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Allosteric inhibitors of Akt1 and Akt2: A naphthyridinone with efficacy in an A2780 tumor xenograft model

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Abstract—A series of naphthyridine and naphthyridinone allosteric dual inhibitors of Akt1 and 2 have been developed. These compounds have been optimized to have potent dual activity against the activated kinase as well as the activation of Akt in cells. One molecule in particular, compound **17**, has potent inhibitory activity against Akt1 and 2 in vivo in a mouse lung and efficacy in a tumor xenograft model.

The serine/threonine kinase Akt (PKB) plays a central role in cancer cell growth and survival. It is a key downstream effector of phosphoinositide 3′-kinase (PI3K) and directly modulates a wide range of pro-apoptotic and metabolism regulating proteins.[1] Inhibition of Akt is a significant therapeutic goal due to the prevalence of activating mutations in the PI3K/Akt pathway.[2,3] Previous papers have described potent dual Akt1 and Akt2 inhibitors. These molecules are PH-domain dependent inhibitors, prevent the activation of Akt, and display excellent selectivity versus kinases outside of the Akt family as well as the highly homologous AGC family of kinases.[4]

The utility of previous allosteric Akt inhibitors for in vivo studies was limited due to the inability to achieve sustained high levels of inhibition of Akt1 and Akt2. Though inhibition of Akt1 and 2 could be demonstrated with compound **1**[4a] (Fig. 1), the high plasma levels necessary for Akt1 and 2 inhibition could not be sustained due to its low solubility and moderate potency. Compound **2**, though more soluble than **1**, was poorly tolerated in mice and only limited plasma exposures could be obtained.[4c,5] This Letter describes the subsequent successful effort to invent a compound with sufficient in vivo activity, pharmacokinetics and tolerability to be used in proof-of-concept tumor xenograft studies.

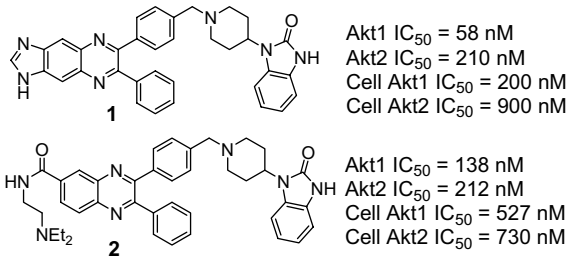

Figure 1. Previously published balanced dual Akt1 and Akt2 inhibitors.

While the dibasic functionality in **2** gave the dihydrochloride salt good aqueous solubility at low pH, this compound has a high MW (653 Da) and the dibasic functionality may contribute to its poor tolerability. The goal was to improve the potency of the lead series and at the same time lower molecular weight by focusing on the key structural elements of these molecules. These efforts fell into two categories, variation of the quinoxaline core and broad exploration of alternate piperidines.

Keywords: Akt; Oncology.
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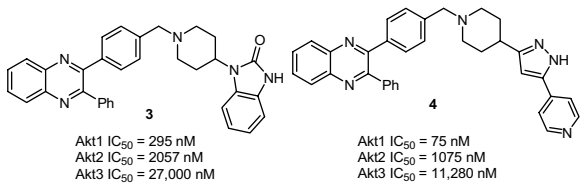


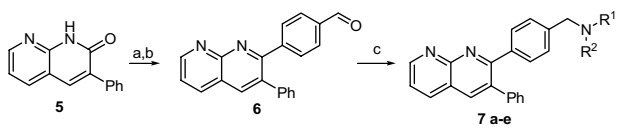
Figure 2. Quinoxaline lead compounds.

Screening of alternate amines began around the quinoxaline **3** (Fig. 2).[4a] The first break in this wide-ranging approach to find alternate piperidines was the identification of the pyrazolylpiperidine motif in **4**.[6] This compound displayed improved activity over **3** albeit with still relatively weak Akt2 activity.[7,8] It also had a respectable MW (522 Da) and good physical properties (log D = 2.71).

