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Bioorganic & Medicinal Chemistry Letters 15 (2005) 905–909
Bioorganic & Medicinal Chemistry Letters

Discovery of 2,3,5-trisubstituted pyridine derivatives as potent Akt1 and Akt2 dual inhibitors

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Received 22 October 2004; revised 16 December 2004; accepted 21 December 2004
Available online 19 January 2005

Abstract—This letter describes the discovery of a novel series of dual Akt1/Akt2 kinase inhibitors, based on a 2,3,5-trisubstituted pyridine scaffold. Compounds from this series, which contain a 5-tetrazolyl moiety, exhibit more potent inhibition of Akt2 than Akt1.
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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 Akt is a major contributor to tumorigenesis and a potential 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 identity homology.[1–3]

In a recent letter, we reported a novel series of potent and selective allosteric Akt kinase inhibitors based on a 2,3-diphenylquinoxaline core **1** (Fig. 1).[4] An optimized dual Akt1/Akt2 inhibitor **2** (Fig. 1) was developed through an iterative analog library synthesis approach that was shown to sensitize tumor cells to apoptotic stimuli and inhibit the phosphorylation of both Akt1 and Akt2 in vitro (Table 1). Importantly, these Akt inhibitors displayed selectivity versus the closely related AGC family (PKA, PKC, SGK) of kinases as well as selectivity with respect to the individual Akt isozymes.

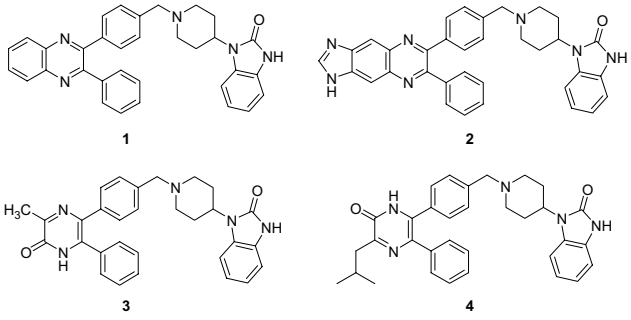

Figure 1. Structures of Akt1 and Akt2 inhibitors.

Table 1. Akt activities of **1**–**4**

Compd	Akt1 IC_{50} (nM)[a]	Akt2 IC_{50} (nM)[a]	Akt3 IC_{50} (nM)[a]
1	295	2057	>50,000
2	58	210	2200
3	760	>20,000	>20,000
4	>20,000	325	>20,000

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

The observed selectivity has been attributed to an allosteric mode of binding, noncompetitive with ATP, wherein Akt inhibition is dependent on the presence of the pleckstrin homology (PH) domain.[5] In the same letter,

Keywords: Pyridine; Tetrazole; Akt; Inhibitor; Kinase.
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doi:10.1016/j.bmcl.2004.12.062

we also reported on the discovery of a series of 5,6-diphenyl-pyrazin-2(1*H*)-one derivatives that, selectively inhibited either Akt1 or Akt2 (**3** and **4**, respectively, Table 1). These isozyme selective inhibitors demonstrated, in caspase-3 assays, that inhibition of both Akt1 and Akt2 were required for a maximal apoptotic response.[4]

Despite this notable advance, **1**–**4** possessed poor physical properties which translated into poor cellular potency. In order to conduct additional in vivo studies, dual Akt1/Akt2 inhibitors with improved physical properties and cellular potency were required. In this letter, we disclose results of our efforts directed toward the development of Akt inhibitors with improved physical properties and the discovery of a series of dual Akt1/Akt2 inhibitors based on a 2,3,5-trisubstituted pyridine scaffold.

One way to improve the physical properties of the 2,3-diphenylquinoxaline series **1** is to incorporate a more basic nitrogen into the core ring system, that is, convert the quinoxaline core to a quinoline core. Based on this idea, the two quinoline regioisomers **5** and **6** (Fig. 2) were prepared wherein either the N4 or N1 nitrogen of **1**, respectively, was replaced with carbon. The synthesis of **5** is shown in Scheme 1.

Microwave-assisted Suzuki coupling[6] of iodide **9**[7] generated **10**, which was converted into triflate **11**. Then, a second microwave-assisted Suzuki coupling, employing 4-formylphenyl boronic acid, followed by a polymer-supported reductive amination sequence[8] afforded quinoline **5**.

