

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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Supporting Information

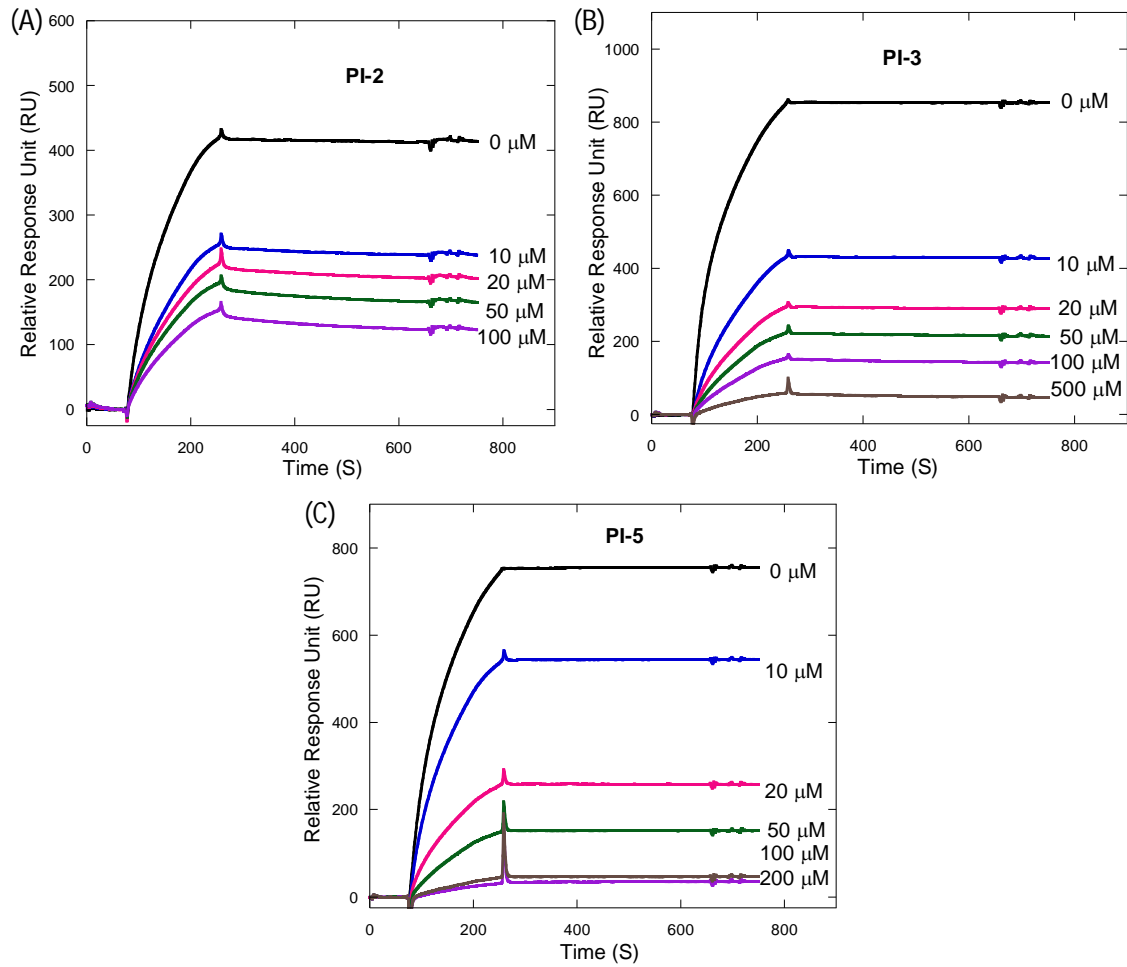


Figure S1: The inhibitory effects of compounds on the interaction between AKT1-PH domain and PI(3,4,5)P₃. Representative SPR sensorgrams of AKT1-PH domain binding to the PI(3,4,5)P₃ containing liposomes in the presence of increasing concentrations of the compounds **PI-2** (A), **PI-3** (B) and **PI-5** (C).

