Bioorganic & Medicinal Chemistry Letters 20 (2010) 684–688

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Tetrasubstituted pyridines as potent and selective AKT inhibitors: Reduced CYP450 and hERG inhibition of aminopyridines

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ARTICLE INFO

Article history:
Received 25 September 2009
Revised 11 November 2009
Accepted 16 November 2009

Keywords:
AKT kinase inhibitors
Aminopyridines

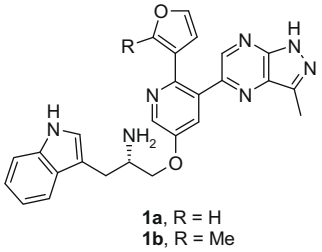
ABSTRACT

The synthesis and evaluation of tetrasubstituted aminopyridines, bearing novel azaindazole hinge binders, as potent AKT inhibitors are described. Compound **14c** was identified as a potent AKT inhibitor that demonstrated reduced CYP450 inhibition and an improved developability profile compared to those of previously described trisubstituted pyridines. It also displayed dose-dependent inhibition of both phosphorylation of GSK3β and tumor growth in a BT474 tumor xenograft model in mice.

In the preceding communications,[1,2] we disclosed the development of a potent and selective series of AKT inhibitors based on a trisubstituted pyridine core and novel azaindazole hinge binders. Our efforts culminated in the identification of compound **1a** (Fig. 1), which displayed improved kinase selectivity and drug-like properties with respect to its indazole analog.[2] However, compound **1a** was observed to be a sub-micromolar CYP3A4 inhibitor, and its methyl analog **1b** was found to be a potent hERG channel inhibitor when tested in a functional assay (IC_{50} = 1.4 μM, see Fig. 1).[3] Despite efforts to reduce lipophilicity of the trisubstituted pyridine derivatives by replacing indazole with azaindazoles,[2] potent CYP3A4 inhibition persisted and this remained a daunting problem. We attempted to replace the pyridine core with pyridazine or pyrazine, but this only led to inactive compounds (data not shown). We then turned our attention to introducing small groups next to the pyridine nitrogen atom to increase steric bulkiness, which we believed might help to reduce the CYP450 liability.

Introduction of an amino group onto the pyridine core led to appreciably reduced CYP450 and hERG inhibition relative to the parent trisubstituted pyridine series. Our efforts resulted in the identification of compound **14c**, which demonstrated an improved developability profile, and achieved a robust pharmacodynamic effect and tumor growth inhibition in BT474 tumor xenograft model in mice. Herein, we wish to report the synthesis and evaluation of compound **14c** and related tetrasubstituted pyridine analogs.

The synthesis of tetrasubstituted pyridines **14a–c** commenced from compound **2** and proceeded via pyridine N-oxide **3**, a versatile intermediate for subsequent introduction of small groups adjacent to the ring nitrogen atom.[4] Taking advantage of this, compound **3** was subjected to Mitsunobu coupling conditions to generate intermediate **5**, which was converted to tetrasubstituted aminopyridine **6a** with tosylsulfonyl chloride (TsCl), pyridine and ethanolamine.[5,6]



1a, R = H
1b, R = Me

Figure 1. Trisubstituted pyridines as AKT inhibitors.

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doi:10.1016/j.bmcl.2009.11.061

A Suzuki coupling reaction of **6a** with indazole boronate ester **7a** afforded compound **8a** and a second Suzuki coupling reaction of **8a** with 2-methyl-furanylboronate ester **9**[1] afforded **10a**. Boc deprotection under standard conditions furnished the target molecules **14a**. 7-Azaindazole **14b** was prepared in a similar manner as that of **14a**. For the Suzuki coupling reactions, it was found that the use of a bi-dentate ligand on the catalyst, such as $Pd(dppf)Cl_2$, worked better for these aminopyridine substrates.

