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Identification of a novel 3,5-disubstituted pyridine as a potent, selective, and orally active inhibitor of Akt1 kinase

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Abstract—Based on lead compounds **2** and **3** a series of 3,5-disubstituted pyridines have been designed and evaluated for inhibition of AKT/PKB. Modifications at the 3 position of the pyridine ring led to a number of potent compounds with improved physical properties, resulting in the identification of **11g** as a promising, orally active Akt inhibitor. The synthesis, structure–activity relationship studies, and pharmacokinetic data are presented in this paper.

The prevalence of enhanced Akt1/protein kinase B (PKB) activity has been observed in a large proportion of human malignancies.[1–4] The initial discovery of Akt1, a serine-threonine kinase, as a viral oncogene[5] was followed by the discovery of homology of Akt1 with PKA and PKC kinases.[6] Three cellular isoforms of Akt1 have been identified.[7,8] Akt plays a pivotal role in several signal transduction pathways[9–11] and is critical in the repression of a number of apoptotic pathways.[12] Overexpression of Akt is linked to an effective survival mechanism for tumor cells and is associated with an increase in tumor progression.[13,14] Hence, inhibition of Akt would be a significant tool in cancer therapy.

We have previously described the discovery of 3,5-disubstituted pyridines as novel small molecule inhibitors of Akt1.[15–17] Considerable exploration of substituents at the 5 position of the pyridine resulted in the identification of several potent and selective inhibitors, compounds **1**–**3** in Figure 1. Optimization of **1** led to **2** and **3** as nanomolar inhibitors of Akt1. In order to further investigate these lead compounds, substituents at the 3 position of the pyridine ring were systematically explored. In this paper, we describe our efforts in this direction and report the synthesis and structure–activity relationship studies of this work.

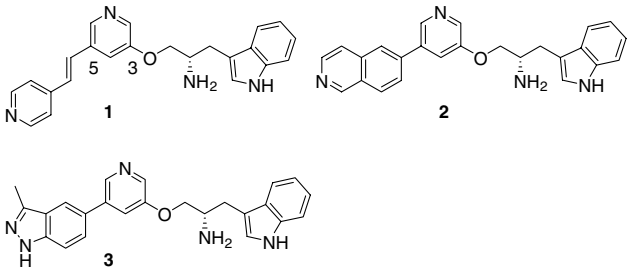

Figure 1. 3,5-Disubstituted pyridines as Akt-1 inhibitors.

The route used to synthesize the 3,5-disubstituted pyridines is shown in Scheme 1. The *N*-Boc phenols were generated using a published procedure.[18] The carboxylic acids used were either commercially available or were prepared by hydrolysis of the corresponding ester with lithium hydroxide. Treatment of the acid with isobutyl chloroformate at −10 °C, followed by a rapid reduction with sodium borohydride, gave high yields of the alcohol **5**. Compound **5** was treated with 3,5-bromohydroxy pyridine under Mitsunobu conditions to generate the 3-oxo-5-bromo-pyridine compound **7**. While DEAD as well as DBAD reagents worked equally well in the Mitsunobu reactions, the purifications of the products

Keywords: AKT/PKB; 3,5-Disubstituted pyridines; AKT inhibitors.
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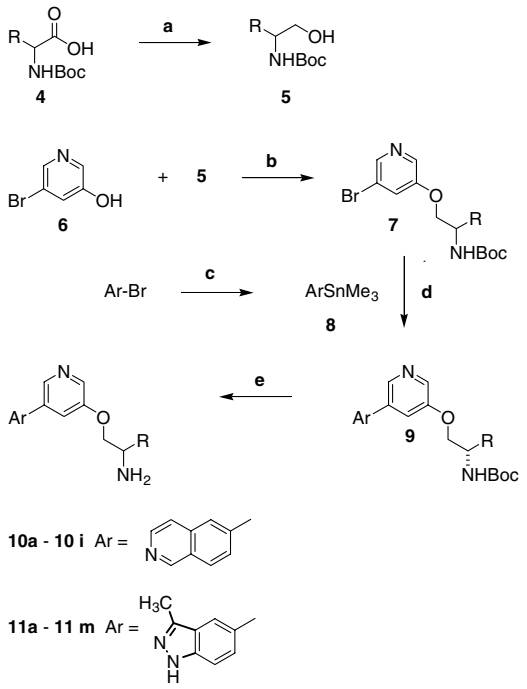
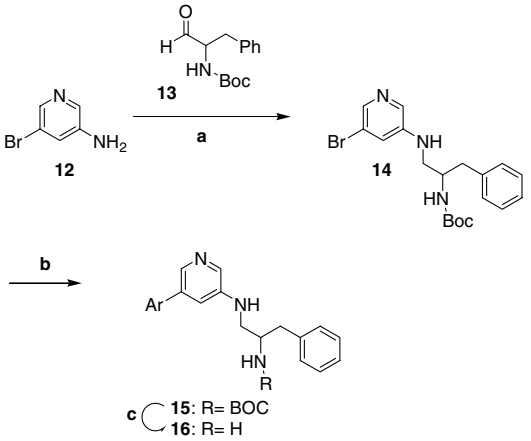


