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2,3,5-Trisubstituted pyridines as selective AKT inhibitors—Part I: Substitution at 2-position of the core pyridine for ROCK1 selectivity

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ABSTRACT

2,3,5-Trisubstituted pyridines have been designed as potent AKT inhibitors that are selective against ROCK1 based on the comparison between AKT and ROCK1 structures. Substitution at the 2-position of the core pyridine is the key element to provide selectivity against ROCK1. An X-ray co-crystal structure of **9p** in PKA supports the proposed rationale of ROCK1 selectivity.

Activation of oncogenes, such as Ras, ErbB-2, and Src or loss of tumor suppressor genes, such as PTEN, can lead to aberrant signaling in the PI3K/AKT signal transduction pathway.[1] There are three isoforms of AKT kinases, known as AKT1 (PKBα), AKT2 (PKBβ) and AKT3 (PKBγ). All three are up-regulated in different types of cancers including NSCLC (non-small cell lung carcinoma), breast and prostate cancers, making them potential oncology targets. Several small molecule AKT inhibitors have recently been reported[2–5] including the phospholipid perifosine, dual AKT1/2-allosteric diphenylquinolines, and ATP competitive inhibitors, such as GSK690693 (**1**) (see Fig. 1).[6,7]

Kinase selectivity is important because long term inhibition of off-target kinases may cause undesired side effects and toxicity. Following up on our work on the development of GSK690963 (**1**), we overlayed **2a**, an ATP-competitive AKT inhibitor that belongs to a novel series of 3,5-disubstituted pyridine analogs having potent in vitro and in vivo activities,[8] with compound **1** in an AKT2 homology model. This modeling suggested that a C-2 substitution of the core pyridine of **2a** could occupy the space where the 3-hydroxy-3-methyl-butynyl substituent resided in compound **1**. The 3-hydroxy-3-methyl-butynyl substituent was observed to function as an important selectivity element to reduce ROCK1 (Rho kinase)[9] inhibitory activity of this chemical class.[7] AKT and ROCK1 belong to AGC superfamily of Ser/Thr protein kinases, and have Met as the gate keeper residue (Met229 and Met153, respectively). There are different amino acids, Leu 204 for AKT and Met 128 for ROCK1, at the back cleft of the ATP binding pocket, an area in close proximity with the gate keeper. This subtle difference suggested that the gate keeper Met of ROCK1 might favor the adoption of a less flexible conformation than that observed for AKT due to a tighter packing in the ATP back-pocket of ROCK1. Since PKA is a common surrogate of AKT for structural studies,[10] we compared crystal structures of ROCK and PKA. Similar to that of AKT, the gate keeper (Met120) of PKA should be more flexible than that of ROCK1 (Met153) by virtue of a smaller Leu95 residue being in close proximity with Met120 in PKA (see Fig. 2). Therefore, it was reasoned that substitution at the 2-position of the pyridine core might be tolerated in AKT or PKA but not in ROCK1.

We believed that we could design ROCK1 selective AKT inhibitors by incorporating substitution at C-2 of the core pyridine. Therefore, we selected the 3,5-disubstituted pyridine series to test this hypothesis as a means of introducing selectivity against ROCK1 by rational design. Herein, we report the results of those efforts.

The synthesis of the trisubstituted pyridines is outlined in Scheme 1. A Mitsunobu reaction of amino alcohol **3** (either commercially available or prepared by BH_3 reduction of the corresponding carboxylic acid) and 3-bromo-2-chloro-5-hydroxypyridine (**4**)[11] afforded pyridine **5**. Boronate ester **6**, prepared from

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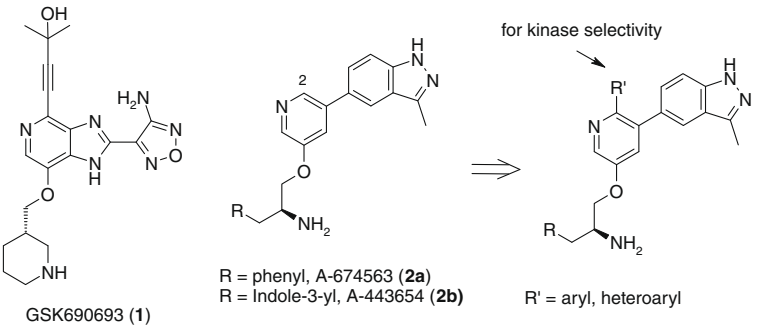


Figure 1. Lead structures.

