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Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors

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Abstract—This letter describes the development of two series of potent and selective allosteric Akt kinase inhibitors that display an unprecedented level of selectivity for either Akt1, Akt2 or both Akt1/Akt2. An iterative analog library synthesis approach quickly provided a highly selective Akt1/Akt2 inhibitor that induces apoptosis in tumor cells and inhibits Akt phosphorylation in vivo.

Akt (Protein kinase B/PKB) is a serine/threonine kinase that has recently garnered a great deal of attention as a promising molecular target for cancer therapy due to its critical role as a regulator of the cell's apoptotic machinery (Fig. 1).[1] Akt belongs to the AGC family of kinases and shares high homology with PKA and PKC. Moreover, the three isozymes of human Akt (Akt1, Akt2, and Akt3) share >85% homology and all possess an amino terminal pleckstrin homology (PH) domain and a kinase domain separated by a 39-amino acid hinge region.[2]

Akt is a critical downstream effector of multiple growth factors and receptors involved in tumorigenesis. Akt is also activated as a result of inactivation of tumor suppressor PTEN, a lipid phosphatase mutated or deleted in >50% of human cancers.[3] Both Akt1 and Akt2 are commonly overexpressed or constitutively active in a large number of human cancers including brain, gastric, colon, breast, lung, and prostate carcinomas and their activation correlates to cancer progression.[2,4]

The study of Akt as a potential therapeutic target has been hampered by a lack of Akt specific inhibitors (versus other AGC family kinases) as well as isozyme selective Akt inhibitors, since recent studies have shown that the three isozymes of Akt are functionally

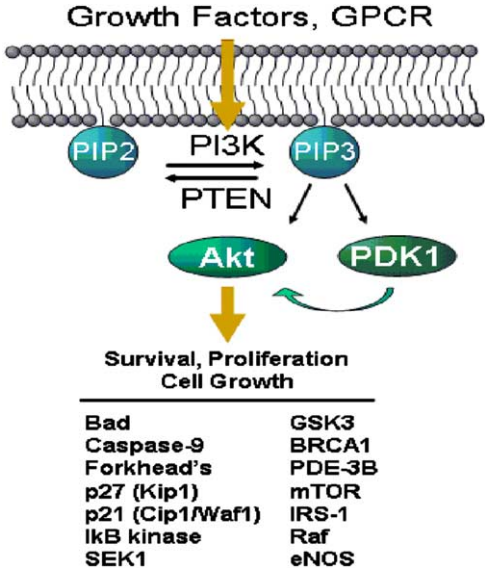

Figure 1. The Akt pathway. Akt can be activated by inactivation of PTEN, stimulation of growth factors and GPCRs or amplification of PI3K. Once activated, Akt increases phosphorylation of a number of downstream substrates involved in cell survival, growth, and proliferation.

Keywords: Akt; PKB; Kinase cancer.
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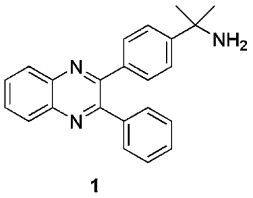
distinct.[1–8] In this letter, we disclose the SAR and optimization of two series of allosteric Akt kinase inhibitors that are specific for Akt versus the AGC family of kinases (PKA, PKC, and SGK). More importantly, these series provided Akt1 selective and Akt2 selective inhibitors that defined the profile for optimal apoptotic effect as well as a dual Akt1/Akt2 inhibitor that sensitized tumor cells to apoptotic stimuli and inhibited Akt phosphorylation in vivo.