Scheme 1. Reagents and conditions: (a) 1—isobutyl chloroformate, NMP, −10 °C; 2—$NaBH_4$ DMF, −10 °C; (b) Ph_3P, DEAD/DBAD, THF, 0 °C to rt; (c) hexamethylditin, tetrakis(triphenylphosphine)palladium(0), toluene, 100 °C; (d) $ArSnMe_3$, Pd_2dba_3, (2′-dicyclohexylphosphanyl-biphenyl-2-yl)-dimethyl-amine, TEA, DMF, 95 °C; (e) TFA, CH_2Cl_2, rt.

were easier when the DEAD reagent was used. Aryl stannanes **8**, prepared by treatment of the bromo aryl compound with hexamethyl ditin, were used for coupling with the bromo pyridines **7** under Stille conditions to form the N-protected compounds **9**. Finally removal of the *N*-Boc group under acidic conditions yielded target compounds **10** and **11**.

Synthesis of the amino compound **16** is outlined in Scheme 2. Introduction of the amino side chain was carried out by reductive amination of amino pyridine **12** with tryptophanal **13**. The 30% yield obtained in this reaction was caused by the deactivation of the aryl amine by the pyridine ring. Introduction of the aryl group and deprotection of the amine were carried out as previously described.

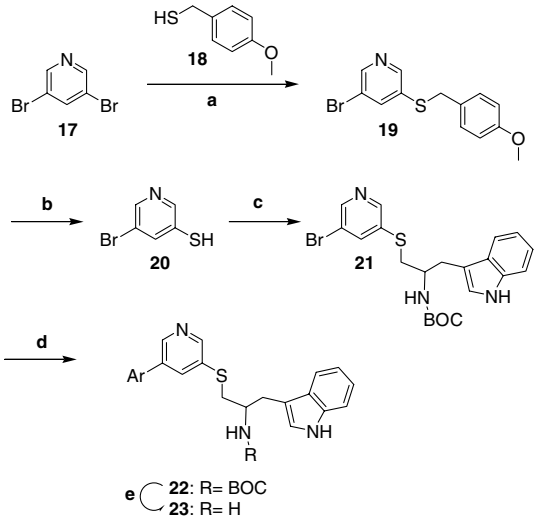


Scheme 2. Reagents and conditions: (a) $NaBH_3CN$, $Ti(iPrO)_4$, EtOH, rt; (b) $ArSnMe_3$, Pd_2dba_3, (2′-dicyclohexylphosphanyl-biphenyl-2-yl)-dimethyl-amine, TEA, DMF, 95 °C; (c) TFA, CH_2Cl_2, rt.

Thio ether **23** was prepared as shown in Scheme 3. Alkylation of the thiophenol **18** with dibromopyridine **17** using sodium hydride, followed by deprotection of the methoxy benzyl group, provided compound **19**. Although Mitsunobu reactions of earlier compounds were generally high yielding, coupling of **19** under Mitsunobu conditions provided only a 41% yield of **21**. Stille coupling followed by removal of the Boc protecting group provided compound **23**.

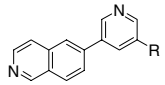
Comparative in vitro studies of the initial compounds prepared are summarized in Tables 1 and 2. The compounds were assayed for their ability to bind to the ATP binding site of Akt.[15] At first we sought to study the effect of replacing the indole group of the lead compound **2** as shown in Table 1. Though these compounds were less active than **2**, the naphthyl analog **10d** and the indazole analog **10b** were reasonably potent. A 5-fold drop in inhibitory potency (**10f** vs **10i**) was observed when the chain length was shortened.