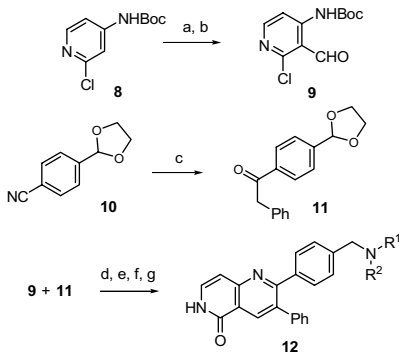
Alternate core heterocycles were also explored to attempt to identify more potent and balanced dual activity. Quinoline and pyridine variations have been described,[4b] and this Letter describes the exploration of naphthyridine derivatives. Naphthyridines were expected to have improved physical properties, since the pK_a of 1,8-naphthyridine is 3.39, versus 1,4-quinoxaline with a pK_a of 0.60. The added basicity was hoped to contribute to increased polarity and better solubility. Previously it was shown that the position of one of the core nitrogens was critical to potency.[4b] In the case of 1,8-naphthyridines, this would correspond to the position of $N-1$, so 2-aryl-3-phenyl-[1,8]-naphthyridines were targeted. Later we explored other 2-aryl-3-phenyl-[1,x]-naphthyridines (vide infra).

Schemes 1 and 2 show the synthesis of naphthyridines described in this Letter. 3-Phenylnaphthyridin-2-ones (**5**) were prepared by utilizing chemistry previously published by this group.[9] Scheme 1 illustrates the chemistry for the 1,8-naphthyridine series but the same chemistry was also utilized for the synthesis of 1,5- and 1,7-naphthyridines. The naphthyridinone **5** was converted to the 2-chloro-1,8-naphthyridine by treatment with $POCl_3$. Suzuki coupling with the appropriate boronic acid then provided the aldehyde **6**. Reductive amination afforded the range of tertiary amines (**7**) described below.

Scheme 2 illustrates the synthesis of 1,6-naphthyridines and naphthyridinones. Lithiation of **8**[10] and Quenching with N,N-dimethylformamide provided the aldehyde **9**. The nitrile **10**[11] was treated with benzyl magnesium chloride to provide the ketone **11**. Condensation of the aldehyde **9** with the ketone **11** in the presence of sodium



Scheme 1. Reagents and conditions: (a) $POCl_3$ (1.5 equiv), MeCN, reflux, 72% yield; (b) p-CHO-PhB(OH)$_2$, PdP(t-Bu)$_3$, Cs_2CO_3, dioxane, reflux, 72% yield; (c) HNR^{1}R^2, NaBH(OAc)$_3$.



Scheme 2. Reagents and conditions: (a) t-BuLi, −70 °C, THF; (b) DMF, 53% yield; (c) BnMgCl, 0 °C to rt, 84% yield; (d) NaOMe, MeOH, reflux, 55% yield; (e) 1 N HCl, THF, water, rt, 85% yield; (f) HNR^{1}R^2, NaBH(OAc)$_3$ (g) pyr-HCl, 150 °C, HCl.

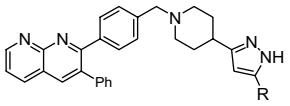
methoxide led to the methoxynaphthyridine. Hydrolysis of the acetal, reductive amination and demethylation provided the 1,6-naphthyridinon-5-one **12**.

Table 1 shows selected additional end groups that were incorporated into this structure. The guiding principles were to find balanced in vitro activity, good cell potency, and to optimize physical properties by keeping molecular weight in check and introducing polar functionality where possible. Pyridine regiochemistry had a moderate impact on Akt1 and Akt2 activities, though the 2-substitution in **7c** provided 3-fold improved Akt1 activity relative to **7a**. Increasing the polarity of the molecules with the 4-pyridone (**7d**) functionality gave improved Akt2 activity. All these heterocyclic substituents impart improved potency relative to phenyl (**7e**). Despite the significant improvements in Akt1 and Akt2 activities observed in this optimization of the amine end group, the cell potency was still moderate. To attain the goal of inventing a compound that inhibits Akt in vivo at reasonable plasma levels further improvements in cell activity were sought.

A significant advance in the series was found when exploration was done around the 2-pyridyl pyrazole. When the pyrazole was replaced with a triazole (Table 2, compound **13**) the potency was similar but the triazole was more active in a caspase induction assay (vide infra). Using this end group an investigation of naphthyridine isomers was performed (Table 2). While the 1,7-naphthyridine **14** gave comparable activity to the 1,8-isomer, increased Akt1 activity was found in the 1,6-naphthyridine (**15**). The most notable isomer proved to be the 1,5-naphthyridine **16**, which possessed significantly enhanced activity against Akt1 and Akt2 relative to **13**. These improvements in Akt1 activity against the activated kinase translated to significantly improved inhibition of Akt1 phosphorylation in cells. However, the apparent improvements in Akt2 activity did not translate directly to improved Akt2 cell activity. The reason for this disconnection is not well understood.

