Published in final edited form as:

Bioorg Med Chem. 2013 September 1; 21(17): 5182–5187. doi:10.1016/j.bmc.2013.06.036.

Improved synthesis of 17 β -hydroxy-16 α -iodo-wortmannin, 17 β -hydroxy-16 α -iodoPX866, and the [131 I] analogue as useful PET tracers for PI3-kinase

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Abstract

An improved method for the synthesis of 17 β -hydroxy-16 α -iodo-wortmannin along with the first synthesis of 17 β -hydroxy-16 α -iodoPX866 and [131 I] radiolabeled 17 β -hydroxy-16 α -[131 I]iodo-wortmannin, as potential PET tracers for PI3K was also described. The differences between wortmannin and its iodo analogue were compared by covalently docking each structure to L833 in PI3K.

Keywords

PI3 kinase; Positron emission tomography (PET); imaging; 17 β -Hydroxy-16 α -iodowortmannin; 17 β -Hydroxy-16 α -iodoPX866; 17 β -Hydroxy-16 α -[131 I]iodowortmannin

1. Introduction

Phosphatidylinositol 3-kinase (PI3-kinase) is over expressed in a number of human cancers, especially ovarian, head and neck, urinary tract, and cervical cancers, where it leads to increased proliferation and inhibition of apoptosis.^{1–4} Inactivating mutations in PTEN phosphatase represent a hallmark of transition between intraepithelial neoplastic lesions to invasive and metastatic carcinomas.

Due to crucial impact of the phosphatidylinositol-3-kinase (PI3K) signaling pathway in many aspects of cell growth and survival,⁵ it became an important target for the development of cancer therapeutics. Developing molecular markers to help early diagnosis the cancer related to this signaling pathway, and to pre-select patients who are likely to respond as well as to identify patients not responding at an early point during treatment and triage them to alternative therapies, will greatly increase the likelihood of demonstrating efficacy and decrease the size, cost and duration of clinical trials while concurrently be greatly benefit to save patients' lives. Since it was first used for oncological studies in the

1970s, positron emission tomography (PET) has been increasingly developed for imaging and quantifying molecular mechanisms in oncology. The inherent sensitivity and specificity of PET is unrivaled because it can image molecular interactions and pathways, providing quantitative kinetic information down to the subpicomolar level.⁶ Therefore, the development of radioactive PET tracers for imaging the PI3K expression will serve this purpose and become a very attractive field.

Wortmannin (**1**, Fig. 1),^{7–9} a fungal metabolite that specifically inhibits PI3-kinase by covalently reacting with Lys-833 in the ATP binding site of the enzyme^{10–13} is regarded as a powerful and selective tool to study the function of PI3-kinase. Highly electrophilic C-21 position of wortmannin was suggested to be responsible for its activity.^{12,14} Till today, several wortmannin analogues (Fig. 2) were synthesized to be used as reporters for detection of PI3-kinase, especially two [¹²⁵I] radiolabeled compounds **2b** and **3** were reported as probes that can be used to monitor the reactivity of wortmannin analogues with proteins in SDS–PAGE.^{15–17} However, none of them were explored as the tracer for PET imaging. In this paper, an improved method for the synthesis of 17 β -hydroxy-16 α -iodo-wortmannin **2a** was provided, [¹³¹I] radiolabeled 17 β -hydroxy-16 α -[¹³¹I]iodo-wortmannin **2c** was first time reported as a promising PET imageable tracer for PI3-kinase. In addition, the synthesis of a modified wortmannin analogue 17 β -hydroxy-16 α -iodo-PX866 was developed as another potential PET tracer for PI3 kinase.

The synthesis of 17 β -hydroxy-16 α -iodo-wortmannin **2b** [¹²⁵I] was originally reported by Shibasaki in total five steps with poor overall yield (<5%).¹¹ The extremely low yields resulted from the preparation of the key intermediate 16 α -bromo-wortmannin **7**, which was achieved by enol acetylation followed by bromination with NBS gave only 12% in first two steps (Scheme 1). The low yields in the first two steps prompted us to explore an alternative route to the target molecule. Herein, the improvement on the cold chemistry toward the synthesis of the molecule **7** is described. Furthermore, along with 17 β -hydroxy-16 α -iodo-wortmannin **2a**, the first synthesis of 17 β -hydroxy-16 α -iodo-PX866 **11** which is a diallylamino wortmannin derivative lacking the furan ring is described.¹⁸

