run with 3 g of **2**, filtered, and dried in vacuo to give a yellow solid (20.66 g, 88.9%). MS, *m/z*: 547.3 $(M + H)^+$. ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3H), 1.43 (d, J = 7.44, 1H), 1.46 (d, J = 7.45, 1H), 1.54 (s, 3H), 1.50–1.78 (m, 5H), 2.07 (s, 3H), 2.18 (s, 7H), 2.23 (bd s, 2H), 2.54 (m, 3H), 2.71 (bd s, 2H), 3.11 (t, J = 6.1, 1H), 3.21 (d, J = 2.1, 1H), 3.22 (s, 3H), 3.39 (bd s, 1H), 3.51 (bd s, 1H), 3.85 (t, J = 5.6, 1H), 4.58 (bd s, 1H), 6.02 t, J = 4.92, 1H), 8.10 (s, 1H). ^{1}H NMR (DMSO-d_6) δ 0.61 (s, 3H), 1.39 (m, 2H), 1.42 (s, 3H), 1.45–1.55 (m, 1H), 1.65–1.75 (m, 2H), 1.90–1.97 (m, 2H), 2.04 (s, 3H), 2.11 (s, 6H), 2.15–2.31 (m, 2H), 2.35–2.45 (m, 2H), 2.48–2.53 (m, 1H), 2.61 (s, 3H), 2.94 (t, J = 6.2, 1H), 3.14 (s, 3H), 3.35–3.41 (m, 1H), 3.46–3.52 (m, 1H), 3.68 (t, J = 5.0, 1H), 4.42 (d, J = 7.6, 1H), 4.66 (s, 1H), 5.88 (t, J = 5.5, 1H), 7.92 (s, 1H), 8.19 (bd s, 1H). Anal. ($C_{29}H_{42}N_2O_8 \cdot 0.5H_2O$) C, H, N.

Synthesis of Libraries. To a solution of **2** in CH_2Cl_2 was added 2 equiv of an amine. After 30 min the solvent was removed in vacuo and the residue triturated with EtOAc/hexane or Et_2O/hexane. The yellow to orange powders were collected by filtration and dried in vacuo. Analysis by LC/MS showed these compounds to be >95% pure.

Acknowledgment. The authors thank Drs. Tarek Mansour, Semiramis Ayral-Kaloustian, and Jay Gibbons for support of this work and useful discussions. Dr. Robert Mallon and Steven Kim provided PI3K α enzyme for the assays. Dr. Ping Cai prepared compounds **S2**, **S27**−**S29**. Dr. Madelene Antane carried out the synthesis of **13** as described in the Experimental Section. Dr. Jack Wang provided the data on the reactivity of **1** with lysine−phenylalanine, and Dr. Inder Chaudhary provided the PK data of **13**. Dr. Douglas Ho (Princeton University) solved the X-ray structure of **11**.

Supporting Information Available: CHN and HRMS data and biological data for compounds prepared by parallel synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Engelman, J. A.; Luo, J.; Cantley, L. C. The Evolution of Phosphatidylinositol 3-Kinases as Regulators of Growth and Metabolism. *Nat. Rev. Genet.* **2006**, *7*, 606–619.

(2) Verheijen, J.; Zask, A. Phosphatidylinositol 3-Kinase (PI3K) Inhibitors as Anticancer Drugs. *Drugs Future* **2007**, *32*, 537–547.

(3) Walker, E. H.; Pacold, M. E.; Perisic, O.; Stephens, L.; Hawkins, P. T.; Wymann, M. P.; Williams, R. L. Structural Determinants of Phosphoinositide 3-Kinase Inhibition by Wortmannin, LY294002, Quercetin, Myricetin, and Staurosporine. *Mol. Cell* **2000**, *6*, 909–919.

(4) Schultz, R. M.; Merriman, R. L.; Andis, S. L.; Bonjouklian, R.; Grindey, G. B.; Rutherford, P. G.; Gallegos, A.; Massey, K.; Powis, G. In Vitro and in Vivo Antitumor Activity of the Phosphatidylinositol-3-kinase Inhibitor, Wortmannin. *Anticancer Res.* **1995**, *15*, 1135–1139.

(5) Norman, B. H.; Shih, C.; Toth, J. E.; Ray, J. E.; Dodge, J. A.; Johnson, D. W.; Rutherford, P. G.; Schultz, R. M.; Worzalla, J. F.; Vlahos, C. J. Studies on the Mechanism of Phosphatidylinositol 3-Kinase Inhibition by Wortmannin and Related Analogs. *J. Med. Chem.* **1996**, *39*, 1106–1111.

