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New thiazole carboxamides as potent inhibitors of Akt kinases

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ABSTRACT

A new series of 2-substituted thiazole carboxamides were identified as potent pan inhibitors against all three isoforms of Akt (Akt1, Akt2 and Akt3) by systematic optimization of weak screening hit *N*-(1-amino-3-phenylpropan-2-yl)-2-phenylthiazole-5-carboxamide (**1**). One of the most potent compounds, **5m**, inhibited the kinase activities of Akt1, Akt2 and Akt3 with IC_{50} values of 25, 196 and 24 nM, respectively. The compound also potently inhibited the phosphorylation of downstream MDM2 and GSK3β proteins, and displayed strongly antiproliferative activity in prostate cancer cells. The inhibitors might serve as lead compounds for further development of novel effective anticancer agents.

Akt, also known as protein kinase B (PKB), is a class of serine/threonine kinases and consists of three isoforms, Akt1 (PKBα), Akt2 (PKBβ) and Akt3 (PKBγ). Although the three isoforms share significant sequence identity, they possess disparate functions.[1,2] Akt represents a key node in the PI3K-PKB-mTOR pathway and is located at the crucial crossroads of various upstream signaling events.[3,4] Almost all the known oncogenic growth factors, angiogenic factors, cytokines and steroid hormones regulate cell growth and proliferation, cell survival protein synthesis, glucose uptake and metabolism by activation of Akt.[5] Deregulation of Akt through mutation or amplification of upstream PI3K, loss of PTEN function and activation of oncogenic receptor kinases results in aberrant signaling.[6–8] Constitutive activation and/or overexpression of Akt have been observed in a variety of human malignancies and render cancer cells resistant to chemotherapy or molecular target drugs.[6] Therefore, Akt has been suggested as one of the most attractive targets for new anticancer drug development. Several classes of ATP-competitive or allosteric small molecular Akt inhibitors[9–18] have been discovered, among which GSK2141795 and MK-2206 have been recently approved by FDA for clinical trials.[19,20]

As a part of our anticancer drug discovery program, a random screening on a self-established focused library was performed to search for new potential Akt inhibitors by a FRET-based Z'-Lyte assay.[21] Among all of the compounds tested, *N*-(1-amino-3-phenylpropan-2-yl)-2-phenylthiazole-5-carboxamide (**1**) (Fig. 1) dose-dependently inhibited the enzymatic activity of Akt1 with an IC_{50} value of 40.0 μM. Interestingly, hydroxyl substituted derivatives **2** and **3** displayed obviously improved activities against Akt1 (IC_{50} values of 8.0 and 1.7 μM, respectively), which might serve as new promising lead compounds for further studies. A structural feature analysis suggested that compounds **1**, **2** and **3** shared great similarity with a well characterized pan Akt inhibitor **4**.[10] Therefore, compound **1** was extensively optimized by taking advantage of the structure–activity relationship information of inhibitor **4** (Fig. 1).

Herein, we report the structural optimization of 2-substituted thiazole carboxamide derivatives as new potent Akt inhibitors.

The synthesis of 2-aminopyrimidine thiazole carboxamide derivatives is depicted in Schemes 1 and 2. Briefly, compound **7** was prepared by a selective Minisci acetylation[22,23] of methyl thiazole-5-carboxylate **6**. Compound **8** was yielded by a condensation of **7** with *N*,*N*-dimethylformamide dimethyl acetal. Both compounds **9** and **12** were readily produced using **8** as a key intermediate under reported procedures.[10,24] Finally, the designed compounds **5a–5k** and **5l** were obtained by coupling **9** and **12** with different amines **19** (Scheme 1), and followed by hydrogenation.

Similarly, the pyrrolopyridinyl thiazole amide derivative **5m** was synthesized[14] using Pd-catalyzed Suzuki coupling reaction as the key step (Scheme 2).