2. Results and discussion

This two-step bromination at C-16 was the only procedure reported during that time for the estradiol and wortmannin systems. However, a more effective one-step bromination at C-16 has recently been reported in the estriol system,⁶ in which the C-16 bromination was achieved in a high yield (>70%) simply by treatment with CuBr₂ in a 1:1 mixture of CHCl₃ and EtOAc under reflux for an hour. In our hands, the treatment of wortmannin with CuBr₂ under the similar conditions resulted to the formation of **8** in 1 h. The ¹H NMR experiment showed that the crude contained about 85% of the 16-bromo-wortmannin **8** (α : β = ~1:1) as well as 15% of an unidentified by-product. The crude **8**, without purification, was directly subjected to epimerization at C-16 with an excess amount of LiBr to furnish exclusive formation of 16 β -bromo ketone **9** in 46% yield after isolation. The structure of **9** was confirmed by MS, ¹H NMR, ¹³C NMR, and COSY experiments.¹⁹ Having the compound **9** in hand, 16 β -bromo-17 β -hydroxy-wortmannin **10**, the precursor for the radiolabeling was smoothly synthesized by following the reported procedure, where the carbonyl group at the C-17 was reduced by treatment with BH₃–THF at 0 °C to give **10** in 61% yield after purification (Scheme 2).

The cold version of halogen exchange on **10** was conducted with an excess amount of NaI in 2-butanone under reflux for 30 min to yield 17 β -hydroxy-16 α -iodo-wortmannin **2a**, the structure of which was fully confirmed by MS, ¹H NMR, ¹³C NMR, NOESY (the observed NOE correlation between 16 β -H and 18-CH₃, and the correlation between 17 α -H and 14 α -

H), and COSY experiments. The treatment of 17 β -hydroxy-16 α -iodo-wortmannin **2a** with diallyl amine in dichloromethane at room temperature gave 17 β -hydroxy-16 α -iodo-PX866 **11** in 77% yield. The compound **11** can also be synthesized directly from **10** in one-pot reaction without isolating compound **2a**, however, the 1,4-addition of diallyl amine to crude **2a** gave only 47% of **11**. (See the Section 6 for the details).

3. Radiolabeling

The [^{131}I] radiolabeled 17 β -hydroxy-16 α -[^{131}I]iodo-wortmannin **2c** was achieved by reacting the precursor 16 β -bromo-17 β -hydroxy-wortmannin **10** with Na[^{131}I] in acetonitrile at 95 °C for 120 min (Scheme 3) in 29% radiochemical yield with radiochemical purity greater than 95% (See the Section 6, Fig. 4).²⁰

4. Docking

The assumption made in modeling of the interaction of **2a** with PI3-K was that it would react in the same way as wortmannin **1**, which seems reasonable since the reaction site is sufficiently far from the point of modification. However, it was not clear what affect that the change would have on the interaction with the binding site in PI3-K, and potentially on activity. In an attempt to address this issue, we ran covalent docking calculations on both structures to determine the configurations and ligand buried surface area, since it has been correlated to activity (with IC₅₀) by others.²¹ The lowest energy structures for **1** and **2a** had buried surface area of 872.2 Å² and 934 Å², respectively. However, when considering all ten poses, structure **2a** buried less surface area on average, 740.1 $\pm$ 167.0 Å², versus 766.9 $\pm$ 149.9 for structure **1**. Therefore, it is difficult to determine whether or not any activity difference should be expected for **2a**. The lowest energy configuration for **1** had a heavy atom RMSD of 0.46 Å to the crystal configuration, whereas it was 1.57 Å for structure **2a** (excluding Iodine and Oxygen). An image of each docked configuration is shown in Figure 3. This difference is likely due to a move to accommodate the larger Iodine, which interacts with T867, V882, F961 and I963. The structure is moved in such a way that the hydrogen bonds to G868 and D964 are broken and replaced by another hydrogen bond is formed on the other side of the molecule to S806.

5. Conclusions

In summary, first time we have synthesized the [^{131}I] radiolabeled 17 β -hydroxy-16 α -iodo-wortmannin as a promising Positron Emission Tomography (PET) tracer for the detection of PI3 kinase along with the synthesis of 17 β -hydroxy-16 α -iodo-PX866, a modified wortmannin analogue as another potential PET tracer for PI3K. We have also improved the bromination at C-16 position of wortmannin. The differences between **1** and its iodo analogue **2a** were compared by covalently docking each structure to L833 in PI3K.