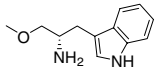
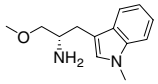
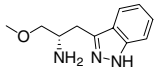
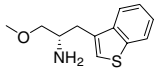
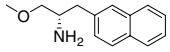
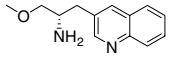
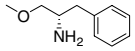
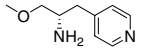
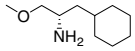
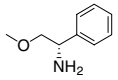
A similar study of analogs of the 3-indazole compound **3** (Table 2) demonstrated the importance of the indole group to the potency of Akt1 inhibition. Analogous to the previous findings, all compounds prepared for this study were less potent than the parent compound **3**. Nonetheless, many of the analogs showed nanomolar Akt1 inhibition. Larger rings such as the naphthyl group (**11c** and **11d**) were tolerated in this position with the 2-methoxynaphthyl derivative **11e** showing 1.1 nM activity. The 2-methyl indole analog **11a** also showed good potency at 2.1 nM and the benzothiophene compound **11b** was a fairly good Akt1 inhibitor (6.6 nM). An



Scheme 3. Reagents and conditions: (a) NaH, DMF, 48 h; (b) TFA, *m*-cresol, reflux; (c) **5**, Ph_3P, DBAD, THF, 0 °C to rt; (d) $ArSnMe_3$, Pd_2dba_3, (2′-dicyclohexylphosphanyl-biphenyl-2-yl)-dimethyl-amine, TEA, DMF, 95 °C overnight; (e) TFA, CH_2Cl_2, rt.

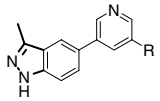
Table 1. Akt1 inhibition of 5-isoquinolinyl pyridines

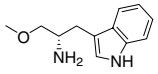
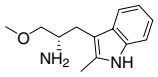
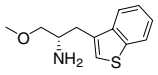
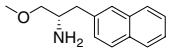
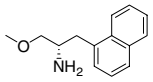
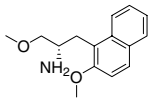
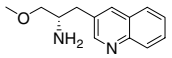
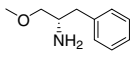
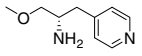
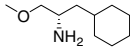
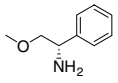


R	Compound	Akt1 inhibition K_i (nM)
	**2**	2
	**10a**	54.6
	**10b**	6.8
	**10c**	63.7
	**10d**	3.5
	**10e**	85
	**10f**	89.6
	**10g**	1525
	**10h**	672
	**10i**	465

Values were measured against Akt1 with ATP concentration of 10 μM.

Table 2. Akt1 inhibition of 5-indazolyl pyridines



R	Compound	Akt1 inhibition K_i (nM)
	**3**	0.16
	**11a**	2.1
	**11b**	6.6
	**11c**	3.2
	**11d**	8.3
	**11e**	1.1
	**11f**	185
	**11g**	11
	**11h**	3282
	**11i**	368
	**11j**	233

Values were measured against Akt1 with ATP concentration of 10 μM.

aromatic ring is required in this position as seen by the loss in potency in going from the phenyl analog **11g** (11 nM) to the cyclohexyl analog (368 nM). The quinoline compounds exhibited diminished activity in both series (**10e** and **11f**) and the 4-pyridyl replacements resulted in a complete loss of activity (**10g** and **11h**).

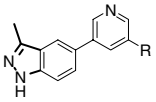
The effect of a decrease or an increase in chain length was demonstrated by **11j**–**11m** in comparison to **11g** (Table 3) and was seen to result in a significant drop in Akt inhibition. Replacement of the ether linkage of compound **11g** with a nitrogen linkage (**16**) resulted in a more potent compound, while replacement of the ether linkage of **3** with sulfur (**23**) proved deleterious to activity.

