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Bioorganic & Medicinal Chemistry Letters 18 (2008) 1274–1279
Bioorganic & Medicinal Chemistry Letters

Development of Pyridopyrimidines as Potent Akt1/2 Inhibitors

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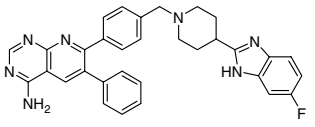
Received 7 November 2007; revised 3 January 2008; accepted 10 January 2008
Available online 19 January 2008

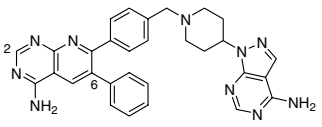
Abstract—This communication reports a new synthetic route of pyridopyrimidines to facilitate their structural optimization in a library fashion and describes the development of pyridopyrimidines that have excellent enzymatic and cell potency against Akt1 and Akt2. This series also shows a high level of selectivity over other closely related kinases and significantly improved caspase-3 activity with the more optimized compounds.

Akt is a serine/threonine kinase that is a key regulator of apoptosis, cell cycle progression, cell proliferation and growth.[1,2] Recently, inhibition of Akt kinase has been recognized as a potential new therapeutic treatment for cancer.[1,3,4] It has been shown that inhibition of both Akt1 and Akt2, but not Akt1 or Akt2 alone, is needed to maximally sensitize tumor cells to certain apoptotic stimuli.[5] Much effort has been dedicated to develop small molecule Akt1 and Akt2 dual inhibitors.[5–9]



1

Akt1 IC_{50} = 18 nM, cell IC_{50} = 277 nM
Akt2 IC_{50} = 239 nM, cell IC_{50} = 1811 nM



2

Akt1 IC_{50} = 14 nM, cell IC_{50} = 295 nM
Akt2 IC_{50} = 99 nM, cell IC_{50} = 468 nM

Previously, we have reported the discovery of pyridylpyrimidines as dual inhibitors of Akt1 and Akt2.[8] These compounds are pH domain dependent and also specific for Akt over other closely related kinases. Therefore, it should be possible to develop highly specific Akt inhibitors for therapeutic use that are devoid of off-target activities. As represented by **1** and **2**, the initial pyridinepyrimidines obtained did not display optimal cell potency.[8] We communicate here our effort to improve their cell potency as well as physical properties.

Since these compounds required the presence of PH domain to be active and they did not compete for ATP binding site, it is unclear where and how they exactly bind to Akt. We decided to use library synthesis to rapidly move the project. While it was found the lower right 6-phenyl group tolerated very limited modification, previous data showed position 2 could be substitued and the terminal group attached to the piperidine ring had a great impact on the Akt activity.[8] However, the terminal groups were introduced at the early stage of the synthetic sequence, thus it was not possible to investigate these groups in an efficient library fashion. To facilitate the optimization process, a new synthetic route was devised (Scheme 1). Hydroxylmethylpyrimidine **3** was oxidized to give the aldehyde **4**. This aldehyde then underwent an aldol reaction with methyl phenylacetate followed by a cyclization to produce a pyrimidylpyridone, which was transformed to the chloropyridopyrimidine **5** with the treatment of phosphorus oxychloride. Suzuki coupling reaction of **5** and 4-formylbenzeneboronic acid afforded aldehyde **6**. Reductive amination of **6** with various amines provided the final product. Since the terminal groups were incorporated at the last step of this new synthetic scheme, they can be investigated in a rapid library fashion.

Keywords: Akt; Kinase; Cancer; Pyridopyrimidine.
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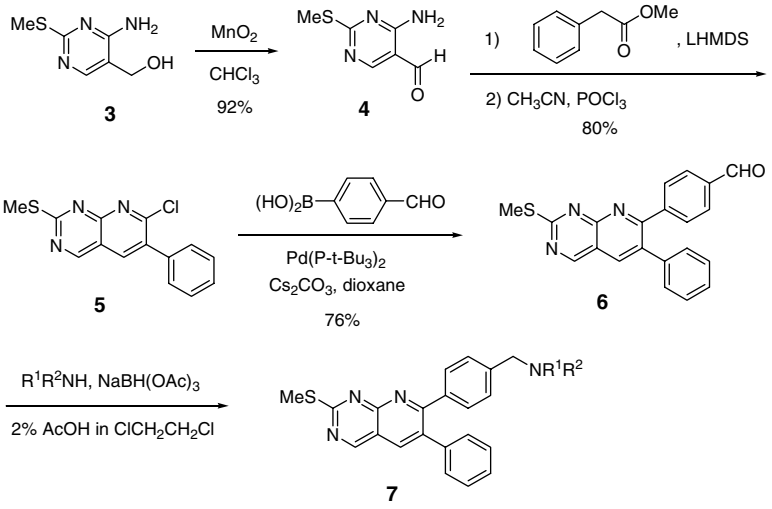
0960-894X/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2008.01.054

