Lead Optimization of Spiropyrzazolopyridones: A New and Potent Class of Dengue Virus Inhibitors

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S Supporting Information

ABSTRACT: Spiropyrzazolopyridone **1** was identified, as a novel dengue virus (DENV) inhibitor, from a DENV serotype 2 (DENV-2) high-throughput phenotypic screen. As a general trend within this chemical class, chiral resolution of the racemate revealed that *R* enantiomer was significantly more potent than the *S*. Cell-based lead optimization of the spiropyrzazolopyridones focusing on improving the physicochemical properties is described. As a result, an optimal compound **14a**, with balanced *in vitro* potency and pharmacokinetic profile, achieved about 1.9 log viremia reduction at 3 × 50 mg/kg (bid) or 3 × 100 mg/kg (QD) oral doses in the dengue *in vivo* mouse efficacy model.

KEYWORDS: Dengue virus (DENV), spiropyrzazolopyridones, structure–activity relationship, lead optimization

Dengue fever is a febrile disease caused by dengue virus (DENV), which is transmitted by *Aedes aegypti*, a mosquito that feeds on humans.¹ DENV threatens up to 2.5 billion people in more than 100 endemic countries. According to a World Health Organization (WHO) report,² there are 50–100 million infections annually (and yet this number could be far underestimated),³ with approximately 500,000 cases of dengue hemorrhagic fever and 22,000 deaths globally.^{4,5} Despite the clear unmet medical need, currently there is no clinically approved vaccine or antiviral therapy available for treatment of dengue.^{6–10} Therefore, it is urgent to develop safe and effective therapeutics.

High-throughput phenotypic screening is a powerful tool to identify compounds that are active against DENV by inhibiting either host pathways and/or viral proteins, which are essential for viral replication.^{11–16} As a result of DENV-2 screening on Novartis compound library, a new chemical class spiropyrzazolopyridone **1** was identified to be an interesting starting point for further optimization (Figure 1). As compound **1** was a racemate, two enantiomers were subsequently separated by chiral high-performance purification chromatography (HPLC). The structure of *R* enantiomer **1a** was unambiguously determined by X-ray crystallography (Figure 2). Interestingly, when tested in the DENV-2 *in vitro* assay, these two individual enantiomers displayed remarkably different potency where *R* enantiomer **1a** was greater than 200-fold more potent than the *S* enantiomer **1b** (Figure 1). Very recently, genetic analysis demonstrated that mutations in dengue viral NS4B conferred

Figure 1. DENV-2 *in vitro* potency of spiropyrzazolopyridone **1** and its enantiomers.

the resistance of compound **1a**, suggesting that this class of compounds inhibited DENV replication by targeting NS4B protein.^{11,17–19} In this report, lead optimization efforts to explore the structure–activity relationships (SARs) and improve the physicochemical properties are described.

Following the identification of compound **1**, which was subsequently found to be poorly soluble in aqueous media, we initiated a lead optimization effort in an attempt to improve the solubility while maintaining the potency. The general synthetic route to the spiropyrzazolopyridone derivatives is outlined in Scheme 1. The synthesis, featuring a three component condensation of aminopyrazoles, isatin derivatives, and Mel-

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Figure 2. X-ray structure of the *R* enantiomer 1a.Scheme 1. Synthetic Route to the Spiropyrazolopyridones^a

^aReagents and conditions: (a) $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$, EtOH, room temperature (rt); (b) i. di-*tert*-butylcarbazate, PPh_3 , THF, 0 °C; ii. HCl in dioxane, rt; (c) (*E*)-ethyl 2-cyano-3-ethoxyacrylate **5**, EtOH, 80 °C; (d) CH_3I , NaH, DMF, 0 °C to rt; (e) i. NaOH, EtOH/ H_2O , reflux; ii. AcOH, reflux; (f) $\text{BH}_3\cdot\text{Me}_2\text{S}$, THF, rt.

drum's acid, provides the access to a series of spiro[pyrazolo[1,5-*a*]pyridone analogues for SAR investigation.^{20,21} Since isatin derivatives (**9**) and Meldrum's acid (**10**) are largely commercially available, our main effort to diversify the class was to make aminopyrazole derivatives (**6** and **7**).

