



Development of sulfonamide AKT PH domain inhibitors

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ABSTRACT

Disruption of the phosphatidylinositol 3-kinase/AKT signaling pathway can lead to apoptosis in cancer cells. Previously we identified a lead sulfonamide that selectively bound to the pleckstrin homology (PH) domain of AKT and induced apoptosis when present at low micromolar concentrations. To examine the effects of structural modification, a set of sulfonamides related to the lead compound was designed, synthesized, and tested for binding to the expressed PH domain of AKT using a surface plasmon resonance-based competitive binding assay. Cellular activity was determined by means of an assay for pAKT production and a cell killing assay using BxPC-3 cells. The most active compounds in the set are lipophilic and possess an aliphatic chain of the proper length. Results were interpreted with the aid of computational modeling. This paper represents the first structure–activity relationship (SAR) study of a large family of AKT PH domain inhibitors. Information obtained will be used in the design of the next generation of inhibitors of AKT PH domain function.

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1. Introduction

Pleckstrin homology (PH) domains containing 100–120 amino acids, are found in over 500 human proteins,¹ and are the 11th most common domain in the human proteome.² A subset of 40 PH domains are known to bind phosphorylated phosphatidylinositol (PI) lipids held in cell membranes. PI phosphorylation and the subsequent binding of PH domain-containing proteins are vital components of signal transduction pathways that regulate cell growth and survival.^{3,4} For example, phosphorylation of PI(4,5)P₂ to produce PI(3,4,5)P₃ (see Fig. 1) by PI3K signals the recruitment and binding of AKT to the inner leaflet of the plasma membrane via recognition of the PH domain.^{5,6} This translocation allows phosphorylation of AKT at Thr³⁰⁸ by the plasma membrane-bound

PDPK1.⁷ Phosphorylation at the Ser⁴⁷³ residue occurs either by the ILK, by the kinase activity of AKT itself, or by rictor-mTOR.⁸ Once fully phosphorylated, AKT translocates through the cytosol to the nucleus where it phosphorylates a variety of downstream targets. AKT promotes cell survival by activating CREB,⁹ and promotes proliferation by activating p70S⁶ kinase¹⁰ and GSK-3β¹¹ which contribute to cyclin D accumulation of cell cycle entry. Furthermore, AKT acts as a mediator for VEGF production and angiogenesis by phosphorylation of mTOR.¹² Given its importance in proliferation and survival signaling, AKT is an important target for cancer drug discovery.¹³

Several previous attempts to develop AKT inhibitors have led to compounds that bind to the ATP-binding pocket¹⁴ or behave as allosteric inhibitors.¹⁵ Due to the similarity of this pocket among serine/threonine kinases, achieving target specificity has been difficult. Our previous studies involving a D-3-deoxyphosphatidylinositol ether lipid, DPIEL (Fig. 1),^{16,17} provided proof-of-principle for using PH domains as drug targets.^{17–24} DPIEL exhibits a high binding affinity and selectivity for the PH domain of AKT. In addition, DPIEL does not bind to other PH domain-containing proteins, including PDPK1, IRS-1, mSOS, and β-ARK. Although DPIEL exhibits significant antitumor activity, unfortunately it is not a useful drug candidate because of its pharmacokinetic and pharmacodynamic properties.¹⁶

Previously, we used a crystal structure of the PH domain of AKT and in silico screening to cull from libraries compounds expected to bind to the target domain.^{25,26} Compounds selected from these

Abbreviations: AKT, protein kinase B; β-ARK, β-adrenergic receptor kinase; CREB, cAMP response element-binding; DMEM, Dulbecco's modified Eagle medium; DPIEL, deoxyphosphatidylinositol ether lipid; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; FBS, fetal bovine serum; HRMS, high resolution mass spectrum; ILK, integrin-linked kinase; IRS-1, insulin receptor substrate-1; LRMS, low resolution mass spectrum; mSOS, mammalian son-of-sevenless; mTOR, mammalian target of rapamycin; PDPK1, 3-phosphoinositide dependent protein kinase-1; PH, pleckstrin homology; PI, phosphoinositol; PI3K, phosphatidylinositol 3-kinase; PI(4,5)P₂, phosphatidylinositol (4,5)-bisphosphate; PI(3,4,5)P₃, phosphatidylinositol (3,4,5)-trisphosphate; RP HPLC, reversed phase high performance liquid chromatography; SAR, structure–activity relationship; TLC, thin-layer chromatography; VEGF, vascular endothelial growth factor.

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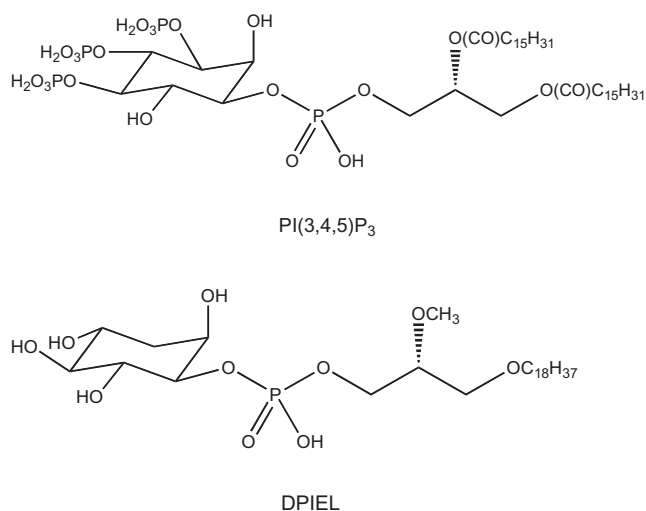


Figure 1. The structures of PI(3,4,5)P₃ and DPIEL.

libraries were purchased or prepared, then tested for binding to AKT and for inhibition of AKT function in several cancer cell lines. Compound **1**, a sulfonamide (see Table 1), was among the leads identified, and so several analogs of **1** were prepared and tested in vitro. The best analog, compound **29** herein, was then tested in vivo where it exhibited good antitumor activity in a mouse xenograft model of pancreatic cancer cells in immunocompromised mice.^{26,27} This work provided for the first time proof-of-principle for further development of sulfonamides as drugs that bind to the PH domain of AKT, inhibit its function, and as a result exhibit antitumor activity. Presented herein are studies with compounds **2–47**, derivatives of **1** that were synthesized and tested in an effort to generate structure–activity relationships (SARs) to guide further development of potential drug candidates.

2. Materials and methods

2.1. Chemical synthesis

2.1.1. General experimental

All commercial reagents were used without further purification. Analytical TLC was carried out on pre-coated Silica Gel F254 plates. TLC plates were visualized with UV light (254 nm). ¹H NMR spectra were recorded at 250, 300, or 500 MHz and ¹³C NMR spectra at 62.5, 75, or 125 MHz. Chemical shifts (δ) are expressed in ppm and are internally referenced (7.26 ppm for ¹H NMR and 77.00 ppm for ¹³C NMR in CDCl₃, 2.50 ppm for ¹H NMR and 39.50 ppm for ¹³C NMR in DMSO-*d*₆). High resolution mass spectra were obtained in the Mass Spectrometry Laboratory in the Department of Chemistry at the University of Arizona. Melting points are uncorrected.

2.1.2. 4-Butyl-*N*-(1,3,4-thiadiazol-2-yl)benzenesulfonamide (**26**)

To a solution of butylbenzene (4.13 g, 30.8 mmol) in CHCl₃ (50 mL) was added chlorosulfonic acid (17.0 mL, 29.8 g, 256 mmol).²⁸ The mixture was stirred at rt for 20 h. The mixture was poured on ice (200 mL) and extracted with EtOAc (3 × 100 mL). The combined extracts were washed with water, aqueous NaHCO₃, and water, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The resulting yellow oily residue, crude *p*-butylbenzenesulfonyl chloride (ca 88% yield), was used without further purification in the next reaction; ¹H NMR (300 MHz, CDCl₃) δ 0.94 (3, t, *J* = 7 Hz), 1.34–1.41 (2, m), 1.62–1.67 (2, m), 2.73 (2, t, *J* = 8 Hz), 7.41 (2, d, *J* = 8 Hz), 7.94 (2, d, *J* = 8 Hz).