6. Experimental

6.1. Materials and instrumentation

All of the starting materials and solvents were used from Sigma–Aldrich, VWR, Fisher or Acros organics without further purification unless specified. Melting points were measured in open capillary tubes on a Buchi Melting point B-545 apparatus and are uncorrected. Microwave reaction was performed on a CEM Microwave Discover S Class with Explorer 48 robot arm. NMR spectra were recorded on an IBM-Bruker Advance 300 (300 MHz for ^1H NMR and 75.48 MHz for ^{13}C NMR), and IBM-Bruker Advance 500 (500 MHz for ^1H NMR and 125.76 MHz for ^{13}C NMR) spectrometers. Chemical shifts (δ) are determined relative to CDCl₃ (referenced to 7.27 ppm (δ) for ^1H NMR and 77.0 ppm for ^{13}C

NMR) or DMSO- d_6 (referenced to 2.49 ppm (δ) for ^1H NMR and 39.5 ppm for ^{13}C NMR). Proton-proton coupling constant (J) are given in Hertz and splitting patterns are designated as singlet (s), doublet (d), triplet (t) quadruplet (q), multiplet or overlapped (m) and broad (br). High resolution mass spectra were acquired on an Agilent TOF Accurate Mass 6220 System. Analytical HPLC was performed on an Agilent LC1200 system with Zorbax sb-c18 2.1×30 mm 3.5-Micron column with buffer A: 0.1% formic acid in water, buffer B: Methanol. 10% B to 95% B in 10 min and 95% B for 5 min with flow rate 0.5 mL/min. Flash chromatography was performed using Merck silica gel 60 (mesh size 230–400 ASTM) or using an isco (lincon, NE) combiFlash Companion or SQ16x flash chromatography system with RedSep columns (normal phase silica gel with mesh size 230–400 ASTM) and Fisher Optima TM grade solvents. Thin layer chromatography (TLC) was performed on E. Merck (Darmstadt, Germany) silica gel F-354 aluminum-backed plates with visualization under UV (254 nm) and by staining with potassium permanganate or ceric ammonium molybdate.

6.1.1. 16 β -Bromo-Wortmannin (9)—A suspension of wortmannin **1** (104 mg, 0.24 mmol) and cupric bromide (240 mg, 1.07 mmol) in a mixture of chloroform and ethyl acetate (1:1, v/v) was heated under reflux for 1 h. Upon cooling to room temperature, the mixture was filtered to remove the solids. Evaporation of the filtrate gave 120 mg of 16 α -bromo-Wortmannin **8** as a white solid. To a solution of **8** (120 mg, 0.24 mmol) in 2-butanone (15 mL) was added anhydrous LiBr_2 (0.23 g, 2.64 mmol). The resultant mixture was allowed to stir overnight. The reaction solution was then diluted with ethyl acetate and washed with water twice. After the evaporation of solvent, the crude was subjected to column chromatography on silica gel (elution with hexane-EtOAc 10–40%) to give 54 mg (44%) of 16 β -bromo-Wortmannin **9** as a white solid. mp: 245–247 °C; R_f = 0.54 (1:1 hexanes:EtOAc); $\alpha^{20}\text{D} +15.1^\circ$; HPLC: t_{ret} = 7.35 min (0–100% MeCN in water containing 10 mM ammonium formate (pH 7.5) in 10 min); UV: λ_{max} = 242, 300 nm; ^1H NMR (500 MHz, CDCl_3): δ 8.25 (1H, s), 6.16 (1H, dd, J = 8.4, 2.4 Hz), 4.75 (1H, dd, J = 4.8, 6.6 Hz), 4.20 (1H, t, J = 8.4 Hz, 16 α -H), 3.87 (1H, ddd, J = 5.4, 8.4, 13.8 Hz), 3.44 (1H, dd, J = 1.8, 11.4 Hz), 3.17 (3H, s), 3.02 (1H, dd, J = 6.6, 10.8 Hz), 2.87 (1H, ddd, J = 2.4, 5.4, 13.2 Hz), 2.67 (1H, dd, J = 12.6, 7.2 Hz), 2.40 (1H, dt, J = 9.0, 13.8 Hz), 2.14 (3H, s), 1.74 (3H, s), 1.64 (1H, dd, J = 8.4, 13.2 Hz), 1.17 (3H, s); ^{13}C NMR (125.7 MHz, CDCl_3): δ 209.6, 172.1, 169.4, 157.4, 150.2, 150.0, 144.5, 143.1, 139.2, 114.3, 88.3, 72.9, 69.3, 59.5, 48.9, 42.1, 40.9, 36.4, 34.4, 26.6, 21.0, 16.4; HRMS ($\text{C}_2\text{H}_2\text{BrO}_8$) calcd 506.0502 found 506.0509 $[\text{M}]^+$.

