Discovery of Pyridopyrimidinones as Potent and Orally Active Dual Inhibitors of PI3K/mTOR

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Supporting Information

ABSTRACT: The identification and lead optimization of a series of pyridopyrimidinone derivatives are described as a novel class of efficacious dual PI3K/mTOR inhibitors, resulting in the discovery of 31. Compound 31 exhibited high enzyme activity against PI3K and mTOR, potent suppression of Akt and p70s6k phosphorylation in cell assays, and good pharmacokinetic profile. Furthermore, compound 31 demonstrated in vivo efficacy in a PC-3M tumor xenograft model.

KEYWORDS: PI3K, mTOR, dual inhibitor, anticancer

The phosphoinositide 3-kinases (PI3Ks) family is a class of lipid kinases that is involved in cell growth, proliferation, differentiation, and survival.^{1–3} The PI3Ks can be divided into three classes (Class I, Class II, and Class III) according to the structural characteristic, and the most important class I PI3Ks, including four isoforms (p110α, p110β, p110δ, and p110γ), can phosphorylate phosphatidylinositol diphosphate (PIP2) to generate its corresponding triphosphate (PIP3), a potent secondary messenger that results in the activation of the downstream serine-threonine kinase Akt.^{4,5} Subsequent phosphorylation of AKT leads to the triggering of the pathway that ultimately activates the mammalian target of rapamycin (mTOR), which promotes cell growth and survival by integrating signals from growth factors, nutrients, and cellular energetics.⁶ Therefore, inhibitors of key kinases in the PI3K/AKT/mTOR signaling pathway have been extensively identified for the treatment of cancer in recent years.⁷

Since dual suppression of PI3K/mTOR may most effectively prevent the PI3K signaling cascade transduction, overcome feedback loops, and block PI3K-independent mTOR activation, the development of PI3K/mTOR dual kinase inhibitors represents a beneficial therapeutic intervention strategy in cancer treatment.⁸ To this end, several research groups have been focusing their efforts on the development of small molecular PI3K/mTOR inhibitors in the literature.^{9–18}

Figure 1. Structure of GSK2126458 and 31.

Among them, GSK2126458 (Figure 1) has been identified as a highly potent and orally bioavailable PI3K/mTOR dual inhibitor and is currently in phase I clinical trials.¹⁴ The reported cocrystal structure of GSK2126458 with PI3Kγ,¹⁴ which is highly homologous to other class I PI3K isoforms, showed that the nitrogen in quinoline forms an important hydrogen bond with Val882 in the hinge region. The pyridyl nitrogen atom has a key interaction with a conserved water molecule. The sulfonamide makes a strong charged interaction with Lys833. In addition, the difluorophenyl group occupies a hydrophobic region in the ATP binding site back pocket of the

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Figure 2. Inhibition of pAKT in MCF-7 cell at 1 μ M concentration.

Figure 3. Modeled structure of compound 4 with PI3K α .

kinase. The pyridazine group seemed to point in the solvent region and not contribute to binding interaction.

On the basis of the binding mode, our design strategy was to keep pyridyl sulfonamide moiety unchanged initially, and to remove the pyridazine group to reduce molecule weight and number of aromatic rings, and to search for a novel hinge binder. Herein, we describe the discovery of a novel pyridopyrimidinone scaffold and our early lead optimization efforts in this series, resulting in *N*-(5-(3-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-7-yl)-2-methoxypyridin-3-yl)-2,4-difluorobenzenesulfonamide **31** as a highly potent dual PI3K/mTOR inhibitor for in vivo studies (Figure 1).

