Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 49–53
Bioorganic & Medicinal Chemistry Letters

Development of potent, allosteric dual Akt1 and Akt2 inhibitors with improved physical properties and cell activity

Zhijian Zhao,[a,*] Ronald G. Robinson,[b] Stanley F. Barnett,[b] Deborah Defeo-Jones,[b] Raymond E. Jones,[b] George D. Hartman,[a] Hans E. Huber,[b] Mark E. Duggan[a] and Craig W. Lindsley[a,c]

[a]*Department of Medicinal Chemistry, Technology Enabled Synthesis Group, Merck & Co., PO Box 4, West Point, PA 19486, USA*
[b]*Department of Cancer Research, Merck Research Laboratories, Merck & Co., PO Box 4, West Point, PA 19486, USA*
[c]*VICB Program in Drug Discovery, Department of Pharmacology, Vanderbilt Medical Center, Nashville, TN 37232, USA*

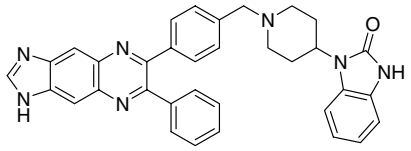
Received 4 September 2007; revised 2 November 2007; accepted 7 November 2007
Available online 13 November 2007

Abstract—This letter describes the development of potent, allosteric dual Akt1 and Akt2 inhibitors with improved aqueous solubility (~18 mg/mL) that translates into enhanced cell activity and caspase-3 induction.

The serine/threonine kinase Akt (PKB) phosphorylates an ever increasing list of downstream substrates that promote cell survival, growth and block pro-apoptotic signals.[1,2] Numerous studies have shown that dysregulation of PI3K/Akt is a major contributor to tumorigenesis and a promising target for cancer therapy.[3] However, the development of inhibitors of Akt as small molecule therapeutics for the treatment of cancer has been hindered by a lack of Akt specific inhibitors (versus the AGC family of kinases) and isozyme selective (Akt1, Akt2, and Akt3) Akt inhibitors due to high sequence homology.[1–3]

Recent reports from our laboratories disclosed a series of novel, allosteric Akt kinase inhibitors that displayed high levels of Akt isozyme selectivity and PH-domain dependent inhibition.[4] Significantly, these allosteric inhibitors were postulated to lock Akt into a closed conformation which not only inhibited the activity of the kinase, but also prevented the activation (phosphorylation) of Akt.[4,5] This was observed both in vitro and in vivo with the dual Akt1 and Akt2 inhibitor **1** (Akt1 IC_{50} = 58 nM, Akt2 IC_{50} = 210 nM, Akt3 IC_{50} = 2200 nM), thereby representing a fundamentally new mechanism of kinase inhibition (Fig. 1).[4,5] Despite this notable advance, **1** possessed limited solubility in most vehicles and poor physical properties which afforded only modest activity in cell-based IPKA Akt assays (Akt1 IC_{50} = 305 nM, Akt2 IC_{50} = 2100 nM, Akt3 IC_{50} = >10,000 nM).[6] These issues limited the utility of **1** for further in vivo experiments. In this letter, we describe the optimization of **1** to provide potent allosteric Akt1 and Akt2 dual inhibitors with improved solubility, physical properties, and cellular activity.

Early work in this series indicated that substitution in the C6 or C7 positions was tolerated and provided improved potency; however, the regioisomeric quinoxalines **2** displayed distinct and variable inhibition against the individual Akt isozymes (Table 1).[4a] For instance, **2a** and **2b** displayed balanced inhibition of Akt1 and Akt2, but **2a** did not inhibit Akt3 whereas **2b** did. Despite significant increases in intrinsic potency with **2c** and **2d**, all of these analogs were not cell permeable likely due to zwitterionic character.[4] As a result, we fo-



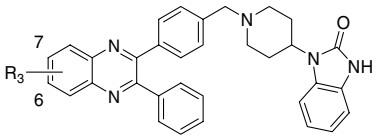
1

Figure 1. Allosteric dual Akt1 and Akt2 inhibitor **1**.