The kinase inhibitory activities against Akts of compounds **5a–m** were evaluated via a well established FRET-based Z'-Lyte assay. Staurosporine (a pan-kinase inhibitor) was included as a positive control to validate the screening assay. Under the screening conditions, staurosporine inhibited the kinase activities of

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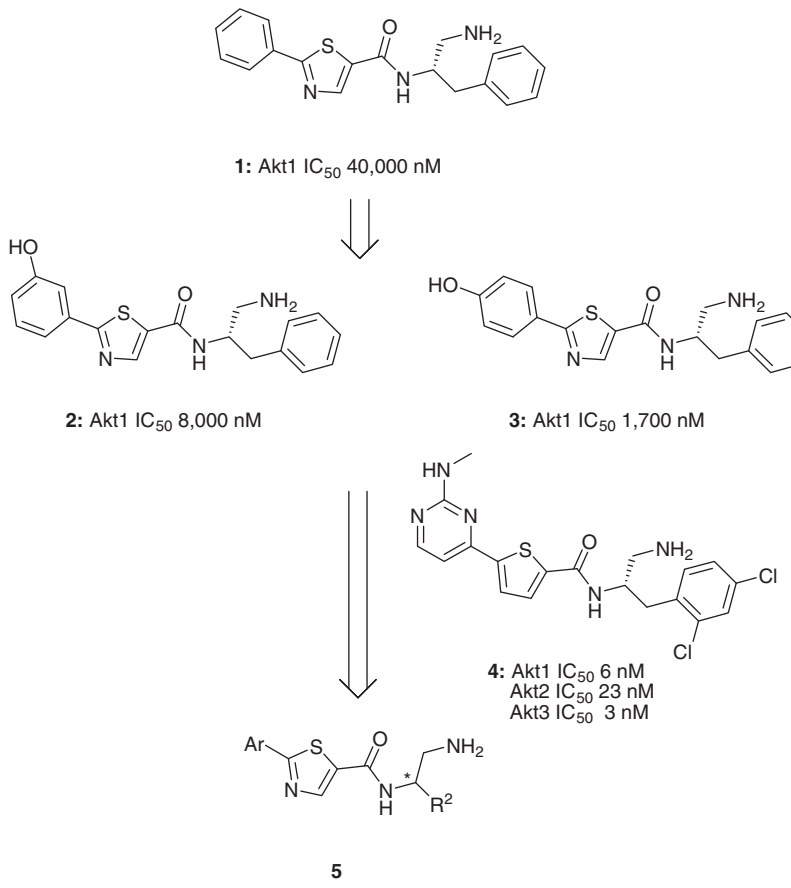


