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Rapid assembly of diverse and potent allosteric Akt inhibitors

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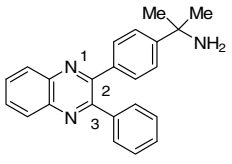
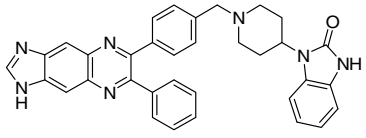
Abstract—This paper describes the rapid assembly of four different classes of potent Akt inhibitors from a common intermediate. Among them, a pyridopyrimidine series displayed the best intrinsic and cell potency against Akt1 and Akt2. This series also showed a promising pharmacokinetic profile and excellent selectivity over other closely related kinases.

Akt is a serine/threonine kinase that is a key regulator of apoptosis and directly phosphorylates several proteins that are part of the cell survival machinery.[1,2] Akt has also been shown to be involved in the regulation of cell cycle progression, cell proliferation, and cell growth.[1,2] Thus Akt activation plays a critical role in tumorigenesis. This is supported by the common observation of dysregulation of Akt in the development of human cancer.[2a–f,3] Therefore, Akt inhibitors have potential as a new therapeutic treatment for cancer and may induce apoptosis alone or in combination with standard cancer chemotherapeutics.

The three isoforms of human Akt (Akt1, Akt2, and Akt3) have an amino terminal pleckstrin homology (PH) domain and a kinase domain separated by a 39-amino acid hinge region. They share more than 80% of their amino acid identities.[2a,b,4] In addition, Akt belongs to the AGC family of kinases and shares high homology with PKA and PKC. Therefore, it is a challenge to develop isozyme selective and Akt specific (vs the AGC family of kinases) inhibitors. Recently, leading compound **1** was found to reversibly inhibit the activity as well as the activation of Akt1 and Akt2, but not Akt3.[5–7] It was shown that inhibition of both Akt1 and Akt2, but not Akt1 or Akt2 alone, was sufficient to maximally sensitize tumor cells to apoptotic stimuli. This sensitization was not reversed by over-expression of a functionally active Akt3. In addition, compound **1** is PH domain-dependent and also specific for Akt over other closely related kinases. Our goal is to develop highly specific Akt inhibitors for therapeutic use that are devoid of off-target activities.



1
Akt1 IC_{50} = 3,400 nM
Akt2 IC_{50} = 2,3000 nM
AKt3 IC_{50} > 50,000 nM



2
Akt1 IC_{50} = 58 nM
Akt2 IC_{50} = 210 nM
AKt3 IC_{50} > 2,119 nM

Optimization of potency for **1** by library synthesis led to compound **2** which induced apoptosis,[5–7] but displayed a high molecular weight and limited solubility. To eliminate these problems as well as improve potency and physical properties, we modified the quinoxaline template. We communicate here a rapid assembly of potent Akt1 and Akt2 dual inhibitors with diversified core structures from a common intermediate.

The earlier SAR studies of quinoxalines pointed out the importance of 2,3-diphenyl substituents and the N-1

Keywords: Akt inhibitor; Allosteric; Rapid assembly; Cancer; Kinase.
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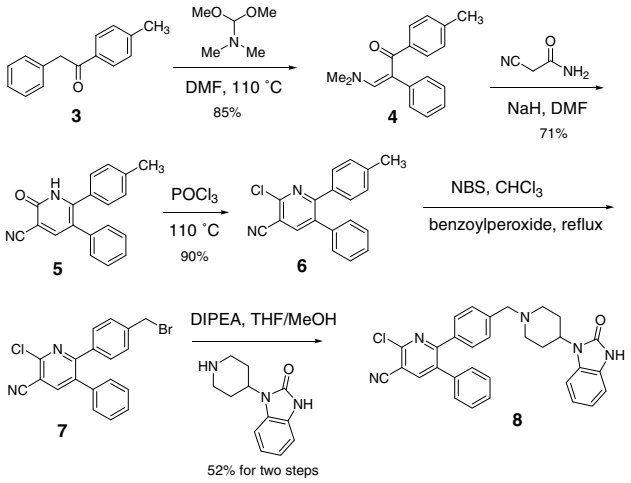
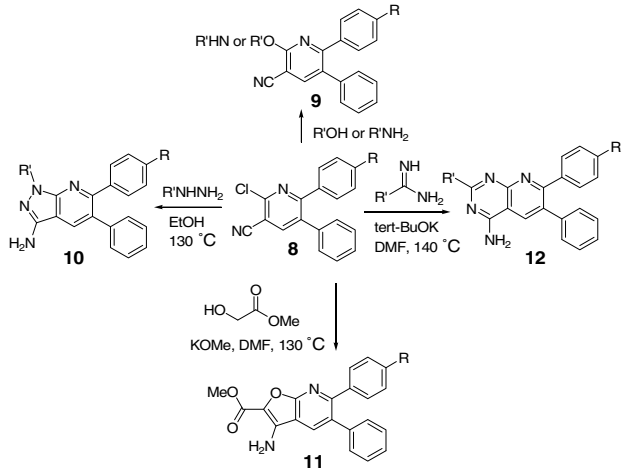