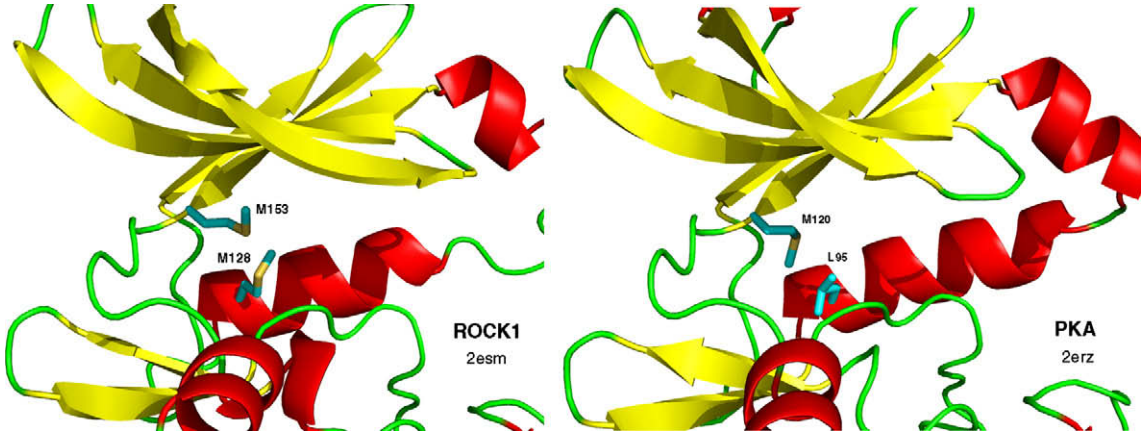
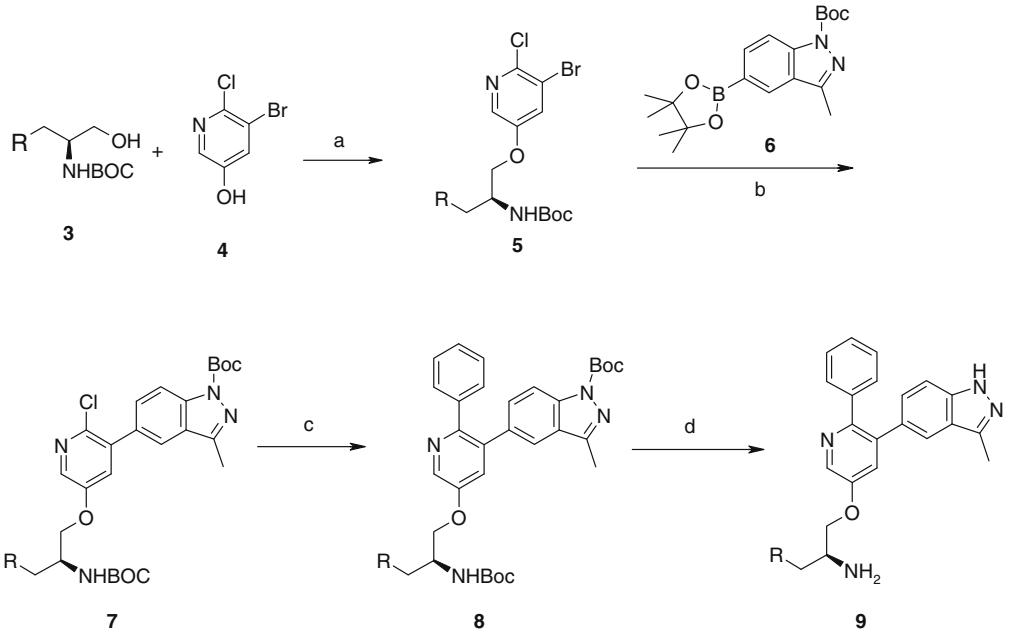


Figure 2. Comparison of the gate keeper Met153 in ROCK1 and Met120 in PKA, a common structural surrogate for AKT.