The final improvement in this progression occurred when an additional polar element was added to the core.

Table 1. 1,8-Naphthyridine series SAR



	R	Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)	Cell Akt1 IC_{50} (nM)	Cell Akt2 IC_{50} (nM)
7a		120	260	369	1454
7b		250	900	nd	nd
7c		44	280	149	995
7d		34	83	132	613
7e		470	1600	nd	nd

All compounds >50,000 nM versus PKA, PKC, SGK.
[a]Average of at least three measurements; enzyme protocol.[8,12]

Previously in the quinoxaline series several examples of potency-enhancing polar hydrogen bond donating substituents were identified including the fused imidazole in **1**.[4a] Here, polarity and hydrogen bond donating functionalities were added directly to the core without significantly increasing molecular weight by creating a 1,6-naphthyridin-5-one (**17**). This change resulted in enhancement in both Akt1 and Akt2 activities. In particular, compound **17** shows significantly improved potency while having a lower molecular weight (539 Da) than **2**.

Consistent with the allosteric mode of inhibition, **17** is dependent on the PH-domain for Akt inhibition, is selective for Akt1/2 over Akt3 (IC_{50} = 1900 nM), and is highly selective over other members of the AGC family of kinases (>50 μM vs PKA, PKC, SGK). Compound **17** has moderate activity in an hERG binding assay (IC_{50} = 5610 nM) and is a substrate for human P-glycoprotein (MDR1 Directional Transport Ratio (B to A/A to B) = 17.6). It also showed good physical properties with a human plasma protein binding of 97.3% and a log P = 3.51. It showed good pharmacokinetics in rat with a low clearance of 4.6 mL/min/kg and a half-life of 3.8 h.

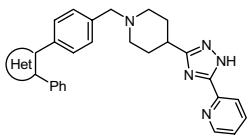
Due to the improved cell potency, physical properties, and rodent pharmacokinetics of compound **17**, tolerability and Akt inhibition were assessed in mice. Using an acute dosing schedule (IP dosing of 50 mg/kg at times 0, 3, and 8 h), administration of **17** was well tolerated in mice and showed high levels of Akt inhibition in mouse lung. The levels of Akt1 and Akt2 activities in mouse lung were determined by isozyme-specific immunoprecipitation from lysates, followed by in vitro kinase assays (Table 3). At the 10 h timepoint, a blood concentration of 13 μM of **17** resulted in 79% inhibition of Akt1 and 59% inhibition of Akt2 in mouse lung. At later timepoints lower blood concentrations, and correspondingly lower levels of Akt inhibition were observed. Overall, the extent of Akt inhibition responded well to the blood levels of **17**. Importantly, compound **17** was well tolerated and high levels of inhibition of Akt1 and Akt2 were seen in vivo at readily achievable blood levels.

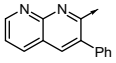
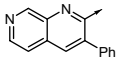
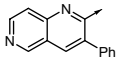
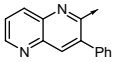
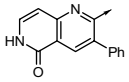
The properties of compound **17** were sufficient to warrant a multiple-dose efficacy study in tumor xenograft mice. A tumor xenograft efficacy study using the A2780 ovarian carcinoma cell line in nude mice was conducted. It included an experiment to determine pharmacokinetics and in vivo Akt1 and 2 inhibition in lung and tumor samples during the course of dosing [13].

In the study, A2780 cells were implanted on day 1. Animals were weighed on day 7, and dosing was commenced on day 12. Compound **17** was dosed subcutaneously at 50 mg/kg three times per day (at t = 0, 3, and 7 h), twice a week for five cycles. Figure 3 shows the tumor weight data for vehicle and compound **17** treatment (21 days from when animals were initially weighed). A statistically significant decrease in mean tumor weight as compared to the vehicle control was noted for mice treated with **17** (p = 0.007).[14] Compound **17** was well tolerated and there was no significant effect on animal weight relative to vehicle treated animals.

