Bioorganic & Medicinal Chemistry Letters 18 (2008) 4191–4194

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The design and synthesis of potent and cell-active allosteric dual Akt 1 and 2 inhibitors devoid of hERG activity

Tony Siu [a,*], Yiwei Li [b], Johnny Nagasawa [a], Jun Liang [a], Lida Tehrani [a], Peter Chua [a], Raymond E. Jones [b], Deborah Defeo-Jones [b], Stanley F. Barnett [b], Ronald G. Robinson [b]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, Merck & Co., 3535 General Atomics Court, San Diego, CA 92129, USA
[b] Department of Cancer Research, Merck Research Laboratories, Merck & Co., PO Box 4, West Point, PA 19486, USA

ARTICLE INFO

Article history:
Received 3 March 2008
Revised 18 May 2008
Accepted 19 May 2008
Available online 24 May 2008

Keywords:
hERG
Allosteric
Akt
Kinase

ABSTRACT

This letter details the attenuation of hERG in a class of Akt inhibitors through heteroatom insertions into aromatic rings. The development of a cell-active dual Akt 1 and 2 inhibitors devoid of hERG activity is discussed using structure–activity relationships.

Akt is a serine/threonine kinase belonging to the AGC family of kinases. Activation of Akt is responsible for multiple cellular events including cell survival, energy metabolism, proliferation, and growth. In an earlier letter, we identified potent dual Akt 1 and 2 inhibitors by incorporating solubilizing amines.[1a] However, these inhibitors as well as other inhibitors discovered in our program represented by **1**[1b] and **2**[1a] (Fig. 1) possess off-target binding affinity to the I_{kr} potassium channel hERG (human Ether-a-go-go-Related Gene) with IC_{50}s in the micromolar range. Affinity for the hERG channel has the potential to alter cardiac repolarization through prolongation of the QT interval,[2] and alterations of the QT interval could potentially trigger torsades de pointes, a life-threatening ventricular tachyarrhythmia. Because of the potential for cardiac death associated with QT prolongation, optimization of the hERG binding window is a goal in the development of a drug. This letter outlines our strategy to identify a cell-based potent dual Akt 1 and 2 inhibitors devoid of hERG activity.

Earlier work at Merck Research Laboratories has shown that minor heteroatom additions to aromatic rings can attenuate hERG binding.[3] Presumably these modifications work by decreasing the lipophilicity of the aromatic ring, thus destabilizing π stacking interactions with the hERG-binding environment.[4] However, it was not clear whether these changes in polarity would affect the ability of our inhibitors to penetrate cells. Our strategy was to incorporate a variety of polar group modifications to the B ring of **1** to address hERG binding and to evaluate their effects on cellular potency.

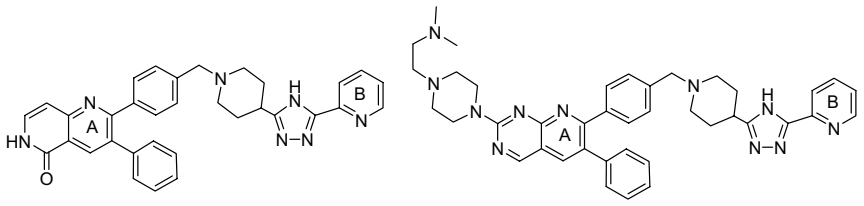
The analogs for our strategy commenced with hydrazide formation **3** using carbonyl diimidazole and hydrazine illustrated in Scheme 1. Heating hydrazide **4** with a variety of nitriles together in the presence of sodium methoxide provided access to an assortment of polar aromatic end groups as represented by **5**. These compounds were attached to subunit pyridone **6**[1b] by reductive amination to afford **7**.