A single tractable lead, 2,3-diphenylquinoxaline **1**, resulted from a high throughput screening effort to identify compounds capable of inhibiting the three Akt isozymes (Fig. 2). Interestingly, **1** displayed a high degree of isozyme selectivity (IC_{50}s: Akt1 = 3400 nM, Akt2 = 23,000 nM, and Akt3 >50,000 nM) and was found be specific for Akt (>50,000 nM vs PKA, PKC, and SGK). In assays with Akt mutants lacking the PH domain, **1** displayed no inhibition; moreover, Akt inhibition by **1** was not competitive with ATP, suggesting an allosteric binding site. The selectivity observed has been attributed to this allosteric mode of inhibition, for which a model has been proposed.[7] All of the Akt inhibitors described herein are PH-domain dependent and not competitive with ATP.

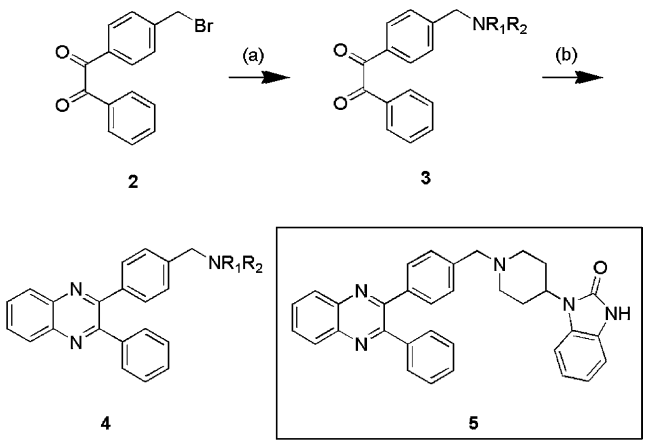
An iterative analog library synthesis approach was employed to rapidly develop SAR for **1**. The initial strategy to analog the 2,3-diphenylquinoxaline core focused on replacing the primary amine in **1** with a diverse collection of functionalized amines, while at the same time deleting the *gem*-dimethyl functionality. Commercially available *p*-bromomethyl benzil **2** was treated with 200 different primary and secondary amines, followed by fluorous scavenging to provide 200 amino-functionalized benzils **3**.[9] Each benzil **3** was heated with 1,2-diamino benzene under microwave irradiation (160 °C, 10 min) to afford 200 amino-functionalized quinoxalines **4** (Scheme 1).[11] Significantly, **5**, possessing a GPCR privileged structure[12] and lacking the *gem*-dimethyl moiety, increased potency at Akt1 (IC_{50} = 290 nM) and Akt2 (IC_{50} = 2090 nM) by over 10-fold, relative to **1**, while maintaining selectivity versus Akt3 and the AGC family of kinases (IC_{50} >50,000 nM). Despite this advance, **5** displayed poor solubility and lacked cell activity.

A key objective for this nascent program was to evaluate the apoptotic response of Akt1, Akt2, and dual Akt1/Akt2 inhibition. In an effort to provide small molecule tools with these selectivity profiles and improved physical properties, subsequent libraries were designed to maximize structural diversity. To enable these efforts, our laboratory developed protocols employing micro-



1

Figure 2. HTS screening lead.



Scheme 1. Synthesis of amino-functionalized quinoxalines **4**. Reagents and conditions: (a) (i) HNR_1R_2, PS-DIEA, DCM, rt, (ii) R_f–SH, (iii) Fluoroit Flash™ SPE, 62–95%; (b) 1,2-diaminobenzene, EtOH/HOAc (9:1), 160 °C, 10 min, microwave, 89–99%. All compounds purified by mass-guided HPLC.[10]

wave-assisted organic synthesis (MAOS), amenable to an iterative analog library approach, to access a number of diverse heterocyclic templates from common 1,2-diketone intermediates (Fig. 3).[11] While library work continued around **5**, new libraries of 5,6-diphenylpyrazin-2(1*H*)-ones **10** were prepared employing a vast assortment of commercial available α-aminocarboxamide building blocks.