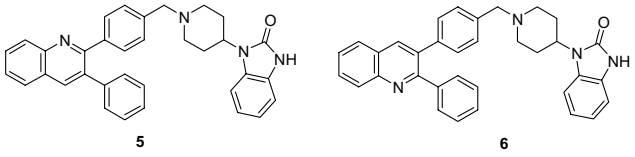
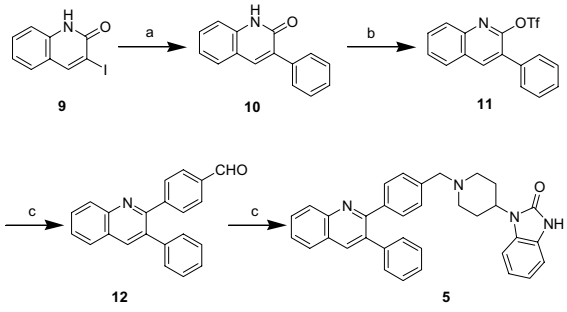


Figure 2. Structures of quinolines **5** and **6**.

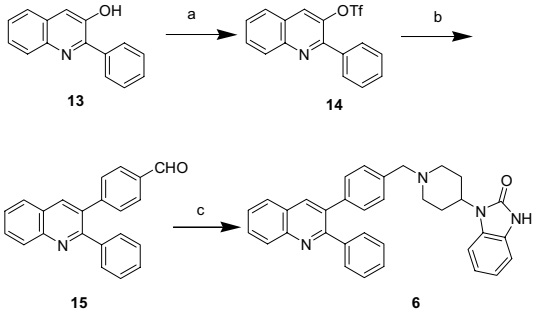


Scheme 1. Reagents and conditions: (a) $PhB(OH)_2$, $Pd(dppf)Cl_2$, Cs_2CO_3, $THF–H_2O$, microwave 150 °C, 10 min, 91%; (b) $(CF_3SO_2)_2O$, pyridine, 0 °C–rt, overnight, 93%; (c) (4-formylphenyl)boronic acid, $Pd(dppf)Cl_2$, Cs_2CO_3, $THF–H_2O$, microwave 150 °C, 10 min, 87%; (d) 1-piperidin-4-yl-1,3-dihydro-2*H*-benzimidazol-2-one, $MP\text{-}BH_3(CN)$, 95%.

A similar sequence was applied to advanced intermediate **13**[7] to deliver quinoline **6** (Scheme 2). As summarized in Table 2, the in vitro Akt1 inhibition activity of quinoline **5** (IC_{50} Akt1 = 365 nM) is almost equal to that of the quinoxaline **1** (Table 1, IC_{50} Akt1 = 295 nM). The Akt2 inhibition by **5** (Table 2, IC_{50} Akt2 = 1205 nM) is even more potent than **1** (Table 1, IC_{50} Akt2 = 2057 nM). Interestingly, regioisomer **5** is 8-fold more potent than the regioisomeric quinoline **6** (Table 2, IC_{50} Akt1 = 3136 nM) for Akt1 inhibition, and this trend is even more pronounced with respect to Akt2 inhibition, wherein **5** is 30 times more potent than **6** (Table 2, IC_{50} Akt2 = 33,660 nM). The large difference in the activities of regioisomers **5** and **6** clearly indicates the importance of the nitrogen position in the quinoline core. Importantly, neither quinoline **5** nor **6** displayed inhibition against Akt3, PKA, PKC or SGK, or the delta-PH Akt mutants, suggesting the allosteric mode of inhibition observed with **1**–**4** was maintained.

The dual Akt1/Akt2 inhibition observed with quinoline **5** prompted us to further increase the basicity of the core heterocyclic template. In an effort to both reduce molecular weight and provide a basic nitrogen, capable of salt formation for solubility studies, the quinoline core was truncated to provide a pyridine template. Based on the SAR generated within **1**–**4**, our initial design placed a cyano group in the 5-position to provide a handle for further manipulation.