For the synthesis of 4,7-diazaindazole derivative **14c**, a modified approach was applied since preparation of the corresponding boronate ester of **12** was not successful and the corresponding stannane reagent could not be conveniently prepared on large scale with sufficient purity. Thus, tetrasubstituted aminopyridine **6a** was converted to boronate ester **11**, which was used as a crude reaction mixture without purification since it was prone to proteo-deboronation. A Suzuki coupling reaction of compound **11** and 5-bromo-4,7-diazaindazole **12**[2] afforded a mixture of Boc-protected **13a** and deprotected **13b** under these basic conditions.[7] This mixture was subjected to a subsequent Suzuki reaction with compound **9**, followed by Boc deprotection to afford target molecule **14c**.

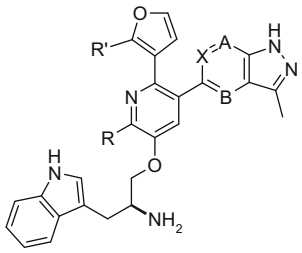
The cyano and methyl group were introduced at the 6-position of the pyridine core via N-oxide **5** according to the literature procedures[8,9] and the targeted molecules (i.e., compound **15** and **16** in Table 1) were prepared following the route depicted in Scheme 1.

The 6-amino group was also introduced by regio-selective nitration of compound **2**. This alternative synthetic route produced a number of analogs, such as 6-azaindazole **14d** exemplified in Scheme 2. The pyridine alcohol **2** was first nitrated at the 6-position using standard conditions, followed by reduction of the nitro group with iron in acetic acid to furnish compound **17** in good overall yield. Boc protection of the 6-amino group followed by a Mitsunobu reaction with *N*-Boc protected amino alcohol **4** furnished compound **18** in excellent yields. Conversion of compound **18** to the corresponding boronate ester **19** was performed under similar conditions to those described for preparing **11**. Subsequent introduction of the 6-azaindazole **20**[10] under Suzuki conditions furnished the elaborated compound **21** in moderate to good yields. A final Suzuki coupling between compound **21** and boronate ester **9**, followed by deprotection of the benzenesulfonyl and Boc protecting groups using standard methods, furnished compound **14d** in good overall yields.

We also prepared tetrasubstituted pyridine analogs with the substituents at the 2-position of the indole to evaluate the impact of such a modification on AKT activity and CYP450 inhibition (compounds **25a–c** in Table 2).

Synthesis of requisite side chains **24a** and **24b** is depicted in Scheme 3. Following efficient conversion of tryptophanol **4** to the fully protected derivative **22**, regio-controlled lithiation/deprotonation of the 2-position of the indole ring became possible upon treatment with LDA at low temperature. This process was conveniently facilitated by the directing effect of the neighboring benzenesulfonamide group.[11] Quenching the anion derived from **22** with benzenesulfonyl chloride or iodine then led to **23a** and **23b**, respectively. The latter was then converted to nitrile **23c** with $Zn(CN)_2$ under microwave irradiation conditions in the presence of Fu's catalyst.[12] Deprotection of the acetonide under acidic conditions also removed the Boc protecting group, which was put back on with Boc_2O to give **24a** or **24b**. These two chiral aminoalcohols were subjected to the chemistry highlighted in Scheme 2 to afford the final products **25a** and **25b**. The cyano group of **25b** was partially hydrolyzed to give carboxamide **25c**.

The effects of small substitution adjacent to the pyridine nitrogen atom on CYP450 inhibition and potency are summarized in Table 1. In general, introduction of an amino group maintained or slightly increased the enzymatic potency against all three AKT isoforms, as well as cellular potency in both anti-proliferation and mechanistic assays (**14a**, **14b**, **14c**).[13] The amino group also helped to reduce CYP450 3A4 inhibition by about eightfold (**1b** and **14c**). Compound **14c** demonstrated reduced hERG channel inhibitory potency (IC_{50} = 10.4 μM, see Fig. 2) in comparison to compound **1b**.[3] The analogs containing either a cyano or methyl group at the 6-position of the core pyridine did not demonstrate cellular potency even though these analogs were potent AKT inhibitors in enzymatic assays. Furthermore, these compounds did not appreciably reduce CYP450 inhibition.