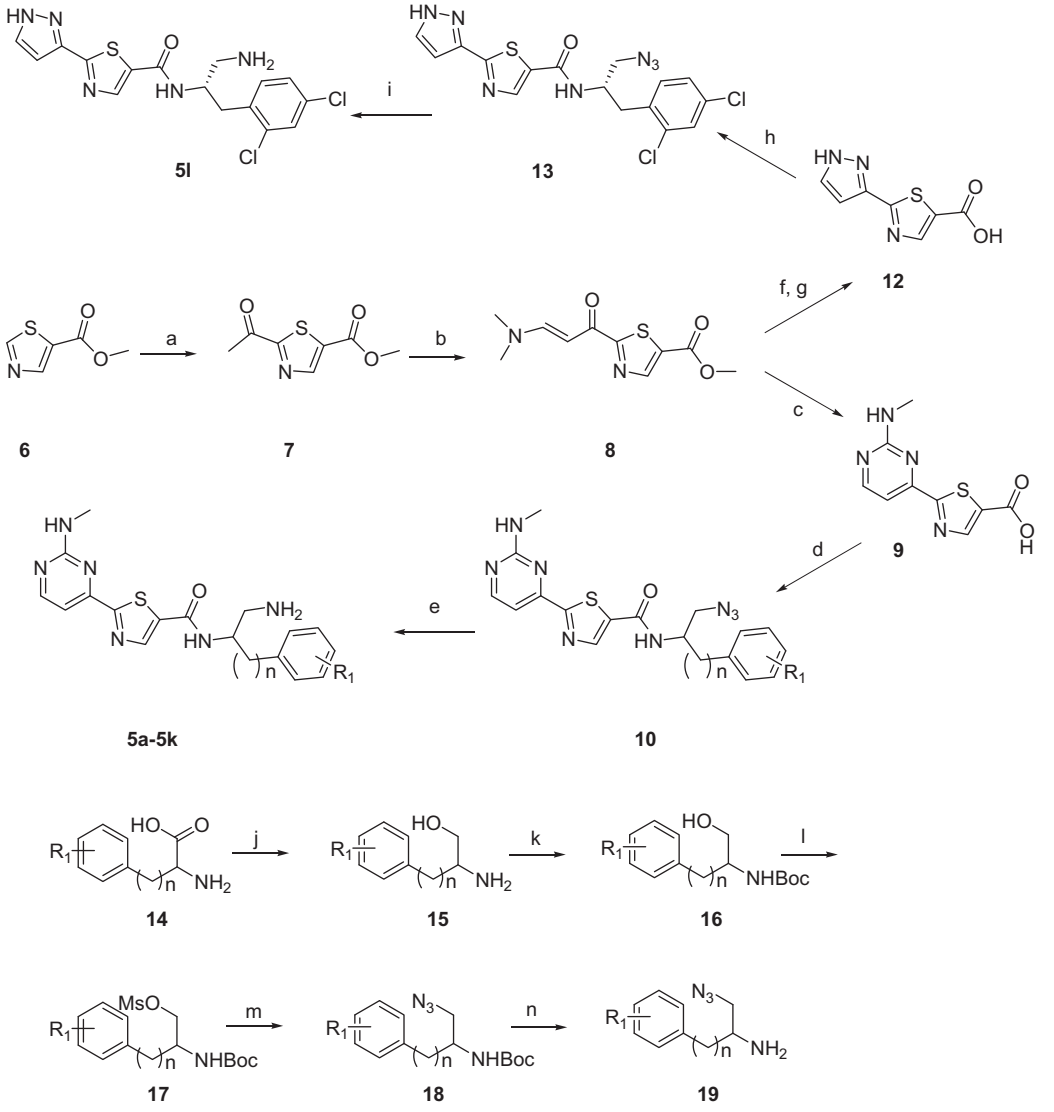
Figure 1. 2-Substituted thiazole carboxamides as new Akt inhibitors.

Akt1, Akt2 and Akt3 with IC_{50} values of 0.12, 0.22 and 0.065 μM, respectively, which showed great consistency with the reported data.[10] As shown in Table 1, when the phenyl group in compound **1** was replaced by a 4-(2-*N*-methylamino) pyrimidinyl moiety, the resulting compound **5a** strongly inhibited the kinase activities of Akt1, Akt2 and Akt3 with IC_{50} values of 0.18, 1.30 and 0.17 μM, respectively. In addition, when the *S*-configuration in **5a** was switched to *R*-configuration (**5b**), about 6-10-time potency was lost, which suggested that the stereochemistry of the compound **5a** might be critical for its kinase inhibitory activity. Moreover, the investigation also demonstrated that the replacement of the benzyl group in **5a** with a phenyl group caused a 3–4-fold decrease of the Akt inhibitory activity (**5c**). Further structure–activity relationship studies showed that the substituted group on the benzyl group in **5a** had great impact on the kinase inhibitory activities. For instance, when a 4-hydroxyl group was introduced (**5g**), the potency was attenuated by about 50 times, while 2-chloro derivative (**5i**) was about 6- or 10-times more potent than the original compound **5c**. 4-Chloro substituted compound (**5f**) was also more potent than **5c**. Combinedly, 2,4-dichloro benzyl analogue **5k** was further designed and synthesized to display improved potency.

