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Allosteric inhibitors of Akt1 and Akt2: Discovery of [1,2,4]triazolo[3,4-f][1,6]naphthyridines with potent and balanced activity

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ABSTRACT

A series of [1,2,4]triazolo[3,4-f][1,6]naphthyridine allosteric dual inhibitors of Akt1 and 2 have been developed. These compounds have been shown to have potent dual Akt1 and 2 cell potency. The representative compound **13** provided potent inhibitory activity against Akt1 and 2 in vivo in a mouse model.

The serine/threonine kinase Akt (PKB), a member of the AGC super family of kinases, plays a key role in the regulation of cell survival, proliferation and growth.[1] Activation of Akt has been considered an important step in the development and maintenance of cancers, and consequently the inhibition of Akt through small molecule perturbation could lead to novel oncology therapies.[2,3]

The Akt family kinases contain an N-terminal pleckstrin homology (PH) domain in addition to the kinase catalytic domain. The kinase domains of the Akt isozymes, denoted Akt1, Akt2, and Akt3, are highly conserved but the PH domains are notably divergent. Akt1 and Akt2 are ubiquitously expressed while Akt3 is the major isozyme in tissues of neuroendocrine origin. Since each of these Akt isozymes has distinct functions and expression profiles, compounds that are isozyme selective may provide optimum impact on cancer cell survival and minimum toxicity.[4] Previous disclosures from these research laboratories have reported a strategy for targeting Akt isozyme 1 and 2 via allosteric inhibitors. These molecules are PH-domain dependent and display excellent selectivity against kinases outside of the Akt family.[5,6] Continuing our studies in this important research area we report in this communication our successful efforts to develop a novel tricyclic compound series with potent and balanced Akt1 and Akt2 activities in vivo and with an improved hERG activity profile.

A recent communication described the development of compound **1** (Fig. 1).[6b] This potent Akt inhibitor exhibits in vivo inhibitory activity against Akt1 and Akt2 in a mouse model and is

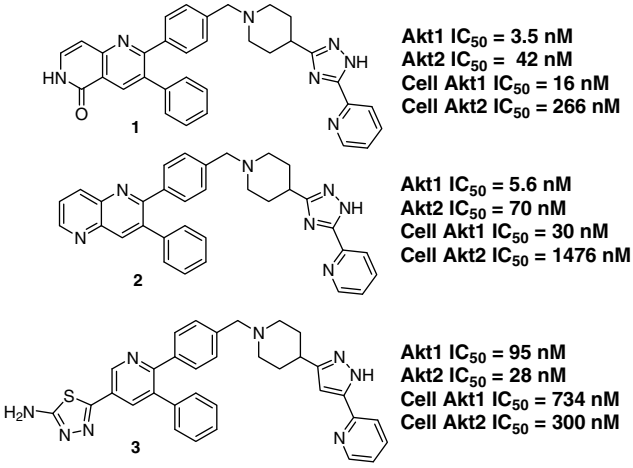

Figure 1. Previously published balanced dual Akt1 and Akt2 inhibitors.

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efficacious in a tumor xenograft model. However, compound **1** possesses off-target binding affinity to the I_{kr} potassium channel hERG (human Ether-a-go-go-Related Gene) (**1**: hERG binding IC_{50} = 5610 nM).[6b] Our objective was to develop compounds with attenuated hERG affinity and more balanced activity against the Akt1 and Akt2 isozymes as a means to optimize in vivo efficacy. To achieve these goals, we decided to revisit the naphthyridine compounds that showed increased potency against the Akt1 isozyme and through SAR exploration enhance the activity against the Akt2 isozyme.