Keywords: Kinases; Akt; Inhibitors; Cancer.
* Corresponding author. E-mail: zhijian_zhao@merck.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.11.015

Table 1. Structures and activities for quinoxalines **2**



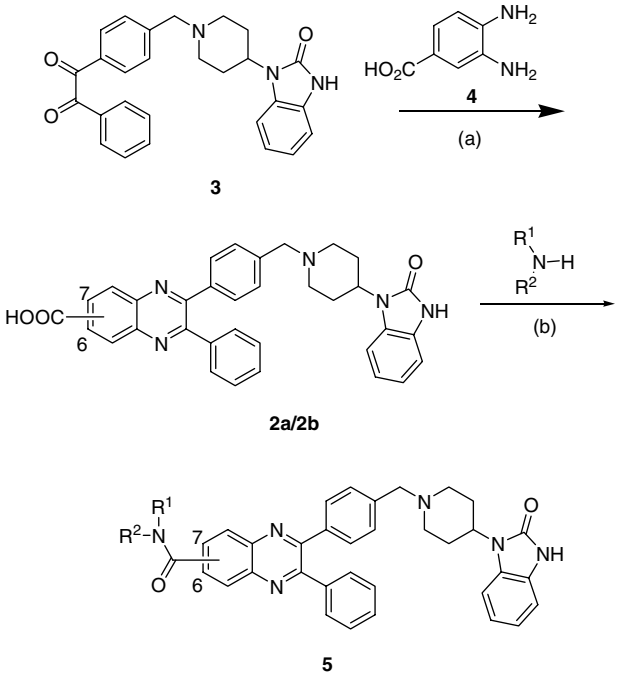
2

Compound	R_3	Akt1 IC_{50}[a] (nM)	Akt2 IC_{50}[a] (nM)	Akt3 IC_{50}[a] (nM)
2a	6-COOH	240	281	>50,000
2b	7-COOH	166	388	3200
2c	6-(2*H*-Tetrazole)	63	65	1228
2d	7-(2*H*-Tetrazole)	20	144	1613

[a] Average of at least three measurements; enzyme protocol[5]; all compounds >50,000 nM versus PKA, PKC, SGK.

cused our attention on increasing the aqueous solubility and cell penetration of **2** by other means, in particular, through the incorporation of amine-containing substituents in the C6/C7 positions.

As shown in Scheme 1, our initial strategy focused on a microwave-assisted condensation of benzil **3** with 3,4-diaminobenzoic acid **4** to deliver regioisomeric quinoxalines **2a**/**2b**.[4,7] The isomers could be separated by HPLC; however, initial libraries employed a 1:1 mixture of **2a**/**2b** to afford, after amide coupling protocols, **5**. As shown in Table 2, this 48-membered library provided compounds that maintained potency, variable Akt isozyme inhibitions and the same allosteric mechanism of Akt inhibition as our earlier series.[4,6] In general, cyclic diamines, such as **5a**, were selective for Akt2 but provided only modest inhibition. Aliphatic diamines afforded more balanced inhibition of both Akt 1 and Akt2; however, the nature of the diamine had dramatic effects on the degree of individual Akt isozyme inhibition. For instance, **5c** and **5d** show a 2- to 3-fold preferential inhibition of Akt2 over Akt1, a pattern of inhibition previously observed only in our pyridine series of allosteric Akt inhibitors.[4b] Interestingly, the addition of a single methyl group to **5e** (IC_{50}s: Akt1 = 5028 nM, Akt2 = 4182 nM, and Akt3 > 10,000 nM) affords **5f**, a submicromolar inhibitor (IC_{50}s: Akt1 = 715 nM, Akt2 = 816 nM, and Akt3 = 5200 nM). Despite similar in vitro potency and uniformly improved aqueous solubility (>10 mg/mL), a number of analogs of **5** showed a significant potency shift in cells. With respect to cell potency, compound **5i** stood out with a minimal potency shift in our cell-based IPKA assay (IC_{50}s: Akt1 = 526 nM, Akt2 = 730 nM, and Akt3 > 10,000 nM).



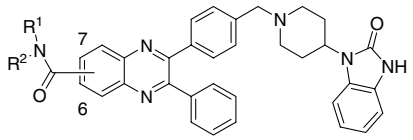
Scheme 1. Synthesis of amine-tethered quinoxalines **5**. Reagents and conditions: (a) EtOH/HOAc (9:1), 160 °C, 10 min, microwave, 87%; (b) i—PS-DCC, HOBt, DCM:DIEA (9:1); ii—MP–CO_3^{2-}, 75–95%. All compounds purified by mass-guided HPLC.[7]

Prior to further evaluation, **5i** was separated into pure C6 and C7 regioisomers, **6** and **7**, respectively (Fig. 2). The C7 regioisomer **7** proved to be more potent in an enzymatic as well as in the cell-based IPKA assay than the C6 regioisomer **6** as displayed in Figure 2. Notably, for **7**, this was the first case in which there was such a negligible shift between the in vitro IC_{50}s and those determined in the cell-based IPKA (LNCAP cells) assay in our program. In addition, both **6** and **7** were dependent on the PH-domain for Akt inhibition, non-competitive with ATP and selective versus the AGC family of kinases (>50 μM vs PKA, PKC, SGK).