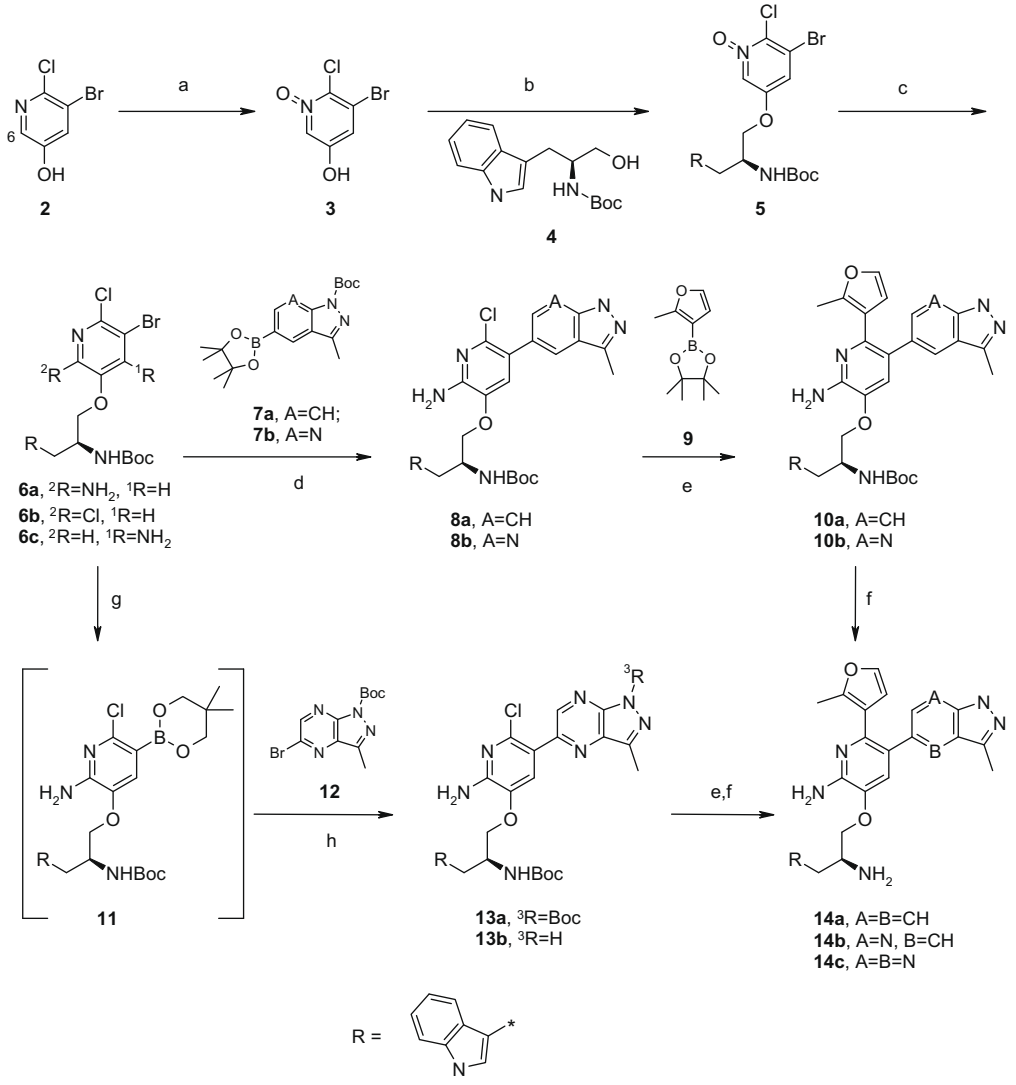


Encouraged by the tolerability of this amino group and the advantage it brought to reduce CYP450 inhibition, substituted amine analogs at the 6-position of the core pyridine were prepared through standard reductive amination reactions. Unfortunately, this position did not tolerate any steric bulkiness, as even the simple ethylamine analog **26b** lost about 100-fold potency (Table 2). Alternatively, substitution of small group at the 2-position of the indole ring seems to be well tolerated as compounds **25a–c** were potent AKT inhibitors in enzymatic assays. However, cyano analog **25b** and carboxamide analog **25c** seemed to lose cellular potency. Furthermore, they were observed to have similar potency against p450 3A4 compared to compound **14c**.