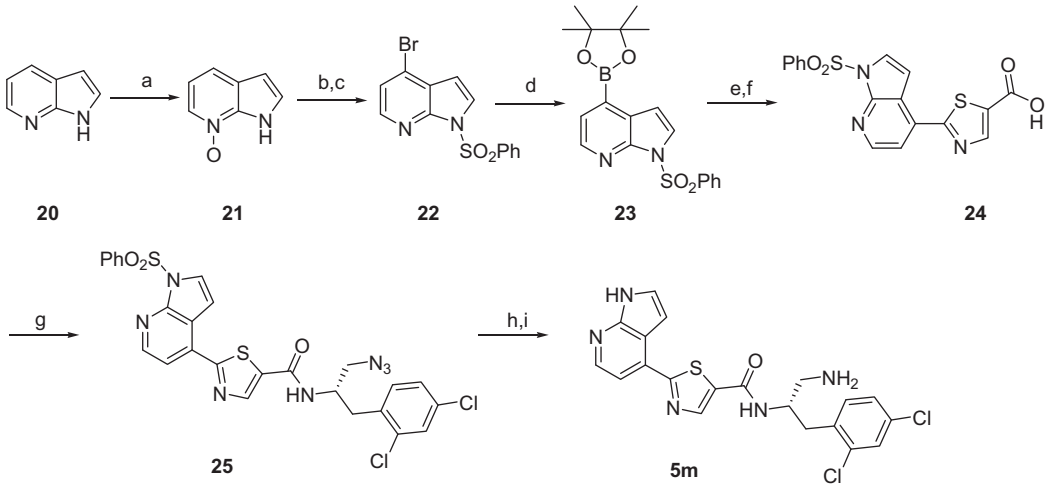
Taking compound **5k** as a template, pyrazol thiazole amide **5l** and pyrrolopyridinyl thiazole amide **5m** were designed and synthesized by replacing 4-(2-*N*-methylamino) pyrimidine with small pyrazole and rigid 7-azaindole rings. The results showed that compound **5l** was almost totally inactive against Akts, while the pyrrolopyridinyl thiazole derivative **5m** displayed the best potencies against 3 isoforms of Akt kinase.

In order to rationalize the affinity observed for our inhibitors, we studied their binding modes within the ATP binding site of Akt1 (PDB: 3OCB) by using computational docking method. Several important features of possible interactions could be derived from the predicted binding mode of compound **5m**. As shown in Fig. 2A, the rigid pyrrolopyridine core was deeply buried in the ATP binding pocket. It could form two essential hydrogen bonds with carbonyl oxygen of Glu228 and nitrogen of Ala230 in the kinase hinge region like other reported crystal structures of Akt inhibitors.[14] The pyrrolopyridine core of compound **5m** also occupied the hydrophobic region which was composed of residues Met227, Tyr229, Thr291, Thr211, and Phe438. In addition, the 2,4-dichlorobenzyl stretched out of the ATP binding pocket and could have an intense hydrophobic interaction with Phe161. Hence compounds with strong hydrophobic groups in R^2 had notably better activity (Table 1). Thiazole ring worked as a hydrophobic linker and the nitrogen atom of amide attached to thiazole could interact with Asp292 via a hydrogen bond. Primary amine side chain might interact with Asp292, Asn279 and Glu278 in the carbonyl-rich region via three pairs of hydrogen bonds, which are same as the hydrogen bond interactions in the reported X-ray complex structure of (2*S*)-2-(4-chlorobenzyl)-3-oxo-3-[4-(7*H*-pyrrolo[2,3,*d*]pyrimidin-4-yl) piperazin-1-yl]propan-1-amine and Akt1 kinase domain.[17] Compared with compound **5m**, compound **5k** (Fig. 2b) with a smaller amimopyrimidine core would gain less hydrophobic interactions and van der Waals (VDW) contacts, and the entropy will increase because of the additional degree of freedom of the rotational bond in Ar group, which all probably caused the decrease of activity. Similarly, pyrazol core in compound **5l** was a much smaller group than amimopyrimidine and pyrrolopyridine, which might be too short to form the two essential hydrogen bonds with the residues in the kinase hinge region.

