Concise SAR Exploration Based on the “Head-to-Tail” Approach: Discovery of PI4KIII α Inhibitors Bearing Diverse Scaffolds

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S Supporting Information

ABSTRACT: In typical kinase inhibitor programs, a hinge binder showing best potency with preferential specificity is initially selected, followed by fine-tuning of the accompanying substituents on its core module. A shortcoming of this approach is that the exclusive focus on a single chemotype can endanger all the analogues in the series if a critical shortcoming is revealed. Thus, an early evaluation of structure–activity relationships (SARs) can mitigate unforeseen outcomes within a series of multiple compounds, although there have been very few examples to follow such a policy. PI4KIII α is one of four mammalian phosphatidylinositol-4 kinases and has recently drawn significant attention as an emerging target for hepatitis C virus (HCV) treatment. In this letter, a novel “head-to-tail” approach to discover a diverse set of PI4KIII α inhibitors is reported. We believe this method will generate distinct core scaffolds, a rational strategy to circumvent potential risks in general kinase programs.

KEYWORDS: HCV, PI4KIII α , head-to-tail approach, diverse scaffolds

Hepatitis C virus (HCV) is a leading cause of chronic liver disease, and 130–150 million people are considered to be infected worldwide.^{1,2} Patients with persistent infection of HCV are at high risk to develop serious liver damage typified by hepatocellular carcinoma, which can eventually necessitate liver transplantation.^{3,4} Although the therapeutic options are being improved after the approval of newly developed direct acting antivirals,⁵ significant concerns remain about drug resistance as well as inadequate coverage of all existing HCV genotypes. Due to these unmet needs, development of a new broad-spectrum antiviral strategy managing such potential problems are of great importance. To achieve such goals, the disruption of the viral lifecycle is a viable option so that several host proteins, which are implicated in HCV reproduction, have been intensely pursued.⁶

PI4KIII α is known as one of four mammalian phosphatidylinositol-4 kinases and is responsible for formation of phosphatidylinositol 4-phosphate (PI4P) at cytoplasmic membranes, *cis*-Golgi compartments, and nucleus.⁷ One of

the nonstructural HCV protein, NS5A, was reported to hijack the PI4P production machinery, which facilitates the viral replication by forming unique membranous web-like structures. Subsequently, silencing the PI4KIII α gene was confirmed to abrogate HCV's replication as well as its production.^{8,9} Therefore, a strategy targeting this enzyme is a rational approach for treatment of this virus based on the emerging mechanism of action.

While the concept of targeting this lipid kinase seems valid, one must take into account that interference with such an essential cellular function might cause some serious damage to the host cells themselves. Fortunately, there are other PI4 kinases such as PI4KIII β , which are responsible for maintenance of PI4P homeostasis,¹⁰ and thus specific inhibition of PI4KIII α holds great promise to suppress HCV replication

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while keeping basal cell trafficking functions via PI4P intact through bypassing such redundant machinery.

Toward this end, we started a SAR investigation to obtain selective PI4KIII α inhibitors. Among the inhibitors reported to date, we focused our attention on compound **1** reported by a GSK research group¹¹ since this compound showed good PI4KIII α inhibitory activity (pIC_{50} = 8.3) with preferable selectivity over PI4KIII β as high as 200-fold (Figure 1).

The compound possessed a 2-aminoquinazolinone skeleton as a hinge binder, on which a disubstituted pyridine and an atropisomeric benzene ring were attached. Although the selectivity profile of this compound was reasonably assured, we felt confident in implementing another core module having reduced affinity since the hinge domain is known to be a highly conserved region across many kinases. So, we decided to initiate investigation from its monodentate¹² analogue **2** (Table 1) on which a simple benzene ring was implemented to avoid any stereoisomeric complications. To further mitigate an unexpected risk,¹³ we undertook an exploration of distinct classes of hinge-binding modules because we expected such an approach to subsequently result in generation of a diverse set of the inhibitors. In typical kinase programs, a hinge binder showing the best potency with preferential specificity is initially recruited, followed by fine-tuning of the substituents on this core motif. A shortcoming of such a classical approach is the excessive commitment to a sole chemotype, which, once a critical problem is revealed during the screening, could endanger further investigation of all the corresponding congeners. It is quite evident that a wide variety of compounds will be generated if the core scaffold can be modified, particularly if there are numerous potential substituents available. Aside from the hinge region, the affinity pocket¹⁴ is an ideal place to obtain good binding. Therefore, as the initial step of our investigation, we searched for a motif that displayed a favorable affinity for this pocket.