Our primary optimization is focused on probing different scaffolds that resemble a quinoline core, such as cinnoline, pyrido[3,4-*d*]pyrimidine, 1,8-naphthyridine, and pyridopyrimidinone. All synthesized compounds **1–4** were initially evaluated for in vitro activities against PI3K α by testing the corresponding inhibition of pAKT in MCF-7 cell at a single concentration (1 μ M). Among the various heterocycles, compound **4** was very attractive with a pAKT inhibition of 98% at 1 μ M. Further replacing the “CH” of the pyridopyrimidinone ring with a “N” led to 4*H*-pyrimido[1,2-*b*]pyridazin-4-one derivative **5** and 4*H*-pyrazino[1,2-*a*]pyrimidin-4-one derivative **6**, which exhibited a slightly higher

Table 1. Structure–Activity Relationship with R¹ and R² Groups

4, 7–28

compd	R ¹	R ²	MCF-7 cell p-Akt % inhibition at 1 μ M	MCF-7 cell p-Akt IC ₅₀ (nM)	MCF-7 cell p-p70s6k IC ₅₀ (nM)
7	OCH ₃	Ph	95	98.8	197.9
8	OCH ₃	4-FC ₆ H ₄	95	80.7	105.8
9	OCH ₃	4-ClC ₆ H ₄	96	48.8	223.4
10	OCH ₃	4-CNC ₆ H ₄	94	57.4	245.7
11	OCH ₃	4-CF ₃ C ₆ H ₄	94	48.7	163.3
12	OCH ₃	4-OMeC ₆ H ₄	95	54.1	138.0
13	OCH ₃	3-FC ₆ H ₄	96	67.0	64.0
14	OCH ₃	2-FC ₆ H ₄	94	141.0	>400
15	OCH ₃	2-MeC ₆ H ₄	94	23.9	153.4
4	OCH ₃	2,4-FC ₆ H ₃	98	16	66
16	OCH ₃	3,5-FC ₆ H ₃	98	78.2	77.2
17	OCH ₃	2,5-FC ₆ H ₃	96	45	108.5
18	OCH ₃	cyclopropyl	76		
19	OCH ₃	Et	53		
20	OCH ₃	CF ₃ CH ₂	78		
21	OEt	2-FC ₆ H ₄	60		
22	Me	2,4-FC ₆ H ₃	81		
23	Me	2-FC ₆ H ₄	82		
24	Me	4-FC ₆ H ₄	85		
25	Et	2-FC ₆ H ₄	81		
26	Et	2,4-FC ₆ H ₃	86		
27	Cl	2,4-FC ₆ H ₃	92	32.8	>400
28	Cl	2-FC ₆ H ₄	91	34.4	>400

TPSA and lower cLogP value, but showed poor inhibition of pAKT relative to compound **4** (Figure 2).

Docking study of compound **4** with PI3K α was carried out.¹⁹ As illustrated in Figure 3, the nitrogen atom of pyridopyr-

Table 2. Structure–Activity Relationship with R³ Group

compd	R ³	MCF-7 cell p-Akt % inhibition at 1 μ M	MCF-7 cell p-Akt IC ₅₀ (nM)	MCF-7 cell p-p70s6k IC ₅₀ (nM)
29	OEt	76		
30	Me	90	27.9	119
31	Cl	93	11.6	89.2

Table 3. In Vitro Activities of Compounds 4 and 31

compd		4	31
enzyme IC ₅₀ (nM)	PI3K α / β / δ / γ	3.1/-/-/-	3.4/34/16/1
	mTOR	19.2	4.7
MCF-7 cell IC ₅₀ (nM)	pAkt	16	11.6
	p-p70s6k	66	89.2

Table 4. Mouse PPB and PK Profiles of Compounds 4 and 31

compd		4	31
PPB % bound (h, r, m)		>99, >99, 99.5	99.5, 99.2, 98.1
iv (mouse) @ 1 mg/kg	Cl (mL/min/kg)	5.15	1.4
	Vd _{ss} (L/kg)	0.443	0.472
	T _{1/2} (h)	1	3
	AUC _{0-last} (ng·h/mL)	3285	11883
po (mouse) @ 3 mg/kg	C _{max} (ng/mL)	4083	5450
	T _{max} (h)	0.333	2.67
	AUC _{0-last} (ng·h/mL)	13755	38568
	F%	139	108

imidinone formed a key hydrogen bond with hinge residue Val850. The methoxy group from the pyridine moiety formed a hydrogen bond with Lys802. Besides the hydrogen bonds, pyridopyrimidinone also formed a π - π stacking interaction with gatekeeper Tyr836.