To a stirred solution of 2-amino-1,3,4-thiadiazole (2.0 g, 19.7 mmol) in pyridine (30 mL) under argon at –20 °C was added crude *p*-butylbenzenesulfonyl chloride (4.89 g, ca. 21 mmol) over 10 min. The reaction mixture was allowed to attain rt and stirred for 16 h. Water (300 mL) was added to quench the reaction. The mixture was extracted with CH₂Cl₂, the organic extracts washed with 2 N HCl (2 × 150 mL), brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The residue was subjected to flash chromatography on silica gel eluted with CH₂Cl₂/MeOH 33:1 to give the product **26** (3.46 g, 11.6 mmol, 59% yield) as a solid, mp 120–121 °C; RP HPLC *t*_R 6.06 min (4.6 × 250 mm C₁₈ column eluted with CH₃CN, 0.5 mL/min); ¹H NMR (300 MHz, CDCl₃) δ 0.91 (3, t, *J* = 7 Hz), 1.29–1.37 (2, m), 1.56–1.61 (2, m), 2.65 (2, t, *J* = 7 Hz), 7.27 (2, d, *J* = 8 Hz), 7.84 (2, d, *J* = 8 Hz), 8.25 (1, s); ¹³C NMR (75 MHz, CDCl₃) 13.9, 22.3, 33.2, 33.6, 126.5, 129.1, 138.1, 142.7, 148.6, 167.4; HRMS (Q-TOF) calcd for C₁₂H₁₆N₃O₂S₂ 298.0684, observed 298.0695 (M+H)⁺; calcd for C₁₂H₁₅N₃NaO₂S₂ 320.0503, observed 320.0361 (M+Na)⁺.

2.1.3. 4-Hexyl-*N*-(1,3,4-thiadiazol-2-yl)benzenesulfonamide (**27**)

In a similar manner, hexylbenzene (5.00 g, 30.8 mmol) and chlorosulfonic acid (17.0 mL, 29.8 g, 256 mmol) gave crude *p*-hexylbenzenesulfonyl chloride as a yellow oily residue (ca. 81% yield); ¹H NMR (300 MHz, CDCl₃) δ 0.88 (3, t, *J* = 7 Hz), 1.30–1.35 (6, m), 1.55–1.63 (2, m), 2.59 (2, t, *J* = 8 Hz), 7.38 (2, d, *J* = 8 Hz), 7.89 (2, d, *J* = 8 Hz). Reaction of 2-amino-1,3,4-thiadiazole (2.0 g, 19.7 mmol) with *p*-hexylbenzenesulfonyl chloride (5.48 g, ca 21 mmol) afforded the product **27** (3.72 g, 11.4 mmol, 58% yield) as a solid, mp 125–126 °C, after flash chromatography on silica gel eluted with CH₂Cl₂/MeOH 33:1; RP HPLC *t*_R 6.79 min (4.6 × 250 mm C₁₈ column eluted with CH₃CN, 0.5 mL/min); ¹H NMR (300 MHz, CDCl₃) δ 0.88 (3, t, *J* = 7 Hz), 1.28 (6, m), 1.58 (2, m), 2.63 (2, t, *J* = 7 Hz), 7.27 (2, d, *J* = 8 Hz), 7.83 (2, d, *J* = 8 Hz), 8.24 (1, s); ¹³C NMR (75 MHz, CDCl₃) 14.1, 22.6, 28.9, 31.1, 31.6, 35.9, 126.5, 129.0, 138.1, 142.6, 148.6, 167.4; HRMS (Q-TOF) calcd for C₁₄H₂₀N₃O₂S₂ 326.0997, observed 326.0931 (M+H)⁺; calcd for C₁₄H₁₉N₃NaO₂S₂ 348.0816, observed 348.0816 (M+Na)⁺.

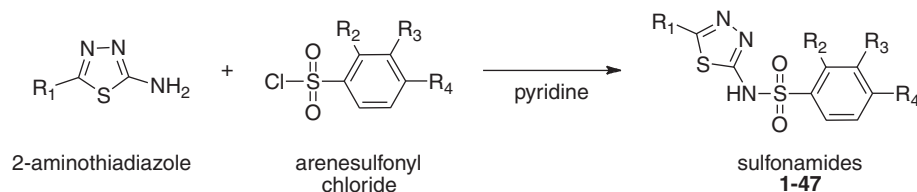
2.1.4. 4-Octyl-*N*-(1,3,4-thiadiazol-2-yl)benzenesulfonamide (**28**)

In a similar manner, 1-phenyloctane (5.86 g, 30.8 mmol) and chlorosulfonic acid (17.0 mL, 29.8 g, 256 mmol) gave crude *p*-octylbenzenesulfonyl chloride as a yellow oily residue (ca. 80% yield); ¹H NMR (300 MHz, CDCl₃) δ 0.87 (3, t, *J* = 7 Hz), 1.27–1.32 (10, m), 1.64–1.66 (2, m), 2.72 (2, t, *J* = 8 Hz), 7.42 (2, d, *J* = 8 Hz), 7.93 (2, d, *J* = 8 Hz). Reaction of 2-amino-1,3,4-thiadiazole (2.0 g, 19.7 mmol) with *p*-octylbenzenesulfonyl chloride (6.06 g, ca. 21 mmol) afforded the product **28** (3.83 g, 10.8 mmol, 55% yield) as a solid, mp 123–124 °C, after flash chromatography on silica gel eluted with CH₂Cl₂/MeOH 33:1; RP HPLC *t*_R 8.17 min (4.6 × 250 mm C₁₈ column eluted with CH₃CN, 0.5 mL/min); ¹H NMR (300 MHz, CDCl₃) δ 0.87 (3, t, *J* = 7 Hz), 1.36 (10, m), 1.59 (2, m), 2.63 (2, t, *J* = 7 Hz), 7.27 (2, d, *J* = 8 Hz), 7.82 (2, d, *J* = 8 Hz), 8.23 (1, s); ¹³C NMR (75 MHz, CDCl₃) 14.1, 22.6, 29.2, 29.3, 29.4, 31.1, 31.8, 35.9, 126.5, 129.0, 138.1, 142.6, 148.7, 167.3; HRMS (Q-TOF) calcd for C₁₆H₂₄N₃O₂S₂ 354.1310, observed 354.1211 (M+H)⁺; calcd for C₁₆H₂₃N₃NaO₂S₂ 376.1129, observed 376.1154 (M+Na)⁺.

2.1.5. 4-Dodecyl-*N*-(1,3,4-thiadiazol-2-yl)benzenesulfonamide (**29**)

The syntheses of *p*-dodecylbenzenesulfonyl chloride and compound **29** have previously been described; see the supplementary data associated with Ref. 26.