6.1.2. 16 β -Bromo-17 β -hydroxy-wortmannin (10)—To a solution of 16b-bromo-Wortmannin **9** (120 mg, 0.24 mmol) in 15 mL of THF at 0 °C, was added BH_3 –THF solution (1 mL, 1 mmol, 1 M in THF) over 15 min. The resultant solution was stirred at 0 °C for 3 h, then heated to 30 °C for 12 h. Ice and saturated NH_4Cl was added to quench the reaction. After dilution with ethyl acetate, the organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic phase was dried over Na_2SO_4 . After evaporation of the solvent, the crude material was purified by flash column chromatography on silica gel with elution of MeOH/DCM from 0–2% to obtain 70 mg (57%) of 16 β -bromo-17 β -hydroxy-wortmannin **10** as a white solid and 40 mg of 16 β -Br-Wortmannin **9** was recovered. $\alpha^{20}\text{D} +17.0^\circ$; mp 224–226 °C; R_f = 0.60 (MeOH/DCM = 1/20 on silica gel); HPLC: t_{ret} = 7.20 min (0–100% MeCN in water containing 10 mM ammonium formate in 10 min); UV: λ_{max} = 248, 297 nm; ^1H NMR (500 MHz, CDCl_3): δ 8.24 (1H, s), 6.12 (1H, m), 4.76 (1H, dd, J = 1.8, 7.2 Hz), 4.69 (1H, ddd, J = 8.4, 6.6, 8.4 Hz, 16 α -H), 3.79 (1H, ddd, J = 6.6, 8.4, 14.4 Hz), 3.57 (1H, dd, J = 8.4, 9.0 Hz, 17 α -H), 3.45 (1H, dd, J = 1.8, 11.4 Hz), 3.19 (3H, s), 2.99 (1H, dd, J = 11.4, 7.2 Hz), 2.69 (1H, dd, J = 7.8, 12.6 Hz), 2.45 (1H, ddd, J = 3, 6.6, 13.8 Hz), 2.31 (1H, d, J = 9 Hz, $-\text{OH}$), 2.27 (1H,

ddd, $J = 6.0, 13.8, 13.8$ Hz), 2.15 (3H, s), 1.73 (3H, s), 1.42 (1H, dd, $J = 9.6, 12.6$ Hz), 1(3H, s); ^{13}C NMR (125.7 MHz, CDCl_3): δ 172.5, 169.5, 157.6, 150.0, 149.2, 144.8, 142.9, 140.4, 114.3, 88.8, 76.7, 72.9, 69.9, 59.4, 53.6, 45.8, 42.5, 40.8, 40.6, 38.0, 26.6, 21.1, 12.7.; HRMS ($\text{C}_{23}\text{H}_{25}\text{BrO}_8$) calcd 508.0661 found 508.0659 $[\text{M}]^+$.

6.1.3. 17 β -Hydroxy-16 α -iodo-wortmannin (2a)—A mixture of 16 β -bromo-17 α -hydroxy-wortmannin (**4**) (56 mg, 0.11 mmol) and anhydrous NaI (210 mg, 1.3 mmol) in 2-butanone (10 mL) was heated with stirring for 30 min at 90 °C in a microwave reactor. After the reaction was completed (monitored by TLC, silica gel, MeOH/DCM 1:19, $R_f = 0.53$, substrate = 0.60), the reaction was brought to room temperature and the solvent was removed and then EtOAc (30 mL) was added to the residue. The organic phase was washed with aqueous solution of $\text{Na}_2\text{S}_2\text{O}_3$ (1 M, 35 mL) twice. The separated organic phase was dried over Na_2SO_4 . Evaporation of the solvent gave 47 mg of a white solid, which was purified by flash column chromatography on silica gel (MeOH/DCM 0–0.02%) to obtain 35 mg (57%) of 17 β -hydroxy-16 α -iodo-wortmannin (**2a**) as a white solid. mp: 241–243 °C; $R_f = 0.53$ (MeOH/DCM = 1/20 on silica gel); $\alpha^{20}\text{D} +58.2^\circ$; HPLC: $t_{\text{ret}} = 8.02$ min (0–100% MeCN in water containing 10 mM ammonium formate in 10 min); UV: $\lambda_{\text{max}} = 244, 297$ nm; ^1H NMR (500 MHz, CDCl_3): δ 8.23 (1H, s), 6.11 (1H, ddd, $J = 3.0, 7.2, 10$ Hz), 4.76 (1H, dd, $J = 1.8, 7.2$ Hz), 4.23 (1H, br, 17 α -H), 4.14 (1H, m, 16 β -H), 3.46 (1H, dd, $J = 1.8, 10.8$ Hz), 3.30 (1H, ddd, $J = 3.0, 7.2, 10.2$ Hz), 3.19 (3H, s), 3.01 (1H, m), 2.99 (1H, dd, $J = 7.2, 11.4$ Hz), 2.64 (1H, dd, $J = 7.2, 12$ Hz), 2.58 (1H, m), 2.14 (3H, s), 2.12 (1H, br, OH), 1.72 (3H, s), 1.48 (1H, dd, $J = 9.6, 12$ Hz), 0.84 (3H, s); ^{13}C NMR (125.7 MHz, CDCl_3): δ 172.4, 169.6, 157.6, 150.0, 149.0, 144.8, 142.8, 140.7, 114.3, 90.6, 88.8, 72.9, 70.1, 59.4, 45.6, 42.5, 40.8, 39.8, 38.9, 28.2, 26.6, 21.1, 11.4; HRMS ($\text{C}_{23}\text{H}_{25}\text{IO}_8$) calcd 555.0523 found 555.0526 $[\text{M}-\text{H}]^-$.

