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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2194–2197
Bioorganic & Medicinal Chemistry Letters

Optimization of 2,3,5-trisubstituted pyridine derivatives as potent allosteric Akt1 and Akt2 inhibitors

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Received 29 October 2007; revised 14 December 2007; accepted 17 December 2007
Available online 23 December 2007

Abstract—This letter shows inhibitor SAR on a pyridine series of allosteric Akt inhibitors to optimize enzymatic and cellular potency. We have optimized 2,3,5-trisubstituted pyridines to give potent Akt1 and Akt2 inhibitors in both enzyme and cell based assays. In addition, we will also highlight the pharmacokinetic profile of an optimized inhibitor that has low clearance and long half-life in dogs.

Akt (protein kinase B) is a serine threonine kinase belonging to the AGC superfamily of kinases. There are three isoforms of the enzyme with >80% homology to one another. Akt is a critical downstream effector in the PI3K signal transduction pathway. This pathway is frequently activated in tumors by growth factor overexpression and mutation in tumor suppressor PTEN (phosphatase and tensin homologue). Activation of Akt can lead to cell growth, survival, and tumor progression.[1–3] Thus, the selective inhibition of Akt1 and Akt2 by small molecule inhibitors could be critical in the development in novel cancer therapy.

A novel series of potent and selective Akt kinase inhibitors based on 2,3,5-trisubstituted pyridine inhibitors was reported in a recent letter (Fig. 1).[4b] The optimization of molecule **1** was accomplished through library synthesis. It was discovered that the conversion of the nitrile moiety in **1** to the tetrazole moiety in **2** led to a more potent compound. Further optimization of the compounds containing the tetrazole moiety led to compound **3**.

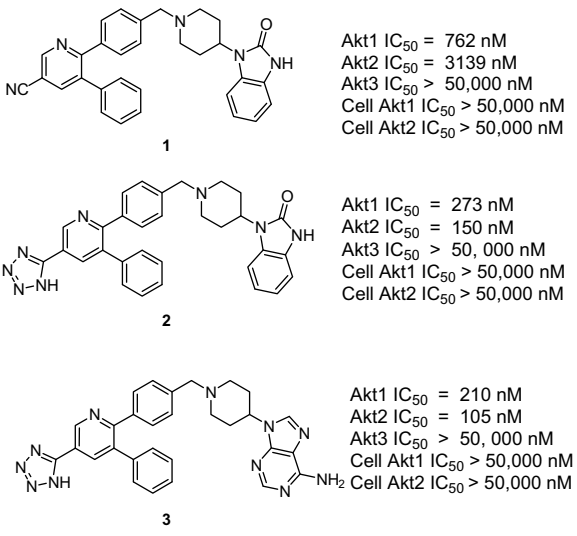

Figure 1. Structures of Akt1 and Akt2 inhibitors.

There were two important discoveries with the 2,3,5-trisubstituted pyridines. The first was these molecules displayed selectivity over the Akt3 isozyme. The second was this series displayed a different selectivity profile than compounds previously reported.[4a,c] When this series was substituted with a heterocycle in the 5 position, it was more Akt2 potent than Akt1.