Table 1
Structures, yields, and melting points of compounds **1–47**



Compound	R ₁	R ₂	R ₃	R ₄	Yield (%)	Mp (°C)
1 ^a	H	H	H	NH ₂	49 ^b	224–225
2	CH ₃	H	H	NH ₂	72 ^b	207–208
3	CH ₂ CH ₃	H	H	NH ₂	69 ^b	190–191
4	C(CH ₃) ₃	H	H	NH ₂	74 ^b	220–221
5	CH ₂ CO ₂ H	H	H	NH ₂	82 ^c	209–210
6	CH ₂ OH	H	H	NH ₂	68 ^b	89–90
7	SO ₂ NH ₂	H	H	NH ₂	57 ^b	241–242
8 ^a	H	H	H	NH(CO)CH ₃	95	216–217
9	CH ₃	H	H	NH(CO)CH ₃	97	239–240
10	CH ₂ CH ₃	H	H	NH(CO)CH ₃	70	197–198
11	C(CH ₃) ₃	H	H	NH(CO)CH ₃	84	137–138
12	CH ₂ CO ₂ H	H	H	NH(CO)CH ₃	88 ^d	206–207
13	CH ₂ CO ₂ CH ₂ CH ₃	H	H	NH(CO)CH ₃	76	156–157
14	CO ₂ CH ₂ CH ₃	H	H	NH(CO)CH ₃	73	201–202
15	CH ₂ OH	H	H	NH(CO)CH ₃	82	101–102
16	SO ₂ NH ₂	H	H	NH(CO)CH ₃	63	280–281
17 ^a	H	H	H	NH(CO)(CH ₂) ₈ CH ₃	95 ^e	151–152
18	CH ₃	H	H	NH(CO)(CH ₂) ₈ CH ₃	95 ^e	141–142
19	CH ₂ CH ₃	H	H	NH(CO)(CH ₂) ₈ CH ₃	97 ^e	121–122
20	C(CH ₃) ₃	H	H	NH(CO)(CH ₂) ₈ CH ₃	98 ^e	156–157
21	CH ₂ CO ₂ H	H	H	NH(CO)(CH ₂) ₈ CH ₃	83 ^d	190–191
22	CH ₂ CO ₂ CH ₂ CH ₃	H	H	NH(CO)(CH ₂) ₈ CH ₃	63	89–90
23	CO ₂ CH ₂ CH ₃	H	H	NH(CO)(CH ₂) ₈ CH ₃	65	101–102
24	CH ₂ OH	H	H	NH(CO)(CH ₂) ₈ CH ₃	73	69–70
25	SO ₂ NH ₂	H	H	NH(CO)(CH ₂) ₈ CH ₃	60	242–243
26	H	H	H	(CH ₂) ₃ CH ₃	59	120–121
27	H	H	H	(CH ₂) ₅ CH ₃	58	125–126
28	H	H	H	(CH ₂) ₇ CH ₃	55	123–124
29 ^a	H	H	H	(CH ₂) ₁₁ CH ₃	51	126–127
30	H	H	H	(CH ₂) ₁₃ CH ₃	47	116–117
31	H	H	H	(CH ₂) ₁₅ CH ₃	46	118–119
32	H	H	H	(CH ₂) ₁₇ CH ₃	51	116–117
33	CH ₃	H	H	(CH ₂) ₁₁ CH ₃	84	149–150
34	CH ₂ CH ₃	H	H	(CH ₂) ₁₁ CH ₃	59	93–94
35	C(CH ₃) ₃	H	H	(CH ₂) ₁₁ CH ₃	87	117–118
36	CH ₂ CO ₂ H	H	H	(CH ₂) ₁₁ CH ₃	86 ^d	194–195
37	CH ₂ CO ₂ CH ₂ CH ₃	H	H	(CH ₂) ₁₁ CH ₃	54	110–111
38	CO ₂ CH ₂ CH ₃	H	H	(CH ₂) ₁₁ CH ₃	78	134–135
39	CH ₂ OH	H	H	(CH ₂) ₁₁ CH ₃	65	138–139
40	SO ₂ NH ₂	H	H	(CH ₂) ₁₁ CH ₃	63	249–250
41	H	H	H	C ₆ H ₅	57	250–251
42	CH ₂ CH ₃	H	H	C ₆ H ₅	43	146–147
43	CH ₂ OH	H	H	C ₆ H ₅	52	239–240
44 ^a	H	H	H	NH(CO)CH ₂ O(CH ₂ CH ₂ O) ₂ CH ₃	62	oil
45					68	54–55
46	H	(CH ₂) ₁₁ CH ₃	H	H	13	159–161
47	H	H	(CH ₂) ₁₁ CH ₃	H	91	98–99

Details of the syntheses are given in the Section 2.1 (compounds **26–40**) or in the Supplementary data (compounds **1–25** and **41–47**).

^a Compound previously reported; see Ref. 26.

^b Yield of hydrolysis of the corresponding acetamide derivative.

^c Yield of hydrolysis of compound **13**.

^d Yield of hydrolysis of the corresponding ethyl ester.

^e Yield of acylation of the corresponding aniline derivative.

2.1.6. 4-Tetradecyl-N-(1,3,4-thiadiazol-2-yl)benzenesulfonamide (**30**)

In a similar manner, 1-phenyltetradecane (0.69 g, 2.5 mmol) and chlorosulfonic acid (0.50 mL, 7.5 mmol) gave *p*-tetradecylbenzenesulfonyl chloride as a white solid (0.63 g, 1.7 mmol,

68%), *R*_f 0.18 (50% EtOAc/hexanes), mp 32–33 °C, after chromatography on silica gel (70–230 mesh) with hexanes/EtOAc (49:1); ¹H NMR (300 MHz, CDCl₃) δ 0.88 (3, t, *J* = 7.2 Hz), 1.25 (22, m), 1.65 (2, m), 2.72 (2, t, *J* = 7.8 Hz), 7.42 (2, d, *J* = 8.4 Hz), 7.93 (2, d, *J* = 8.4 Hz); ¹³C NMR (75 MHz, CDCl₃) 14.1, 22.6, 29.1, 29.3, 29.5,

29.6, 29.7, 30.9, 31.9, 36.0, 126.9, 129.5, 141.7, 151.6. Reaction of 2-amino-1,3,4-thiadiazole (179 mg, 1.77 mmol) with *p*-tetradecylbenzenesulfonyl chloride (440 mg, 1.18 mmol) afforded the product **30** (240 mg, 0.55 mmol, 47% yield), R_f 0.46 (5% MeOH/ CH_2Cl_2), as a solid, mp 116–117 °C, after chromatography on silica gel (70–230 mesh) eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 19:1; ^1H NMR (300 MHz, CDCl_3) δ 0.88 (3, t, J = 6.9 Hz), 1.25 (22, m), 1.60 (2, m), 2.64 (2, t, J = 7.2 Hz), 7.29 (2, d, J = 8.4 Hz), 7.84 (2, d, J = 8.4 Hz), 8.23 (1, s); ^{13}C NMR (75 MHz, CDCl_3) 14.1, 22.6, 29.2, 29.3, 29.4, 29.5, 29.6, 31.1, 31.9, 35.9, 126.5, 128.9, 138.1, 142.6, 148.6, 167.4; HRMS (ESI^+ , m/z) calcd for $\text{C}_{22}\text{H}_{36}\text{N}_3\text{O}_2\text{S}_2$ 438.2243, observed 438.2243 ($\text{M}+\text{H}$) $^+$.

2.1.7. 4-Hexadecyl-*N*-(1,3,4-thiadiazol-2-yl)benzenesulfonamide (31)

In a similar manner, 1-phenylhexadecane (0.76 g, 2.5 mmol) and chlorosulfonic acid (0.50 mL, 7.5 mmol) gave *p*-hexadecylbenzenesulfonyl chloride as a white solid (0.71 g, 1.8 mmol, 72%), mp 35–36 °C, after chromatography on silica gel eluted with hexanes/EtOAc (49:1); ^1H NMR (300 MHz, CDCl_3) δ 0.88 (3, t, J = 7.2 Hz), 1.25 (26, m), 1.62 (2, m), 2.72 (2, t, J = 7.8 Hz), 7.42 (2, d, J = 8.4 Hz), 7.95 (2, d, J = 8.4 Hz); ^{13}C NMR (75 MHz, CDCl_3) 14.4, 22.9, 29.4, 29.64, 29.8, 29.9, 31.2, 32.2, 36.3, 127.3, 129.8, 142.0, 151.9. Reaction of 2-amino-1,3,4-thiadiazole (228 mg, 2.25 mmol) with *p*-hexadecylbenzenesulfonyl chloride (600 mg, 1.50 mmol) afforded the product **31** (320 mg, 0.69 mmol, 46% yield), R_f 0.46 (5% MeOH/ CH_2Cl_2), as a solid, mp 118–119 °C, after chromatography on silica gel (70–230 mesh) eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 19:1; ^1H NMR (300 MHz, CDCl_3) δ 0.88 (3, t, J = 6.9 Hz), 1.25 (26, m), 1.59 (2, m), 2.64 (2, t, J = 8.1 Hz), 7.29 (2, d, J = 7.8 Hz), 7.84 (2, d, J = 7.8 Hz), 8.23 (1, s); ^{13}C NMR (75 MHz, CDCl_3) 14.1, 22.7, 29.2, 29.3, 29.4, 29.6, 29.7, 31.1, 31.9, 35.9, 126.5, 128.9, 138.1, 142.5, 148.7, 167.5; HRMS (ESI^+ , m/z) calcd for $\text{C}_{24}\text{H}_{40}\text{N}_3\text{O}_2\text{S}_2$ 466.2556, observed 466.2558 ($\text{M}+\text{H}$) $^+$.