Benzil **11**, containing the key piperidinyl benzimidazolone privileged structure,[12] was treated with 24 functionalized α-aminocarboxamides **12** under our microwave-assisted protocol to deliver 48 regioisomeric 5,6-diphenylpyrazin-2(1*H*)-ones **13/14** (Scheme 2) in good yields. As highlighted in Table 1, the degree of inhibition of the individual Akt isozymes was highly variable, providing Akt1 selective, Akt2 selective, and various Akt combination inhibitors. Not only did each regioisomer, **13** and **14**, display a distinct inhibition profile, but the nature of the 3-position substituent also impacted the isozyme selectivity. Of the two regioisomers derived from H-Ala-NH_2, **13b** (IC_{50}s: Akt1 = 760 nM, Akt2 = 24,000 nM, Akt3 >50,000 nM) proved to be specific for Akt1, whereas regioisomer **14b** (IC_{50}s: Akt1 = 1003 nM, Akt2 = 1179 nM, Akt3 > 50,000 nM) exhibited the same degree of inhibition for both Akt1 and Akt2. Quite unexpectedly, **13f** was uniformly inactive (IC_{50}s: Akt1 > 20,000 nM, Akt2 = 18,000 nM, Akt3 > 20,000 nM) while isomer **14f** was a potent and selective

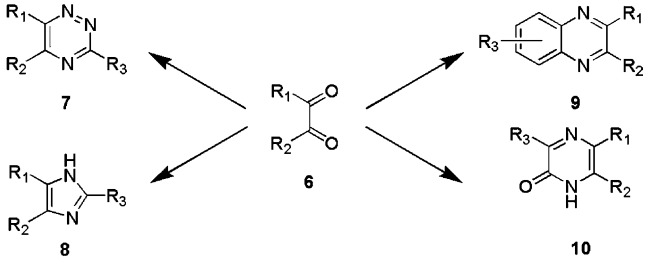
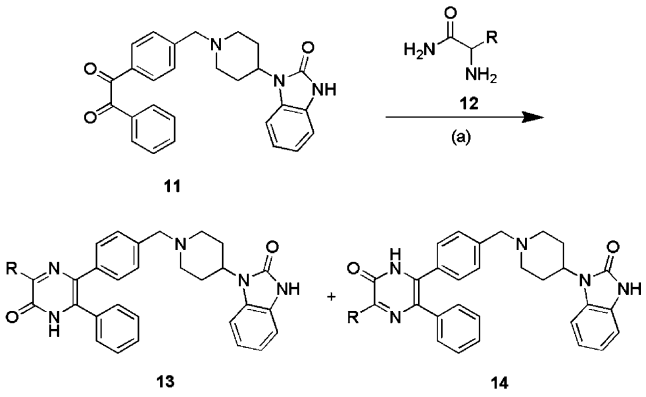
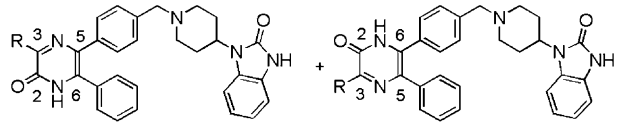


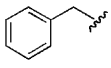
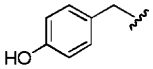
Figure 3. 1,2-Diketone approach to diverse heterocycles.



Scheme 2. Library synthesis of diverse pyrazinones **12**. Reagents and conditions: (a) 3.0 equiv **12**, 9:1 EtOH/HOAc, 160 °C, 20 min., microwave, 45–73%. Compounds purified by mass-guided HPLC.[10]

Table 1. Structures and activities for pyrazinones **13/14**



Compd	R	Akt1 IC_{50} (nM)[a]	Akt2 IC_{50} (nM)[a]	Akt3 IC_{50} (nM)[a]
13a	H	3029	15,700	>50,000
14a		1500	>50,000	>50,000
13b	CH_3	760	24,000	>50,000
14b		1003	1179	33,100
13c		17,000	>50,000	>50,000
14c		>50,000	1755	3973
13d		21,670	45,270	>50,000
14d		>50,000	5407	>50,000
13e		17,000	>50,000	>50,000
14e		>50,000	4517	>50,000
13f		>50,000	18,000	>50,000
14f		21,200	325	21,870

[a] Average of at least three measurements; enzyme protocol.[7] All compounds >50,000 nM versus PKA, PKC, SGK Regioisomer structures assigned by 2-D NMR and NOE.