As shown in Scheme 3, applying the same chemistry employed for the synthesis of quinoline **5**, a library of cyanopyridine derivatives **7** (32 compounds total, **7a**–**g** shown in Table 3) was prepared from **16**.[7] It should be mentioned that the use of polymer-supported

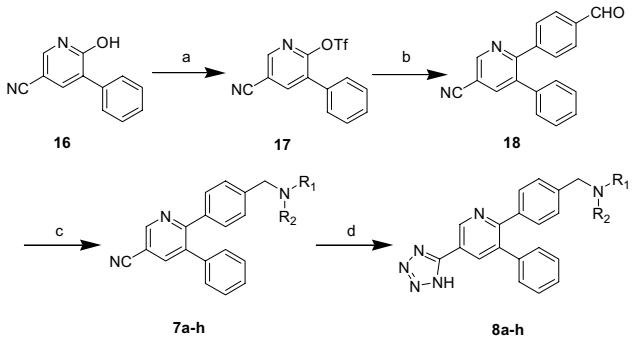


Scheme 2. Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, 0 °C–rt, overnight, 90%; (c) (4-formylphenyl)boronic acid, $Pd(dppf)Cl_2$, Cs_2CO_3, $THF–H_2O$, microwave 150 °C, 10 min, 92%; (c) 1-piperidin-4-yl-1,3-dihydro-2*H*-benzimidazol-2-one, $MP\text{-}BH_3(CN)$, 94%.

Table 2. Activities of quinolines **5** and **6**

Compd	Akt1 IC_{50} (nM)[a]	Akt2 IC_{50} (nM)[a]	Akt3 IC_{50} (nM)[a]
5	365	1205	>50,000
6	3136	33,660	>50,000

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

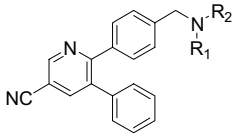


Scheme 3. Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, 0 °C–rt, overnight, 91%; (b) (4-formylphenyl)boronic acid, $Pd(dppf)Cl_2$, Cs_2CO_3, THF–H_2O, microwave 150 °C, 10 min, 87%; (c) amine, MP-$BH_3(CN)$, 82–95%; (d) NaN_3, $ZnBr_2$, H_2O, microwave 185 °C, 20 min, 75–91%.

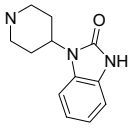
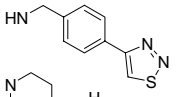
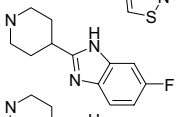
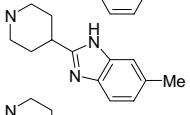
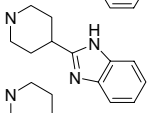
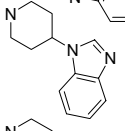
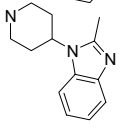
cyanoborohydride reductive amination sequence greatly enhanced the parallel synthesis of the cyanopyridine library **7**. Utilizing a microwave enhanced [3 + 2] cycloaddition of nitrile with NaN_3 under Sharpless conditions,[9] the cyanopyridine library **7** was rapidly converted to another tetrazolylpyridine library **8** (32 compounds total, **8a**–**g** shown in Table 4).

To our delight, the truncated cyanopyridine core derivatives maintain the Akt1/Akt2 inhibitory activities. Comparison of the cyanopyridine **7a** (Table 3, IC_{50} Akt1 = 762 nM, Akt2 = 3139 nM) with the analogous 2,3-diphenylquinoxaline **1** (Table 1, IC_{50} Akt1 = 295 nM, Akt2 = 2057 nM) and 2,3-diphenylquinoline **5** (Table 2, IC_{50} Akt1 = 365 nM, Akt2 = 1205 nM), shows a maintenance of potency and selectivity. Moreover, the tetrazolylpyridine **8a** (Table 4, IC_{50} Akt1 = 273 nM, Akt2 = 157 nM) is a more potent inhibitor of both Akt1 and Akt2 as compared to **1** and **5**. Remarkably, the Akt2 inhibition by tetrazolylpyridine **8a** is 14 and 8 times more potent than that of **1** and **5**, respectively. When comparing the profiles of Akt1/Akt2 inhibition in Tables 3 and 4, it is interesting that the conversion of the cyano group to a tetrazole ring enhances Akt2 activity 6–24 times and Akt1 activity 1–7 times. It should be noted that with the exception of **8b** and **8c**, all other tetrazolylpyridine derivatives in Table 4 are more potent inhibitors of Akt2 than Akt1. This is in contrast to our previous observations in the 2,3-diphenylquinoxalines, quinolines, and cyanopyridines wherein inhibition of Akt1 was more pronounced than Akt2. As with our other series of Akt inhibitors **1**–**6**, **7a**–**g** and **8a**–**g** displayed no inhibition of Akt3, PKA, PKC or SGK, or the delta-PH Akt mutants, suggesting the