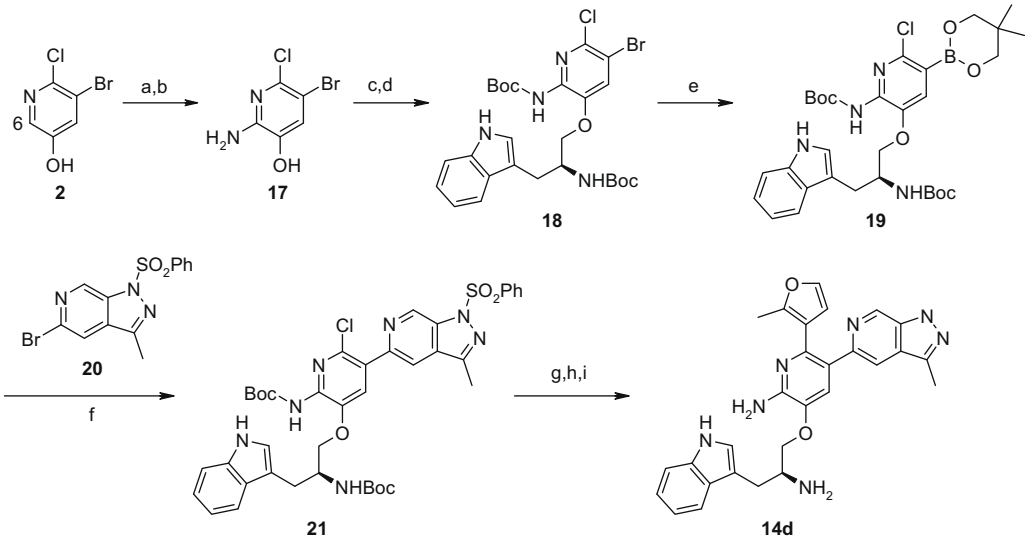
Table 1
Tetrasubstituted pyridine analogs with indazole and azaindazole hinge binders

Compd	A	B	X	R	R′	AKT1/2/3[a] IC_{50} (μM)	BT474[b] IC_{50} (μM)	p-GSK3β BT474[c] IC_{50} (μM)	CYP450 3A4 IC_{50} (μM)
1b	N	N	CH	H	Me	0.001/0.025/ND	0.29	0.21	0.63
14a	CH	CH	CH	NH_2	Me	0.003/0.015/0.001	0.20	0.22	0.10
14b	N	CH	CH	NH_2	Me	0.001/0.015/0.002	0.47	0.47	1.0
14c	N	N	CH	NH_2	Me	0.001/0.019/0.002	0.31	0.56	5.0
14d	CH	CH	N	NH_2	Me	0.001/0.013/0.003	0.30	0.68	1.3
15	N	N	CH	CN	H	0.010/0.25/0.019	ND	>10	0.79
16	N	N	CH	Me	H	0.003/0.063/0.010	1.26	0.28	0.63

[a] $n \geqslant 2$.
[b] Inhibition of cell proliferation.
[c] Inhibition of phosphorylation of GSK3β.