Akt2 inhibitor (IC_{50}s: Akt1 >20,000 nM, Akt2 = 325 nM, Akt3 >20,000 nM). As with **5**, Akt inhibition was PH domain dependent and noncompetitive with ATP, providing specificity against the AGC family of kinases (>50,000 nM vs PKA, PKC, SGK).

With **13b** and **14f** in hand, we were poised to determine if either Akt1 inhibition or Akt2 inhibition alone was sufficient to induce apoptosis. As shown in Figure 4, when A2780 cells were pretreated with either **13b** or **14f**, and then incubated with doxorubicin (Dox), a modest 3-fold increase (versus doxorubicin alone) in caspase-3 activity was observed. However, a 1:1 mixture of **13b**:**14f** elicited a significant 10-fold increase in caspase-3 activity. Similar results were obtained in LnCaP cells with a number of chemosensitizing agents, suggesting that inhibition of both Akt1 and Akt2 were required for a maximal apoptotic response.

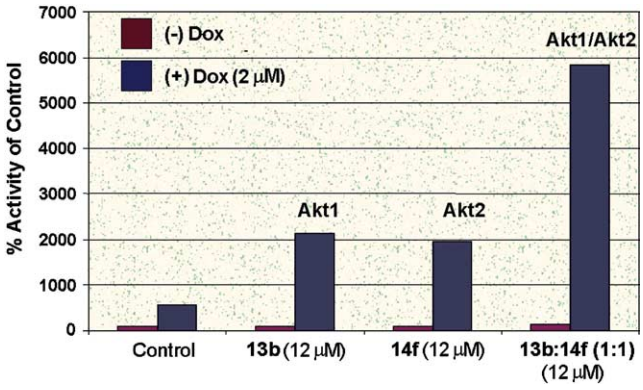
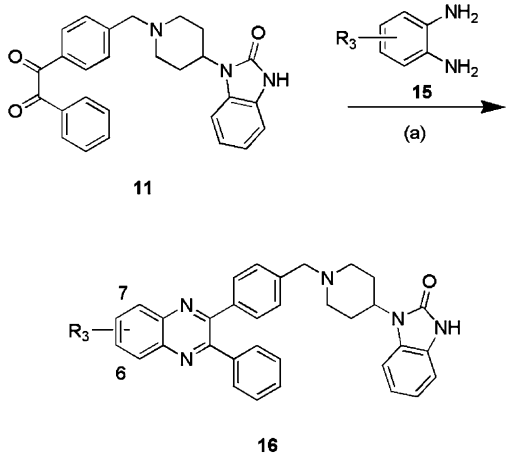


Figure 4. Caspase-3 assay: A2780 cells treated with **13b** and **14f** alone or in combination with ± doxorubicin. Each experiment employed a 12 μM total dose of inhibitor(s).

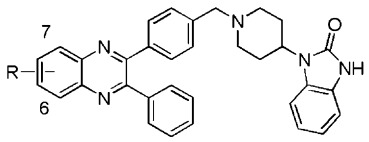
Our attention now focused on the development of a potent Akt1/Akt2 dual inhibitor by further optimization of **5**. As shown in Scheme 3, benzil **11** was heated, under microwave irradiation, with 50 different functionalized aryl-1,2-diamines **15** to deliver C6/C7-functionalized and tricyclic quinoxalines **16** in excellent yields (Scheme 3).