Table 3. Structures and Akt1/Akt2 inhibition of cyanopyridines **7**



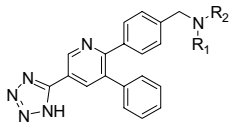
7

Compd	NR_1R_2	Akt1 IC_{50} (nM)[a]	Akt2 IC_{50} (nM)[a]	Akt3 IC_{50} (nM)[a]
7a		762	3139	>50,000
7b		1288	4811	>50,000
7c		865	6650	>50,000
7d		2874	11,410	>50,000
7e		1471	8740	>50,000
7f		4397	13,000	>50,000
7g		7407	18,360	>50,000

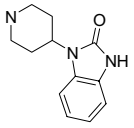
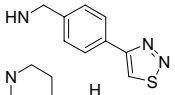
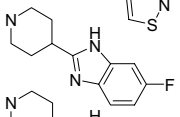
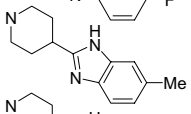
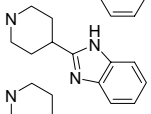
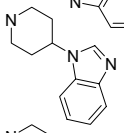
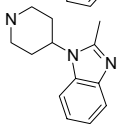
All compounds >50,000 nM versus PKA, PKC, SGK.

[a] Average of at least three measurements; enzyme protocol.[5]

Table 4. Structures and Akt1/Akt2 inhibition of tetrazolylypyridines **8**



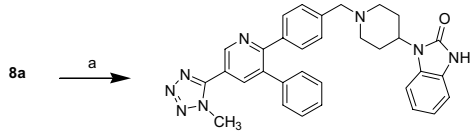
8

Compd	NR_1R_2	Akt1 IC_{50} (nM)[a]	Akt2 IC_{50} (nM)[a]	Akt3 IC_{50} (nM)[a]
8a		273	157	>50,000
8b		177	248	>50,000
8c		239	1720	>50,000
8d		763	422	>50,000
8e		3323	1501	>50,000
8f		4237	1956	>50,000
8g		6915	2746	>50,000

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

allosteric mode of inhibition observed with **1**–**4** was once again maintained.

Importantly, both the cyanopyridine analogs **7a**–**g** and the tetrazolylpyridine analogs **8a**–**g**, displayed excellent aqueous solubility at pH 4.5; however, while **7a**–**g** were cell permeable, **8a**–**g** were not cell permeable possibly due to zwitterionic character and afforded no inhibition of the Akt isozymes in our cell-based IP assay.[10] To further test this theory, **8a** was alkylated with methyl iodide to provide **19**, thereby removing any zwitterionic character (Scheme 4). This modification did improve cell permeability, and **19** was active in the cell-based IP assay; however, inhibition of Akt1 was diminished 8-fold (Akt1 IC_{50} = 1772 nM) while inhibition of Akt2 was reduced by only 3-fold (Akt2 IC_{50} = 613 nM).



19

Scheme 4. Reagents and conditions: (a) MeI, MP-CO_3^{2-}, CH_2Cl_2, 0 °C, 48%.

In conclusion, we have discovered a novel series of potent, allosteric Akt (PKB) kinase inhibitors based on a 2,3,5-trisubstituted pyridine core with improved aqueous solubility, cell permeability and reduced molecular weight relative to our previously reported inhibitors. Further refinements to the pyridine core and in vivo experiments with pyridine analogs are in progress and will be reported in due course.

Acknowledgments

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

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10. Similar behavior was observed with a carboxylate in the 5-position, which was alleviated when the corresponding methyl ester analog was tested, providing further evidence of zwitterionic character affording poor cell permeability in this series.