As shown in Table 1, changes in polarity to the aromatic B ring attenuated hERG-binding affinity. For example, significant improvements were observed with pyrimidine **8**, pyridone **9**, and amino pyridine **10**. These compounds all displayed binding affinity of IC_{50} = 10 μM or greater to hERG.[5] In addition, these substitutions were not detrimental to the overall intrinsic or cell-based potency for Akt 1 and 2. Other heteroaromatic derivatives such as pyrazine **11** (hERG IC_{50} = 8.5 μM) achieved modest gains in attenuating hERG binding, while no improvements were obtained with 2-methoxy pyridine **12** (hERG IC_{50} = 1220 nM). Gratifyingly, in all instances the intrinsic and cell-based potencies were within 3-fold of **1** while modifying hERG binding.

With hERG binding attentuated through heteroatom additions to the aromatic B ring, we turned our attention to merging this key finding with our strategy to enhance cell-based potency. Our conception was that the potency optimized piperazines discovered in the previous communication[1a] would confer cellular potency

* Corresponding author at present address: Department of Drug Optimization, Merck Research Laboratories, Merck & Co., 33 Avenue Louis Pasteur, Boston, MA 02115, USA.
E-mail address: tony_siu@merck.com (T. Siu).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.084



1

Akt 1/2 IC_{50} = 3.5/42 nM

Cell Akt 1/2 IC_{50} = 16/266 nM

hERG IC_{50} = 2500 nM

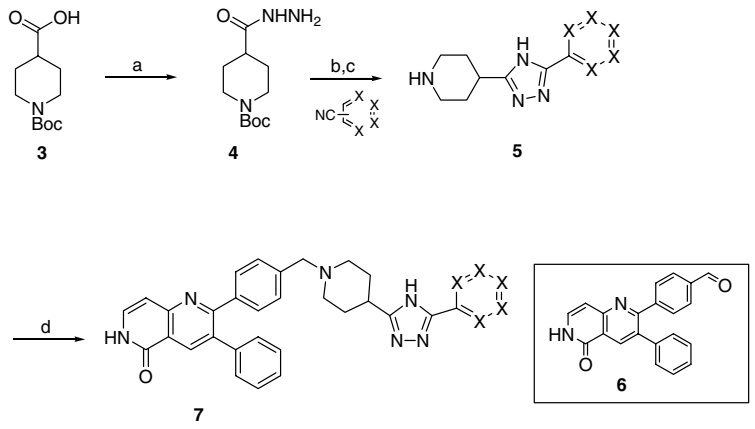
2

Akt 1/2 IC_{50} = 4/4 nM

Cell Akt 1/2 IC_{50} = 10/39 nM

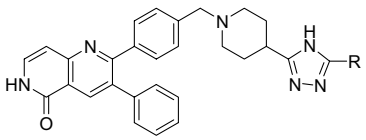
hERG IC_{50} = 2100 nM

Figure 1. Merck allosteric Akt inhibitors.

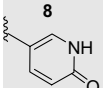
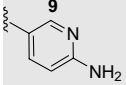
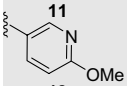


Scheme 1. Synthesis of pyridone analogs. Reagents and conditions. (a) Hydrazine, carbonyl diimidazole, CH_2Cl_2; (b) nitrile, 25% sodium methoxide in MeOH, 2-ethoxyethanol, 100 °C; (c) TFA, CH_2Cl_2; (d) **6**, sodium triacetoxyborohydride, DMF.

Table 1
SAR of hERG binding with polar end groups



7

R	Enzyme IC_{50} (nM)		Cell IC_{50} (nM)		hERG IC_{50} (nM)
	Akt 1	Akt 2	Akt 1	Akt 2	
 **1**	4	26	18	224	2500
 **8**	7	44	23	69	>10,000
 **9**	1	8	62	284	>10,000
 **10**	4	33	26	165	10,000
 **11**	4	21	44	405	8428
 **12**	3	16	40	159	1220

whereas the polar aromatic end groups would impart favorable hERG-binding properties on the pyridopyrimidine core (Fig. 2).