Table S1: Relative Inhibitory Activity of the Compounds Measured by Competitive-Surface Plasmon Resonance Analysis

Compound	Relative inhibitory activity (%)		IC ₅₀ values (μM)	
	Tapp1-PH	PLCδ1-PH	Tapp1-PH	PLCδ1-PH
PI-1	28 ± 3	54 ± 6	8.5 ± 0.99	9.6 ± 1.71
PI-3	18 ± 2	46 ± 3	22.1 ± 2.49	3.4 ± 0.41
PI-4	5 ± 2	28 ± 3	286 ± 19.6	34 ± 3.39

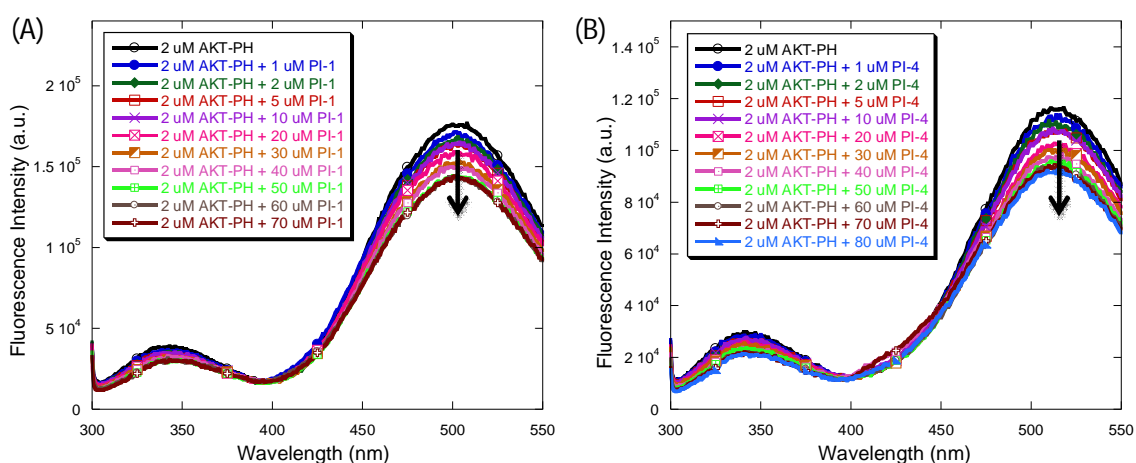


Figure S2: Representative protein-to-membrane FRET experiment under liposomal environment. Addition of increased concentration of compounds, **PI-1** (A) and **PI-4** (B) to AKT1-PH domain (2 μM) bound to the active liposome (PC/PE/PS/dPE/PI(3,4,5)P₃ (56/20/20/1/3)) decreases the FRET signal at 509 nm. 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.