6.1.4. 17 β -Hydroxy-16 α -iodo-PX866. (11) (Method A)—To a solution of 17 β -hydroxy-16 α -iodo-wortmannin **2a** (20 mg, 0.036 mmol) in 15 mL of DCM (freshly distilled over P_2O_5) at 0 °C, was added 5.3 μL (4.2 mg, 0.043 mmol) of allylamine (freshly distilled over CaH_2) in DCM (2 mL) drop wise while stirring. After 5 min, the cold bath was removed and then the reaction mixture was warmed to room temperature and stirred for 1 h, then again another portion of 5.3 μL (4.2 mg, 0.043 mmol) of allylamine in DCM (2 mL) was added and the stirring continued for another 1 h. Such operation was repeated two more times until the starting material was completely consumed. All the volatiles were removed under reduced pressure and the crude was purified by flash column chromatography over silica gel (MeOH/DCM 0–5%) to obtain 18 mg (77%) of 17 β -hydroxy-16 α -iodo-PX866 **11** as a yellow gum. $\alpha^{20}\text{D} +63.5^\circ$; mp 235–237 °C (decom.); TLC: $R_f = 0.41$ (MeOH/DCM = 1/20); HPLC: $t_{\text{ret}} = 8.08$ min (0–100% MeCN in water containing 10 mM ammonium formate in 10 min); UV: $\lambda_{\text{max}} = 415, 320, 252$ nm; ^1H NMR (500 MHz, CDCl_3) δ 8.06 (s, 1H), 6.67 (s, 1H), 5.93 (m, 1H), 5.75 (s, br, 1H), 5.48 (s, br, 1H), 5.28 (s, br, 1H), 5.17 (s, br, 2H), 5.05 (s, br, 1H), 4.49 (s, 1H), 4.17 (m, 1H), 4.04 (m, 1H), 3.88 (s, br, 2H), 3.35–3.37 (m, br 2H), 3.17 (s, 3H), 3.00 (m, 2H), 2.52 (m, 2H), 2.17 (s, br, 1H), 2.02 (s, 3H), 1.51 (m, 2H), 1.44 (s, 3H), 1.24 (m, 1H), 0.66 (s, 3H); ^{13}C NMR (125.7 MHz, CDCl_3) δ 195.9, 178.2, 170.2, 164.3, 152.7, 148, 139.5, 136.9, 131, 121.3, 119.5, 91.2, 86.2, 81.5, 73.4, 67.9, 59.9, 59.3, 46.6, 46.0, 42.2, 38.4, 29.0, 27.0, 21.0, 12.5; HRMS ($\text{C}_{29}\text{H}_{36}\text{INO}_8$) calcd 654.1507 found 654.1548 $[\text{M}+\text{H}]^-$.