Further in vitro biomarker studies demonstrated potent suppression of Akt-S473 and p70s6k-T389 phosphorylation (IC₅₀ = 16 nM and 66 nM) in MCF-7 cells after 4 h of incubation with compound 4, using ADP-Glo assay. Encouraged by this result, we commenced with an investigation on the pyridyl moiety with different sulfonamides at 3-position and substituents at 2-position of pyridine ring to obtain potent PI3K/mTOR inhibitors. The biological data for compounds 4 and 7–28 are compiled in Table 1. All pyridopyrimidinone derivatives (7–17) with substituted aryl sulfonamide exhibited excellent inhibition of pAKT at 1 μ M concentration. The unsubstituted benz sulfamide 7 had 98.8 nM for PI3K cellular activity and 197.9 nM for mTOR cellular activity. *para*-Substitution with a small group, such as F (8), Cl (9), CN (10), CF₃ (11), or OMe (12), all increased PI3K cellular activity, but with mixed effect on mTOR. *meta* and *ortho*-Substitution were studied with less examples. *meta*-F-substituent (13) increased both PI3K and mTOR activity to be equally potent (67 and 64 nM, respectively), while an *ortho*-F (14) decreased both activities. *ortho*-Me (15) increased PI3K cellular activity by approximately 5 $\times$ with little effect on mTOR. Disubstitution

Figure 4. Modeled structures of compound 31 with PI3K α and mTOR.

Figure 5. PC-3M xenograft model tumor growth curve and body weight change (%).

patterns were studied with fluoro groups. All 2,4-(4), 2,5-(17), and 3,5-(16) difluorosubstitutions increased PI3K and mTOR

Scheme 1. Synthetic Route of Compounds 4 and 7–28

Scheme 1a. (i) B_2Pin_2 , $Pd(dppf)_2Cl_2$, KOAc, dioxane, 100 °C; (ii) Pd/C, MeOH, RT; (iii) Sulfonyl chloride **35**, pyridine.

4 $R^1 = OMe$, $R^2 = 2,4-FC_6H_3$	18 $R^1 = OMe$, $R^2 = Cyclopropyl$
7 $R^1 = OMe$, $R^2 = C_6H_5$	19 $R^1 = OMe$, $R^2 = Ethyl$
8 $R^1 = OMe$, $R^2 = 4-FC_6H_4$	20 $R^1 = OMe$, $R^2 = CF_3CH_2$
9 $R^1 = OMe$, $R^2 = 4-ClC_6H_4$	21 $R^1 = OEt$, $R^2 = 2-FC_6H_4$
10 $R^1 = OMe$, $R^2 = 4-CNC_6H_4$	22 $R^1 = Me$, $R^2 = 2,4-FC_6H_3$
11 $R^1 = OMe$, $R^2 = 4-CF_3C_6H_4$	23 $R^1 = Me$, $R^2 = 2-FC_6H_4$
12 $R^1 = OMe$, $R^2 = 4-OMeC_6H_4$	24 $R^1 = Me$, $R^2 = 4-FC_6H_4$
13 $R^1 = OMe$, $R^2 = 3-FC_6H_4$	25 $R^1 = Et$, $R^2 = 2-FC_6H_4$
14 $R^1 = OMe$, $R^2 = 2-FC_6H_4$	26 $R^1 = Et$, $R^2 = 2,4-FC_6H_3$
15 $R^1 = OMe$, $R^2 = 2-MeC_6H_4$	27 $R^1 = Cl$, $R^2 = 2,4-FC_6H_3$
16 $R^1 = OMe$, $R^2 = 3,5-FC_6H_3$	28 $R^1 = Cl$, $R^2 = 2-FC_6H_4$
17 $R^1 = OMe$, $R^2 = 2,5-FC_6H_3$	

Scheme 1b. (i) Triethyl orthoformate, 2,2-dimethyl-1,3-dioxane-4,6-dione, EtOH, reflux; (ii) Diphenyl oxide, 220 °C; (iii) **36**, $Pd(dppf)_2Cl_2$, K_2CO_3 , dioxane/ H_2O , 100 °C.