Additionally, the kinase inhibitory activities of these new Akt inhibitors were further validated by western blot analysis. The results were illustrated in Figure 3. It was clearly that compound **5m**

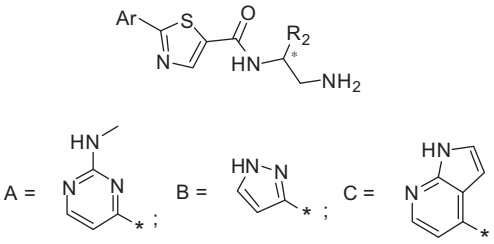


Scheme 1. Reagents and conditions (n = 0 or 1): (a) 40% acetaldehyde, H_2SO_4, $FeSO_4$–$7H_2O$, t-BuOOH, 0 °C to rt, 73%; (b) DMF-DMA, toluene, reflux, 84%; (c) EtONa (2.1 equiv), methyl guanidine hydrochloride (2.0 equiv), EtOH, 70 °C,16 h, 79%; (d) compound **19**, EDC, HOBt, DIPEA, DMF, rt; (e) H_2, Pd/C, MeOH, rt; or PPh_3, THF/H_2O; (f) hydrazine hydrochloride, AcOH, reflux, 1 h, 77%; (g) NaOH, THF/H_2O, rt; (h) EDC, HOBt, DIPEA, **19k**, DMF, rt, 66%; (i) H_2, Pd/C, MeOH, rt, 69%; (j) $LiBH_4$, Me_3SiCl, THF, rt; (k) $(Boc)_2O$, $CHCl_3$, rt; (l) MsCl, Et_3N, DCM, rt; (m) NaN_3, DMF, 60 °C, 16 h; (n) 3 M HCl, MeOH, 60 °C, overnight, quant.



Scheme 2. Reagents and conditions: (a) MCPBA, DME, 48%; (b) $(CH_3SO_2)_2O$, $(CH_3)_4NBr$, DMF, 56%; (c) $PhSO_2Cl$, NaH, DMF, 72%; (d) $Pd(dppf)_2Cl_2$, bis(pinacolato)diboron, KOAc, DMF, 80 °C, 67%; (e) $Pd(PPh_3)_4$, K_2CO_3, ethyl 2-bromothiazole-5-carboxylate, DMF, 80 °C, 48%; (f) LiOH, THF/H_2O, rt; (g) EDC, HOBt, DIPEA, **19k**, DMF, rt, 85%; (h) NaOH, THF/H_2O, rt; (i) H_2, Pd/C, MeOH, rt, 78%.

Table 1
Structure–activity relationship of 2-substituted thiazole carboxamide derivatives as new potent Akt inhibitors[a]



Compd	R^2	Ar	*R*/*S*	Kinase inhibition IC_{50} (μM)			Cell growth inhibition IC_{50} (μM)		
				Akt1	Akt2	Akt3	LnCaP	PC3	Du145
5a	Benzyl	A	*S*	0.18	1.30	0.17	1.36	7.50	2.54
5b	Benzyl	A	*R*	1.85	8.42	1.06	13.9	31.0	14.1
5c	Phenyl	A	*S*	0.55	4.69	0.50	2.32	8.32	2.89
5d	4-Me-benzyl	A	*S*	0.58	5.70	0.52	7.16	14.9	6.63
5e	4-MeO-benzyl	A	*S*	1.39	7.32	0.63	8.13	46.0	15.0
5f	4-Cl-benzyl	A	*S*	0.15	6.24	0.11	0.89	7.45	7.54
5g	4-OH-benzyl	A	*S*	10.9	20.5	10.86	24.8	49.2	9.59
5h	4-NO_2-benzyl	A	*S*	0.74	21.8	0.34	5.05	20.5	12.6
5i	2-Cl-benzyl	A	*S*	0.033	1.95	0.083	0.26	6.72	10.1
5j	4-Ph-benzyl	A	*S*	4.00	>50	5.47	1.75	2.66	2.66
5k	2,4-Dichlorobenzyl	A	*S*	0.15	0.90	0.074	0.70	8.06	4.09
5l	2,4-Dichlorobenzyl	B	*S*	>50	>50	>50	11.93	10.99	12.1
5m	2,4-Dichlorobenzyl	C	*S*	0.025	0.20	0.024	0.23	0.90	2.84
STSP				0.122	0.215	0.065	NT	NT	NT

[a] Akt activity assays were performed using the FRET-based Z'-Lyte assay according to the manufacturer's instructions. The antiproliferative activities of the compounds were evaluated using MTT assay. The data were means from at least 3 independent experiments. STSP: Staurosporine, NT, not tested.