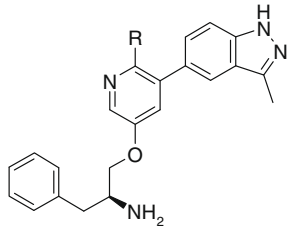


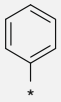
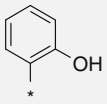
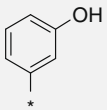
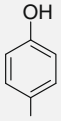
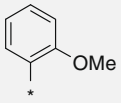
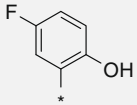
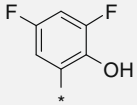
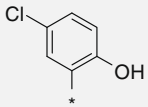
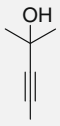
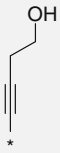
Scheme 1. Synthesis of 2,3,5-trisubstituted pyridine analogs. Reagents and conditions:[13] (a) Ph_3P, DEAD, THF, 1 h (87%); (b) $Pd(PPh_3)_4$, 2 M Na_2CO_3, 1,4-dioxane, 100 °C, overnight (80%); (c) phenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, 1,4-dioxane, microwave irradiation, 160 °C, 20 min (57%); (d) TFA, DCM, 30 min (78%).

its corresponding bromoindazole,[12] was coupled with **5** in a Suzuki coupling reaction to provide **7**. A second Suzuki coupling to install the phenyl ring at the 2-position of the pyridine core was carried out under microwave irradiation conditions, furnishing **8** in good yield. The final de-protection of the BOC group afforded the desired tri-substituted pyridine derivative **9**.

A variety of analogs with a range of C-2 substituents were prepared by this route. A Stille or Negishi coupling reaction was also used if the corresponding organo-tin or organo-zinc reagents were available. In the cases of **9n** and **9o**, a Sonogashira reaction was applied as previously described.[7] The representative analogs are listed in Table 1.

Table 1
2-Substitution to improve selectivity



Compd	R	IC_{50} (μM)	
		AKT1	ROCK1
2a	H	0.125[a]	0.079
9a		0.050[a]	>10
9b		0.015[a]	9.5
9c		0.079[a]	>10
9d		0.50[a]	1.4
9e		0.10[a]	>10
9f		0.010[c]	9.8
9g		0.063[b]	>10
9h		0.003[a]	7.6
9i		0.79[c]	1.29
9j		0.010[a]	5.2
9k		0.079[a]	2.5
9l		0.050[a]	6.0
9m		0.015[a]	>10
9n		10.5[c]	>10
9o		0.72[b]	>10

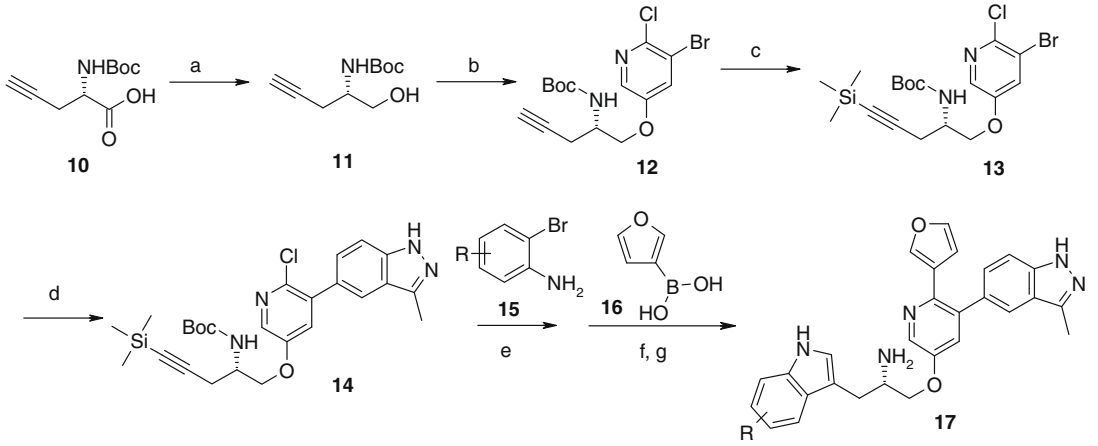
[a] Values were measured at or below K_m for ATP; $n \geqslant 2$.
[b] $n = 1$.
[c] Data were generated using truncated AKT1 enzyme. For this chemical series, the data generated from the truncated AKT1 (without PH domain) correlate well with those from the full length AKT1; $n \geqslant 2$.