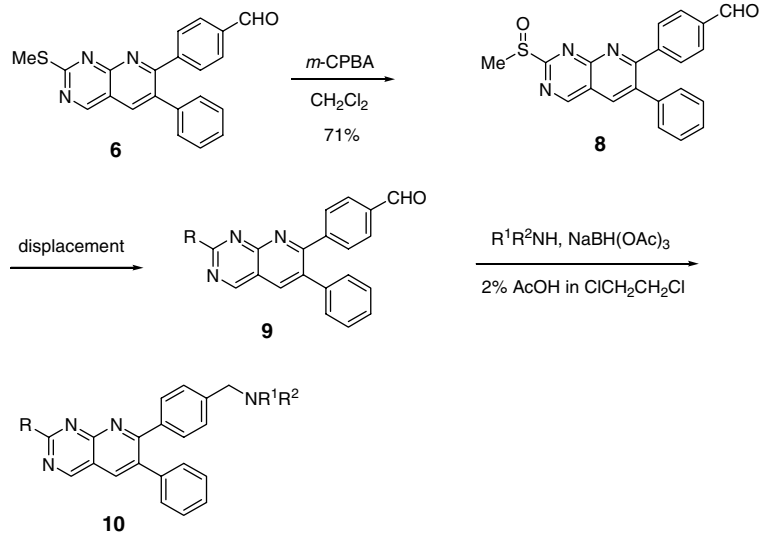


Scheme 1. Synthesis of pyridopyrimidines.

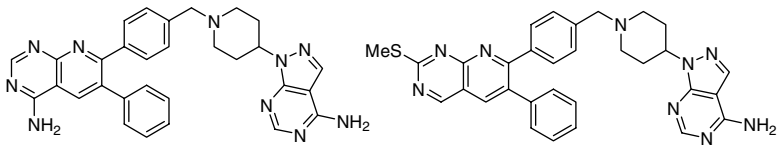
In addition, 2-methylthio group can be easily transformed to other functionalities, which allows SAR of this position to be readily explored (Scheme 2). Methylthio **6** was oxidized to sulfoxide **8** which was then displaced with various nucleophiles including amines, alkoxide or cyano groups to give **9**. Compound **9** was converted via reductive amination reactions to the final product **10** with varied 2-substituents.

It should be noted that the substitution pattern is different between this new generation of pyridopyrimidines **7** and **10** and the original compounds **1** and **2**. Compounds **7** and **10** have a 2-substituent while **1** and **2** contain a 4-amino group. A direct comparison is made between **2** and **11** in Figure 1. With the same terminal groups, the Akt1 activity of 2-substituted **11** is 5-fold more potent than that of 4-substituted **2** and their Akt2 activity is similar.[10] As a result, the newly generated 2-substituted pyridopyrimidine provided a suitable template to develop Akt inhibitors.

With the new template and optimal synthetic route, we modified systematically the terminal groups by varying the functional groups attached to the piperidine ring and changing the piperidine to an acyclic amine. The guiding principle was to improve in vitro activity and cell potency and the amines are selected to optimize physical properties by keeping molecular weight in check and introducing polar functionality where possible. First, the Akt activities of selected pyridopyrimidines with acyclic amines are shown in Table 1. The very simple primary amide **12a** gave a promising starting point (Akt1 $IC_{50} = 226$ nM, Akt2 $IC_{50} \sim 1300$ nM).[10] Replacing the amide with a piperazine ring (**12b**) decreased activities against both Akt1 and Akt2 3- to 5-fold. On the other hand, replacement with aromatic heterocycles such as, imidazole (**12c**), triazole (**12d**), thiazole (**12e**) and pyridine (**12f**) resulted in slightly improved Akt1 activity and similar Akt2 activity. Apparently the H-bond donor or acceptor properties of the heterocycles were not critical for these compounds



Scheme 2. Synthesis of pyridopyrimidines with different 2-substituents.