cellular activity with 2,4-(4) being the most active for both kinases. The aromaticity of benzene seemed to be required for PI3K activity since replacement of phenyl with Et (**19**), trifluoroethyl (**20**), or cyclopropyl (**18**) gave only 53–78% inhibition at 1 μM . Replacement of the methoxy group on the pyridine ring with methyl, ethyl, or ethoxyl all caused a decrease of PI3K percent inhibition at 1 μM : methyl (**22** vs **4**, **23** vs **14**, and **24** vs **8**), ethyl (**25** vs **14**, **26** vs **4**), and ethoxyl (**21** vs **14**). However, replacement by a Cl group resulted in an increase of PI3K activity to about 30 nM (**27** and **28**) but destroyed mTOR activity.

Based on the structure of compound **4**, we conducted further SAR studies on the pyridopyrimidinone moiety. In particular, we examined the effect of ethoxyl, methyl, and chlorine at the *ortho*-position of the carbonyl group on potency. As can be seen from Table 2, the introduction of a chlorine or methyl group had negligible effect on cell potencies. However, this effect depends on the size of the substituent group, so the larger ethoxy group substituted at the same position showed a lower potency relative to compounds **30** and **31**.

Among the various pyridopyrimidinone derivatives, compounds **4** and **31** looked the most promising because of their high potency in cell assays. In the meantime, their enzyme activities against PI3K α and mTOR were also examined, using a HTRF assay.²⁰ It is found that each of the two compounds possessed appreciable in vitro inhibition of the enzyme, especially **31** represents a novel scaffold for a potent dual pan-PI3K/mTOR inhibitor, exhibiting strong IC_{50} values of 3.4/34/16/1 nM for PI3K $\alpha/\beta/\delta/\gamma$ and 4.7 nM for mTOR (Table 3).

After this preliminary optimization of enzymatic and cellular activities, we next evaluated in vitro and in vivo pharmacokinetic (PK) profiles (**4** and **31**). As presented in Table 4, they each showed a high level of plasma protein-binding in human, rat, and mouse. When the two compounds were administered intravenously (iv) to mice at 1 mg/kg (in 10% DMSO/65% PEG400/25% H_2O for compound **4**; in 10% DMSO/80% PEG400/10% H_2O for compound **31**), compound **31** exhibited a low clearance (1.4 mL/min/kg), a similar volume of distribution (0.472 L/kg), a long half-life (3 h), and a high AUC exposure relative to compound **4**. When dosed orally (po) in mouse at 3 mg/kg (in 10% DMSO/65% PEG400/25% H_2O for compound **4**; in 10% DMSO/80% PEG400/10% H_2O for compound **31**), a high C_{max} (5450 ng·h/mL) and plasma exposure with an excellent oral bioavailability were also observed.

Further analysis of **31** docked into the models of PI3K α and mTOR indicated that an extra halogen bond with Cys2243 in mTOR was formed, thereby explaining the increased enzyme activity against mTOR (Figure 4).¹⁹

On the basis of its enzyme and cellular potencies and its pharmacokinetic profile, compound **31** was taken forward for in vivo efficacy studies (Figure 5). Evaluation of in vivo efficacy of **31** was performed in nude mice bearing PC-3M cancer tumors. Compound **31** was administered @ 1, 3, and 10 mg/kg, po doses, daily for 21 days (10 mice per group). In this PC-3M xenograft tumor model, compound **31** exhibited dose-dependent tumor growth inhibition, and significant in vivo efficacy was observed when dosed 10 mg/kg, qd (as shown in Figure 5). In these studies, compound **31** was also well tolerated, and no

Scheme 2. Synthetic Route of Compound 29–31

Scheme 2a. (i) Brederick's reagent, 80 °C; (ii) **37**, HOAc, 90 °C; (iii) **36a**, Pd(dppf)₂Cl₂, K₂CO₃, dioxane/H₂O, 100 °C.

Scheme 2b. (i) Diethyl 2-methyl-3-oxosuccinate, EtOH, reflux; (ii) Diphenyl oxide, reflux; (iii) **36a**, Pd(dppf)₂Cl₂, K₂CO₃, dioxane/H₂O, 100 °C.