As shown in Scheme 2, the phenyl group in the amino-aryl side chain was also replaced by a substituted indole ring, which was introduced via Larock's indole synthesis as the key step, starting from substituted bromoanilines and silylated alkynes.[14] Commercially available chiral amino acid **10** was reduced with $LiAlH_4$ to afford alcohol **11**, which was coupled with hydroxypyridine under Mitsunobu reaction conditions to afford compound **12**. The TMS group was introduced onto the terminal alkyne to give **13**, which was coupled with boronate ester **6** under microwave assisted Suzuki reaction conditions to afford **14** in good yield. Treatment of compound **14** with substituted bromoanilines followed by a second Suzuki coupling with 3-furanyl boronic acid were carried out in one pot under microwave irradiation at 170 °C. TFA deprotection of the Boc group gave the target molecules **17**. Additional azaindole analogs (**18a–c**, see Table 2) were prepared in a similar manner from compound **14** and different regioisomers of ortho- amino-bromo-pyridines.

Consistent with our hypothesis that substitution at the 2-position of the core pyridine would diminish ROCK1 inhibition, a >200-fold improvement in selectivity over ROCK1 was achieved when comparing compound **9a** and compound **2a** (see Table 1). In addition, compound **9a** demonstrated nearly 3-fold higher AKT1 potency, while compound **2a** was observed to be approximately equipotent against ROCK1 and AKT1.

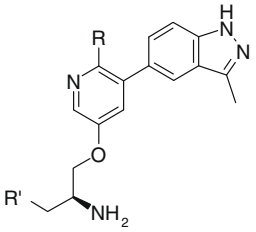
Table 1 shows the SAR preferences of the substitution at C-2 of the pyridine core. Besides phenyl, *ortho*-phenol of **9b** was also tolerated, providing excellent selectivity against ROCK1. The halogenated *ortho*-phenol analogs (compounds **9f-h**) were observed as being potent and selective, especially compound **9h**, which demonstrated single digit nanomolar potency against AKT1 and greater than 2,000-fold selectivity over ROCK1. However, the *meta*- and *para*-phenol derivatives (**9c** and **9d**, respectively) were less potent AKT1 inhibitors. Likewise, the *ortho*-anisole **9e**, demonstrated reduced potency against AKT1.

Incorporation of five membered heterocycles at C-2 position was also investigated. The 3-furanyl derivative **9j** was observed to be much more potent and selective than the 2-furanyl regio iso-



Scheme 2. Synthesis of substituted tryptophanol analogs. Reagents and conditions: (a) $LiAlH_4$, THF, rt; (b) **4**, Ph_3P, DEAD, THF, 0 °C, rt, overnight (82% for 2 steps); (c) EtMgBr, −36 °C, then TMSCl, (72%); (d) **6**, $Pd(Ph_3P)_4$, aq Na_2CO_3, dioxane, microwave irradiation, 160 °C, 30 min (83%); (e) **15**, $Pd(Ph_3P)_4$, aq Na_2CO_3, dioxane, microwave irradiation, 170 °C, 30 min; (f) **16**, microwave irradiation, 170 °C, 30 min; (g) TFA, DCM, RP-HPLC purification.