Briefly, substituted hydrazine **4** was made either by substitution of chloride **2** with hydrazine or Mitsunobu reaction of the alcohol **3** with di-*tert*-butylcarbazate followed by deprotection in the presence of acid. Then, condensation of hydrazine **4** with (*E*)-ethyl 2-cyano-3-ethoxyacrylate **5** in ethanol afforded aminocarboxylate **6**,²² which could be methylated to deliver compound **7**. Lastly, hydrolysis of the esters **6** or **7** in the presence of NaOH gave the corresponding sodium salts, which were directly used for the three component condensation with substituted isatins **8** and Meldrum's acid **9** in acetic acid at reflux to afford the spiro[pyrazolo[1,5-*a*]pyridones **1**, **10–19**, and **22–26**. Reduction of the compound **1** in the presence of borane–dimethyl sulfide gave a mixture of compounds **20** and **21**, which were separated by chromatography. Finally, those enantiomers mentioned in this report were obtained by chiral HPLC separation of their corresponding racemates.

The structure–activity relationship (SAR) of this spiro[pyrazolo[1,5-*a*]pyridone class is summarized in Table 1. For all the chiral separated pairs (compounds **13a/b**, **14a/b**, and **23a/b**),

Table 1. Structure–Activity Relationship and Cytotoxicity

cmpd	Structure	DENV-2 EC ₅₀ (μM) ^a	HepG2 CC ₅₀ (μM)	Chirality
1a		0.012 (0.012) ^b	>10	<i>R</i>
10		3.9	>10	racemate
11		1.7	>10	racemate
12		6.2	>10	racemate
13		0.025	>10	racemate
13a		0.021	>10	<i>R</i>
13b		3.9	>10	<i>S</i>
14		0.67	33	racemate
14a		0.23 (0.042) ^b	33	<i>R</i>
14b		18.4	>10	<i>S</i>
15		32.8	>10	racemate
16		85.3	>10	racemate
17		22.5	>10	racemate
18		32.5	>10	racemate
19		7.2	>10	racemate

Table 1. continued

cmpd	Structure	DENV-2 EC ₅₀ (μM) ^a	HepG2 CC ₅₀ (μM)	Chirality
20		4.0	8.1	racemate
21		1.1	>10	racemate
22		0.42	>10	racemate
23		0.011	>10	racemate
23a		0.006	>10	R
23b		0.772	>10	S
24		0.05	>10	racemate
25		1.3	>10	racemate
26		2.3	>10	racemate

^aEC₅₀ values are determined in the DENV-2 CFI assay (see Supporting Information). ^bDetermined in the DENV-2 viral-titer reduction assay.⁷

Table 2. Summary of the Key *in Vitro* Properties

cmpd	solubility (μM, pH 6.8)	LogP	mouse microsomal stability	
			ER ^a (%)	T _{1/2} (min)
1a	11	4.9	66	31
13a	27	4.1	49	62
23a	161	4.6	91	5.8
14a	504	2.8	71	24

^aHepatic extraction ratio.

similarly to what has been described for **1a** and **1b**, *R* enantiomers were markedly more potent than *S* enantiomers.

Table 3. Pharmacokinetic Parameters Following 25 mg/kg Oral and 5 mg/kg Intravenous Dosing in Rats^a

cmpd	oral PK ^b parameters				intravenous PK ^c parameters		
	C _{max} (μg/mL)	T _{max} (h)	AUC (h·μg/mL)	F (%)	V _{ss} (L/kg)	CL (mL/min/kg)	T _{1/2} (h)
1a	0.78	2.0	9.5	33	4.3	15.1	3.8
14a	3.86	0.17	13.8	63	3.0	18.8	1.3

^aC_{max}, maximum concentration of drug in plasma; T_{max}, time to maximum concentration of drug in plasma; AUC, area under the curve (*t* = 0 to 24 h); V_{ss}, volume of distribution at steady state; CL, plasma clearance; T_{1/2}, terminal half-life; F, absolute oral bioavailability. ^bFormulation for oral dose: suspension of 0.5% Tween80 and 0.5% methylcellulose in phosphate buffer, pH 6.8. ^cFormulation for intravenous dose: 10% NMP and 90% rat plasma.