Scheme 2c. (i) NCS, DMF, RT; (ii) **36a**, Pd(dppf)₂Cl₂, K₂CO₃, dioxane/H₂O, 100 °C.

significant body weight loss was observed for all different dosages.

Compounds 7–31, which are shown in the present Letter, were prepared starting from commercially available materials, as outlined in Schemes 1 and 2. Compounds 32 were reacted with bis(pinacolato)diboron in the presence of Pd(dppf)₂Cl₂ and KOAc in dioxane at 100 °C to afford the corresponding pyridineboronic acid pinacol esters 33. Reduction of 33 with Pd/C under hydrogenation conditions gave the pyridinamines 34, which were treated with different sulfonyl chlorides 35 in pyridine to give the key intermediates 36 (Scheme 1a). This synthetic sequence can be applied to the synthesis of various substituted pyridineboronic acid pinacol ester by varying the primary material or sulfonyl chloride. Treatment of 5-bromopyridin-2-amine **37** with 2,2-dimethyl-1,3-dioxane-4,6-dione in the presence of triethyl orthoformate generated the imine **38**, followed by cyclization in diphenyl oxide at 220 °C leading to the 7-bromo-4H-pyrido[1,2-a]pyrimidin-4-one **39**. Suzuki reaction of different pyridineboronic acid pinacol esters

36 with **39** afforded a series of 4H-pyrido[1,2-a]pyrimidin-4-one derivatives **4** and 7–28 (Scheme 1b).

A similar route was also applied to synthesize compounds 29–31. As shown in Scheme 2, under Brederick reaction conditions, commercially available ethyl 2-ethoxyacetate **40** was converted to the enamine product **41**, and subsequent treatment with 5-bromopyridin-2-amine **37** at 90 °C provided 7-bromo-3-ethoxy-4H-pyrido[1,2-a]pyrimidin-4-one **42** (Scheme 2a). Cyclization of 5-bromopyridin-2-amine **37** with diethyl 2-methyl-3-oxosuccinate in refluxing EtOH, followed by decarboxylation led to 7-bromo-3-methyl-4H-pyrido[1,2-a]pyrimidin-4-one **44** (Scheme 2b). In addition, treatment of 7-bromo-4H-pyrido[1,2-a]pyrimidin-4-one **39** with NCS gave the 7-bromo-3-chloro-4H-pyrido[1,2-a]pyrimidin-4-one **45** (Scheme 2c). Finally, ethoxyl-, methyl-, and chlorine-substituted 7-bromo-4H-pyrido[1,2-a]pyrimidin-4-one **42**, **44**, and **45** were respectively reacted with pyridineboronic acid pinacol ester **36a** to provide the desired products 29–31.

In summary, we have designed and synthesized a new series of 4H-pyrido[1,2-a]pyrimidin-4-one derivatives as potent and orally active PI3K/mTOR dual inhibitors utilizing structure-based design strategy. Through the exploration of different sulfonamides in a pyridine ring and substituents at the 2-position of the pyridine ring and at the *ortho*-position of carbonyl group of 4H-pyrido[1,2-a]pyrimidin-4-one, we successfully developed the SAR profile around this series, resulting in the discovery of compound **31**, which has shown good enzyme activity against PI3K and mTOR and potent suppression of Akt and p70s6k phosphorylation in cellular assays. Furthermore, compound **31** also demonstrated significant *in vivo* efficacy in a PC-3M tumor xenograft model.

■ ASSOCIATED CONTENT

S Supporting Information

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Experimental and characterization data for all new compounds and all biological data (PDF)

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Author Contributions

The manuscript was written by L.Y. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

PK, pharmacokinetics; SAR, structure–activity relationship; PPB, plasma protein binding; IV, intravenous; PO, per oral; AUC, area under curve; mpk, mg/kg

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- (19) The figures were prepared by Pymol and Maestro packages, see: (a) *The PyMOL Molecular Graphics System*, version 1.8.6.2; Schrödinger. (b) *Maestro*, release 2017-3; Schrödinger, LLC: New York, 2017. For details see, [Supporting Information](#).
- (20) The activities of **GKS2126458** against PI3K α and mTOR are 0.55 and 0.68 nM, respectively.