Table 2
Side chain modification to improve potency



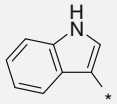
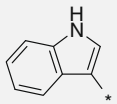
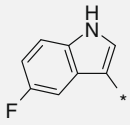
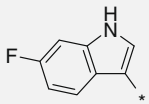
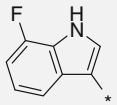
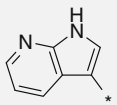
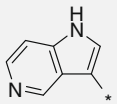
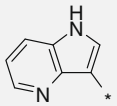
Compd	R	R'	IC_{50} (μM)	
			AKT1[a]	pGSK3 BT474
9p			0.001	0.50
9q			0.0008	0.084
17a			0.001	0.36
17b			0.0008	0.22
17c			0.001	0.26
18a			0.008	0.93
18b			0.013	6.8

Table 2 (*continued*)

Compd	R	R'	IC_{50} (μM)	
			AKT1[a]	pGSK3 BT474
18c			0.010	4.3

[a] Values were measured at or below K_m for ATP; $n \geqslant 2$.

mer **9i**. The 2-pyrrolyl analog **9m** was also very potent and selective, while thienyl derivatives **9k** and **9l** were less potent against AKT1. The 3-hydroxy-3-methyl-butynyl substituent, which was present in GSK690693 (**1**), was introduced at C-2 (**9n**). However, this compound was significantly less potent within this compound class whereas the 4-hydroxyl-butynyl substituted compound **(9o)** was better tolerated (see the discussion on X-ray co-crystal structures of GSK690693 and **9p**).

To further improve enzymatic potency of this chemical class against AKT, the *S*-phenylalaninol side chain was replaced with *S*-tryptophanol following the same chemistry scheme as described in Scheme 1.[15] Compared to compound **9j**, compound **9p** displayed a 10-fold increase of potency against AKT1. Further SAR studies revealed that the 2-methyl-3-furan[16] group at 2-position of the core pyridine provided the most significant increase in activity (e.g., **9q** vs **9p** in Table 2), wherein compound **9q** was highly potent in cellular mechanistic assay (BT474 cell line) measuring the reduction of pGSK3β.[7] As shown in Table 2, the 5-, 6-, and 7-fluorinated indole analogs (**17a**, **17b**, and **17c**) were equipotent against AKT1 and in the cellular assay, compared to the parent compound **9p**. However, azaindole modification was less tolerated, as most derivatives lost cellular potency (**18a**–**18c**).

Compound **9p** was successfully co-crystallized with PKA.[18,19] As shown in Figure 3, the binding interactions of **9p** were similar to those reported for **2b**. The indazole ring acts as a two-point hinge binder, the core-pyridine nitrogen interacts with Lys72, and the charged amino group on the side chain forms H-bond interactions with both Asn171 and Asp184. As predicted, the furan ring at the 2-position of the core pyridine pointed to the gate keeper Met120 and pushed its side chain back towards Leu95. Comparison with the co-crystal structure of compound **1** in PKA showed that