(6) Yu, K.; Lucas, J.; Zhu, T.; Zask, A.; Gaydos, C.; Toral-Barza, L.; Gu, J.; Li, F.; Chaudhary, I.; Cai, P.; Lotvin, J.; Petersen, R.; Ruppen, M.; Fawzi, M.; Ayral-Kaloustian, S.; Skotnicki, J.; Mansour, T.; Frost, P.; Gibbons, J. PWT-458, a Novel Pegylated-17-Hydroxywortmannin, Inhibits Phosphatidylinositol 3-Kinase Signaling and Suppresses Growth of Solid Tumors. *Cancer Biol. Ther.* **2005**, *5*, 538–545.

(7) Zhu, T.; Gu, J.; Yu, K.; Lucas, J.; Cai, P.; Tsao, R.; Gong, Y.; Li, F.; Chaudhary, I.; Desai, P.; Ruppen, R.; Fawzi, M.; Gibbons, G.; Ayral-Kaloustian, S.; Skotnicki, J.; Mansour, T.; Zask, A. Pegylated Wortmannin and 17-Hydroxywortmannin Conjugates as Phosphoinositide 3-Kinase Inhibitors Active in Human Tumor Xenograft Models. *J. Med. Chem.* **2006**, *49*, 1373–1378.

(8) Haefliger, W.; Kis, Z.; Hauser, D. Selective Functionalization of Wortmannin with the Help of a Masked Furan Ring. *Helv. Chim. Acta* **1975**, *58*, 1620–1628.

(9) Norman, B. H.; Shih, C.; Toth, J. E.; Ray, J. E.; Dodge, J. A.; Johnson, D. W.; Rutherford, P. G.; Schultz, R. M.; Worzalla, J. F.; Vlahos, C. J. Studies on the Mechanism of Phosphatidylinositol 3-Kinase Inhibition by Wortmannin and Related Analogs. *J. Med. Chem.* **1996**, *39*, 1106–1111.

(10) Wipf, P.; Minion, D. J.; Halter, R. J.; Berggren, M. I.; Ho, C. B.; Chiang, G. G.; Kirkpatrick, L.; Abraham, R.; Powis, G. Synthesis and Biological Evaluation of Synthetic Viridins Derived From C(20)-Heteroalkylation of the Steroidal PI-3-Kinase Inhibitor Wortmannin. *Org. Biomol. Chem.* **2004**, *2*, 1911–1920.

(11) Ihle, N. T.; Williams, R.; Chow, S.; Chew, W.; Berggren, M. I.; Paine-Murrieta, G.; Minion, D. J.; Halter, R. J.; Wipf, P.; Abraham, R.; Kirkpatrick, L.; Powis, G. Molecular Pharmacology and Antitumor Activity of PX-866, a Novel Inhibitor of Phosphoinositide-3-Kinase Signaling. *Mol. Cancer Ther.* **2004**, *3*, 763–772.

(12) Isosaki, M. Inhibition of Wortmannin Activities by Amino Compounds. *Biochem. Biophys. Res. Commun.* **2004**, *324*, 1406–1412.

(13) Yuan, H.; Luo, J.; Weissleder, R.; Cantley, L.; Josephson, L. Wortmannin-C20 Conjugates Generate Wortmannin. *J. Med. Chem.* **2006**, *49*, 740–747.

(14) Holleran, J. L.; Egorin, M. J.; Zuhowski, E. G.; Parise, R. A.; Musser, S. M.; Pan, S. Use of High-Performance Liquid Chromatography to Characterize the Rapid Decomposition of Wortmannin in Tissue Culture Media. *Anal. Biochem.* **2003**, *323*, 19–25.

(15) Yuan, H.; Barnes, K. R.; Weissleder, R.; Cantley, L.; Josephson, L. Covalent Reactions of Wortmannin under Physiological Conditions. *Chem. Biol.* **2007**, *14*, 321–328.

(16) Yu, K.; Toral-Barza, L.; Shi, C.; Zhang, W.-G.; Zask, A. Response and Determinants of Cancer Cell Susceptibility to PI3K Inhibitors: Combined Targeting of PI3K and Mek1 as an Effective Anticancer Strategy. *Cancer Biol. Ther.*, in press.

(17) Toral-Barza, L.; Zhang, W. G.; Lamison, C.; Larocque, J.; Gibbons, J.; Yu, K. Characterization of the Cloned Full-Length and a Truncated Human Target of Rapamycin: Activity, Specificity, and Enzyme Inhibition as Studied by a High Capacity Assay. *Biochem. Biophys. Res. Commun.* **2005**, *332*, 304–310.

(18) Yu, K.; Toral-Barza, L.; Discafani, C.; Zhang, W. G.; Skotnicki, J.; Frost, P.; Gibbons, J. mTOR, a Novel Target in Breast Cancer: The Effect of CCI-779, an mTOR Inhibitor, in Preclinical Models of Breast Cancer. *Endocr.-Relat. Cancer* **2001**, *8*, 249–258.

JM7012858