To gain a structure-based inspiration, a homology model of PI4KIII α was generated by using MOE (Chemical Computing Group, Montreal, Canada) using the known crystal structure¹⁵ of PI3K γ as a template (Figure 2a). Compound **2** was placed manually into the ATP-binding pocket of PI4KIII α with the 1-nitrogen atom of the quinazolinone acting as a hinge binder, and the other parts of the molecule forming hydrophobic contacts in the pocket. The resultant complex model suggested that the substituted pyridine moiety of **2** projected toward a site corresponding to the affinity pocket so that exploration of this part of **2** was prioritized. In the preceding paper, the GSK group reported that reversal of the aminosulfonyl linkage resulted in an improvement of potency against PI4KIII α ,¹¹ and so we synthesized two analogues having the reversed orientation (**3** and **4**, Table 1). The results provided quite a contrast. Compound **3** showed significant improvement in

Figure 1. Structure of PI4KIII α inhibitor reported by GSK.

Table 1. SAR of the Head Motif

Compd	R ¹	R ²	Enzyme (IC ₅₀ , nM) ^a		Fold (α/β)	Replicon (EC ₅₀ , nM) ^a
			PI4K III α	β		
2	MeO-		190	>30000	>160	99
3	MeO-		14	780	56	-
4	MeO-		>1000	>30000	>30	>30000
5	MeO-		4.1	4900	1200	20
6^b			100	>30000	>3000	400

^aValues of IC₅₀ and EC₅₀ are mean values determined from at least three replicates. ^bRacemic.

Figure 2. (a) PI4KIII α homology model with compound **2** based on using the PI3K γ crystal structure as a template. (b) Comparison of the affinity pocket of PI4KIII α (cyan) and PI4KIII β (white) with **5**.

PI4KIII α inhibitory potency, while its sulfone analogue **4** showed complete loss of the potency. It is quite plausible that the NH part of the sulfonamide moiety acquired a crucial electrostatic interaction with some residues in the pocket, suggesting that the exploration of core motifs could be feasible once we strengthened the compound's PI4KIII α selectivity without losing its robust potency. Actually, our homology and

Table 2. SAR of the Core Motif

Compd	Core	R ³	Enzyme (IC ₅₀ , nM) ^a		Fold (α/β)	Replicon (EC ₅₀ , nM) ^a
			PI4KIIIα	PI4KIIIβ		
5			4.1	4900	1200	20
7			8.0	8200	1030	68
8			2.2	1400	640	41
9			9.1	200	22	610
10			13	410	32	880
11			50	3700	74	1000
12			93	>10000	>110	780
13			260	18000	96	700
14			3.1	98	32	50
15			2.0	39	20	29
16			16	920	58	78
17			5.7	300	53	31

^aValues of IC₅₀ and EC₅₀ are mean values determined from at least three replicates.

sequence comparison model pointed out a pocket adjacent to the methoxy group of **3** in PI4KIIIα. Compared to other closely related enzymes, this binding pocket was more spacious, suggesting that this difference in binding site might be a source of improved isomer selectivity. Driven by this hypothesis, we synthesized two compounds possessing a slightly larger substituent as the R¹ group. Gratifyingly, compound **5** completely satisfied our expectation, and >1000-fold selectivity over PI4KIIIβ along with further improvement of PI4KIIIα inhibitory potency was achieved.

According to our homology model (Figure 2b),¹⁶ the methoxymethyl group of compound **5** may be forced too closely to the Glu584 and Leu581 residues of PI4KIIIβ. Contrastingly, their counterparts of PI4KIIIα are Asp1800 and Cys1797, respectively, more compact amino acids that afford

Table 3. SAR of the Tail Motif

Compd	R ³	R ⁴	Enzyme (IC ₅₀ , nM) ^a		Fold (α/β)	Replicon (EC ₅₀ , nM) ^a
			PI4KIIIα	PI4KIIIβ		
18	<i>n</i> -Pr		1.9	230	120	10
19	(CH ₂) ₂ OH	Me	2.5	660	260	81
20	(CH ₂) ₂ OMe		1.0	130	130	13
21	(CH ₂) ₂ SO ₂ Me		1.6	160	100	88
22	(CH ₂) ₂ OMe	<i>c</i> -Pr	0.31	220	710	6.8