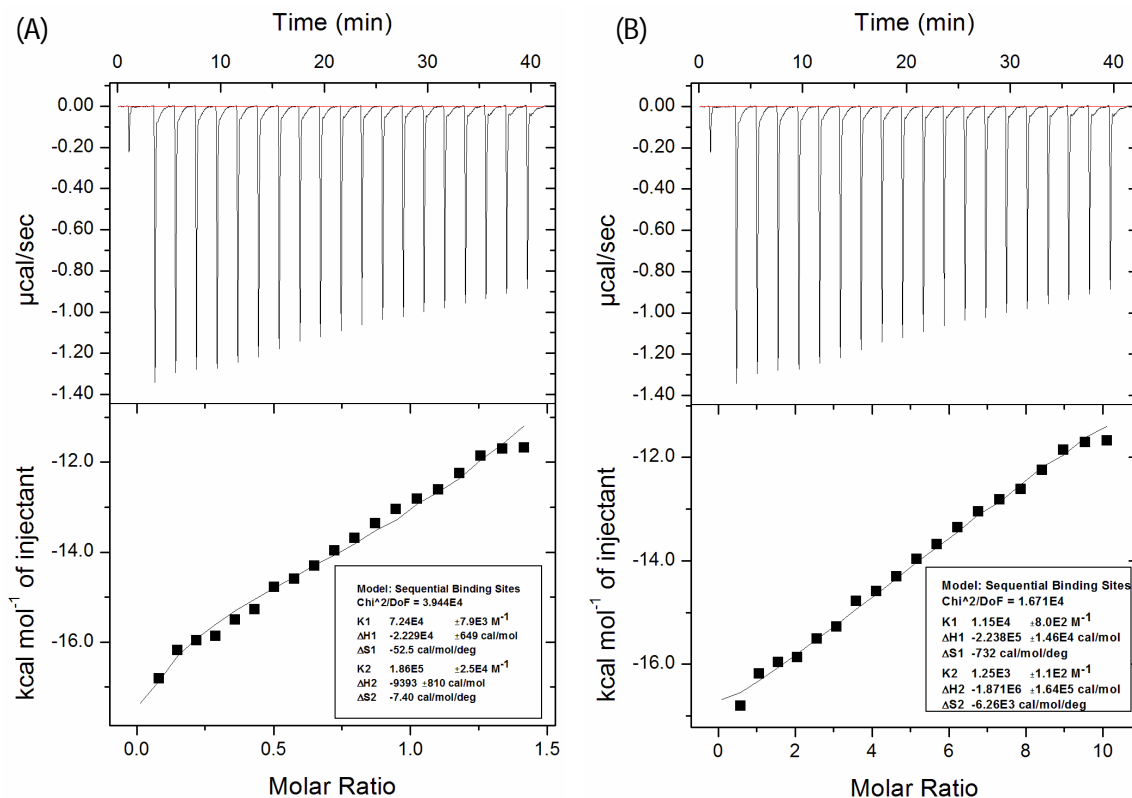
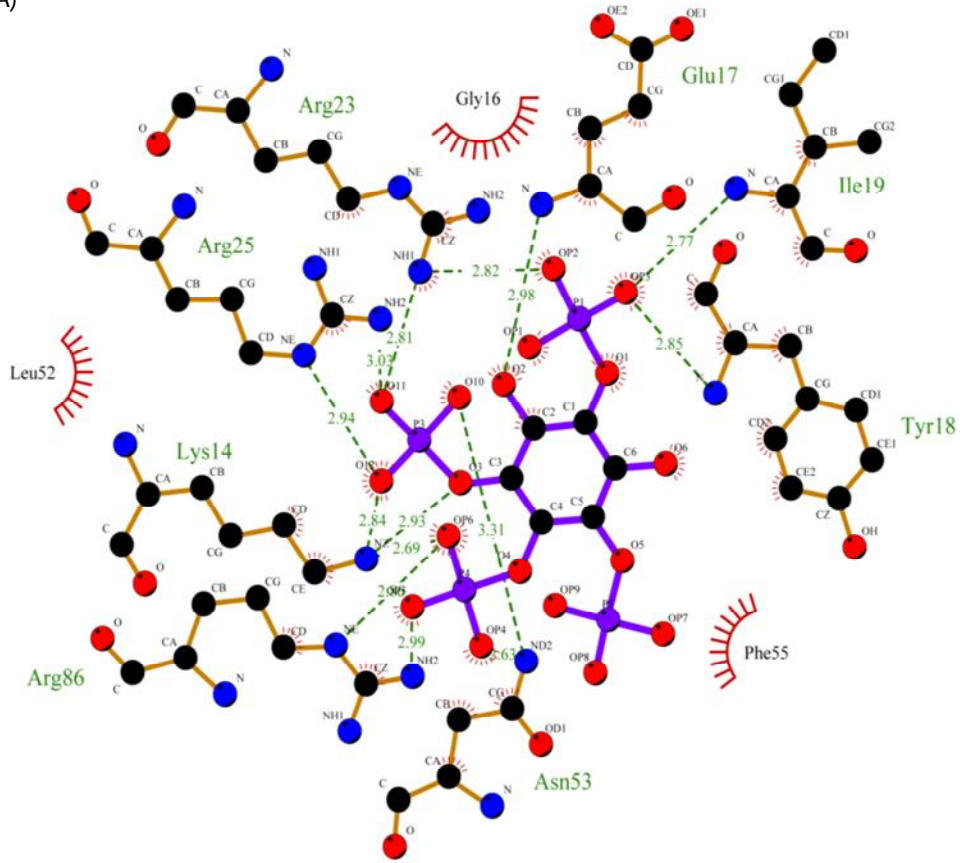