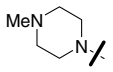
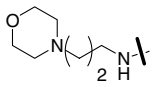
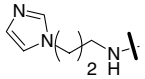
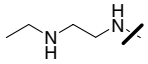
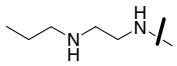
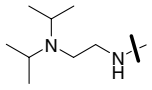
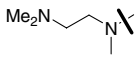
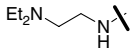
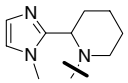
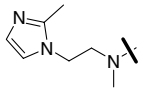
Based on the improvements in solubility and cell activity with the amine-tethered quinoxalines **5**, we synthesized additional libraries that incorporated other polar substituents in place of basic amines. Following the same basic synthetic route outlined in Scheme 1, a number of potent compounds (represented by a general structure **8**) resulted from this endeavor (Table 3). While in vitro potency, physical properties, and aqueous solubility remained favorable in **8a**–**8f**, these analogs suffered significant 10- to 20-fold potency shifts in the cell-based IPKA assay affording micromolar levels of inhibition, which prevented further in vivo experiments.

Because of its minimal potency shift between in vitro and cell-based IPKA assay, **7** was selected for further evaluation. By way of comparison, our earlier proof of concept (POC) molecule **1** was 10-fold less potent on Akt2 in the IPKA assay than **7**, had poor solubility in most vehicles, and had no measurable aqueous solubility.[4,6] Despite a molecular weight of 654, **7** possessed good physical properties. As the di-HCl salt, **7** was soluble (~18 mg/mL) in 98% saline. Based on these data, **7** was evaluated in caspase-3 assays as a surrogate for apoptosis induction. Similar to **1**, **7** significantly increased caspase-3 activity (6- to 10-fold) with a variety of chemotherapeutic agents (herceptin, camptothecin, doxorubicin, and TRAIL) and against a number of tu-

Table 2. Structures and activities for quinoxalines **5**



5

Compound	R^1 R^2-N-	Akt1 IC_{50}[a] (nM)	Akt2 IC_{50}[a] (nM)	Akt3 IC_{50}[a] (nM)	Solubility in 98% saline[b] (mg/mL)
5a		>10,000	805	>10,000	10
5b		461	910	7200	8
5c		173	92	5400	n.d.
5d		720	225	6400	10
5e		5028	4182	>10,000	n.d.
5f		715	816	5200	n.d.
5g		521	423	6800	9
5h		6381	3097	>10,000	n.d.
5i		560	390	7800	18
5j		4391	2411	n.d.	n.d.
5k		8494	2040	n.d.	n.d.

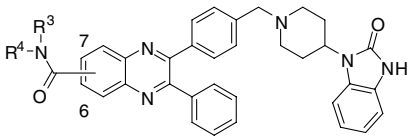
[a] Average of at least three measurements; enzyme protocol[5]; all compounds assayed as a 1:1 C6:C7 mixture; all compounds >50,000 nM versus PKA, PKC, SGK.
[b] Solubilities were determined at pH 7.4; n.d., not determined.

mor cell lines (LnCaP, HT29, MCF7, and A2780). As shown in Figure 3, **7** afforded a similar level of induction of caspase-3 activity as **1**, but at a 3-fold lower dose (4 μM for **7** vs 12 μM for **1**) reflecting the improved aqueous solubility and cell activity despite roughly equivalent in vitro enzyme inhibition profiles.

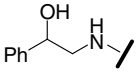
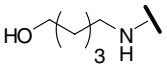
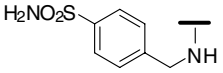
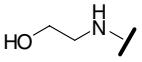
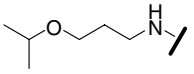
Encouraged by the profile of **7**, a mouse PD study was undertaken to see if **7** could potentially allow for a 24-hour tolerability (Akt1 and Akt2 knockdown) study.[4,6] As with **1**, **7** inhibited Akt1 and Akt2 phosphorylation in vivo. However, **7** also induced significant behavioral effects in rodents which precluded further in vivo experiments from being conducted.