Scheme 1. Synthesis of the common intermediate **8**.

heteroatom.[5] Taken together with the known versatility of 2-chloro-3-cyanopyridine functionality,[8] we envisioned pyridine **8** (Scheme 1) as a common intermediate to generate analogs with different heterocyclic cores. Compound **8** was easily obtained in five steps from ketone **3**[9] (Scheme 1). Condensation of **3** with *N*,*N*-dimethylformamide dimethylacetal gave **4**, which cyclized with 2-cyanoacetamide to afford pyridone **5**. Treatment of **5** with phosphorus oxychloride produced chloropyridine **6**. Radical bromination followed by displacement with suitably substituted amines generated the proposed intermediate **8**.

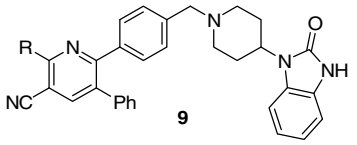
Compound **8** served as a powerful common intermediate and afforded different heterocyclic systems in just one further step including: (a) tetrasubstituted aminopyridines or alkoxypyridines (**9**) via the displacement of the chlorine atom; (b) pyrazolopyridines (**10**) through the reaction with hydrazines; (c) furopyridines (**11**) via the reaction with glycolates; and (d) pyridopyrimidines (**12**) through the reaction with amidines (Scheme 2).

The Akt activities[10] of selected tetrasubstituted pyridines are shown in Table 1. Chloropyridine **8** provided



Scheme 2. Synthesis of different core structures from **8**.

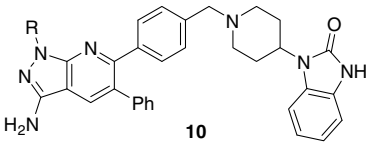
Table 1. Akt activities of tetrasubstituted pyridines



Compound	R	Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)
8	Cl	253 ± 47	7356 ± 5104
9a	NH_2	495 ± 240	2062 ± 1021
9b	NHMe	2249 ± 915	8141 ± 2584
9c	NMe_2	8457 ± 975	15390 ± 692
9d	OMe	5548 ± 1660	37720 ± 8779

encouraging results (Akt1 IC_{50} = 253 nM, Akt2 IC_{50} = 7356 nM). However, its analogs, aminopyridines (**9a**–**c**) or alkoxypyridine (**9d**), offered lower potency against both Akt1 and Akt2.

Table 2. Akt activities of pyrazolopyridines



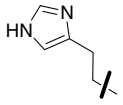
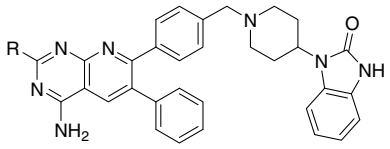
Compound	R	Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)
10a	H	86 ± 9	7356 ± 5104
10b	Me	121 ± 22	556 ± 26
10c	CH_2CH_2OH	131 ± 12	1228 ± 26
10d		21 ± 6	839 ± 53

Table 3. Akt activities of pyridopyrimidines

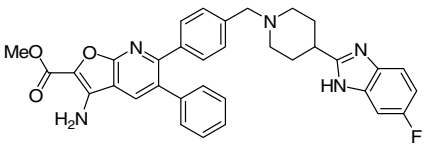


Compound	R	Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)	Caspase 3-fold induction*
12a	OH	244 ± 146	577 ± 300	1.4
12b	NH_2	107 ± 76	274 ± 148	2.2
12c	H	81 ± 48	259 ± 150	2.7

* @ 4000 nM.

We then turned our attention to bicyclic cores. Shown in Table 2 are the Akt activities of selected pyrazolopyridines. In this series, the Akt1 potency was greatly improved with the best compound (**10d**) displaying an Akt1 IC_{50} = 21 nM. However, its Akt2 IC_{50} was still 839 nM. These compounds were not active in cells as they were not able to sensitize LNCaP cells toward apoptosis in a caspase 3 induction assay (no induction was observed at 4000 nM in the presence of TRAIL).[11] Their weak Akt2 activities might be responsible for the lack of cell potency.