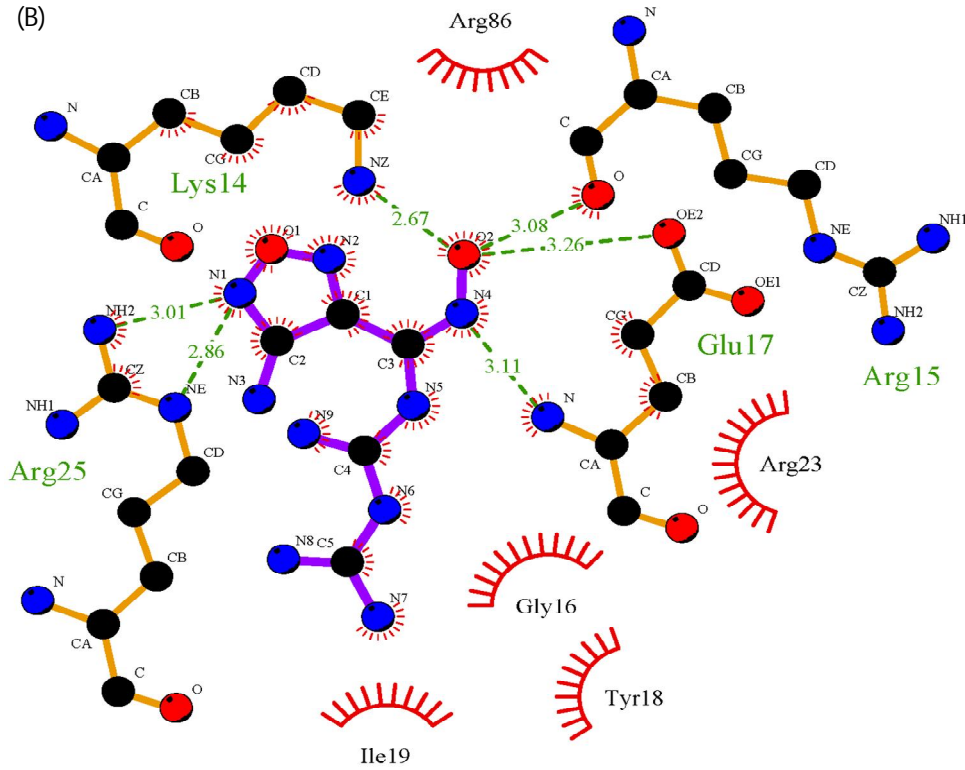