2

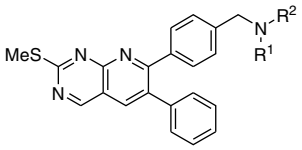
Akt1 IC_{50} = 14 nM, cell IC_{50} = 295 nM
Akt2 IC_{50} = 99 nM, cell IC_{50} = 468 nM

11

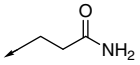
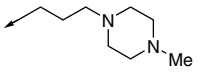
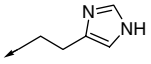
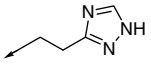
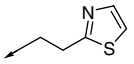
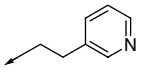
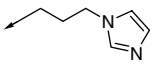
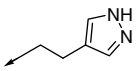
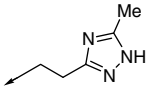
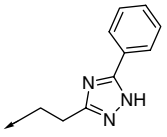
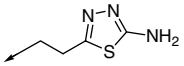
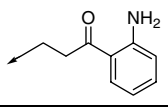
Akt1 IC_{50} = 4 nM, cell IC_{50} = 60 nM
Akt2 IC_{50} =44 nM, cell IC_{50} = 828 nM

Figure 1. Comparison of the new and original pyridopyrimidines.

Table 1. Akt activities of pyridopyrimidines with acyclic amines



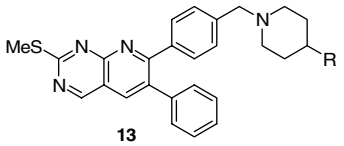
12

Compound	R^1	R^2	Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)
12a	H		226	1299
12b	H		765	8923
12c	H		99 ± 13	1242 ± 215
12d	H		139	1709
12e	H		198	1381
12f	H		90 ± 20	1160 ± 313
12g	H		137	1514
12h	Me		1521	17470
12i	H		162	1792
12j	H		63 ± 26	494 ± 75
12k	H		46 ± 2	529 ± 1
12l	H		15 ± 3	90 ± 19

to inhibit Akt. Changing the chain length between the amine nitrogen and the heterocycle from two to three also made little difference (**12g**). Surprisingly, unlike the cyclic tertiary piperidyl compound **11**, tertiary amine was not compatible with the acyclic version, leading to a great loss of potency against both Akt1 and Akt2 (**12h**). Next we investigated the effect of the substituent on the heterocycle. While a methyl group had no effect on Akt inhibitory ability (**12i** vs. **12d**), a phenyl (**12j**) or an amino group (**12k**) enhanced both Akt1 and Akt2 activities 2- to 3-fold. Finally, we were pleased to find that an anilineketone terminal group (**12l**) offered satisfactory results (Akt1 IC_{50} = 15 nM, Akt2 IC_{50} = 90 nM), with a 10-fold increase in potency over the initial **12a**. The compound **12l** was also very potent in the cell based assay (Akt1 cell IC_{50} = 78 nM, Akt2 cell IC_{50} = 388 nM).[11]

We also examined the terminal groups in the 4-position of the piperidine and the results of selected examples are shown in Table 2. The simple amide (**13a**) or urea (**13b**) provided encouraging results (Akt1 $IC_{50} < 100$ nM, Akt2 $IC_{50} \sim 1000$ nM). Further effort to explore more acyclic functional groups (**13c** and **13d**) offered no improvement. However, when an aromatic heterocycle was put on the piperidine ring directly (**13e**) or attached to the amide nitrogen (**13f**), significant enhancement of Akt1 activity was observed (IC_{50} less than 10 nM). The Akt2 activity was also increased to about an IC_{50} of 300 nM for these compounds. Compounds **13e** and **13f** had good cell potency too. The results from **13e**, **13f**, and previous leading compounds represented by **1** and **2** indicated the potential to include two aromatic heterocycles (**13g**, **13h** and **13i**). While the pyridyloxadiazole produced a less potent compound (**13g**), the more polar pyridylpyrazole (**13h**) and pyridyltriazole (**13i**) with a hydrogen donor provided compounds with excellent activity against Akt1 and Akt2 (Akt1 $IC_{50} \sim 5$ nM, Akt2 $IC_{50} \sim 50$ nM). These two compounds were also shown to penetrate cells, and it is worth noting the remarkable Akt1 cell potency of **13i**