As highlighted in Table 2, the degree of inhibition of the individual Akt isozymes was conserved in the quinoxaline series, providing a number of potent dual Akt1/Akt2 inhibitors. Notably, the regioisomeric C6/C7 tetrazoles, **16c** and **16d**, were potent dual inhibitors, but possibly due to zwitterionic character, neither were cell permeable. *N*-alkylation of the tetrazole moiety removed the zwitterionic character; however, **16e** lost over 15-fold potency for Akt1/Akt2 and **16f** displayed poor physical properties. The tricyclic analog **16h** was selected for further evaluation as it displayed improved potency (IC_{50}s: Akt1 = 58 nM, Akt2 = 210 nM, and Akt3 = 2119 nM), solubility and cell permeability. In our cell-based IPKA (C33A) assay, **16h** was found to possess a similar pattern of inhibition for Akt1 and Akt2 (IC_{50}s: Akt1 = 305 nM, Akt2 = 2086 nM) while having no inhibition of Akt3 (IC_{50} > 25,000 nM).



Scheme 3. Synthesis of functionalized quinoxalines **16**. Reagents and conditions: (a) EtOH/HOAc (9:1), 160 °C, 10 min, microwave, 85–99%. All compounds purified by mass-guided HPLC.[10]

Table 2. Structures and activities for quinoxalines **16**



16

Compd	R	Akt1 IC_{50} (nM)[a]	Akt2 IC_{50} (nM)[a]	Akt3 IC_{50} (nM)[a]
16a	6-COOH	240	281	>50,000
16b	7-COOH	166	388	3200
16c	6-(2*H*-tetrazole)	63	65	1228
16d	7-(2*H*-tetrazole)	20	144	1613
16e	6-(2-Me-tetrazole)	1089	1877	>50,000
16f	7(-2-Me-tetrazole)	55	332	>50,000
16g		85	300	2400
16h		58	210	2119

[a] Average of at least three measurements; enzyme protocol.[7] All compounds >50,000 nM versus PKA, PKC, SGK Regioisomers assigned by 2-D NMR and NOE.

As anticipated from data obtained in caspase-3 assays with a 1:1 mixture of **13b**:**14f** (Fig. 4), the dual Akt1/Akt2 inhibitor **16h** displayed a similar profile. When LnCaP cells were pretreated with **16h** and then incubated with TRAIL, a dramatic increase in caspase-3 activity (6–10-fold relative to control or TRAIL alone) was observed.[8,13] This sensitization of tumor cell lines with **16h** was not limited to LnCaP cells as similar apoptosis induction was observed in HT29, MCF7, and A2780 cells, among others, with chemosensitizers such as camptothecin, herceptin, and doxorubicin.[8] Based on these encouraging data, a mouse PD study was undertaken to determine if **16h** could inhibit Akt phosphorylation in vivo. Mice were dosed with **16h** (ip, 50 mpk, 3 doses, every 90 min) achieving plasma concentrations of 1.5–2.0 μM, and then the animals were tail vein injected with IGF to stimulate Akt phosphorylation. By IP Western, both basal and IGF stimulated Akt1 and Akt2 phosphorylation were inhibited in mouse lung, with no effect on Akt3 phosphorylation.[8]

In summary, two novel series of selective, allosteric Akt (PKB) kinase inhibitors have been developed based on either a 2,3-diphenylquinoxaline core or a 5,6-diphenylpyrazin-2(1*H*)-one core. These potent Akt1 selective and Akt2 selective inhibitors demonstrated that both Akt1 and Akt2 must be inhibited for a maximal apoptotic response. An optimized dual Akt1/Akt2 inhibitor was shown to sensitize tumor cells to induce apoptosis when combined with a number of chemotherapeutic agents and biologicals, such as TRAIL, and inhibit the phosphorylation of Akt1 and Akt2 in vivo. Moreover, this work has demonstrated that it is possible to develop specific Akt inhibitors (versus PKA, PKC, and SGK) as well as isozyme selective Akt inhibitors.

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