Keywords: Inhibitors; Akt1; Akt2; Enzymatic; Cellular.
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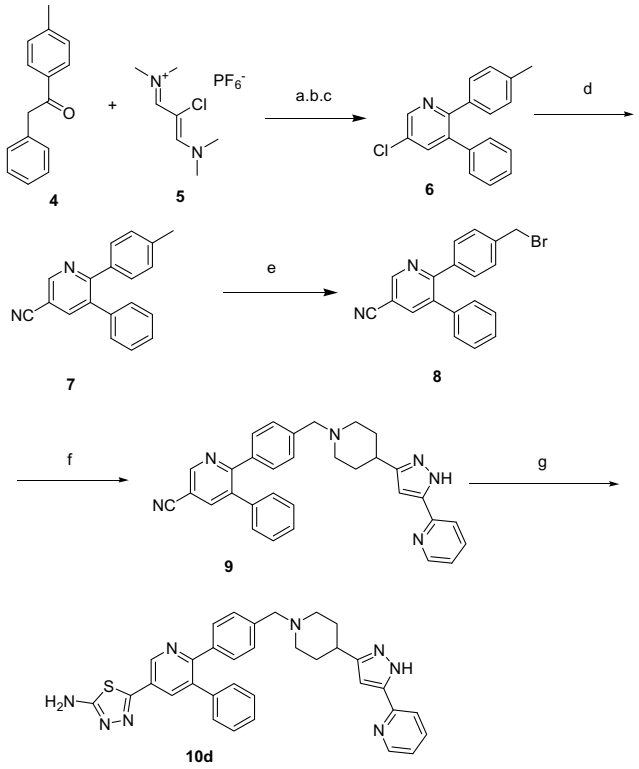
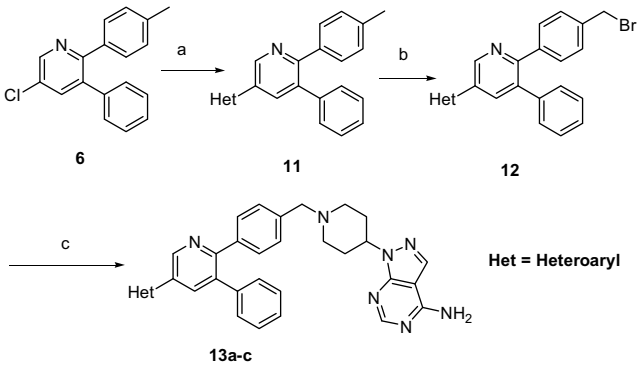
doi:10.1016/j.bmcl.2007.12.040



Scheme 1. Reagents and conditions: (a) *t*-BuOK, THF, 0 °C→ rt, 1 h; (b) **5**, THF, 45 °C, 3 h; (c) NH_4OAc, THF, 70 °C, 18 h, 36%; (d) $Zn(CN)_2$, $Pd_2(dba)_3$, dppf, Zn, DMA, 150 °C, 20 h, 89%; (e) NBS, $(BnO_2)_2$, $CDCl_3$, 20 h, 70 °C, 93%; (f) 2-(5-piperidin-4-yl-1*H*-pyrazol-3-yl)pyridine, DIPEA, 1:1 MeOH THF, rt, 1 h, 57%; (g) thiosemicarbazide, TFA, 60 °C, 16 h, 79%.



Scheme 2. Reagents and conditions: (a) bis(pinacolato)diboron, KOAc, $Pd_2(dba)_3$, $P(Cy)_3$, 160 °C, 1 h microwave, then Het-Br, 2 M aq Na_2CO_3, $Pd(PPh_3)_4$, 150 °C, 0.5 h microwave, 59–69%; (b) NBS, $(BnO_2)_2$, $CDCl_3$, 68 °C, 20 h, 100%; (c) 1-piperidin-4-yl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine, DIPEA, 1:1 MeOH THF, rt, 1 h, 35–100%.

Although these leads exhibited excellent potency against the Akt1 and Akt2 isozymes, they lacked activity in cells.[5] To improve the activity of this series, the overall physical properties of these molecules needed to be improved. This was achieved by replacing the acidic tetrazole moiety with non-acidic heterocycles and by optimizing the eastern basic amine moiety.

Two different reaction schemes were developed to vary both the heterocycle on the western portion of the molecule in the final step (Scheme 1) as well as the basic amine moiety on the eastern portion in the final step (Scheme 2).

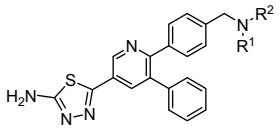
In Scheme 1 the synthesis of the pyridine core is shown. In this scheme the penultimate step was to vary the basic amine moiety and then convert the nitrile to a heterocycle in the final step. Condensation of the ketone **4** with the iminium[6] salt **5** afforded the pyridine core **6**.[7] Using palladium coupling conditions, the chlorine of **6** was converted to the nitrile in compound **7**. Bromination of the methyl group in **7** gave bromomethyl **8**, which could be displaced with a variety of amines to synthesize molecules such as **9**. Finally, the nitrile was then converted to a heterocycle in the final step. In this sequence thiosemicarbazide was used under acidic conditions to make the aminothiadiazole, **10d**.[8]