Earlier work in these laboratories indicated that the regiochemistry of the two *N* atoms in the naphthyridine core had significant impact on Akt1 and Akt2 activity. In the cases of 1,6-naphthyridin-5-one **1** and 1,5-naphthyridine **2**, heteroatoms occupying the southwest corner of the naphthyridine core were shown to provide enhanced potency.[6b] The relationship between the substitution pattern and potency can be further corroborated by earlier work exploring pyridine and other heterocycles. In particular, studies on 2,3,5-substituted pyridine core showed that Akt2 activity was more sensitive toward substitution at the southwest corner.[6a] For example, compound **3** exhibits more potent Akt2 activity relative to that of Akt1 with an aminothiadiazole moiety occupying the southwest corner. Based on our SAR analysis, we envisioned that by merging the core structures of compound **1** and **3** together to give a tricyclic structure we could further enhance the inhibitory activity against the desired Akt isozymes.

The chemistry that was utilized in this work is outlined in Scheme 1. 1,6-naphthyridin-5-one **4** was converted to the 5-chloro-1,6-naphthyridine **5** by the treatment with $POCl_3$. Displacement of chlorine with hydrazine afforded the hydrazide **6**. Treatment of **6** with diimidazole derivatives **8**[7] (compound **9–12**) or orthoesters **7** (compound **13–17**) provided the desired [1,2,4]triazolo[3,4-f][1,6] naphthyridine analogs.

To our gratification, improvement was observed with the tricyclic compounds. Compared to the lead naphthyridinone compound **1**, introduction of a 7-amino-[1,2,4]triazolo[3,4-f][1,6]naphthyridine core in **9** provided ~4-fold increased enzymatic Akt2 activity while maintaining similar Akt1 activity (Table 1). The improved intrinsic potency of **9** translated to significantly enhanced cell activities (cell Akt1/2 IC_{50} = 18.1/47.7 nM). The balanced activities against Akt 1 and 2 and the improvement in the cellular activities led to the continued exploration of the southwest corner of the tricyclic core series.

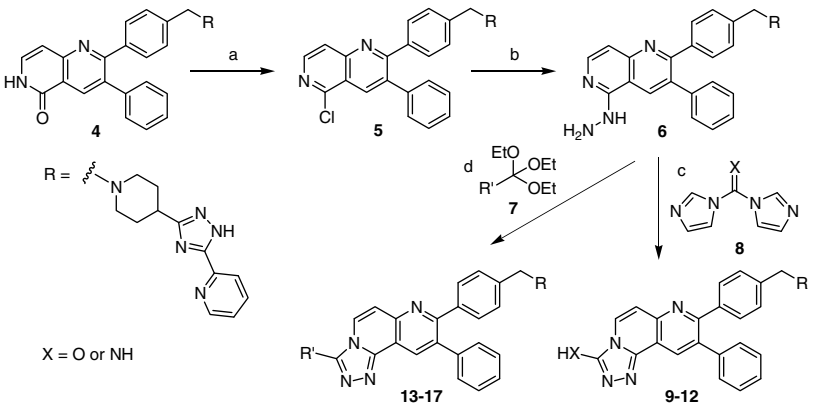
Table 1 shows the analogs synthesized on the triazole core. Variation of the heteroatom at the 7-position of the tricyclic core provided limited impact on Akt1 and Akt2 activity. Increasing polarity in the form of urea such as **12** led to a loss in Akt1 and Akt2 intrinsic potency (Akt 1/2 IC_{50} = 8.5/24.1 nM) which translated to a notable decrease of the cell activities (cell Akt1/2 IC_{50} = 265.5/1213 nM).

To investigate whether polar functionality is required at the 7-position of the tricyclic core, hydrogen (**13**), methyl (**14**) and chloromethyl (**15**) substituents were introduced. Interestingly, no significant loss of activity was observed when a polar heteroatom was replaced by a non-polar group such as methyl. Deterioration of cellular activities, however, was noticed when the size of the substituents was increased.[10] To further examine whether the southwest portion of the molecule was exposed to an aqueous environment, water solubilizing groups were introduced in **16** and **17**. In both cases a loss in potency was observed, suggesting that likely the southwest corner of the inhibitors occupy an area that is at least partially occluded.