2.1.8. 4-Octadecyl-*N*-(1,3,4-thiadiazol-2-yl)benzenesulfonamide (32)

In a similar manner, 1-phenyloctadecane (0.84 g, 2.5 mmol) and chlorosulfonic acid (0.50 mL, 7.5 mmol) gave *p*-octadecylbenzenesulfonyl chloride as a white solid (0.60 g, 1.4 mmol, 56%), mp 43–44 °C, after chromatography on silica gel eluted with hexanes/EtOAc (49:1); ^1H NMR (300 MHz, CDCl_3) δ 0.86 (3, t, J = 6.9 Hz), 1.25 (30, m), 1.65 (2, m), 2.72 (2, t, J = 7.8 Hz), 7.42 (2, d, J = 8.4 Hz), 7.93 (2, d, J = 8.4 Hz); ^{13}C NMR (75 MHz, CDCl_3) 14.1, 22.7, 29.2, 29.4, 29.5, 29.7, 30.9, 31.9, 36.0, 127.1, 129.6, 141.8, 151.7. Reaction of 2-amino-1,3,4-thiadiazole (177 mg, 1.75 mmol) with *p*-octadecylbenzenesulfonyl chloride (500 mg, 1.17 mmol) afforded the product **32** (296 mg, 0.60 mmol, 51% yield), R_f 0.46 (5% MeOH/ CH_2Cl_2), as a solid, mp 116–117 °C, after chromatography on silica gel (70–230 mesh) eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 19:1; ^1H NMR (300 MHz, CDCl_3) δ 0.86 (3, t, J = 6.9 Hz), 1.25 (30, m), 1.60 (2, m), 2.64 (2, t, J = 7.8 Hz), 7.29 (2, d, J = 7.8 Hz), 7.82 (2, d, J = 7.8 Hz), 8.21 (1, s); ^{13}C NMR (75 MHz, CDCl_3) 14.0, 22.7, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.1, 31.9, 35.9, 126.5, 128.9, 138.1, 142.6, 148.6, 167.4; HRMS (ESI^+ , m/z) calcd for $\text{C}_{26}\text{H}_{44}\text{N}_3\text{O}_2\text{S}_2$ 494.2869, observed 494.2869 ($\text{M}+\text{H}$) $^+$.

2.1.9. 4-Dodecyl-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (33)

A suspension of 2-amino-5-methyl-1,3,4-thiadiazole (150 mg, 1.3 mmol) in pyridine (0.5 mL) was stirred and cooled in an ice bath while *p*-dodecylbenzenesulfonyl chloride (300 mg, 0.87 mmol) was added slowly. The reaction mixture was allowed to attain rt, then heated in an oil bath at 95 °C for 1 h. The reaction mixture was then cooled, added to aqueous 10% HCl (5 mL),

and the resulting mixture extracted with EtOAc (3 \times 10 mL). The organic extracts were washed with water, brine, dried over anhydrous Na_2SO_4 , filtered, and volatiles evaporated in vacuo to yield a solid mass. Chromatography on silica gel (70–230 mesh) eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 49:1 gave the product **33** (310 mg, 0.73 mmol, 84%), R_f 0.23 (50% EtOAc/hexanes). Recrystallization from EtOAc/hexanes 7:3 gave an analytical sample, mp 149–150 °C; ^1H NMR (500 MHz, CDCl_3) δ 0.88 (3, t, J = 7.0 Hz), 1.20–1.36 (18, m), 1.54–1.63 (2, m), 2.51 (3, s), 2.63 (2, t, J = 7.5 Hz), 7.25 (2, d, J = 7.5 Hz), 7.82 (2, d, J = 7.5 Hz), 12.36 (1, br s); ^{13}C NMR (125 MHz, CDCl_3) δ 14.1, 16.5, 22.7, 29.2, 29.3, 29.4, 29.5, 29.6, 31.1, 31.9, 35.9, 126.4, 128.8, 138.3, 148.3, 154.1, 168.6; HRMS (ESI^+ , m/z) calcd for $\text{C}_{21}\text{H}_{34}\text{N}_3\text{O}_2\text{S}_2$ 424.2092, observed 424.2085 ($\text{M}+\text{H}$) $^+$.

2.1.10. 4-Dodecyl-*N*-(5-ethyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (34)

In a similar manner, reaction of 2-amino-5-ethyl-1,3,4-thiadiazole (169 mg, 1.3 mmol) and *p*-dodecylbenzenesulfonyl chloride (300 mg, 0.87 mmol) gave the product **34** (225 mg, 0.51 mmol, 59%), R_f 0.27 (50% EtOAc/hexanes), after chromatography on silica gel (70–230 mesh) eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 49:1. Recrystallization from EtOAc/hexanes 7:3 gave an analytical sample, mp 93–94 °C; ^1H NMR (500 MHz, CDCl_3) δ 0.88 (3, t, J = 6.5 Hz), 1.20–1.36 (18, m), 1.33 (3, t, J = 7.5 Hz), 1.54–1.63 (2, m), 2.63 (2, t, J = 7.5 Hz), 2.84 (2, q, J = 7.5 Hz), 7.25 (2, d, J = 8.5 Hz), 7.83 (2, d, J = 8.5 Hz), 12.30 (1, br s); ^{13}C NMR (125 MHz, CDCl_3) δ 12.6, 14.1, 22.7, 24.4, 29.2, 29.3, 29.4, 29.5, 29.6, 31.1, 31.9, 35.9, 126.5, 128.8, 138.4, 148.2, 160.1, 168.2; HRMS (ESI^+ , m/z) calcd for $\text{C}_{22}\text{H}_{36}\text{N}_3\text{O}_2\text{S}_2$ 438.2249, observed 438.2247 ($\text{M}+\text{H}$) $^+$.

2.1.11. *N*-(5-*tert*-Butyl-1,3,4-thiadiazol-2-yl)-4-dodecylbenzenesulfonamide (35)

In a similar manner, reaction of 2-amino-5-*tert*-butyl-1,3,4-thiadiazole (204 mg, 1.3 mmol) and *p*-dodecylbenzenesulfonyl chloride (300 mg, 0.87 mmol) gave the product **35** (350 mg, 0.75 mmol, 86%), R_f 0.45 (50% EtOAc/hexanes), after chromatography on silica gel (70–230 mesh) eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 49:1. Recrystallization from EtOAc/hexanes 7:3 gave an analytical sample, mp 117–118 °C; ^1H NMR (500 MHz, CDCl_3) δ 0.88 (3, t, J = 6.5 Hz), 1.20–1.36 (18, m), 1.38 (9, s), 1.56–1.64 (2, m), 2.63 (2, t, J = 7.5 Hz), 7.25 (2, d, J = 8.0 Hz), 7.86 (2, d, J = 8.0 Hz), 12.24 (1, br s); ^{13}C NMR (125 MHz, CDCl_3) δ 14.1, 22.7, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.1, 31.8, 35.8, 36.5, 126.5, 128.7, 138.5, 148.1, 167.8, 168.0; HRMS (ESI^+ , m/z) calcd for $\text{C}_{24}\text{H}_{40}\text{N}_3\text{O}_2\text{S}_2$ 466.2562, observed 466.2562 ($\text{M}+\text{H}$) $^+$.

2.1.12. 2-(5-(4-Dodecylphenylsulfonamido)-1,3,4-thiadiazol-2-yl)acetic Acid (36)

Distilled water (3.0 mL) and 10% aqueous NaOH (0.65 mL) were added to compound **37** (200 mg, 0.40 mmol) and the mixture was heated under reflux for 2 h. The pH of the solution was then adjusted to 4.0 by addition of 1.0 M HCl, the resulting precipitate was isolated by filtration, washed with cold water, and dried to give the product **36** (161 mg, 0.34 mmol, 85%), R_f 0.34 (20% MeOH/ CH_2Cl_2), as a light yellow solid, mp 194–195 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 0.85 (3, t, J = 6.6 Hz), 1.23 (18, m), 1.53 (2, m), 2.57 (2, t, J = 7.5 Hz), 3.74 (2, s), 7.24 (2, d, J = 8.1 Hz), 7.61 (2, d, J = 7.8 Hz); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 14.0, 22.1, 28.8, 28.9, 29.1, 30.7, 31.3, 34.9, 37.4, 125.8, 128.4, 141.2, 146.0, 153.3, 168.9, 170.8; HRMS (ESI^+ , m/z) calcd for $\text{C}_{22}\text{H}_{34}\text{N}_3\text{O}_4\text{S}_2$ 468.1991, observed 468.1977 ($\text{M}+\text{H}$) $^+$.