Figure S3: Representative isothermal titration calorimetric plots of AKT1 PH-domain with potent compounds. Original titration and integrated peak areas of AKT1 PH-domain with **PI-1** (A) and **PI-4** (B).

(A)



(B)



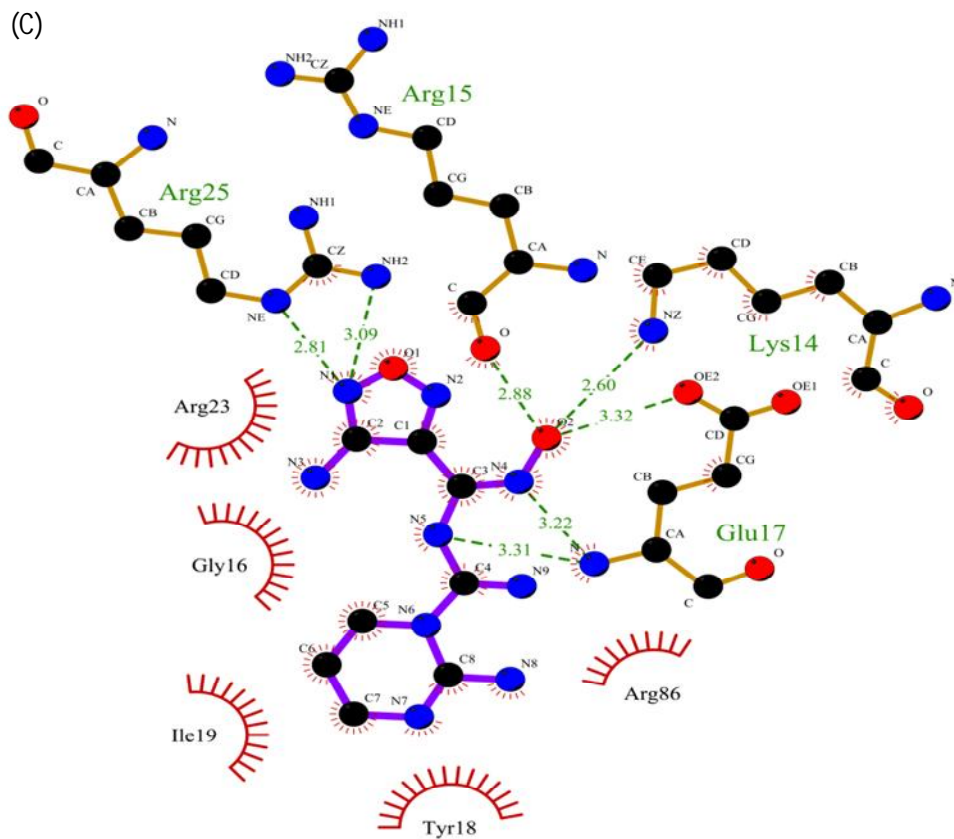


Figure S4: Lig-plot analysis of IP4 bound to AKT1 PH (1H10) domain (A). Lig-plot analysis of **PI-1** (B) and **PI-4** (C) docked into the PIP-binding site of AKT1-PH domain. Residues involved in interactions through hydrogen bond formation are shown using dashed lines.

Table S2: Theoretically calculated Interaction Energy between Ligand and PH-domains

Protein	Ligand	Interaction energy between ligand and Protein (kcal/mol)		Hydrogen bonding energy (kcal/mol)	
		PIP-binding site	Non PIP-binding site	PIP-binding site	Non PIP-binding site
AKT1-PH Domain	PI-1	-096.413	-086.471	-18.895	-14.628
		-094.055	-086.289	-17.347	-15.559
		-092.162	-083.097	-18.140	-13.026
	PI-2	-118.573	-108.961	-23.223	-27.636
		-111.341	-108.642	-20.653	-16.525
		-110.922	-103.784	-19.252	-18.999
	PI-3	-127.659	-118.369	-24.080	-27.361
		-126.770	-112.789	-28.646	-15.482
		-125.818	-120.384	-23.513	-23.033
	PI-4	-118.527	-115.097	-25.753	-15.362
		-119.380	-116.788	-18.622	-21.403
		-117.506	-111.003	-16.348	-14.046
	PI-5	-122.965	-114.062	-24.391	-12.146
		-115.767	-109.465	-19.299	-15.455
		-115.395	-109.266	-23.840	-08.255
Grp1-PH Domain	PI-1	-92.8201	-	-21.959	-
		-90.6866	-	-19.842	-
		-89.9794	-	-22.223	-
	PI-2	-113.564	-	-24.156	-
		-112.836	-	-22.030	-
		-112.737	-	-25.896	-
	PI-3	-131.210	-	-28.362	-
		-129.856	-	-28.363	-
		-122.495	-	-29.276	-
	PI-4	-126.288	-	-23.785	-
		-124.609	-	-18.588	-
		-124.401	-	-22.979	-
	PI-5	-131.210	-	-28.362	-
		-129.856	-	-28.363	-
		-122.495	-	-29.276	-
BTK-PH Domain	PI-1	-112.621	-140.259	-23.995	-20.284
		-110.963	-138.152	-23.616	-23.426
	PI-4	-101.816	-136.625	-10.688	-20.842
		-127.118	-152.321	-21.303	-20.958
		-126.359	-141.947	-23.870	-23.407
		-125.421	-141.484	-25.414	-25.204