Encouraged by the improved intrinsic and cell activity of the tricylic compounds, we proceeded to explore variations of the pyridyl triazole end groups. Replacement of the 2-pyridyl triazole end group in **9** with a 2-pyridyl imidazole moiety led to compound **18** (Fig. 2). Although compound **18** exhibits a slight enhancement in Akt2 enzyme activity (Akt 1/2 IC_{50} = 3.3/2.8 nM) relative to **9**, its cell potency did not show proportional improvement.

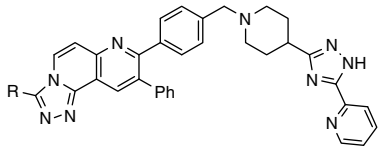
Table 2 shows hERG binding affinity data for selected tricyclic compounds. As has been demonstrated in other Akt inhibitor series, increase in polarity tends to attenuate hERG binding affinity.[5h] In the tricyclic series, the more hydrophobic methyltriazole **14** and chloromethyl **15** exhibit low micromolar hERG activity. The more polar aminotriazole **9** and hydroxytriazole **10** display hERG binding affinity of IC_{50} = 5 μM or greater. In particular, compound **13** shows significantly attenuated hERG binding affinity with an IC_{50} greater than 10 μM.

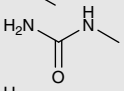
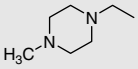
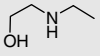
Due to improved cell potency and hERG binding affinity of compound **13**, tolerability and Akt inhibition in mice was assessed in a PK/PD experiment.[12] Under a mini-pump format, the levels of Akt1 and Akt2 phosphorylation activities in mouse lung were measured while the drug concentration in the blood of the subject was maintained at a steady level (Table 3). At a blood concentration of 1.8 μM, compound **13** provided 92% inhibition of Akt1 and 41% inhibition of Akt2 in mouse lung. As a comparison, compound **1** was also tested using the same format. At a blood concentration of 3.0 μM, compound **1** provided 85% inhibition of Akt1 while little Akt2 inhibition was observed. These experiments showed a clear correlation between the in vivo activities and the in vitro enzyme and cell potencies of the Akt inhibitors, and the higher Akt2 inhibition levels exhibited by compound **13** was likely due to its improved intrinsic activity. Our attempts to achieve greater Akt2 inhibition level, however, were not successful as the blood concentration of compound **13** could not be increased with a larger dose.[13] Overall, this study showed that compound **13** was well tolerated and inhibition of both Akt1 and Akt2 were seen in vivo at the blood levels achieved.



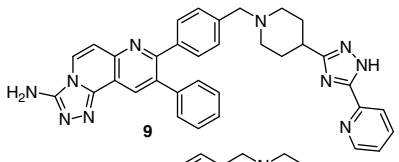
Scheme 1. Reagents and conditions: (a) $POCl_3$, MeCN, reflux, 85% yield; (b) NH_2NH_2, dioxane, 75 °C, 65% yield; (c) **8**, DMF, 85 °C, 84% yield; (d) **7**, DMF, 85 °C, 70% yield.

Table 1
[1,2,4]Triazolo[3,4-f][1,6]naphthyridine series SAR[a]

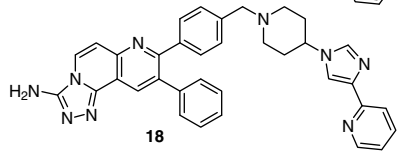


	R	Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)	Cell Akt1 EC_{50} (nM)	Cell Akt2 EC_{50} (nM)
9		2.8	9.4	18.1	47.7
10		2.6	6.7	27.0	167.7
11		10.0	10.4	17.0	107.6
12		8.5	24.1	265.5	1213
13		4.0	10.0	5.0	41.0
14		4.6	14.6	24.6	176.5
15		26.7	10.5	113.5	483.3
16		9.9	55.1	62.5	376.1
17		17.0	77.9	41.8	251.4

[a] Average of at least two measurements; enzyme protocol.[8,9] All compounds >50,000 nM versus PKA, PKC, SGK.