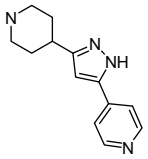
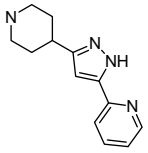
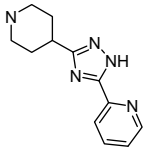
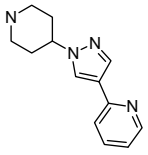
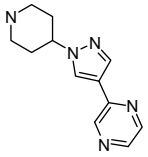
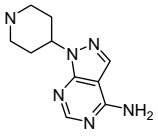
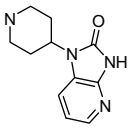
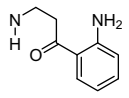
In a similar sequence (Scheme 2) intermediate **6** was coupled to a variety of heterocycles using a Suzuki reaction. First, a boronic acid was formed from **6** under palladium coupling conditions in the microwave. Then, in the same pot, a heteroaryl bromide was added along with an aqueous base and $Pd(PPh_3)_4$ to form molecule **11**. With the western heterocycle in place, the methyl group in **11** was converted to the bromomethyl **12**. The bromide was then displaced by basic amine moieties to provide molecules **13a**–**c**.

Early efforts to find a heterocyclic replacement for the tetrazole in the western portion led to the discovery of the aminothiadiazole. Like the tetrazole, it could be easily synthesized in high yield from the nitrile in **9**. It was found to have similar potency to the tetrazole enzymatically, but more importantly it helped to gain cell potency for these 2,3,5-trisubstituted pyridines. In Table 1, the aminothiadiazole moiety was used as a template on the western portion to vary the basic amine moiety on the eastern portion.

Table 1 shows how various different functionalities were substituted and the molecules still retained potency both enzymatically and in cells. In compounds **10a**–**b**, 2-pyridine and 4-pyridine substitution displayed similar activity to one another. In molecule **10c**, the pyrazole was substituted with a triazole and potency was maintained. Also the C-linked pyrazoles were replaced with N-linked pyrazoles **10d**–**e**. Although some of the potency in cells was lost, the compounds retained their enzymatic potency. Changing the structure from biaryl to bicyclic resulted in the discovery of **10f** and **10g**. **10g** contains a very similar eastern amine portion to **2**. Although **10g** lacks the potency in cells that the others in the table exhibit, it had far better cell potency than **2**. On the other hand, **10f** (having a similar eastern portion to **3**) showed similar potency to the other molecules in the table both enzymatically and in cells, but more importantly, it also showed much better potency in cells than **3**. Finally, we replaced the tertiary amines with a secondary amine. This molecule, **10h**, retained the desired enzymatic and cellular activity of all the molecules in this table.

Table 1. Structures and Akt1/Akt2 enzymatic and cellular inhibition of aminothiadiazolopyridines **10a–h**

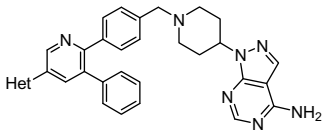


Compound	NR^1R^2	AKT1/2 enzymatic and cell inh[a]
10a		Akt1 IC_{50} = 123 nM Akt2 IC_{50} = 38 nM Cell Akt1 IC_{50} = 407 nM Cell Akt2 IC_{50} = 153 nM
10b		Akt1 IC_{50} = 95 nM Akt2 IC_{50} = 28 nM Cell Akt1 IC_{50} = 734 nM Cell Akt2 IC_{50} = 300 nM
10c		Akt1 IC_{50} = 85 nM Akt2 IC_{50} = 22 nM Cell Akt1 IC_{50} = 516 nM Cell Akt2 IC_{50} = 330 nM
10d		Akt1 IC_{50} = 214 nM Akt2 IC_{50} = 90 nM Cell Akt1 IC_{50} = 1352 nM (1) Cell Akt2 IC_{50} = 1349 nM (1)
10e		Akt1 IC_{50} = 172 nM Akt2 IC_{50} = 51 nM Cell Akt1 IC_{50} = 1378 nM Cell Akt2 IC_{50} = 2028 nM
10f		Akt1 IC_{50} = 126 nM Akt2 IC_{50} = 22 nM Cell Akt1 IC_{50} = 621 nM Cell Akt2 IC_{50} = 416 nM
10g		Akt1 IC_{50} = 434 nM Akt2 IC_{50} = 73 nM Cell Akt1 IC_{50} = 12,650 nM Cell Akt2 IC_{50} = 3957 nM
10h		Akt1 IC_{50} = 114 nM Akt2 IC_{50} = 30 nM Cell Akt1 IC_{50} = 618 nM Cell Akt2 IC_{50} = 472 nM