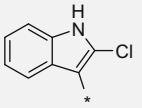
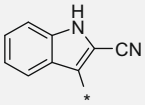
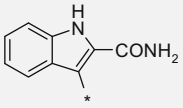


Scheme 1. Synthesis of tetrasubstituted pyridine analogs via pyridine N-oxide intermediates. Reagents and conditions: (a) TFAA, urea·H_2O_2 (UHP), DCM, 0 °C to rt, 2 h, 88%; (b) Ph_3P, DEAD, THF/DMF (6:1); (c) TsCl, pyridine, ethanolamine; (d) $Pd(Ph_3P)_4$, 2 N Na_2CO_3 aq, 90 °C, 4 h; (e) $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, 5 mol %, 2 N Na_2CO_3, 110 °C, 4 h; (f) TFA, DCM, RP-HPLC purification; (g) 5,5,5′,5′-tetramethyl-2,2′-bi-1,3,2-dioxaborinane, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, KOAc, dioxane, 80 °C, 16 h; (h) $Pd(Ph_3P)_4$, 2 N Na_2CO_3 aq, 90 °C, 4 h.

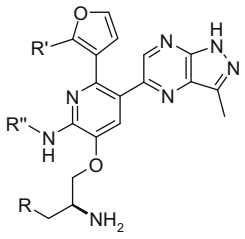


Scheme 2. Synthesis of tetrasubstituted aminopyridine analog via regio-selective nitration. Reagents and conditions: (a) HNO_3/H_2SO_4, 0 °C to rt, 16 h, 65%; (b) Fe, acetic acid, 5 °C, 1.5 h, 97%; (c) NaHMDS, $(Boc)_2O$, THF, −78 °C to rt, 3 h, 79%; (d) **4**, DIAD, PPh_3, DMF/THF (10:1), 0 °C to rt, 4 h, 92–100%; (e) $Pd(dppf)Cl_2$, KOAc, THF, reflux, 7 h, 83–87%; (f) $Pd(dppf)Cl_2$, 2 N aq Na_2CO_3, THF, reflux, 2 h, 48–71%; (g) **9**, $Pd(PPh_3)_4$, 2 N aq Na_2CO_3, THF, reflux, overnight, 47–82%; (h) 6 N NaOH, THF/isopropanol (1:2), rt to 60 °C, 1 h; (i) TFA, CH_2Cl_2, rt, 2 h, 54–81% overall.

Table 2
SAR of aminopyridine 4,7-diazaindazole analogs

Compd	R	R′	R″	AKT1/2/3[a] IC_{50} (μM)	BT474[b] IC_{50} (μM)	p-GSK3β BT474[c] IC_{50} (μM)	P450 3A4 IC_{50} (μM)
26a		Me	H	0.006/0.13/0.006	0.58	0.28	3.2
26b		H	Et	0.50/6.3/0.50	3.9	>10	5.0
25a		Me	H	0.001/0.012/0.002	0.15	0.30	0.50
25b		Me	H	0.001/0.007/0.001	2.8	>30	1.6
25c		Me	H	0.001/0.015/0.001	>10	>30	4.0

[a] $n \geqslant 2$.
[b] Inhibition of cell proliferation.
[c] Inhibition of phosphorylation of GSK3β.



Despite the introduction of an amino group adjacent to the core pyrinde nitrogen atom, compound **14c** maintained selectivity when compared to the corresponding trisubstituted analog **1b**. As shown in Figure 3, compound **14c** displays greater than 1000-fold selectivity over representative kinases from many different families except for PAK1. However, achieving selectivity over PKA, a very close relative of AKT in AGC superfamily, remains challenging.

The pharmacokinetic profile of compound **14c** was evaluated in mouse, rat, dog and monkey (see Table 3). Higher exposure and lower clearance in all species were observed with **14c** upon intravenous (iv) or intraperitoneal (ip) administration. Like **1a**, this compound was not observed to be orally available.