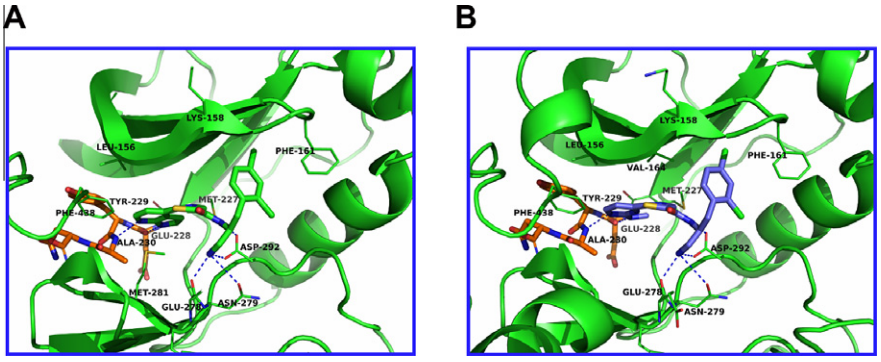


Figure 2. (A) Predicted binding mode of compound **5m** at the ATP binding site of Akt1. (B) Predicted binding mode of compound **5k** at the ATP binding site of Akt1. The coloring is described as follows: Green, Akt1 kinase domain; Cyan, compound **5m**; Purple, compound **5k**; Orange, hinge region of Akt1 kinase domain; Red, oxygen atom, Blue, nitrogen atom; Yellow, sulfur atom; Light green, chlorine atom. The hydrogen bonds are denoted with blue dashed lines. All structure figures were prepared using PyMol 0.99 (http://pymol.sourceforge.net/).

barely affected the protein level of phosphorylated Akt, but dose-dependently inhibited the phosphorylation of MDM2 and GSK3β, which were both Akt downstream phosphorylating substrates,[25]

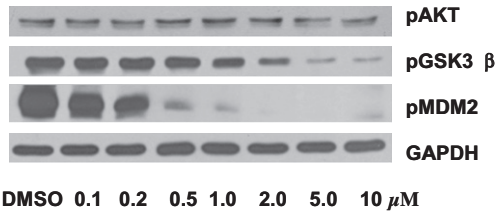


Figure 3. Compound **5m** dose-dependently inhibits the phosphorylation of MDM2 and GSK3β in LnCap cells (four-hour treatment). Results are representative of at least 3 independent experiments.

after four-hour treatment. The results were highly consistent with the observation on many other selective Akt inhibitors.[29,30]

The in vitro anti-tumor activities of the designed compounds were also evaluated by MTT assay in three prostate cancer cell lines (LnCap, PC3 and Du145) with different p53 status (Table 1).[26,27] Corresponding to their potent Akt kinase inhibitory activities, the compounds strongly suppressed the growth of three different types of human prostate cancer cells. The most potent compound **5m** inhibited the growth of LnCap, PC3 and Du145 with IC_{50} values of 0.23, 0.90 and 2.84 μM, respectively. Further data analysis suggested that almost all the new Akt inhibitors preferred to inhibit the growth of LnCap cells which possess wild-type p53. However, significantly lower potencies were observed for PC3 and Du145 cancer cells which lack p53 or bear mutated form of p53. The selectivity on LnCap cells might be due to the consequent inhibitory activity against MDM2 imposed by Akt inhibitors, which could potentate the tumor suppression of p53 subsequently.[28]

In summary, a new series of 2-substituted thiazole carboxamides were initially identified as potent pan inhibitors against all three isoforms of Akt (Akt1, Akt2 and Akt3). As one of the most potent compounds, **5m** inhibited the kinase activities of Akt1, Akt2 and Akt3 with IC_{50} values of 25, 196 and 24 nM, respectively. The Akt inhibition of compound **5m** was further validated using cell lysate assays. Furthermore, the compounds also potently inhibited the cell growth of three different types of human prostate cancer cells. Theses inhibitors may be taken as new potential lead compounds for further development of novel anticancer agents.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.080.

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