We also investigated furopyridines as another[6,5] fused system represented by compound **11**. This compound gave good Akt activity: Akt1 IC_{50} = 40 ± 18 nM, Akt2 IC_{50} = 237 ± 123 nM. More importantly, it displayed good caspase 3 induction activity (fold induction = 5.2 at 4000 nM).[11] Further quantitative measurement of its ability to inhibit Akt phosphorylation in cells showed encouraging results: Akt1 IC_{50} = 256 nM and Akt2 IC_{50} = 1200 nM).[12]

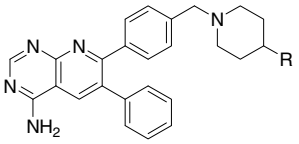


11

Fused [6,6] pyridopyrimidines also yielded satisfactory results. Shown in Table 3 are pyridopyrimidines with different 2-substituents. These compounds offer potent activity against Akt1 and Akt2. They also showed good cell activity in the caspase-induction assay.[11] The 2-non-substituted **12c** and the 2-amino substituted **12b** displayed better intrinsic potency and greater fold induction in the caspase 3 assay.

With the initial encouraging results of 2-unsubstituted pyridopyrimidines, we further optimized this series by modifying the terminal benzimidazolone group (Table 4). Clearly, the terminal groups had a great impact on the compounds' Akt activity. While purine (**13a**) and

Table 4. Optimization of pyridopyrimidines



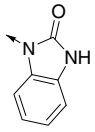
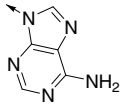
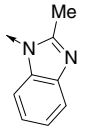
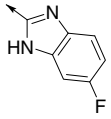
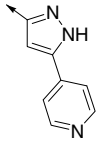
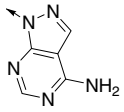
Compound	R	IC_{50} (nM)		Cell IC_{50} (nM)	
		Akt1	Akt2	Akt1	Akt2
12c		81 ± 48	259 ± 150	1834	5084
13a		129 ± 58	696 ± 5	nd	nd
13b		69 ± 31	281 ± 26	221 ± 63	344 ± 112
13c		18 ± 3	239 ± 18	277	1811
13d		21 ± 6	85 ± 30	463	1198
13e		14 ± 0.1	99 ± 8	295	468

Table 5. Akt1 fold-selectivity of pyridopyrimidines

Compound	Akt2	Akt3	Akt1-δPH	SGK	PKA	PKC
13c	13	105	>2800	>2800	nd	nd
13e	7	40	>3400	>3400	>2700	1862

methylbenzimidazole (**13b**) gave similar results to benzimidazolone (**12c**), fluorobenzimidazole (**13c**), pyridopyrazole (**13d**), and isopurine (**13e**) displayed much more potent enzyme activities against Akt1 ($IC_{50} \leqslant 20$ nM) and Akt2 ($IC_{50} \approx 100$ nM). Further, they were also very active at inhibiting Akt phosphorylation in cells.[12] It should be noted that pyridopyrimidine **13c** and furopyridine **11** share the same terminal fluorobenzimidazole group and have similar Akt activity. Further, these compounds generally have good solubility in common organic solvents and acidic aqueous solution (pH < 4).

Initial pharmacokinetic studies in dogs showed promising profiles for the pyridopyrimidines. For example, compound **13c** had a long half-life ($t_{1/2}$ = 2.8 h) with a low clearance (Cl = 5.6 mL/min/kg).[13]

We also examined the selectivity of these compounds for Akt1 versus Akt2, Akt3, and other closely related kinases. Akt1 fold selectivities for **13c** and **13e** over related kinases are shown in Table 5. In general, these compounds behaved similarly to compound **2**. They displayed a modest level of selectivity against Akt2 and a high level of selectivity against Akt3. Their activity against Akt1 required the presence of the PH domain and they were not active against other closely related kinases such as SGK, PKA, and PKC.

In summary, we have rapidly synthesized four classes of compounds from a common pyridine intermediate. Among them, furopyridines and pyridopyrimidines have been identified as potent dual inhibitors of Akt1 and Akt2. In addition, pyridopyrimidine compounds have shown a promising pharmacokinetic profile and an excellent selectivity profile. Future investigation will focus on further improving potency to develop clinical candidates for the treatment of cancer. Efforts along this line will be reported in due course.