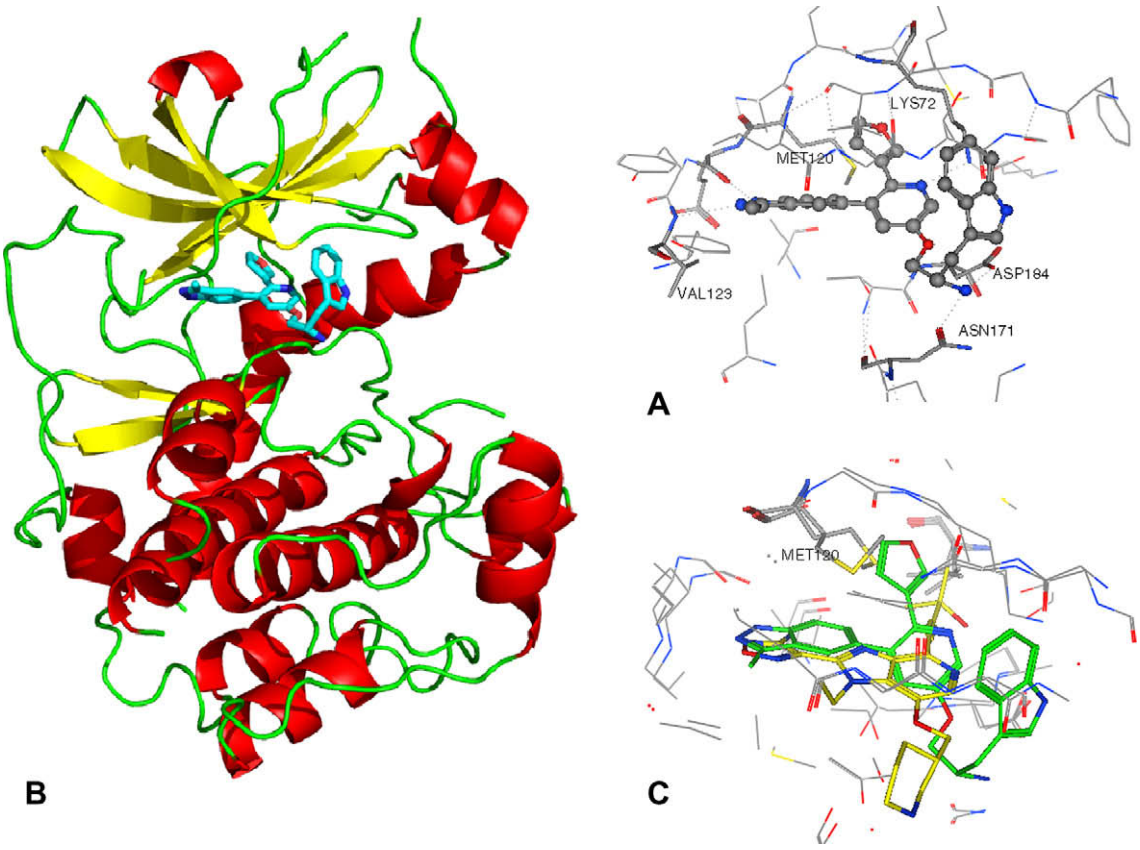


Figure 3. Co-crystal structure of **9p** in PKA and overlay of co-crystal structures of **9p** and **2** in PKA. (A) Compound **9p** co-crystal structure in PKA. Hydrogen bonds are indicated as dotted lines. Key interactive residues are shown in stick. (B) Ribbon diagram representation of PKA co-crystallized with **9p**. (C) Overlay of co-crystal structures of **9p** (green) and **2** (yellow) in PKA.

the furan ring and the propargyl alcohol have different trajectories, so it is not surprising that the latter does not give any benefit in terms of potency or selectivity in the pyridine series (compounds **9n** and **9o** in Table 1).

In summary, we have prepared potent AKT inhibitors from a tri-substituted pyridine series. We have successfully improved selectivity against ROCK1 based on rational design, wherein an X-ray co-crystal structure supported our rationale for selectivity.

Acknowledgments

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12. Reagents and conditions for this transformation: Pd_2dba_3, PCy_3, bis(pinacolato)diboron, KOAc, dioxane, 80 °C, 24h (74%).
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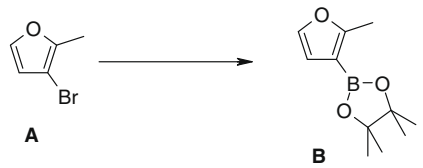
15. This modification did not affect ROCK1 selectivity. For example, compound **9p** was a very weak ROCK1 inhibitor with IC_{50} of 1.58 μM.



16. 2-Methylfuran-3-yl was introduced via a Suzuki coupling reaction with boronate ester **B**, which was prepared by a sequence of halogen- metal exchange and quenching the anion with 4,4,5,5-tetramethyl-2-[(1-methylethyl)oxy]-1,3,2-dioxaborolane on about 150 mmol scale of 3-bromo-2-methylfuran (**A**).[17] This boronate ester is volatile and can be purified either by flash column chromatography or by vacuum distillation.
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18. Crystallographic data for compound **9p** has been deposited to the RSBC Protein Data Bank with deposition code 3kkv.
19. Distorsion around Phe54 was observed in the co-crystal structure of compound **1** in PKA indicating the flexibility of this residue.