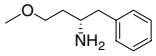
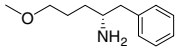
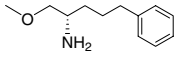
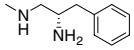
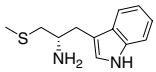
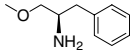
While enzymatic potencies of compounds **2** and **3** were extremely high, we have reported earlier that they exhibited extremely poor aqueous solubility and this was detrimental to the potential development of these compounds. The mouse pharmacokinetic profile of the more potent currently described compounds was evaluated and is shown in Table 4. In this study, **11g** displayed excellent oral bioavailability (67%). While the naphthyl derivatives, in particular **11c**, did exhibit improved PK over the parent **3**, other more potent compounds demonstrated either lower or no oral bioavailability. Interestingly, the isoquinoline analog of **11g**, compound **10f**, was not orally bioavailable.

Antiproliferative activity of the Akt inhibitors was evaluated in a MTT assay[15] and is illustrated in Table 5. Cell growth inhibition determined in MiaPaCa-2 human pancreatic cancer cells indicated reasonable cellular activity in most cases.

The excellent pharmacokinetic profile of **11g** prompted us to carry out a more detailed study of the pharmacokinetics of this compound in other species as shown in Table 6. The results clearly indicate significant oral bioavailability with a good clearance and half-life across species.

Table 3. 5-Indazolyl pyridine analogs



R	Compound	Akt1 inhibition K_i (nM)
	**11k**	289
	**11l**	197
	**11m**	70.6
	**16**	8.3
	**23**	244
	**24**	626

Values were measured against Akt1 with ATP concentration of 10 μM.

Table 4. PK profile of select Akt1 inhibitors in mouse (10 mg/kg po)

Compound	Akt1 inhibition IC_{50} (nM)	PK PO AUC	F (%)
2	2	0	0
3	0.16	0	0
10d	3.5	0.073	3.9
10f	89.6	0	0
11a	2.1	0	0
11b	6.6	0.42	4.2
11c	3.2	0.524	27.6
11e	1.1	0.394	17
11g	11	2.01	67
16	8.3	0	0

Table 5. Cellular activity in MiaPaCa-2

Compound	**2**	**3**	**10d**	**10f**	**11a**	**11b**	**11c**	**11e**	**11g**	**16**
EC_{50} (μM)	0.4	0.1	18	1.0	0.2	0.4	1.9	0.3	0.4	0.2

Table 6. PK profile of **11g** across different species

Species	Dose (mg/kg)	$T_{1/2}$	V_b	Cl	PO AUC	F (%)
Mouse	10	1.0	7.6	5.0	2.0	67
Rat	5	3.6	8.4	1.6	0.94	30
Dog	2.5	3.1	3.9	0.9	1.83	63
Monkey	2.5	NC*	NC*	<0.49	0.68	13

* Not calculated.

Since, as described in our earlier work, the *S*-enantiomer was responsible for Akt1 potency, we had prepared only the *S*-isomers of our analogs. However, the promising profile of **11g** prompted us to prepare the *R*-enantiomer (**24**) as well. We used the route depicted in Scheme 1 for the preparation of **24**. As seen in Table 3, compound **24** exhibited greatly diminished Akt1 inhibition, reiterating the role played by the chirality of the amine toward Akt1 inhibition.

Table 7. Selectivity profile of **11g** for different kinases

Kinase	AKT-1 inhibition K_i (nM)
AKT-1	11
PKA	16
PKCy	1200
PKCd	360
PDK1	>20,000
CDK2	46
ERK2	260
GSK3b	110
MAPK-AP2	1100
CK2	5400
KDR	>3700
SRC	13,000
cKIT	5800
FLT1	>2200
CHK1	2600
RSK2	580

The selectivity profile of **11g** was assessed against a large panel of kinases and is shown in Table 7. Selectivity is always a challenge for kinase inhibitors and the results we obtained were as we had expected. While **11g** exhibited considerable selectivity against less closely related kinases, the high degree of homology between PKA and Akt1 was reflected in the lack of selectivity against PKA.

In summary, we have demonstrated that replacement of the indole moiety in 3,5-disubstituted pyridine analogs can lead to compounds retaining Akt1 inhibitory activity. We selected several compounds for further evaluation and found a number of compounds with improved pharmacokinetic properties. We further identified a compound, **11g**, as a novel, potent, selective, and orally bioavailable inhibitor of Akt1 kinase.

Acknowledgment

We thank Dr. Thomas Penning for helpful discussions and for critical reading of the manuscript.

References and notes

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