2.1.13. Ethyl 2-(5-(4-Dodecylphenylsulfonamido)-1,3,4-thiadiazol-2-yl)acetate (**37**)

A suspension of ethyl 2-(5-amino-1,3,4-thiadiazol-2-yl)acetate²⁹ (2.0 g, 11.2 mmol) in pyridine (50 mL) was stirred and cooled in an ice bath while *p*-dodecylbenzenesulfonyl chloride (4.7 g, 13.5 mmol) was added slowly. The mixture was stirred at rt for 48 h, then heated in an oil bath at 90 °C for 15 min. The reaction mixture was then cooled to rt, poured into aqueous 10% HCl, and extracted with EtOAc (3 × 75 mL). The combined extracts were washed with water (2 × 50 mL), brine (2 × 50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The residue was subjected to chromatography on silica gel (70–230 mesh) eluted with CH₂Cl₂/MeOH 95:5 to give the product **37** (2.9 g, 5.9 mmol, 53%), *R*_f 0.64 (10% MeOH/CH₂Cl₂). Recrystallization from EtOAc/hexanes gave an analytical sample, mp 110–111 °C; ¹H NMR (500 MHz, CDCl₃) δ 0.88 (3, t, *J* = 7.0 Hz), 1.20–1.28 (18, m), 1.32 (3, t, *J* = 7.0 Hz), 1.58–1.66 (2, m), 2.64 (2, t, *J* = 7.0 Hz), 3.87 (2, s), 4.25 (2, q, *J* = 7.0 Hz), 7.26 (2, d, *J* = 8.0 Hz), 7.82 (2, d, *J* = 8.0 Hz), 10.91 (1, br s); ¹³C NMR (125 MHz, CDCl₃) δ 14.0, 14.1, 22.6, 29.2, 29.3, 29.4, 29.5, 29.6, 31.1, 31.9, 35.9, 36.4, 62.2, 126.4, 128.8, 138.2, 148.3, 151.2, 167.3, 168.6; HRMS (ESI⁺, *m/z*) calcd for C₂₄H₃₈N₃O₄S₃ 496.2304, observed 496.2295 (M+H)⁺.

2.1.14. Ethyl 5-(4-Dodecylphenylsulfonamido)-1,3,4-thiadiazol-2-carboxylate (**38**)

In a similar manner, reaction of ethyl 5-amino-1,3,4-thiadiazole-2-carboxylate³⁰ (1.8 g, 10.4 mmol) with *p*-dodecylbenzenesulfonyl chloride (4.3 g, 12.5 mmol) gave the product **38** (3.9 g, 8.1 mmol, 78%), *R*_f 0.44 (10% MeOH/CH₂Cl₂), after chromatography on silica gel (70–230 mesh) eluted with CH₂Cl₂/MeOH 9:1. Recrystallization from EtOAc/hexanes gave an analytical sample, mp 134–135 °C; ¹H NMR (600 MHz, DMSO-*d*₆) δ 0.84 (3, t, *J* = 7.0 Hz), 1.17–1.30 (18, m), 1.31 (3, t, *J* = 7.0 Hz), 1.50–1.51 (2, m), 2.62 (2, t, *J* = 7.0 Hz), 4.36 (2, q, *J* = 7.0 Hz), 7.38 (2, d, *J* = 8.0 Hz), 7.72 (2, d, *J* = 8.0 Hz); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 14.3, 22.6, 29.1, 29.2, 29.3, 29.4, 29.5, 31.1, 31.8, 35.4, 63.4, 126.4, 129.4, 139.1, 147.7, 148.3, 157.9, 168.1; HRMS (ESI⁺, *m/z*) calcd for C₂₃H₃₆N₃O₄S₃ 482.2140, observed 482.2134 (M+H)⁺.

2.1.15. 4-Dodecyl-N-(5-(hydroxymethyl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (**39**)

In a similar manner, reaction of 2-amino-5-hydroxymethyl-1,3,4-thiadiazole³¹ (4.0 g, 30.5 mmol) with *p*-dodecylbenzenesulfonyl chloride (11.6 g, 33.6 mmol) gave the product **39** (8.7 g, 19.8 mmol, 65% yield), *R*_f 0.58 (10% MeOH/CH₂Cl₂), as a solid, mp 138–139 °C, after chromatography on silica gel (70–230 mesh) eluted with CH₂Cl₂/MeOH 19:1; ¹H NMR (300 MHz, DMSO-*d*₆) δ 0.84 (3, t, *J* = 6.6 Hz), 1.22 (18, m), 1.54–1.57 (2, m), 2.64 (2, t, *J* = 7.8 Hz), 4.57 (2, s), 6.05 (1, br s), 7.35 (2, d, *J* = 8.1 Hz), 7.67 (2, d, *J* = 7.8 Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 13.9, 22.1, 28.6, 28.7, 28.8, 29.0, 30.6, 31.3, 34.9, 58.4, 125.8, 128.9, 139.2, 147.5, 161.1, 167.5; LRMS (ESI⁺) calcd for C₂₁H₃₄N₃O₃S₂ 440.2, observed 440.2 (M+H)⁺; HRMS (ESI⁺, *m/z*) calcd for C₂₁H₃₄N₃O₃S₂ 440.2042, observed 440.2029 (M+H)⁺.

2.1.16. 5-(4-Dodecylphenylsulfonamido)-1,3,4-thiadiazole-2-sulfonamide (**40**)

A suspension of 5-amino-1,3,4-thiadiazole-2-sulfonamide³² (4.10 g, 22.8 mmol) in anhydrous acetonitrile (100 mL) was stirred and cooled in an ice bath. Triethylamine (2.5 g, 25 mmol) and a solution of *p*-dodecylbenzenesulfonyl chloride (8.6 g, 25 mmol) in anhydrous acetonitrile (60 mL) were added, the reaction mixture allowed to attain rt, and then stirred for 48 h. Volatiles were removed in vacuo and the residue was washed with 10% aqueous HCl (100 mL) and water (100 mL) in order to eliminate salts. The residue was subjected to chromatography on silica gel (70–230

mesh) eluted with CH₂Cl₂/MeOH 19:1 to give the product **40** (7.0 g, 14.3 mmol, 63%), *R*_f 0.56 (10% MeOH/CH₂Cl₂). Recrystallization from absolute ethanol and a second chromatography gave an analytical sample, mp 249–250 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 0.85 (3, t, *J* = 6.6 Hz), 1.23 (18, m), 1.55 (2, m), 2.58 (2, t, *J* = 7.2 Hz), 7.23 (2, d, *J* = 7.8 Hz), 7.34 (2, s), 7.59 (2, d, *J* = 8.1 Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 13.9, 22.1, 28.7, 28.9, 29.0, 29.1, 30.8, 31.3, 34.9, 126.2, 127.8, 143.3, 145.1, 161.2, 170.9; HRMS (ESI⁺, *m/z*) calcd for C₂₀H₃₁N₄O₄S₃ 487.1513, observed 487.1514 (M–H)[–].

2.2. Biological studies

2.2.1. Expression of recombinant AKT PH domain

Recombinant mouse AKT1 PH domain amino acids 1–111 (UBI/Millipore, Charlottesville, VA) was cloned by PCR into EcoRI/XhoI sites in pGEX-4T1 inducible bacterial expression plasmid (GeneStorm, InVivoGen, Carlsbad, CA) transformed into BL21(DE3) *Escherichia coli*. Expression and purification of the protein were performed as previously described.¹⁵

2.2.2. Surface plasmon resonance (SPR) spectroscopy binding assays

Competitive binding assays were performed with a Biacore 2000, using the Biacore 2000 Control Software v3.2 and BIAevaluation v4.1 analysis software (Biacore, Piscataway, NJ) as previously described.²⁶ Briefly, PI-3,4,5-phosphates-biotin labeled liposomes (Echelon Biosciences, Salt Lake City, UT) were immobilized on SA chips (BR-1000-32) at a level of 600 response units (RUs). Small molecule analytes at concentrations ranging from one tenth to ten times the predicted *K*_D were co-injected with 80nM PH domain GST-fusion protein (AKT1) at a flow rate of 30 μL/min. Dimethylsulfoxide (DMSO) concentrations in all samples and running buffer were 1% (v/v) or less.