^aValues of IC₅₀ and EC₅₀ are mean values determined from at least three replicates.

more room near the identical substituent of the inhibitor **5**. Based on this analysis, we considered that the slightly larger two residues in PI4KIIIβ would cause steric congestion, while a favorable hydrophobic contact between the Cys1797 of PI4KIIIα and the terminal methoxy portion of **5** might have occurred. Moreover, the size of the methoxymethyl group was almost optimal, and compound **6** enlarged at a position adjacent to the pyridine ring showed significant loss of potency against either of the PI4KIII enzymes.

Since we had a suitable headgroup in hand, we embarked on an exploration of the core motif. We examined a diverse set of heterocycles whose substituents could project into a different location (Table 2). Rather surprisingly, compound **7** being almost identical to the reference compound **5** showed reduced PI4KIIIα potency, while compound **8** whose R³ substituent was directed to a different vector showed comparable potency. Further dissimilar analogues having a 5,6-fused heteroaromatic group as the core unit generally showed reduced PI4KIIIα potency (**9**–**13**). However, significant transformations of the R³ portion restored the inhibitory potency, allowing even the least potent analogue **13** to reach a single-digit nanomolar value of IC₅₀ against PI4KIIIα (**17**). It was also notable that the SAR trend of the R³ portion was different depending on the core modules (**9** to **15** vs **13** to **17**), suggesting that each core structure had a distinct preference for the accompanying tail portion R³. Following a typical optimization protocol, fine-tuning in the head and the tail region was complementarily conducted with one of the most potent compounds, **5**.

Starting from an analogue bearing a simple tail-like substructure (**18**), we introduced a functional group at the terminus (**19**–**21**) to lower the lipophilicity to increase the drug-like properties (Table 3).¹⁷ In accordance with the previous results represented by **15** and **17**, compound **20** having an ether moiety showed better potency; by a slight modification of the headgroup (**22**), the PI4KIIIα selectivity was further strengthened. Toward this end, we have successfully discovered a novel PI4KIIIα inhibitor having superb PI4KIIIα potency. This compound is composed of three new modules, the head, core, and tail groups, which are distinct from those of PI4KIIIα inhibitors reported to date (It is also noteworthy that GSK reported that replacement of the benzene tail part with an

Scheme 1. Synthesis of Diverse Sets of PI4KIII α Inhibitors^a

^aReagents and conditions: (a) (1) MsCl, Et₃N, CH₂Cl₂, 0 °C to r.t.; (2) 2,4-difluorothiophenol, *i*-Pr₂NEt, THF, 0 °C to r.t.; (3) *m*CPBA, CH₂Cl₂, 0 °C to r.t.; (b) (1) NaH, CH₃I, THF, 0 °C; (2) 2,4-difluorobenzene-1-sulfonyl chloride, DMAP, pyridine, 60 °C then 4 M NaOH, 1,4-dioxane, H₂O, 90 °C; (c) (1) 2,6-dimethoxybenzylamine, DMF, 120 °C, MW; (2) NaBH₄, MeOH, 0 °C to r.t.; (3) NaH, CH₃I, THF, 0 °C to r.t.; (4) 2,4-difluorobenzene-1-sulfonyl chloride, DMAP, pyridine, 60 °C; (d) (1) trichloroisocyanuric acid, PhCONH₂, CHCl₃, 75 °C; (2) *c*-PrOH, NaH, THF, r.t. then H₂O, r.t.; (3) DPPA, Et₃N, *t*-BuOH, toluene, 100 °C; (4) TFA, CHCl₃, r.t.; (5) 2,4-difluorobenzene-1-sulfonyl chloride, pyridine, 60 °C then 4 M NaOH, 1,4-dioxane, H₂O, 60 °C; (6) (Bpin)₂, PdCl₂(dppf)-CH₂Cl₂, KOAc, 1,4-dioxane, 100 °C; (e) (Bpin)₂, PdCl₂(dppf)-CH₂Cl₂, KOAc, 1,4-dioxane, 100 °C; (f) 3-Bpin-5-R²-6-R¹-pyridine or 3-Br-5-R²-6-R¹-pyridine, PdCl₂(dppf)-CH₂Cl₂, Cs₂CO₃, 1,4-dioxane, H₂O, 100 °C; (g) 29, corresponding aryl bromide,¹⁸ (XPhos)Pd-G1, K₃PO₄, 1,4-dioxane, H₂O, 100 °C, MW (h) 28, corresponding arylBpin,¹⁹ PdCl₂(amphos), 1.5 M K₂CO₃, 1,4-dioxane, 100 °C; (i) 29, corresponding aryl iodide,²⁰ PdCl₂(dppf)-CH₂Cl₂, 1.5 M K₂CO₃, 1,4-dioxane, 100 °C; (2) PhB(OH)₂ or 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, PdCl₂(dppf)-CH₂Cl₂, 1.5 M K₂CO₃, 1,4-dioxane, 100 °C; (j) 10% Pd(OH)₂/C, H₂ (1 atm), THF, MeOH, r.t.