Acknowledgments

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References and notes

1. For excellent reviews on Akt, see: (a) Graff, J. R. *Expert Opin. Ther. Targets* **2002**, *6*, 103; (b) Nicholson, K. M.; Anderson, N. G. *Cell. Signalling* **2002**, *14*, 381; (c) Li, Q.; Zhu, G.-D. *Curr. Top. Med. Chem.* **2002**, *2*, 939; (d) Barnett, S. F.; Bilodeau, M. T.; Lindsley, C. W. *Curr. Top. Med. Chem.* **2005**, *5*, 109.
2. (a) Hanks, S.; Hunter, T. *FASEB* **1995**, *9*, 576; (b) Zinda, M. J.; Johnson, M. A.; Paul, J. D.; Horn, C.; Konicek, B. W.; Lu, Z. H.; Sandusky, G.; Thomas, J. E.; Neubauer, B. L.; Lai, M. T.; Graff, J. R. *Clin. Cancer Res.* **2001**, *7*, 2475; (c) Cheng, J. Q.; Ruggeri, B.; Klein, W. M.; Sonoda, G.; Altomare, D. A.; Watson, D. K.; Testa, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3636; (d) Haas-Kogan, D.; Shalev, N.; Wong, M.; Mills, G.; Yount, G.; Stokoe, D. *Curr. Biol.* **1998**, *8*, 1195; (e) Staal, S. P. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5034; (f) Brognard, J.; Clark, A. S.; Ni, Y.; Dennis, P. A. *Cancer Res.* **2001**, *61*, 3986; (g) Kozikowski, A. P.; Sun, H.; Brognard, J.; Dennis, P. A. *J. Am. Chem. Soc.* **2003**, *125*, 1144; (h) Breitenlechner, C. B.; Wegge, T.; Berillon, L.; Graul, K.; Marzenell, K.; Friebe, W.; Thomas, U.; Huber, R.; Engh, R. A.; Masjost, B. *J. Med. Chem.* **2004**, *47*, 1375.
3. (a) Hsu, J. H.; Shi, Y.; Hu, L. P.; Fisher, M.; Franke, T. F.; Lichtenstein, A. *Oncogene* **2002**, *21*, 1391; (b) Page, C.; Lin, H.; Jin, Y.; Castle, V. P.; Nunez, G.; Huang, M.; Lin, J. *Anticancer Res.* **2000**, *20*, 407; (c) Cheng, J. Q.; Lindsley, C. W.; Cheng, G. Z.; Yang, H.; Nicosia, S. V. *Oncogene* **2005**, *24*, 7482.
4. Masure, S.; Haefner, B.; Wesselink, J. J.; Hoefnagel, E.; Mortier, E.; Verhasselt, P.; Tuytelaars, A.; Gordon, R.; Richardson, A. *Eur. J. Biochem.* **1999**, *265*, 353.
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.
6. 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.
7. Defeo-Jones, D.; Barnett, S. F.; Fu, S.; Hancock, P. J.; Haskell, K. M.; Leander, K. R.; McAvoy, E.; Robinson, R. G.; Duggan, M. E.; Lindsley, C. W.; Zhao, Z.; Huber, H. E.; Jones, R. E. *Mol. Cancer Ther.* **2005**, *4*, 271.
8. (a) Witherington, J.; Bordas, V.; Gaiba, A.; Garton, N. S.; Naylor, A.; Rawlings, A. D.; Slingsby, B. P.; Smith, D. G.; Takle, A. K.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, 3055, and references therein; (b) Pochat, F.; Lavelle, F.; Fizames, C.; Zerial, A. *Eur. J. Med. Chem. Chim. Ther.* **1987**, 135; (c) Schoenfeld, F.; Troschuetz, R. *Heterocycles* **2001**, 1979, and references therein.
9. Renault, O.; Dallemagne, P.; Rault, S. *Org. Prep. Proced. Int.* **1999**, *31*, 324.
10. Akt IC_{50} represents biochemical inhibition of peptide phosphorylation with a full length of Akt enzyme. Detection was performed by homogeneous time resolved fluorescence (HTRF) using an europium chelate (Perkin-Elmer) [Eu(K)]-labeled phospho(S21)-GSK3α antibody (Cell Signaling Technologies) and streptavidin-linked XL665 fluorophore which binds to the biotin moiety on the substrate peptide (biotin-GGRARTSSFAEPG). For detail, see Ref. 6. Values are reported as single determinations or as the average of at least two determinations ± standard deviation.
11. Caspase assay measures the ability of Akt inhibitors to sensitize LNCaP cells to tumor necrosis factor related apoptosis inducing ligand (TRAIL) induced apoptosis. As a surrogate for apoptosis induction we determine the enzymatic activity of the effector caspase 3 in compound-treated and untreated cells and values are reported as the fold induction. For details, see Ref. 7.
12. Cell Akt IC_{50} represents the ability of inhibitors to block the phosphorylation of Akt isozymes in C33 A cells (human cervical carcinoma). For detail, see Ref. 7. Values are reported as single determinations or as the average of at least two determinations ± standard deviation.
13. Compound dosed 0.25 mpk iv as a solution in DMSO and the data are the average of two dogs.