2.2.3. Cell assays

Cellular proliferation: A standard 96-well micro-cytotoxicity assay was performed by plating cells at 5000–10,000 cells per well (depending on cell doubling time) for a growth period of 4 days. Drugs were added directly to the media, dissolved in DMSO at various concentrations ranging from 1 to 50 μM. The endpoint was spectrophotometric determination of the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Detection of pAKT: Human BxPC-3 pancreatic cancer cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in bulk culture in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 4.5 g/L glucose, 100 U/mL penicillin and 100 μg/mL streptomycin in a 5% CO₂ atmosphere. Cells were passaged using 0.25% trypsin and 0.02% EDTA. Cells were confirmed to be mycoplasma free by testing them with an ELISA kit (Roche-Boehringer Mannheim, Indianapolis, IN). Drugs were freshly prepared in DMSO at a stock concentration of 10 mM and then added at 10 μM final concentration directly into the culture media of the cells for 4 h. Following this treatment, cells were lysed as previously published²⁶ and equal amounts of total cell lysate were loaded on a pSer⁴⁷³-AKT/Total AKT Meso Scale Discovery plate. The plate was read using a Sector™Imager 2400A instrument (Meso Scale Discovery protein profiling system, Gaithersburg, MD).

2.3. Computational modeling

In a previous study³³ GOLD docking and scoring³⁴ was reported as the best combination for the analysis of interactions between the AKT PH domain and small molecules. Therefore, the GOLD package was employed for molecular docking studies in this work. During docking, the protein was kept as rigid while the flexibility

of the ligand was explored. Flipping of the ring corners in the ligands was considered, and carboxylic acids were deprotonated. No early termination was allowed in docking. Other parameters and procedures were applied as previously described.³³

3. Results

3.1. Chemistry

The structures, yields, and melting points of compounds **1–47** are given in Table 1. Compounds **1–4** and **8–10** are listed as being commercially available, but only **2** is inexpensive. Compounds **1–5**, **7–10**, and **16** are known and were prepared for this study according to literature procedures. Most of the remaining compounds were prepared by condensation of a 2-amino-1,3,4-thiadiazole nucleophile with an arenesulfonyl chloride electrophile in the presence of pyridine. The 2-amino-1,3,4-thiadiazoles and arenesulfonyl chlorides used were either obtained from commercial sources or were prepared by known methods. Yields of these condensations were generally good to excellent. In some cases, substituent modification followed condensation to produce additional analogs (e.g., saponification of esters **13**, **22**, and **37** gave acids **12**, **21**, and **36**, respectively). Compounds were purified by column chromatography and/or crystallization. Structures were confirmed by spectroscopic methods and purity ascertained to be $\geq 95\%$ by TLC analysis or by RP HPLC analysis. Details of the syntheses and compound characterization for compounds **26–40** are given in the Section 2.1. Details of the syntheses and compound characterization data for compounds **1–25** and **41–47** are given in the Supplementary data that accompanies this paper.

3.2. Bioassays

Results from bioassays of compounds **1–47** are given in Table 2 and Figure 2. Compounds were tested for their ability to compete with the binding of PI(3,4,5)P₃ to the PH domain of AKT (reported as K_i) using a previously described surface plasmon resonance (SPR) competitive binding assay.^{26,27} Compounds **1** and **8** that bear hydrogen atoms at positions R₁, R₂, and R₃ and an amino or acetamido substituent at position R₄, respectively, exhibited no ability to block PI(3,4,5)P₃ binding ($K_i > 50 \mu\text{M}$). As a result, K_i values for compounds **2–7** and **9–16** were not determined. Compounds **17–24** that bear hydrogen atoms at positions R₂ and R₃ and a decamido substituent at position R₄, but vary at R₁, were shown to compete with the binding of PI(3,4,5)P₃ with most K_i values near $10 \mu\text{M}$. Replacement of the hydrophobic decamido substituent of **17** with a more hydrophilic PEG-containing amide in **44** reduced inhibition of PI(3,4,5)P₃ binding. Compounds **26–32** bear hydrogen atoms at positions R₁, R₂, and R₃ and a normal alkyl substituent of variable length at position R₄. As previously reported, an aliphatic chain of the proper length ($\sim \text{C}_{12}\text{H}_{25}$) is necessary for optimal inhibition of PI(3,4,5)P₃ binding.²⁷ The K_i values for compounds **33–40** that bear hydrogen atoms at positions R₂ and R₃, a $\text{C}_{12}\text{H}_{25}$ substituent at position R₄, and an additional substituent (R₁) at position 5 of the thiadiazole ring were unimproved relative to **29**. Replacement of the $\text{C}_{12}\text{H}_{25}$ chain at R₄ with a phenyl (compare **29** with **41**, **34** with **42**, and **39** with **43**) or replacement of the thiadiazole ring with a phenyl (compare **29** with **45**) was detrimental to inhibition of PI(3,4,5)P₃ binding. Movement of the $\text{C}_{12}\text{H}_{25}$ chain from the *para*-position to the *ortho*- or *meta*-position relative to the sulfonamide (compare **29** with **46** and **47**) also reduced inhibition of PI(3,4,5)P₃ binding.

Following binding experiments, cellular assays were conducted: cellular proliferation (see Table 2, IC₅₀ values) and inhibition of AKT activation in BxPC-3 cells (Fig. 2) were measured in the pres-

Table 2

Bioassay data and GOLD docking scores for compounds **1–47**

Compound	$K_i \times 10^{6a}$ (M)	$\text{p}K_i^{a,b}$	Cell survival ^c IC ₅₀ ^d (μM)	GOLD docking score
1	>50 ^e	IA	>300	50
2	ND	ND	>300	52
3	ND	ND	>300	54
4	ND	ND	>300	55
5	ND	ND	>300	61
6	ND	ND	>300	53
7	ND	ND	>300	62
8	>50 ^e	IA	>300	50
9	ND	ND	>300	51
10	ND	ND	>300	53
11	ND	ND	>300	55
12	ND	ND	>300	62
13	ND	ND	176 \pm 8	62
14	ND	ND	>300	51
15	ND	ND	>300	51
16	ND	ND	>300	57
17	22 \pm 2 ^e	4.7	180 \pm 5	57
18	11 \pm 2	5.0	149 \pm 7	60
19	8 \pm 1	5.1	146 \pm 11	63
20	12 \pm 3	4.9	>300	60
21	10 \pm 2	5.0	>300	64
22	9 \pm 2	5.1	170 \pm 12	69
23	9 \pm 1	5.1	117 \pm 6	58
24	11 \pm 1	5.0	189 \pm 28	60
25	>50	IA	>300	61
26	>50 ^f	IA	>300	57
27	>50 ^f	IA	266 \pm 9	60
28	>50 ^f	IA	100 \pm 4	57
29	2.4 \pm 0.6 ^e	5.6	57 \pm 10	62
30	5.6 \pm 0.4 ^f	5.2	53 \pm 8	60
31	7.6 \pm 0.3 ^f	5.1	41 \pm 9	43
32	12 \pm 1 ^f	4.9	39 \pm 11	38
33	>50	IA	51 \pm 14	63
34	7 \pm 1	5.2	41 \pm 1	66
35	>50	IA	41 \pm 15	66
36	5.0 \pm 0.4	5.3	117 \pm 7	68
37	4.3 \pm 0.1	5.4	63 \pm 7	70
38	19 \pm 2	4.7	34 \pm 3	59
39	7.5 \pm 0.6	5.1	33 \pm 3	62
40	6 \pm 1	5.2	85 \pm 9	63
41	19 \pm 2	4.7	>300	51
42	17 \pm 1	4.8	268 \pm 5	54
43	40 \pm 8	4.4	>300	54
44	41 \pm 12	4.4	>300	52
45	>50	IA	38 \pm 10	37
46	18 \pm 6	4.8	74 \pm 8	55
47	8 \pm 1	5.1	71 \pm 12	63
PI(3,4,5)P ₃	0.5 \pm 0.1 ^f	6.3	ND	119
DPIEL	1.6 \pm 0.2 ^f	5.8	ND	40

^a ND = not determined.

^b IA = inactive.

^c This assay employed BxPC-3 cells.

^d The IC₅₀ is the concentration of compound required to cause 50% of the cells to die.

^e Taken from Ref. 26.