Compound **14c** was then evaluated in mice using BT474 tumor xenograft model. As shown in Figure 3A, compound **14c** demonstrated a significant pharmacodynamic effect in a dose dependent manner. To further establish the anti-tumor activity in vivo, compound **14c** was dosed intraperitoneally (ip) for 21 days (QD) in the same xenograft model (Fig. 4B). Dose-dependent inhibition of

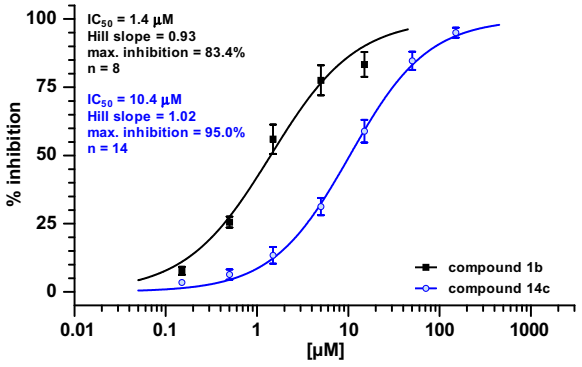
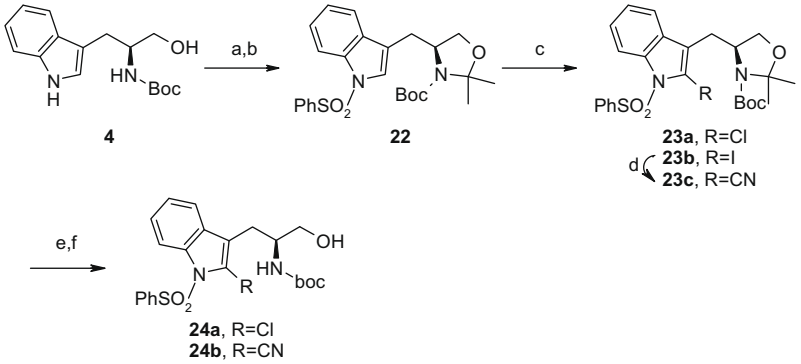


Figure 2. Compound **1b** and **14c** mediated block of hERG current.



Scheme 3. Synthesis of the substituted tryptophanols with small groups at 2-position of the indole ring. Reagents and conditions: (a) 2,2-bis(methyloxy)propane, DMF, $TsOH{\cdot}H_2O$, rt, 3 h, 74%; (b) NaH, $PhSO_2Cl$, DMF, 0 °C, 70%; (c) LDA, THF, −78 °C, $PhSO_2Cl$ (**23a**, 79%) or I_2 (**23b**, quantitative); (d) $Zn(CN)_2$, $Pd(tBu_3P)_2$, DMF, microwave irradiation, 150 °C, 30 min, 85%; (e) 1 N HCl (aq)/MeOH/THF 3:2:1 v/v/v, 80 °C; (f) Boc_2O, $NaHCO_3$ (satd aq), DCM, 82% over two steps.

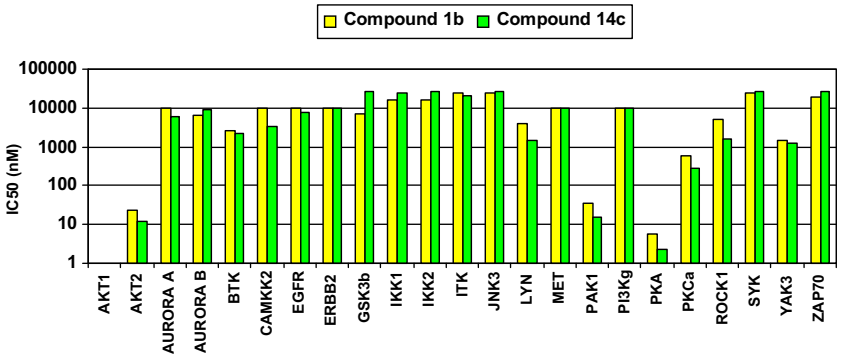


Figure 3. Comparison of the kinase selectivity between compound **1b** and aminopyridine analog **14c**.