Akt1 IC_{50} = 2.8 nM
Akt2 IC_{50} = 9.4 nM
Cell Akt1 IC_{50} = 18.1 nM
Cell Akt2 IC_{50} = 47.7 nM



Akt1 IC_{50} = 3.3 nM
Akt2 IC_{50} = 2.8 nM
Cell Akt1 IC_{50} = 18.3 nM
Cell Akt2 IC_{50} = 74.8 nM

Figure 2. 2-Pyridyl end group modification.

Table 2
hERG binding affinity for selected compounds[a]

Compound	**9**	**10**	**13**	**14**	**15**
hERG binding IC_{50} (nM)	5319	5312	>10,000	2778	2018

[a] IC_{50} values are reported as the averages of at least two independent determinations;[11] standard deviations are within 25–50% of IC_{50} values.

Table 3
Inhibition of Akt1 and Akt2 in mouse lung with compound **13** and **1**[a]

Compound	Dose (mg/mL)	Blood conc (μM)	% Akt1 inhibition in lung	% Akt2 inhibition in lung
13	18	1.8 ± 0.6	92 ± 3	41 ± 20
	70	1.7 ± 0.5	89 ± 19	35 ± 15
1	10	3.0 ± 0.9	85 ± 6	4 ± 7

[a] Compounds were delivered via mini-pump to nude mice (3 per concentration); Blood concentrations are the average over the course of the experiment; Akt inhibition data are from 20-h time points and the Akt phosphorylation activity was determined by IP kinase assay in lung.

This paper outlined the discovery of [1,2,4]triazolo[3,4-f][1,6]naphthyridine inhibitors of Akt. Introduction of the tricyclic structure significantly improved Akt2 intrinsic and cellular activities which led to the improved inhibition of Akt1 and Akt2 in a mouse model.