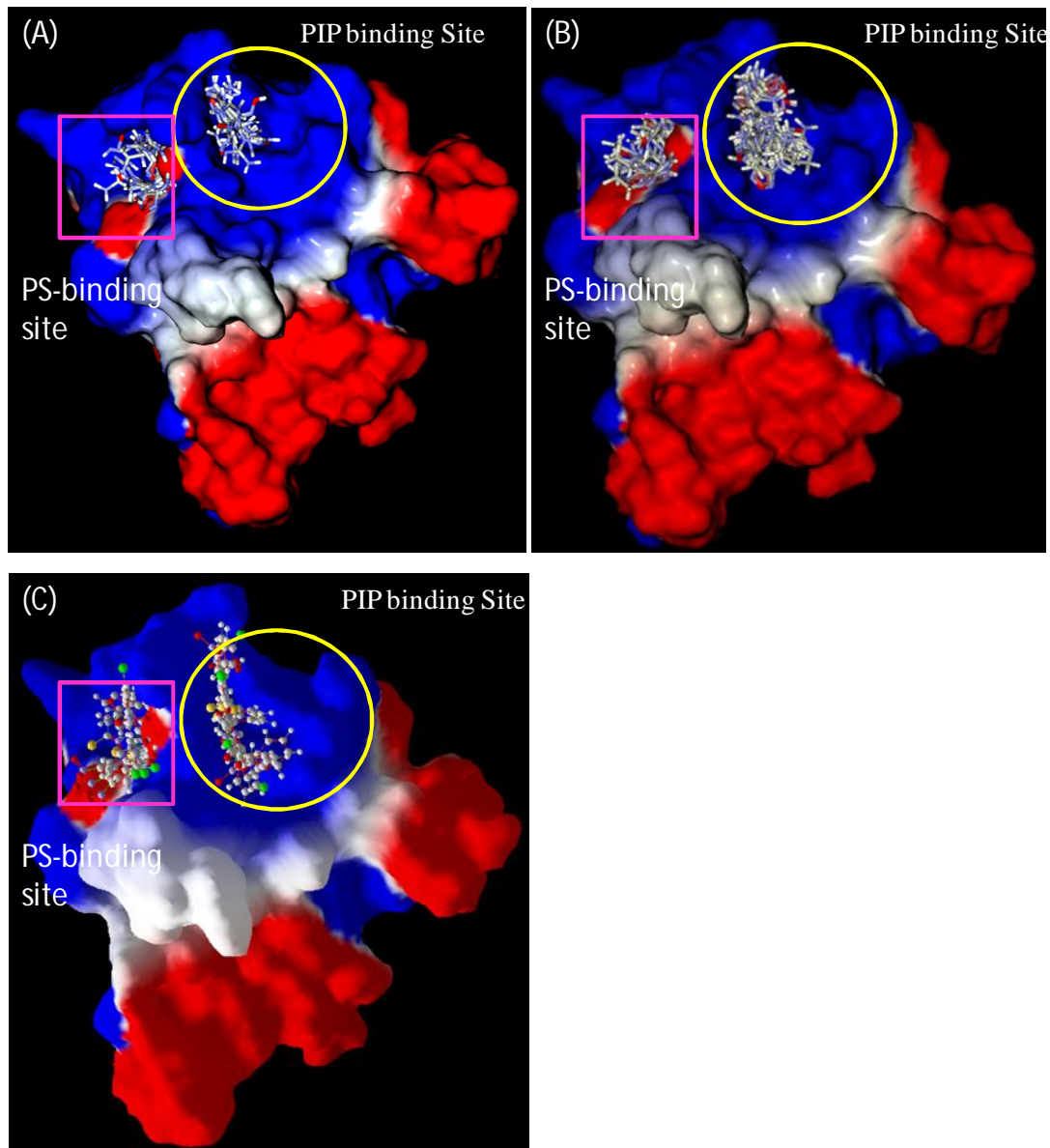


Figure S5: Surface representation of the docking complexes AKT1-PH/**PI-1** (A), AKT1-PH/**PI-4** (B) and AKT1-PH/**PIT-1** (C) both in PIP (yellow circle) and PS- (pink square box) binding sites. Overall surface of AKT1-PH domain was colored according to the electrostatic potential (blue, positively 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

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

Values in parentheses indicate standard deviations.