Table 2. Akt activities of pyridopyrimidines with 4-substituted piperidines



13

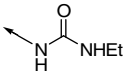
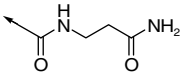
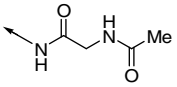
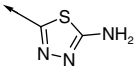
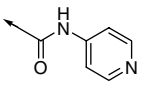
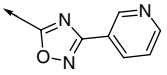
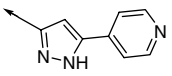
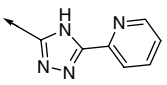
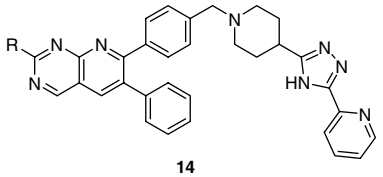
Compound	R	Intrinsic IC_{50} (nM)		Cell IC_{50} (nM)	
		Akt1	Akt2	Akt1	Akt2
13a		29 ± 5	928 ± 118	560 ± 107	9259
13b		81 ± 4	1909 ± 357	795 ± 330	5296
13c		91 ± 20	2106 ± 60	nd	nd
13d		286 ± 5	5127 ± 459	nd	nd
13e		8.5 ± 1.5	267 ± 19	79 ± 1	1572 ± 306
13f		9.7 ± 0.5	301 ± 2	133	1131
13g		64 ± 5	1304 ± 374	nd	nd
13h		4.5 ± 2.9	57 ± 7	85 ± 52	635 ± 208
13i		3.8 ± 1	26 ± 5	9.3 ± 2.6	589 ± 8

Table 3. Akt activities of pyridopyrimidines with various 2-substituents



14

Compound	R	Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)	Cell	
				Akt1 IC_{50} (nM)	Akt2 IC_{50} (nM)
14a	–OMe	23.6	107.2	42.6	2295
14b	–CN	24 ± 1.4	59 ± 22	100 ± 35	586± 420
14c	$–CONH_2$	6.2	35	96.4 ± 55	3706 ± 2458
14d	–NHMe	6.0 ± 0.3	94.4 ± 2.6	20.3 ± 10.1	899 ± 202

(Akt1 IPKA IC_{50} < 10 nM, Akt2 IC_{50} ~ 600 nM). However, this improvement in Akt1 cell activity did not translate directly to Akt2 cell activity. The reason for this disconnection is not well understood.

With successful modification of the western terminal group, we turned our attention to the modification of 2-methylthio group. Several 2-substituents were examined with the best pyridyltriazole terminal group (Table 3). 2-methoxy (**14a**), 2-cyano (**14b**), 2-aminocarbonyl (**14c**), and 2-methylamine (**14d**) all gave satisfactory results. These substituents make the compounds more polar with better physical properties, but did not decrease the Akt activities significantly. For example, the methylamine **14d** is comparable to **13i** regarding to both the intrinsic and cell potency.

We investigated the selectivity of these compounds for Akt1 and Akt2 versus Akt3 and other closely related kinases. In general, these compounds maintained the excellent selectivity profiles of leading compounds **1** and **2**. For example, **14d** has an IC_{50} = 2474 nM for Akt3 and not active against other closely related kinases such as SGK, PKA, and PKC (IC_{50} > 50,000 nM).

Compounds **13i** and **14d** significantly increased caspase-3 activity in LnCaP cells treated in combination with TRAIL (Table 4).[12] Compared to compound **2** which showed a 2-fold increase in caspase-3 at 2 μM, **13i** and **14d** gave a 3-fold induction at 0.1 μM.

In summary, we have described the development of pyridopyrimidines that are potent and selective Akt1/2 dual inhibitors. Compound **12l** with a simple acyclic secondary amine as the terminal group displayed promising potency. Finally, modification of the piperidine and pyridopyrimidine substituents resulted in compounds (**13i** and **14d**) with excellent potency and greatly improved caspase-3 activity.

Table 4. Fold increase of Caspase-3 activity in LnCaP cells with TRAIL[a]

Compound	0.1 μM	0.5 μM	1 μM
13i	3.2-fold	6.8-fold	8-fold
14d	3-fold	6-fold	7.8-fold

[a] Caspase-3 assay: LnCaP cells treated with compound at the given compound concentration in combination with +/- TRAIL (0.5 μg/mL) expressed as a fold difference versus TRAIL alone.

Acknowledgments

The authors thank Dr. Art Coddington, Dr. Charles Ross, and Dr. Harri Ramjit for mass spectral analyses.

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11. Cell-based potency of Akt inhibitors was determined in immunoprecipitation kinase assays (IPKA). IC_{50} values represent the ability of inhibitors to block the phosphorylation of Akt isozymes in C33 A cells (human cervical carcinoma). For detail see Ref. 6. Values are reported as single determinations or as the average of at least two determinations ±standard deviation.
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