In summary, we have developed novel, allosteric Akt1 and Akt2 dual kinase inhibitors with improved aqueous solubility (~18 mg/mL) and balanced in vitro and cell IPKA activity that translate directly into significant improvements in caspase-3 activation (apoptosis). Importantly, the degree and selectivity of inhibition of Akt1 and Akt2 is dependent on both the nature of the C6/C7 substituent and the regiochemistry. Further evaluation of this class of allosteric inhibitors and structural

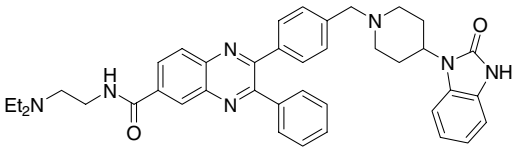
Table 3. Structures and activities for quinoxalines **8**



8

Compound	R^3 / R^4-N-	Akt1 IC_{50}[a] (nM)	Akt2 IC_{50}[a] (nM)	Akt3 IC_{50}[a] (nM)
8a		624	231	4500
8b		460	398	7200
8c		185	316	4600
8d		150	254	7400
8e		8053	1268	>10,000
8f		574	651	5200

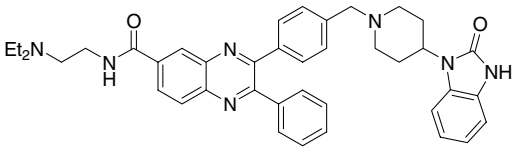
[a] Average of at least three rneasurements; enzyme protocol[5]; all compounds assayed as a 1:1 C6:C7 mixture; all compounds >50,000 nM versus PKA, PKC, SGK.



6

Enzyme assay (IC_{50}s)
Akt1 = 458 nM
Akt2 = 359 nM
Akt3 = 7,400 nM

Cell-based IPKA assay (IC_{50}s)
Akt1 = 368 nM
Akt2 = 617 nM
Akt3 = 8,000 nM



7

Enzyme assay (IC_{50}s)
Akt1 = 138 nM
Akt2 = 212 nM
Akt3 = 7,200 nM

Cell-based IPKA assay (IC_{50}s)
Akt1 = 253 nM
Akt2 = 276 nM
Akt3 >10,000 nM

Figure 2. Allosteric dual Akt1 and Akt2 inhibitors **6** and **7**.

refinements are in progress and will be reported in due course.

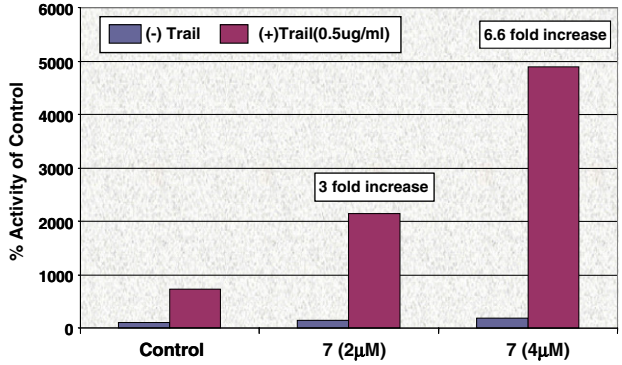


Figure 3. Caspase-3 assay: LnCaP cells treated with **7** alone or in combination with ±TRAIL. Each experiment employed either 2 μM or 4 μM total dose of **7**.

Acknowledgments

The authors wish to thank Dr. Sandor Varga and Dr. Steven Pitzenberger for extensive NMR work to confirm the structural assignments of the regioisomeric quinoxaline analogs, and Dr. Charles W. Ross III for obtaining HRMS data (accurate mass measurements).

References and notes

1. For recent reviews on Akt, see: (a) Barnett, S. F.; Bilodeau, M. T.; Lindsley, C. W. *Curr. Top. Med. Chem.* **2005**, *5*, 109; (b) Cheng, J. Q.; Lindsley, C. W.; Cheng, G. Z.; Yang, H.; Nicosia, S. V. *Oncogene* **2005**, *24*, 7482; (c) Kim, D.; Cheng, G. Z.; Lindsley, C. W.; Yang, H.; Cheng, J. Q. *Curr. Opin. Investig. Drugs* **2005**, *6*, 1250.
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.
4. (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.
5. For Akt enzyme assay details and the proposed model for the allosteric mode of inhibition of these inhibitors, 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.

6. For details on the Akt IPKA assay, the caspase-3 assays, and in vivo experimental details, see: 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.
7. Leister, W. H.; Strauss, K.; Wisnoski, D.; Zhao, Z.; Lindsley, C. *J. Comb. Chem.* **2003**, *5*, 322.