Toward that end, our strategy for optimizing both cell-based potency and reducing hERG affinity was to systematically synthesize 12 combinations of the most potent piperazine subunits with the least potent hERG-binding aromatic B rings in a matrix strategy. This 4 × 3 matrix is detailed in Table 2. The piperazines chosen for this optimization were methyl piperazine, hydroxylethyl piperazine, and dimethyl amino piperazine, whereas the aromatic B ring subunits chosen were pyrimidine, amino pyridine, pyridone, and pyridine N-oxide. The analogs were synthesized using the same route as previously detailed.[1a]

Consistent with observations in Table 1, incorporation of the polar heteroaromatic end groups in general attenuated hERG binding. Of the 12 compounds synthesized, compound **13** proved optimal in terms of cell-based potency (cell Akt 1/2 IC_{50} = 25/40 nM) and hERG binding (hERG IC_{50} > 10,000 nM). The hydroxy ethylpiperazine series (**17–20**) maintained low hERG-binding affinity; however, the cellular potency diminished slightly against Akt 2. On the other hand, the dimethyl amino piperazine series (**21–24**)

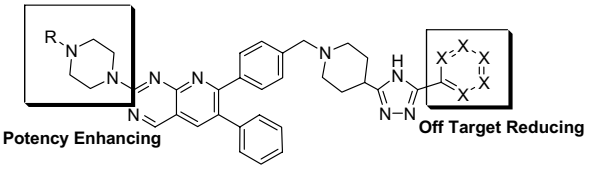
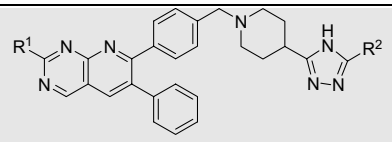
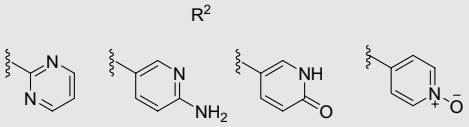


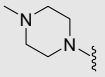
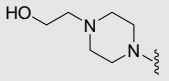
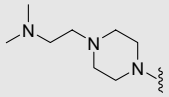
Figure 2. Strategy for combining potency and reducing hERG affinity.

Table 2
Matrix SAR with solubilizing amines and polar end groups



Cell Akt 1/2 nM
(hERG nM)



R^1				
	25/40 (>10000) **13**	20/84 6100 **14**	71/188 NA **15**	88/185 9800 **16**
	37/80 (>10000) **17**	38/180 7400 **18**	127/284 6800 **19**	67/146 >10000 **20**
	35/130 4800 **21**	37/140 3500 **22**	69/98 800 **23**	33/68 7900 **24**

also had slightly diminished cell-based potency, but still possessed low micromolar hERG binding affinity. The loss in cell-based potency in Akt 2 for the ethylhydroxyl and dimethyl amino piperazines might be due to large polarity increase in the molecule when coupled to the aromatic B ring end groups. The methyl piperazine in combination with the pyrimidine end group appears to contain the appropriate amount of polarity to decrease hERG binding, but do not affect the cellular activity of the molecule. This strategy proved useful in optimizing the overall polarity for optimal cell-based potency with decreased hERG binding affinity.

Based on the success of inserting heteroatoms into the B aromatic ring to reduce hERG activity, we also explored whether heteroatom insertion into the A ring can bring the same success. Toward that end, our strategy was to construct a pyridine–pyrazine core as opposed to a pyrimidine–pyridine core (Fig. 3).

The synthesis of Akt inhibitors with a pyridine–pyrazine core commenced with an ammonia displacement of chloride at the 2-position of 2,6-dichloro-3-nitro-pyridine to provide **26**. Reduction of the nitro moiety with tin(II) chloride in ethyl acetate generated the diamine **27**.[6] Condensation of diamine **27** with bromobenzoin **28** afforded a 1:1 mixture of inseparable isomers **29**. With **29** in hand, the piperidine subunit **30**,[1b] was attached to the core by displacement with K_2CO_3 in dimethylformamide to afford **31**. Heating **31** with excess methylpiperazine in dioxane followed by HPLC separation of the regioisomers provided **32** and **33** (Scheme 2).