Table 3
Pharmacokinectic profiles of compounds **1a** and **14c**

Compound	Species	Admin.	Dose mg	DNAUC$_{(0\text{-}t)}$ ng[a] h/mL/mg	Cl mL/min/kg
1a	Mouse	ip[a]	9.6	96	ND
14c		iv[a]	2.2	170	98.9
1a	Rat	iv[b]	10.6	301	53.2
14c		iv[b]	2.4	303	46.0
1a	Dog	iv[b]	2.4	425	34.4
14c		iv[b]	3.1	630	25.3
1a	Monkey	iv[b]	3.1	386	39.1
14c		iv[b]	3.0	853	22.3

[a] Bolus.
[b] Infusion.

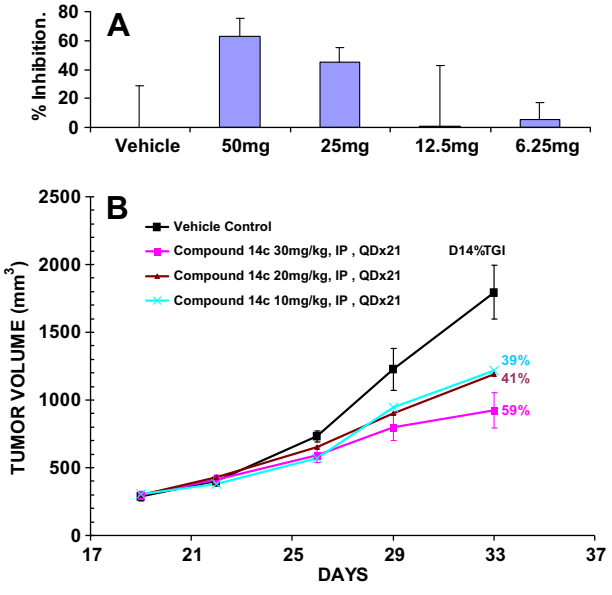


Figure 4. (A) Effect of compound **14c** (IP) on GSK3β phosphorylation in BT474 tumor xenograft mouse model. (B) Effect of compound **14c** on BT474 tumor growth in xenograft mouse model.

BT-474 tumor growth was observed, wherein a 59% reduction in tumor volume, relative to the control group, was observed at 30 mg/kg dose level.

In summary, we have identified the novel tetrasubstituted aminopyridine **14c** as a potent and selective AKT inhibitor. Compound **14c** demonstrated a robust in vivo pharmacodynamic effect and dose-dependent inhibition of tumor growth in a BT474 xenograft model. Furthermore, compound **14c** displayed much reduced CYP450 inhibition, hERG channel inhibition, and improved pharmacokinetic properties compared to the previously described trisubstituted pyridines. Therefore, compound **14c** has a suitable profile to warrant further evaluation as an iv clinical development candidate.

Acknowledgements

The authors thank Dr. Arthur Shu for preparing compound **14c** in large scale, and thank Dr. Karl Erhard for the final purification of compound **14c** to support preclinical toxicity study.

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13. The observed activity of both compound **1b** and **14c** approached to the detection limit in the standard AKT1 enzymatic assay. Hence, the empirical pK_i^* values of 9.30 for compound **1b** and 9.63 for compound **14c** represent a more accurate measure of enzyme potency. For details of the assay conditions, see: Heerding, D. A.; Rhodes, N.; Leber, J. D.; Clark, T. J.; Keenan, R. M.; Lafrance, L. V.; Li, M.; Safonov, I. G.; Takata, D. T.; Venslavsky, J. W.; Yamashita, D. S.; Choudhry, A. E.; Copeland, R. A.; Lai, Z.; Schaber, M. D.; Tummino, P. J.; Strum, S. L.; Wood, E. R.; Duckett, D. R.; Eberwein, D.; Knick, V. B.; Lansing, T. J.; McConnell, R. T.; Zhang, S.-Y.; Minthorn, E. A.; Concha, N. O.; Warren, G. L.; Kumar, R. *J. Med. Chem.* **2008**, *51*, 5663.