6.1.5. 17 β -Hydroxy-16 α -iodo-PX866. (11) (Method B)—A mixture of 16 β -bromo-17 α -hydroxy-wortmannin (6 mg, 0.01 mmol) and anhydrous NaI (21 mg, 0.13 mmol) in 2-butanone (1 mL) was heated with stirring for 30 min at 90 °C in a microwave reactor. The reaction mixture was cooled down to room temperature and 8.0 μ L (6.3 mg, 0.065 mmol) of allylamine (freshly distilled over CaH₂) was added. The reaction mixture was stirred at room temperature for 1 h. The mixture was purified by flash column chromatography over silica gel (C18 column, 10 mM NH₄-OAc/MeCN 0–60%) and the fractions contained compound **11** were pooled and lyophilized to get the pure 17 β -hydroxy-16 α -iodo-PX866 **11** as a fluffy solid (3.1 mg, 47%).

6.1.6. 17 β -Hydroxy-16 α -[¹³¹I]iodo-wortmannin (2c)—A solution of **10** (0.35 mg) in acetonitrile (100 μ L) was added to a vial containing a solution of sodium iodide-131 (Na¹³¹I) in ethanol (215 μ Ci in 100 μ L ethanol). The vial was sealed and heated at 95 °C for 120 min then the solution was cooled to room temperature. The raw iodinated product had 77.5 μ Ci (36% radiochemical yield, radiochemical purity: 96.5%). The crude product was filtered through an anion column (IC-Ag, Alltech, Deerfield, IL) to afford 17 β -hydroxy-16 α -[¹³¹I]iodo-wortmannin **2c** in 29% (62.5 μ Ci) radiochemical yield with radiochemical purity higher than 95% as determined by thin layer chromatographic analysis [silica gel 2 $\times$ 20 cm, hexanes:ethyl acetate (1:2)], *R_f* = 0.24 and the radioiodinated product co-migrated with a cold compound **2a**.

6.2. Modeling

All computational work was completed on a 4-quad core (16-core) 2.4 Ghz AMD Opteron system running the RHEL 5.5 OS and using Maestro,²² Prime,²³ and LigPrep²⁴ modules in the Schrödinger 2011 suite. The structure of PI3-K with Wortmannin (1E7U) was read into Maestro, the bond to Wortmannin was deleted then Wortmannin was removed from the structure. A protein preparation was done with option to fill in missing side chains using Prime and all crystal waters were kept. Further preparation was done to optimize the hydrogen bonding prior to a hydrogen only Impref minimization to RMSD = 0.3 Å. This was followed by an Impref minimization of all atoms to RMSD = 0.5 Å. For the minimization calculations, the OPLS2005 force field²⁵ was utilized with default setting. Finally, the Lys833 residue was modified by removing one of the hydrogens, so that the valency would be correct once it was covalently docked. The 3D SDF file for Wortmannin was downloaded from Pubchem as CID# 312145. It was read into the project and modified to structure with ring opened. Unique names were generated for each atom and residue to avoid conflicts with the protein naming. A Ligprep calculation was done to generate a good 3D structure, with the ionization, Desalt and Generate tautomers options de-selected, and chirality determined from those in the structural file. The same was done for I-Wortmannin. Setting up the covalent docking runs involved identifying the atoms participating in the linkage for the ligand and receptor. For the ligand, a SMARTS pattern was utilized to uniquely select the double bond and hydrogen for leaving atom. For the receptor, the nitrogen was selected as the staying atom and one of the hydrogen's was selected as a leaving atom. The Lys833 and residues within 8 Å of that residue were allowed to move during the docking. Ten poses were requested for each ligand, and the run was written out

and submitted via the command line. After the covalent docking runs were complete, the buried surface was calculated for each ligand pose by deleting the covalent bond, splitting the entry into ligand, water, receptor and using the calculate property function in the table with the default 1.4 Å probe radius.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to acknowledge the Cancer Center support grant CA016672 for the support of the translational chemistry core facility and NMR facility at M. D. Anderson Cancer Center and the authors also thankful to Dr. Khingkan Lertratanangkoon for the help in the mass spectroscopy.

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Figure 1.
Structure of wortmannin.

Figure 2.
C11 and C18-Modified wortmannin probes for PI3 kinase.

Figure 3.
(Top) Structure **1** covalently docked to L833 in PI3-K. (Bottom) Structure **2a** docked to L833 in PI3-K.

Figure 4.
Purification and TLC scan of radiolabeled **2c**.

Scheme 1.

First two steps of Shibasaki's synthesis of the key intermediate 16 α -bromo-wortmannin **7**.

Scheme 2.
Synthesis of **2a** and 17 β -hydroxy-16 α -iodo-PX866 **11**.

Scheme 3.

Synthesis of radioactive 17β-hydroxy-16α-[¹³¹I]iodo-wortmannin **2c**.