^f Taken from Ref. 27.

ence of compounds **1–47**. Cellular activity tracks reasonably well with the K_i values. Compounds **1–16** with an amino or acetamido substituent at R₄ were inactive. Several compounds bearing a decanamide substituent at R₄ showed some activity in one or both assays (e.g., **17**, **19**, **20**, and **23**). The best activities were exhibited by compounds **29**, **30**, **33–35**, and **37** that possess $\text{C}_{12}\text{H}_{25}$ or $\text{C}_{14}\text{H}_{29}$ substituents at R₄ and in some cases an R₁ substituent at position 5 of the thiadiazole ring. Replacement of the R₄ alkyl substituent by phenyl or a PEG-amide gave inactive compounds **41–44**. Interestingly, compound **45**, with a phenyl substituent in place of the thiadiazole ring, did not inhibit phosphorylation of AKT at Ser⁴⁷³ but did inhibit BxPC-3 cell proliferation. The position of the aliphatic chain also mattered, as compounds **46** and **47** with dodecyl groups

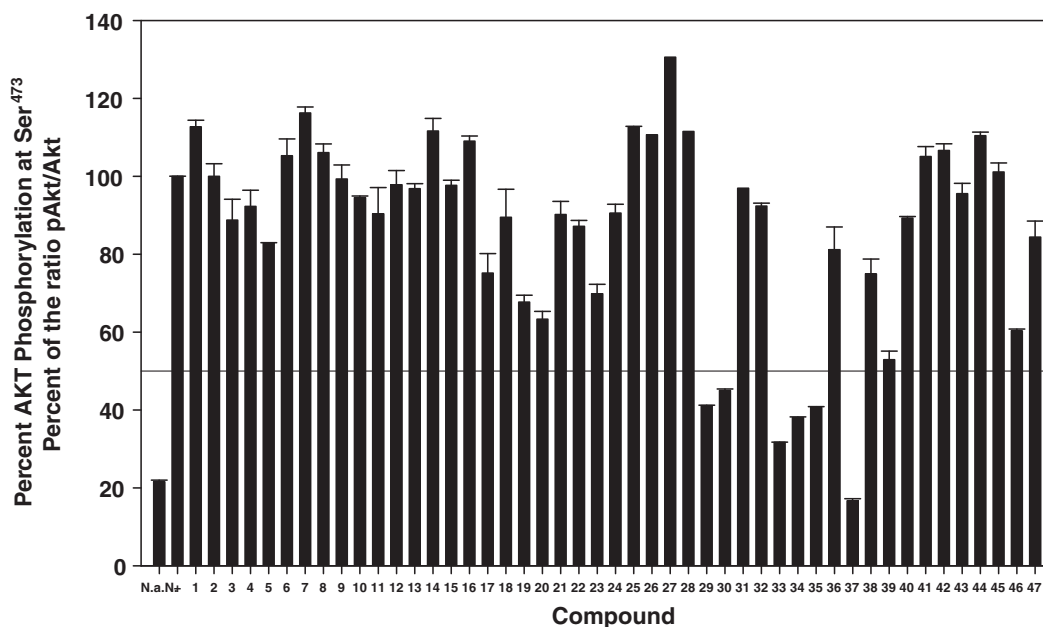


Figure 2. Extent of AKT phosphorylation at Ser⁴⁷³ in the presence of compounds 1–47 at 10 μ M.

ortho and *meta* to the sulfonamide did not greatly inhibit the production of pAKT but did inhibit BxPC-3 cell proliferation.

3.3. Computational modeling

Molecular docking was used to investigate the binding of compounds 1–47 to the AKT PH domain. The best docking pose of each compound was selected according to the GOLD docking scores, the populations of the pose clusters, and their interactions with the binding pocket. For the purposes of this discussion, compounds 1, 17, 29, and 37 were selected to represent molecules in different chemical subgroups based on the substituent present at position R₄. The best docking poses of these four molecules and of inositol(1,3,4,5)tetrakisphosphate (IP₄) with the AKT PH domain are illustrated in Figure 3. In each case, the sulfonamide group interacts with Arg23, Arg25, and Lys14 in a manner similar to the best pose exhibited by compound 1.²⁸ Compounds 2–7 and 8–16 bear amino and acetamido groups at R₄, respectively, and variable substitution at R₁. For the most part, these modifications did not result in large changes in the GOLD docking scores (see Table 2). Increasing the length of the carboxamide chain at R₄ by replacement of the acetamido group with a decanamido group, as in compounds 17–25, improved the GOLD docking scores by an average of 7. This is consistent with the greater activity of these compounds as evidenced by the measured K_i values (Table 2).

Compound 29 is representative of the set of compounds 26–32 that possess alkyl substituents of varying length at R₄. As depicted in Figure 3, the nitrogen atoms of the thiadiazole ring of 29 form hydrogen bonds with residue Glu17. The dodecyl chain extends from the main binding pocket to the protein surface, where it appears to interact with the hydrophobic residue Phe55. Shorter chains, as in 26, do not reach Phe55, thus weakening the binding. Based on our docking studies, the presence of a too-long chain, as in 31 and 32, changes the binding pose of the thiadiazole and sulfonamide moieties (not depicted). Compounds 29 and 30 that possess alkyl chains of optimal length (C₁₂H₂₅ and C₁₄H₂₉, respectively) exhibit stronger binding, as suggested by GOLD docking scores and confirmed by experimental observations (see Table 2).

As with 29, compounds 33–40 bear an optimal C₁₂H₂₅ chain at R₄, and in addition, a substituent R₁ on the thiadiazole ring. The R₁

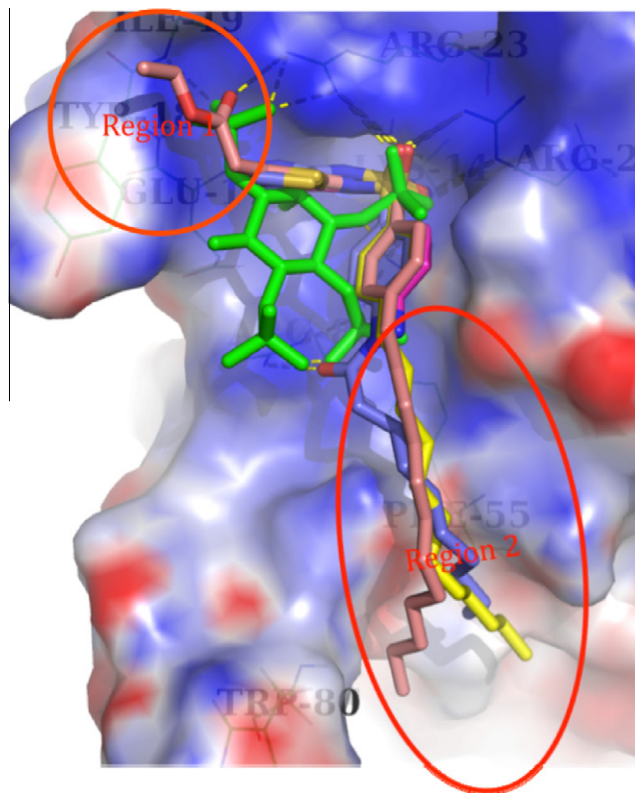


Figure 3. The best GOLD docking poses for IP₄ (green), 1 (magenta), 17 (blue), 29 (yellow), and 37 (salmon). The dashed lines represent hydrogen bonds, and the electrostatic surface is for the protein. The circled regions are those with synthetic modifications.

substituents range from nonpolar alkyl groups that vary in size, to more polar substituents containing carboxylic acid, carboxylate ester, and sulfonamide groups. The more polar groups were expected to mimic the phosphate at C-1 of PI(3,4,5)P₃ and possibly interact with Arg23. Most of the compounds in this series exhibited relatively high GOLD docking scores (60–70) as well as measurable to good K_i values in an SPR-based competitive binding assay. For

example, compounds **36** and **37**, which bear carboxymethyl and 2-ethoxy-2-oxoethyl substituents at R_1 , possess two of the three highest GOLD docking scores obtained and K_i values of 5.0 and 4.3 μM , respectively, indicative of relatively strong competitive binding against $\text{PI}(3,4,5)\text{P}_3$. The best binding poses of these compounds exhibit protein-small molecule interactions similar to those of **29** and also interact with Arg23 via the carbonyl moiety of the R_1 substituent. The ethoxy group of **37** also exhibits hydrophobic interactions with Tyr18 and Ile19.

Structural modifications as in compounds **41–45** were detrimental to binding. Movement of the $\text{C}_{12}\text{H}_{25}$ chain from the *para*-position (compound **29**) to the *ortho* (**46**) or *meta* (**47**) positions also decreased the GOLD docking scores and experimentally measured K_i values.