(a)

Enzyme (IC ₅₀ , nM) ^a		PI3K		LE ^b (PI4KIII α)	Replicon (EC ₅₀ , nM) ^a	
α	β	α	β		1a	1b
0.31	220	240	1400	0.34	10	6.8

^aValues of IC₅₀ and EC₅₀ are mean values determined from at least three replicates. ^bLE = -1.37 log IC₅₀(PI4KIII α)/number of heavy atoms.

(b)

ClogP	MS in liver MS (60 min, remain.%)		iv parameters (0.3 mg/kg)			po parameters (1.0 mg/kg)		
	human	rat	t _{1/2} β (hr)	Cl _{tot} (L/hr/kg)	Vd _{ss} (L/kg)	AUC (h ² M·hr)	MRT (hr)	F (%)
2.4	104	91	3.3	0.040	0.20	42	5.5	91

(c)

Figure 3. Profiles of 22. (a) *In vitro* pharmacological profiles of 22 were shown in tabular form. (b) Both *in vitro* ADME parameters and rat PK parameters for 22 were shown in tabular form. (c) Pharmacokinetic curve of 22 in rat. See the [Supporting Information](#) for detailed protocol.

alkyl group such as tetrahydropyran resulted in reduced PI4KIII α potency).¹¹ Needless to say, brief optimization of the rest of the molecules such as 15 and 17 will lead to generation of more diverse PI4KIII α inhibitors, although the details are beyond the scope of this report.

The PI4KIII α inhibitors shown in this letter were synthesized following a method highlighted in [Scheme 1](#). The route was simple and a diverse set of compounds was prepared through Suzuki-type cross coupling in a convergent manner.

Given its superb PI4KIII α potency along with good on-target selectivity, additional assays were conducted with representative compound 22 ([Figure 3](#)). First, two additional counter assays were carried out to further confirm its nonpromiscuous character, and over 800-fold selectivity was also observed against two typical lipid kinases, PI3K α and PI3K β . More importantly, 22 exhibited a low nanomolar EC₅₀ in both 1b and 1a replicon assays, thus demonstrating broad-spectrum inhibition of HCV genotypes, which was originally expected and now seems highly plausible. Good physicochemical properties as well as high metabolic stability were also notable, leading to preferable plasma half-life and excellent oral bioavailability for 22.

In summary, a novel “head-to-tail” approach has been developed to discover selective PI4KIII α inhibitors. After a concise investigation, we have successfully identified several classes of compounds showing a single-digit nanomolar value of IC₅₀ against PI4KIII α . They are composed of different core

structures, which could further multiply the diversity after the successive tuning of the accompanying substituents. We believe this method is rational yet truly simple and that this overall concept will also be applicable to general kinase programs.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmedchemlett.6b00232](https://doi.org/10.1021/acsmedchemlett.6b00232).

Synthetic procedures, experimental data, assay procedures, and PK profiles of **22** (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

NSSA, hepatitis C virus (HCV) nonstructural protein 5A; PI3K γ , phosphoinositide 3-kinase gamma; PK, pharmacokinetics; LE, ligand efficiency

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- (18) The corresponding arylbromide are as follows: SI-17 for **7**, SI-23 for **8**, SI-24 for **10**, SI-25 for **11**, and SI-22 for **12**. Please also see the Supporting Information.
- (19) The corresponding arylBpin are as follows: SI-27 for **9** and SI-30 for **14**.
- (20) SI-32 is the corresponding aryl iodide for synthesis of **13** as well as **16**.