[a] Average of at least two measurements. For determinations of $n = 1$ number is shown in parentheses. Enzyme and Cell protocol.[5] All compounds >50,000 nM versus SGK except for **10h** where no data are available.

Although the aminothiadiazole showed the desired activity from the start, a thorough SAR study of the western heterocyclic portion of the molecule needed to be done. Since **10f** showed our desired enzymatic and cell activity, its eastern portion was used as a template to examine other heterocycles in the western portion. These molecules are shown in Table 2. Using the Suzuki chemistry developed in Scheme 2, compounds **13a–c** were synthesized. Although these leads maintained enzymatic activity, they lost the cell potency of the aminothiadiazole. An oxadiazole, **13d**, and a triazole, **13e**, were also synthesized. Although these molecules showed better cell activity than **13a–c**, they were not superior to the aminothiadiazole **10f**.

Table 2. Structures and Akt1/Akt2 enzymatic and cellular inhibition of heterocycle replacements **13a–e**



Compound	Het	Akt1/2 enyzmatic and cell inh[a]
13a		Akt1 IC_{50} = 1629 nM Akt2 IC_{50} = 370 nM Cell Akt1 IC_{50} = 10,000 nM (1) Cell Akt2 IC_{50} = 8110 nM (1)
13b		Akt1 IC_{50} = 264 nM Akt2 IC_{50} = 64 nM Cell Akt1 IC_{50} = 1650 nM Cell Akt2 IC_{50} = 3239 nM
13c		Akt1 IC_{50} = 306 nM Akt2 IC_{50} = 62 nM Cell Akt1 IC_{50} = 1954 nM Cell Akt2 IC_{50} = 3459 nM
13d		Akt1 IC_{50} = 180 nM Akt2 IC_{50} = 121 nM Cell Akt1 IC_{50} = 288 nM (1) Cell Akt2 IC_{50} = 1061 nM (1)
13e		Akt1 IC_{50} = 182 nM Akt2 IC_{50} = 101 nM Cell Akt1 IC_{50} = 890 nM (1) Cell Akt2 IC_{50} = 666 nM (1)

[a] Average of at least two measurements. For determinations of $n = 1$ number is shown in parentheses. Enzyme and Cell protocol.[5] All compounds >50,000 nM versus SGK except for **13a** where no data are available.

Table 3. Pharmacokinetic data for **10f**[a]

PK parameter	Dog
$t_{1/2}$ (h)	3.62
Cl (ml/min/kg)	1.37
Vd_{ss} (L/kg)	0.41

[a] Compound dosed iv as a solution in DMSO at a concentration of 0.25 mpk iv.

Due to our desired potency enzymatically and in cells, compound **10f** was chosen for further characterization. First, this compound showed selectivity against a variety of other kinases. It was >10,000 nm versus PKA, PKC, and PKCα. Next, this compound displayed good caspase 3 induction activity (~6-fold induction at

2000 nM).[9] In addition, **10f** showed a desired pharmacokinetic profile in dog (Table 3).

In summary, the search to find compounds that exhibited better cell potency than compounds **1**–**3** led us to many heterocyclic replacements with significantly improved cell potency over the acidic tetrazole. The eastern basic amine moiety was also optimized, and this led us to our most optimal molecule **10f** which showed a desired pharmacokinetic profile in dogs.

Acknowledgments

The authors thank Dr. Art Coddington, Dr. Charles W. Ross III, and Harri Ramjit for obtaining HRMS data (accurate mass measurements).

References and notes

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