In order to improve our inhibitor design capabilities, the flexibility of the PH domain is under study using molecular dynamics and normal mode analysis.³⁶ By examining both backbone and side chain flexibility, we have found that docking predictions using the apo- structure produced results similar to those using more complex structures. In the present case of the AKT PH domain, 12 ns molecular dynamics following rigid docking demonstrated that accounting for protein flexibility did not improve the docking results significantly. The compound **29**-PH domain complex was relatively stable, more or less staying bound in the original docked conformation except for the very end of the alkyl tail (see [Supplementary data](#) for a dynamics movie). This suggests our approach to virtual screening of targets for which complex crystal structures are unavailable, that is, docking ligands into a rigid structure, has validity, at least for the AKT PH domain and this series of compounds related to **29**.³⁷

The availability of the crystal structure of the AKT kinase-PH domain with allosteric inhibitors provides additional understanding of this system.³⁸ Structural alignment with the IP4-PH domain complex indicates that only the far side of loop 3–4 showed a significant difference and the kinase domain disrupts the phospholipid binding site of the PH domain ([Fig. 4](#)). Therefore our inhibitors will not bind to this state of the PH domain.

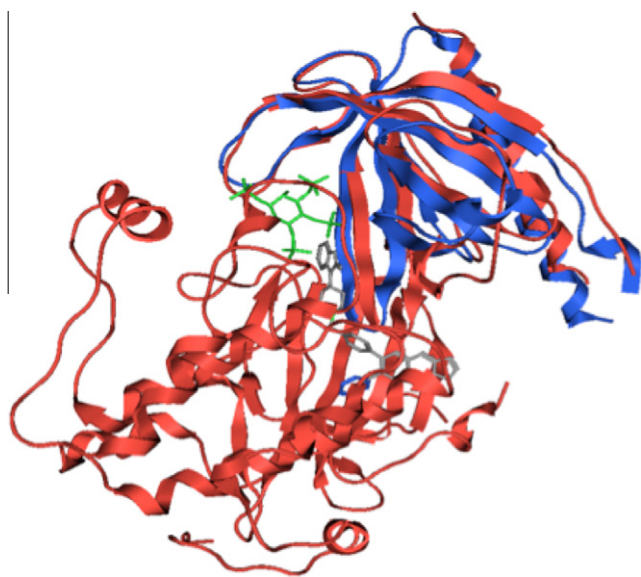


Figure 4. Structural alignment of AKT kinase-PH domain (PDB code 3O96, see Ref. 38) and PH domain (PDB code 1UNQ, see Ref. 37). Blue Ribbons: 1UNQ. Green sticks: $\text{PI}(3,4,5)\text{P}_3$ from 1UNQ. Red Ribbons: 3O96. Grey sticks: inhibitor VIII from 3O96.

4. Discussion

In recent years, we identified a family of sulfonamides as being AKT inhibitors based on their direct binding to the PH domain and their cellular and in vivo activities.^{16,25–27} Although there have been other efforts to develop AKT PH domain inhibitors, few SAR studies based on lead compounds and few studies where antitumor properties have been demonstrated in animals have appeared.^{18,23,39–42} The present study represents the first large SAR study on sulfonamide AKT PH domain inhibitors.

Based on docking of **1** to the PH domain of AKT, analogs **2–47** were designed, synthesized, tested for binding using an SPR-based assay, and tested for cellular activity. Modeling suggested that modification at position R_4 would not disturb the best binding mode of **1** since R_4 points away from the $\text{PI}(3,4,5)\text{P}_3$ binding pocket ([Fig. 3](#)). Compounds with small groups at R_4 , including **1–7** (R_1 variable, $R_2 = R_3 = \text{H}$, $R_4 = \text{NH}_2$), **8–16** (R_1 variable, $R_2 = R_3 = \text{H}$, $R_4 = \text{acetamido}$), and **26–28** ($R_1 = R_2 = R_3 = \text{H}$, $R_4 = \text{C}_4\text{–C}_8$ alkyl chain) do not bind significantly to the AKT PH domain as determined by SPR measurements of competitive binding against $\text{PI}(3,4,5)\text{P}_3$. Some compounds with longer amide-derived substituents at R_4 (decanamides **17–24** and PEG-amide **44**) exhibited the ability to inhibit $\text{PI}(3,4,5)\text{P}_3$ binding ($K_i < 50 \mu\text{M}$), but at best weak cellular activity. SAR analysis also suggested that compounds with an aliphatic chain of the appropriate length at R_4 (e.g., **29** and **30**) exhibited the ability to inhibit $\text{PI}(3,4,5)\text{P}_3$ binding (K_i) and good cellular activity as measured by inhibition of AKT phosphorylation and a cell survival assay using BxPC-3 cells. However, the need to employ both binding and in vitro assays was underscored by the observation that compounds **31** and **32** bearing $\text{C}_{16}\text{H}_{33}$ and $\text{C}_{18}\text{H}_{37}$ chains, respectively, at R_4 , exhibited low μM values of K_i and the lowest IC_{50} values in the cell survival assay, but had little effect on AKT activity, as measured by the pAKT/AKT ratio. In addition, replacement of the thiadiazole ring of **29** with a phenyl ring (compound **45**) decreased the K_i value, and **45** did not inhibit the production of pAKT, but did inhibit BxPC-3 cell proliferation. These observations suggest that cell-based activities depend not only on binding affinity, but also on other properties, such as cell permeability and compound solubility. In addition, off-target effects may occur and confound interpretation of the results. Off target effects of phosphatidylinositol ether lipid analogs (PIAs) led to the discovery that such compounds can activate a single isoform of p38, p38 α , in vitro and in vivo.⁴³ It was concluded that because p38 α activation occurs in cancer cells after chemotherapy and in normal cells during inflammatory processes, activation of p38 α by PIAs could contribute to the efficacy and/or toxicity of PIAs.

Modeling also suggested that the sulfonamide moieties of strongly bound compounds, such as **29**, mimic the phosphate group at C-3 of $\text{PI}(3,4,5)\text{P}_3$ by interacting with Arg23, Arg25, and Lys14 (see [Fig. 3](#)). As $\text{PI}(3,4,5)\text{P}_3$ also interacts with Tyr18, Ile19, and Arg23 through the C-1 phosphate, substituents were introduced at position R_1 to mimic the C-1 phosphate. Compounds **18–25**, **33–40**, and **42–43** were of most interest in this regard, but exhibited K_i and IC_{50} values similar to those of the reference compounds bearing H at R_1 (**17**, **29**, and **41**, respectively). Thus, substitution at R_1 appears to be of lesser importance than was expected.

Although studies have shown that most of the current scoring functions in docking are of limited accuracy and the docking scores usually are not well correlated with experimentally measured binding affinities,³⁵ in the present study a correlation between the GOLD docking scores and pK_i (see [Table 2](#)) is apparent ([Fig. 5](#)). Docking suggested that the two outliers, compounds **31** and **32**, adopt alternate binding poses due to the steric problems caused by the too-long alkyl chains. This agreement between the

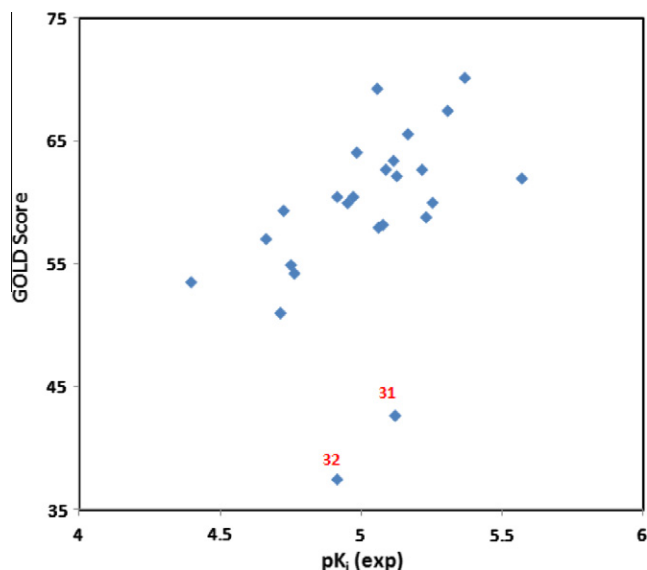


Figure 5. The correlation between experimental pK_i values and GOLD scores. Compounds **31** and **32** are outliers, suggesting their binding mode might be different from the other compounds.

GOLD docking scores and the experimentally determined pK_i values supports our previous assertion that the combination of GOLD docking and GOLD scoring is appropriate for modeling this system.³³

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bmc.2011.01.049](https://doi.org/10.1016/j.bmc.2011.01.049).

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