On the day the study was terminated, three tumor bearing vehicle mice were treated twice with a 50 mg/kg dose of **17** (at 0 and 3 h). Two hours after the last dose, blood, lung, and tumor tissues were taken from these animals as well as two control animals for pharmacokinetic and pharmacodynamic analysis. The extent of Akt1 and 2 inhibition was determined by an IP kinase assay. Inhibition of Akt1 and 2 in lung tissue was 80% and 75%, respectively. Inhibition of Akt1 and 2 in tumor tissue was 95% and 54%, respectively. The average blood concentration from

Table 2. Naphthyridine core variation



	R	Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)	Cell Akt1 IC_{50} (nM)	Cell Akt2 IC_{50} (nM)
13		45	220	83	1077
14		21	160	31	981
15		7.4	210	21	2679
16		5.6	70	30	1476
17		3.5	42	16	266

All compounds >50,000 nM versus PKA, PKC, SGK.
[a]Average of at least three measurements; enzyme protocol.[8,12]

Table 3. Inhibition of Akt1 and Akt2 in mouse lung with **17**[a]

Time (h)	Blood conc (μM)	Akt1 % activity	Akt2 % activity
10	13.0	21 ± 8	41 ± 13
16	2.6	62 ± 34	86 ± 35
24	0.7	100 ± 25	91 ± 30

Akt activity was determined by IP kinase assay at the given timepoints.
[a] Compound **17** was dosed 3 × 50 mg/kg IP at times 0, 3, and 8 h to nude mice (3 per timepoint).

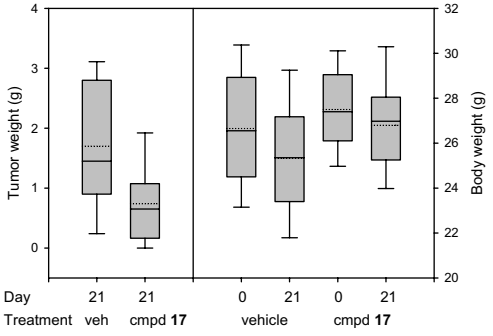


Figure 3. A2780 tumor xenograft study with **17** (solid line: median; dotted line: mean; gray box: 25–75% confidence limits, bars: 5–95% confidence limits).

these animals was 12.8 μM. Both sets of data indicate that high levels of Akt1 inhibition and moderate to high levels of Akt2 inhibition were obtained in the middle of the dosing period in these two tissues. The high levels of Akt1 inhibition are consistent with its potent cellular activity. The levels of Akt2 inhibition in vivo are surprising given the 16-fold decreased activity in cells relative to Akt1. But the levels of Akt1 and 2 inhibition in vivo in this study have been consistently observed in several related in vivo studies.

This Letter has outlined the invention of naphthyridine and naphthyridinone inhibitors of Akt. In particular, naphthyridinone **17** was identified as a potent dual inhibitor of Akt1 and Akt2. This compound is well tolerated in at exposures that provide high levels of Akt1 and 2 inhibition in vivo. It has also been shown to inhibit the growth of A2780 tumors in vivo when used as monotherapy. Additional studies with **17** will be the subject of subsequent publications.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.074.

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5. Upon dosing nude mice with **2** (di-HCl salt, 100 mg/kg ip) significant agitation was observed. No alternate routes of administration were found that showed desired tolerability and exposure.
6. This end-group and optimizations of it were later successfully incorporated into other inhibitor motifs (see Ref. 4d–4f).
7. For Akt enzyme assay details see: Barnett, S. F.; Defeo-Jones, D.; Fu, S.; Hancock, P. J.; Haskell, K. M.; Jones, R. E.; Kahana, J. A.; Kral, A.; Leander, K.; Lee, L. L.; Malinowski, J.; McAvoy, E. M.; Nahas, D. D.; Robinson, R.; Huber, H. E. *Biochem. J.* **2005**, *385*, 399.
8. For all these assays (enzymes Akt1 and Akt2 and cell Akt1 and Akt2 assays) the standard deviation for a positive control is less than 50% of the IC_{50} value.
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13. Experimental design: A2780 tumor cells (9×106 cells per animal) were implanted subcutaneously into the left flank of nude mice. The mice were weighed after 5 days (study day 0). Animals were randomized into groups based on tumor volume and treatment was initiated on study day 5 (12 days from implantation) when tumors were a size of $\sim 100\ cm^3$. Vehicle (25% hydroxypropyl-β-cyclodextrin); **17** hydrochloride dosed subcutaneously at 50 mg/kg 3× per day (at $t = 0$, 3, and 7 h), twice a week for 5 cycles. Animals were euthanized on study day 21 (16 days from beginning of dosing). Animal weights exclude tumor weight.
14. The mean tumor weight and standard error within each dose group were estimated from an analysis of variance (ANOVA) model. The effect of **17** on mean tumor weight was compared to the vehicle group mean and compared via a 1-sided $\alpha = 0.05$ *t*-test.