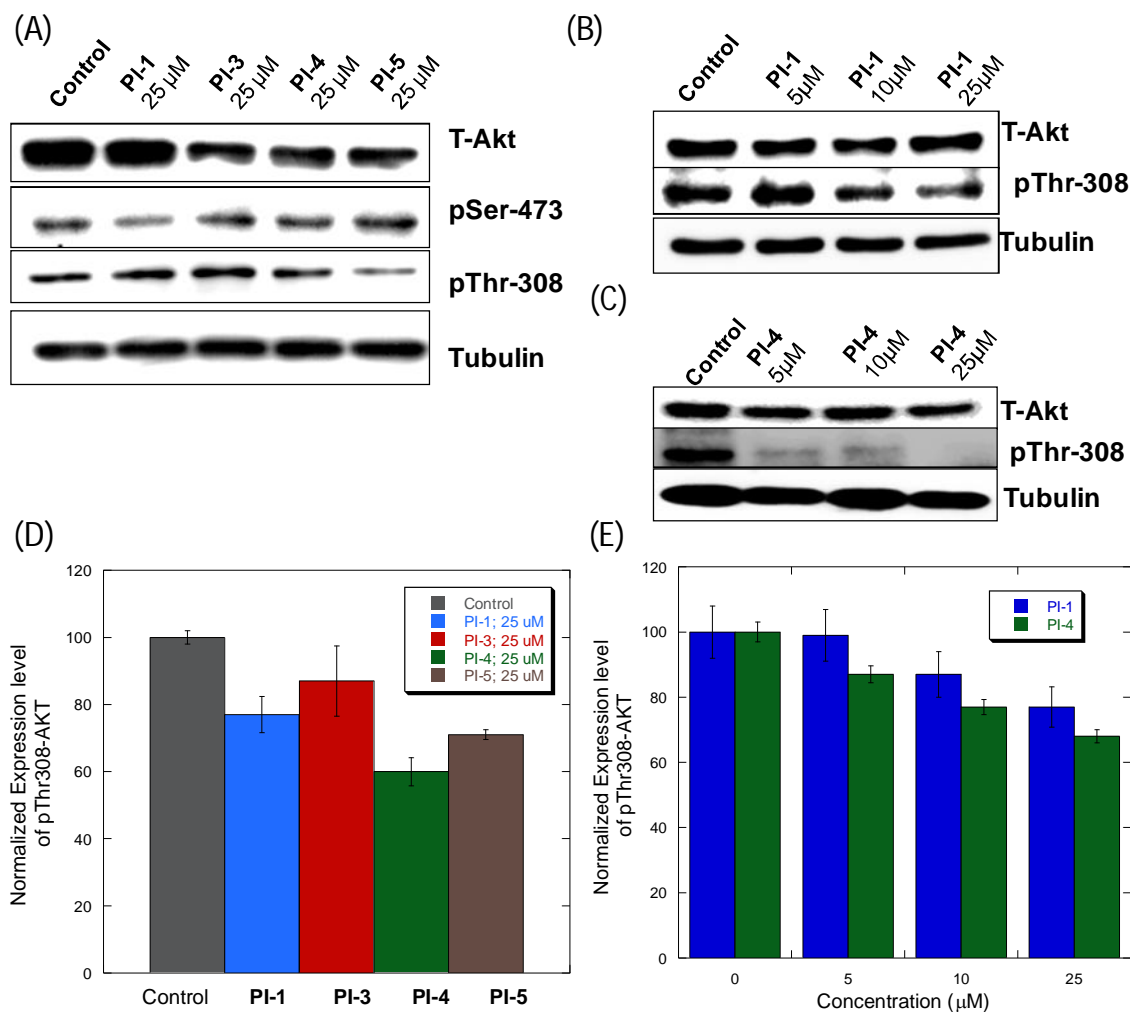


Figure S6: Compounds inhibit AKT signalling pathway in MDA-MB-231 cells. MDA-MB-231 cells were treated with the compounds (25 μM) for 48 h, and the indicated PI(3,4,5)P₃-dependent phosphorylation events were evaluated by immunoblot analysis (A). MDA-MB-231 cells were treated with the **PI-1** and **PI-4** 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 μ M) (E).