From our previous work, it was anticipated that regioisomer **32** would possess the more desirable cell-based potency.[1a] As anticipated, the pyridine–pyrazine core from regioisomer **32** possesses a high level of cell-based potency against both Akt 1 and 2 (18/92 nM) as well as attenuated hERG binding (9100 nM). In contrast, the opposite regioisomer **33** suffered a loss in activity against Akt 1 and 2 (1408/827 nM) and did not attenuate hERG binding when compared to **2**.

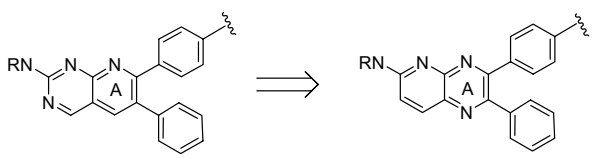
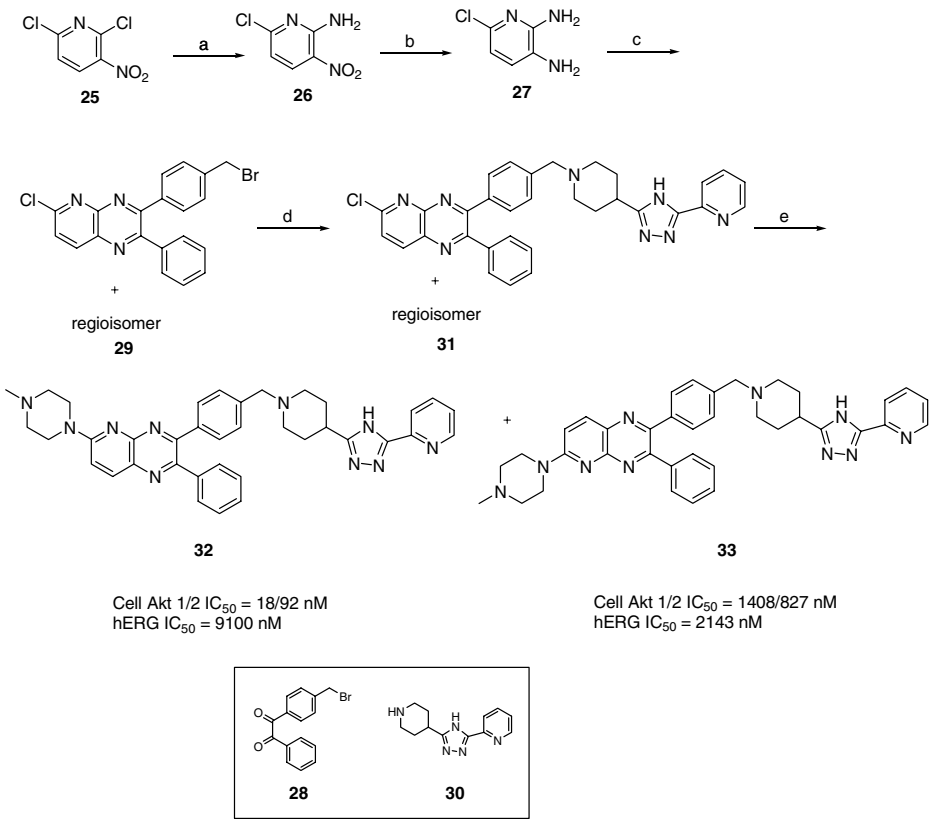


Figure 3. Alternative core heterocycle.