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References and notes

1. (a) Cantley, L. C. *Science* **2002**, *296*, 1655; (b) Vivanco, I.; Sawyers, C. L. *Nat. Rev. Cancer* **2002**, *2*, 489; (c) Hennessy, B. T.; Smith, D. L.; Ram, R. T.; Yiling, L.; Milla, G. B. *Nat. Rev. Drug Disc.* **2005**, *4*, 989; (d) Cully, M.; You, H.; Levine, A. J.; Mak, T. W. *Nat. Rev. Cancer* **2006**, *6*, 184.
2. (a) Li, J.; Simpson, L.; Takahashi, M.; Miliaresis, C.; Myers, M. P.; Tonks, N.; Parsons, R. *Cancer Res.* **1998**, *58*, 5667; (b) Karakas, B.; Bachman, K. E.; Park, B. H. *Br. J. Cancer* **2006**, *94*, 455; (c) Hynes, N. E.; Lane, H. A. *Nat. Rev. Cancer* **2005**, *5*, 341; (d) Engelman, J. A.; Zejnullahu, K.; Mitsudomi, T.; Song, Y.; Hyland, C.; Park, J. O.; Lindeman, N.; Gale, C. M.; Zhao, X.; Christensen, J.; Kosaka, T.; Holmes, A. J.; Rogers, A. M.; Cappuzzo, F.; Mok, T.; Lee, C.; Johnson, B. E.; Cantley, L. C.; Janne, P. A. *Science* **2007**, *316*, 1039.
3. (a) Hennessy, B. T.; Smith, D. L.; Ram, P. T.; Lu, Y.; Mills, G. B. *Nat. Rev. Drug Discov.* **2005**, 988; (b) Lindsley, C. W.; Barnett, S. F.; Yaroschak, M.; Bilodeau, M. T.; Layton, M. E. *Curr. Top. Med. Chem.* **2007**, 1349.
4. (a) Chan, T. O.; Rittenhouse, S. E.; TSichilis, P. N. *An Rev. Biochem.* **1999**, *68*, 965; (b) Barnett, S. F.; Bilodeau, M. T.; Lindsley, C. W. *Curr. Top. Med. Chem.* **2005**, *5*, 109; (c) Page, C.; Lin, J. J.; Jin, Y.; Castle, V. P.; Nunez, G.; Huang, M.; Lin, J. Y. *Anticaner Res.* **2000**, *20*, 407; (d) Laine, J.; Kunstle, G.; Obata, T.; Noguchi, M. *J. Biol. Chem.* **2002**, *277*, 3743.
5. (a) Lindsley, C. W.; Zhao, Z.; Leister, W. H.; Robinson, R. G.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Hartman, G. D.; Huff, J. R.; Huber, H. E.; Duggan, M. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 761; (b) Zhao, Z.; Leister, W. H.; Robinson, R. G.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Hartman, G. D.; Huff, J. R.; Huber, H. E.; Duggan, M. E.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 905; (c) Zhao, Z.; Robinson, R. G.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Hartman, G. D.; Huber, H. E.; Duggan, M. E.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 49; (d) Wu, Z.; Hartnett, J. C.; Neilson, L. A.; Robinson, R. G.; Fu, S.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Kral, A. M.; Huber, H. E.; Hartman, G. D.; Bilodeau, M. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1274; (e) Wu, Z.; Robinson, R. G.; Fu, S.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Kral, A. M.; Huber, H. E.; Kohl, N. E.; Hartman, G. D.; Bilodeau, M. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2211; (f) Siu, T.; Liang, J.; Arruda, J.; Li, Y.; Jones, R. E.; Defeo-Jones, D.; Barnett, S. F.; Robinson, R. E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4186; (g) Siu, T.; Liang, J.; Arruda, J.; Li, Y.; Jones, R. E.; Defeo-Jones, D.; Barnett, S. F.; Robinson, R. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4186; (h) Siu, T.; Li, Y.; Nagasawa, J.; Liang, J.; Tehrani, L.; Chua, P.; Jones, R. E.; Defeo-Jones, D.; Barnett, S. F.; Robinson, R. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4191.
6. (a) Hartnett, J. C.; Barnett, S. F.; Bilodeau, M. T.; Defeo-Jones, D.; Hartman, G. D.; Huber, H. E.; Jones, R. E.; Kral, A. M.; Robinson, R. G.; Wu, Z. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2194; (b) Bilodeau, M. T.; Balitza, A. E.; Hoffman, J. M.; Manley, P. J.; Barnett, S. F.; Haskell, K.; Defeo-Jones, D.; Leander, k.; Jones, R. E.; Robinson, R. G.; Smith, A. M.; Huber, H. E.; Hartman, G. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3178.
7. Wu, Y.-Q.; Hamilton, S. K.; Wilkinson, D. E.; Hamilton, G. S. *J. Org. Chem.* **2002**, *67*, 7553.
8. 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.
9. For all of these assays (enzyme 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.
10. See also reference[6b]: The reason for the apparent disconnect between the enzyme and cell activity for Akt2 isozyme is not well understood.
11. The hERG IC_{50} values were acquired by radioligand binding competition experiments using membrane preparations from human embryonic kidney cells that express hERG. For assay detail see Bell, I. M.; Gallicchio, S. N.; Abrams, M.; Beshore, D. C.; Buser, C. A.; Culberson, J. C.; Davide, J.; Ellis-Hutchings, M.; Fernandes, C.; Gibbs, J. B.; Graham, S. L.; Hartman, G. D.; Heimbrook, D. C.; Homnick, C. F.; Huff, J. R.; Kassahun, K.; Koblan, K. S.; Kohl, N. E.; Lobell, R. B.; Lynch, J. J., Jr.; Miller, P. A.; Omer, C. A.; Rodrigues, A. D.; Walsh, E. S.; Williams, T. M. *J. Med. Chem.* **2001**, *44*, 2933.
12. *Experimental design:* Subjects: Tumor (C33a cervical carcinoma cells) bearing nude mice. Vehicle (25% hydroxypropyl-β-cyclodextrin); **13** hydrochloride dosed via mini-pump (at 70 mg/mL and 18 mg/mL concentrations) at a rate of 8 μL/hr over 20 hrs. Blood draw for PK at 3, 6, 8, 11 and 20 h. Animals were euthanized at 20 h time point.
13. Compound solubility issue was likely the cause for the non-linear proportionality of drug concentrations versus dosage.