Supporting Information

Inhibition of Phosphatidylinositol-3,4,5-trisphosphate Binding to AKT Pleckstrin Homology Domain by 4-Amino-1,2,5-oxadiazole Derivatives

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Figure S1: The inhibitory effects of compounds on the interaction between AKT1-PH domain and PI(3,4,5)P3. Representative SPR sensorgrams of AKT1-PH domain binding to the PI(3,4,5)P3 containing liposomes in the presence of increasing concentrations of the compounds PI-2 (A), PI-3 (B) and PI-5 (C).
Table S1: Relative Inhibitory Activity of the Compounds Measured by Competitive-Surface Plasmon Resonance Analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative inhibitory activity (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; values (μM)</th>
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<tr>
<td></td>
<td>Tapp1-PH</td>
<td>PLCδ1-PH</td>
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<tr>
<td>PI-1</td>
<td>28 ± 3</td>
<td>54 ± 6</td>
</tr>
<tr>
<td>PI-3</td>
<td>18 ± 2</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>PI-4</td>
<td>5 ± 2</td>
<td>28 ± 3</td>
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</table>

Figure S2: Representative protein-to-membrane FRET experiment under liposomal environment. Addition of increased concentration of compounds, PI-1 (A) and PI-4 (B) to AKT1-PH domain (2 μM) bound to the active liposome (PC/PE/PS/dPE/PI(3,4,5)P<sub>3</sub> (56/20/20/1/3)) decreases the FRET signal at 509 nm. All the measurements were performed in 20 mM Tris, pH 7.4 containing 160 mM NaCl. Compound concentrations were varied from 0 to 70 μM.
Figure S3: Representative isothermal titration calorimetric plots of AKT1 PH-domain with potent compounds. Original titration and integrated peak areas of AKT1 PH-domain with PI-1 (A) and PI-4 (B).
Figure S4: Lig-plot analysis of IP4 bound to AKT1 PH (1H10) domain (A). Lig-plot analysis of PI-1 (B) and PI-4 (C) docked into the PIP-binding site of AKT1-PH domain. Residues involved in interactions through hydrogen bond formation are shown using dashed lines.
### Table S2: Theoretically calculated Interaction Energy between Ligand and PH-domains

<table>
<thead>
<tr>
<th>Protein</th>
<th>Ligand</th>
<th>Interaction energy between ligand and Protein (kcal/mol)</th>
<th>Hydrogen bonding energy (kcal/mol)</th>
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<tr>
<td></td>
<td></td>
<td>PIP-binding site</td>
<td>Non PIP-binding site</td>
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<td>PI-1</td>
<td>-096.413</td>
<td>-086.471</td>
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<tr>
<td></td>
<td>-094.055</td>
<td>-086.289</td>
<td>-17.347</td>
</tr>
<tr>
<td></td>
<td>-092.162</td>
<td>-083.097</td>
<td>-18.140</td>
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<td>PI-2</td>
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</tr>
<tr>
<td></td>
<td>-111.341</td>
<td>-108.642</td>
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<tr>
<td>PI-3</td>
<td>-127.659</td>
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<td>-24.080</td>
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<td>AKT1-PH</td>
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Figure S5: Surface representation of the docking complexes AKT1-PH/PI-1 (A), AKT1-PH/PI-4 (B) and AKT1-PH/PI-1 (C) both in PIP (yellow circle) and PS- (pink square box) binding sites. Overall surface of AKT1-PH domain was colored according to the electrostatic potential (blue, positively charged; red, negatively charged; white, neutral) using PyMOL software.
Table S3: Anisotropy Values of the Membrane Sensitive Probes in the Presence and Absence of the compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Anisotropy of DPH</th>
<th>Anisotropy of NBD-PE</th>
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<tbody>
<tr>
<td>liposome</td>
<td>0.2704 ± 0.0128</td>
<td>0.2117 ± 0.0128</td>
</tr>
<tr>
<td>PI-1</td>
<td>0.2599 ± 0.0286</td>
<td>0.1744 ± 0.0262</td>
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<tr>
<td>PI-2</td>
<td>0.2625 ± 0.0122</td>
<td>0.1855 ± 0.0248</td>
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<td>PI-3</td>
<td>0.2595 ± 0.0135</td>
<td>0.1909 ± 0.0011</td>
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<tr>
<td>PI-4</td>
<td>0.2536 ± 0.0025</td>
<td>0.1614 ± 0.0026</td>
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<tr>
<td>PI-5</td>
<td>0.2574 ± 0.0116</td>
<td>0.1704 ± 0.0125</td>
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</table>

Values in parentheses indicate standard deviations.
**Figure S6:** Compounds inhibit AKT signalling pathway in MDA-MB-231 cells. MDA-MB-231 cells were treated with the compounds (25 μM) for 48 h, and the indicated PI(3,4,5)P₃-dependent phosphorylation events were evaluated by immunoblot analysis (A). MDA-MB-231 cells were treated with the **PI-1** and **PI-4** with different concentrations (5, 10 and 25 μM) for 48 h, and the indicated PI(3,4,5)P₃-dependent phosphorylation events were evaluated by immunoblot analysis (B and C). All the immunoblot images were presented in grey scale for better clarity. All cellular measurements were performed more than five times. Normalized expression level of pThr308 of AKT enzyme in the absence and presence of compounds with 25 μM
concentrations (D). Normalized expression level of pThr308 of AKT enzyme in the absence and presence of PI-1 and PI-4 compounds in a concentration dependent manner (0, 5, 10, 25 μM) (E).