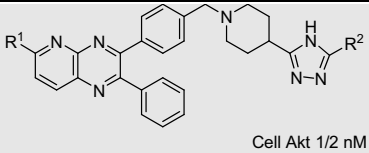
Having established the low hERG-binding affinity for this scaffold, we turned to our matrix strategy to further optimize potency and hERG parameters using the same piperazines and similar heteroaromatic end groups as illustrated in Table 2 (Table 3). From this optimization strategy, compound **36** was identified as possessing favorable hERG binding (IC_{50} hERG >10,000 nM) along with high cellular potency (cell Akt 1 and 2 IC_{50}: 13/48 nM). This compound showed comparable activities to **13**. The combination of the *N*-(2-hydroxyethyl)piperazinyl and the 2-pyridinyl end groups proved to be optimal. Interestingly, *N*-(2-hydroxyethyl) piperazines **37** and **38** also had favorable hERG binding affinities, but had only modest cellular Akt 2 activities. In contrast to the pyrimidine-pyridine scaffold in Table 2, the polar pyrimidine and 2-aminopyridine end groups rendered the molecules too polar when coupled to the pyridine–pyrazine core. This drastic increase in polarity was detrimental to the cellular activity. Presumably, the pyridine–pyrazine core coupled with a piperazine was polar enough to affect the hERG binding without the added polarity of Table 1 end groups. When either the methyl piperazine or the *N,N*-dimethyl amino piperazine was substituted on the A ring, either a lack of cellular potency or increased hERG binding was observed. Compounds **32** and **39** showed good cellular potency against Akt 1/2, but the attenuation of hERG binding was modest when compared to **2**. Similar to the results in Table 2, these data indicate that an overall balanced molecule polarity was necessary for the optimal cell-based potency and hERG attenuation.

In summary, a strategy to reduce hERG activity for dual Akt 1 and 2 allosteric inhibitors was outlined. Increasing the overall polarity of the molecule through small heteroatom insertions into aromatic rings proved successful. By coupling this key finding for modulating hERG activity with our previous discovery of using water solublizing piperazines for potency enhancement, inhibitors **13** and **36** were identified as low-nanomolar cell-based inhibitors



Scheme 2. Synthesis of pyrido-pyrazine core. Reagents and conditions. (a) Ammonium hydroxide, EtOH, 100 °C; (b) Tin (II) Chloride, EtOAc; (c) **28**, AcOH, 100 °C, (d) potassium carbonate, **30**, DMF; (e) methyl piperazine, dioxane, 100 °C.

Table 3
SAR on pyridine–pyrazine analogs



R1			
	18/92 (9100) **32**	52/192 (8300) **34**	70/225 (4700) **35**
HO	13/48 (>10000) **36**	72/190 (>10000) **37**	115/230 (>10000) **38**
	10/43 (6000) **39**	104/116 (>10000) **40**	NA

against Akt 1 and 2 with greater than 10,000 nM binding against hERG.

Acknowledgments

The authors thank Drs. Christopher Cox, Christopher Dinsmore, and Philip Sanderson for careful reading of this manuscript.

References and notes

1. (a) 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; (b) Bilodeau, M. T.; Balitza, A. E.; Hoffman, J. M.; Manley, P. J.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Robinson, R. G.; Smith, A. M.; Huber, H. E.; Hartman, G. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3178.
2. (a) Fermini, B.; Fossa, A. A. *Nat. Rev. Drug Discov.* **2003**, *2*, 439; (b) Roden, D. M. *N. Engl. J. Med.* **2004**, *350*, 1013.
3. (a) 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; For an excellent review of hERG Optimization in Medicinal Chemistry, see: (b) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *49*, 5029.
4. (a) Mitcheson, J. S.; Chen, J.; Lin, M.; Culberson, C.; Sanguinetti, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12329; (b) Fernandez, D.; Ghanta, A.; Kauffman, G. W.; Sanguinetti, M. C. *J. Biol. Chem.* **2004**, *279*, 10120.
5. 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 details, see Ref.[3] and references therein. IC_{50} values are reported as the averages of at least two independent determinations; standard deviations are within ±25–50% of IC_{50} values.
6. Oguchi, M.; Wada, K.; Honma, H.; Tanaka, A.; Kaneko, T.; Sakakibara, S.; Ohsumi, J.; Serizawa, N.; Fuijiwara, T.; Horikoshi, H.; Fujita, T. *J. Med. Chem.* **2000**, *43*, 3052.