Figure 3. DENV-2 *in vivo* mouse efficacy of compound 14a.

So we believe that this phenomenon is a general trend within this class of compounds.²³ As we explored the SAR around the R₁ substitution, we found the *para*-chlorobenzyl was preferred, while *meta*- or *ortho*-chlorobenzyl (**10** and **11**) resulted in a loss of activity on DENV-2. In addition, for the *para*-benzyl substitutions, electron withdrawing group (**13**) was superior to electron donating group (**12**). Interestingly, 3-pyridyl (**14**) was tolerated, and the *R* enantiomer **14a** maintained a similar level of potency in the secondary viral-titer reduction assay⁷ (EC₅₀ = 42 nM) with lower lipophilicity (measured LogP = 2.8). However, the other R₁ substitutions, such as 2-pyridyl (**15**), 3,5-pyrimidine (**16**), cyclohexyl (**17**), phenyl (**18**), or homobenzyl (**19**), resulted in significant loss of potency, suggesting a narrow range of substitutions are tolerated at the R₁-position. Next, we started to investigate the SAR around the core modification. Both of the compounds **20** and **21** resulted in a loss of potency, indicating both of the amide carbonyl groups are important for the activity. Furthermore, compounds **22** and **23** with methyl on either of the amides maintained the same level of potency, indicating that neither of the NH is critical for the potency. In fact, *R* enantiomer **23a** was the most potent compound identified so far; however, it suffered with high LogP and poor metabolic stability (Table 2). Finally, SAR around the R₄ substitutions was investigated. Though bromo (**24**) was well tolerated, both electron-withdrawing (**25**) and electron-donating groups (**26**) diminished the potency, suggesting again a tight range of tolerated substitutions at this position.

The key physicochemical properties of the representative spiropyrazolopyridones are summarized in Table 2. Compound **13a** displayed similar profile to compound **1a** with marginal improvement of solubility, while compound **23a** suffered from poor metabolic stability with high mouse hepatic extraction ratio and short half-life. Gratifying, compound **14a** with lower LogP showed improved solubility, though the microsomal stability was slightly compromised. In order to determine if the

improved solubility would translate to a better *in vivo* PK profile, compounds **1a** and **14a** were evaluated in rats by oral (p.o.) and intravenous (i.v.) routes at 25 and 5 mg/kg, respectively (Table 3). Indeed, compared to compound **1a**, optimized compound **14a** showed higher $C_{\max}$ and exposure (AUC), as well as increased oral bioavailability (63%).

Next, in order to determine the *in vivo* efficacy of this class of compounds, compound **14a** was tested in a DENV-2 viremia mouse model (Figure 3).²⁴ When compound **14a** was administered orally at 50 mg/kg twice daily (bid) for 3 days, compared to vehicle control group, significant viremia reduction (about 1.9 log, P value <0.05) was achieved. Similar efficacy was observed when compound **14a** was orally dosed at 100 mg/kg once daily (QD) for 3 days. These results were similar if not better than the positive control group, which was treated with nucleoside analogue NITD008 (25 mg/kg bid for 3 days).²⁵ In summary, this class of compounds clearly demonstrated a good *in vivo* mouse efficacy and proved that DENV NS4B protein is a druggable target for dengue drug discovery.

In conclusion, a novel spiro-pyrazolopyridone scaffold was identified from dengue phenotypic screening. Chiral HPLC separation of the racemates revealed that *R* enantiomers were significantly more potent than *S* enantiomers. After exploring the SARs, we identified compound **14a** with optimal *in vitro* potency and physicochemical properties, which translated into good *in vivo* PK and efficacy. However, the main drawback for this class of compounds is the relatively weak potency in DENV-1 and -4, though the potency of DENV-3 tracks well with the DENV-2 activity.¹⁷ Further optimization to acquire potency of all four serotypes will be reported in due course.

■ ASSOCIATED CONTENT

■ Supporting Information

Full experimental details for compounds synthesized and descriptions of assays. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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