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

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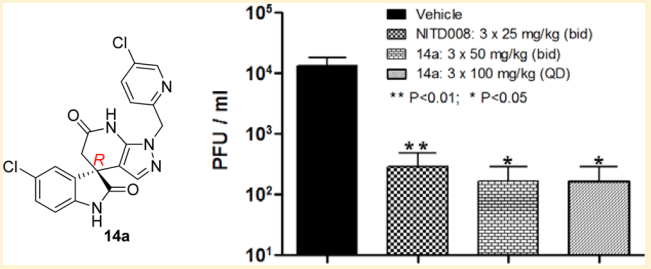
Supporting Information

ABSTRACT: Spiropyrazolopyridone **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 spiropyrazolopyridones 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), spiropyrazolopyridones, 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 spiropyrazolopyridone **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

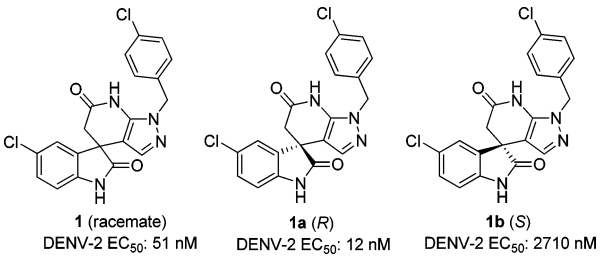


Figure 1. DENV-2 *in vitro* potency of spiropyrazolopyridone **1** and its enantiomers.

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 spiropyrazolopyridone derivatives is outlined in Scheme 1. The synthesis, featuring a three component condensation of aminopyrazoles, isatin derivatives, and Mel-

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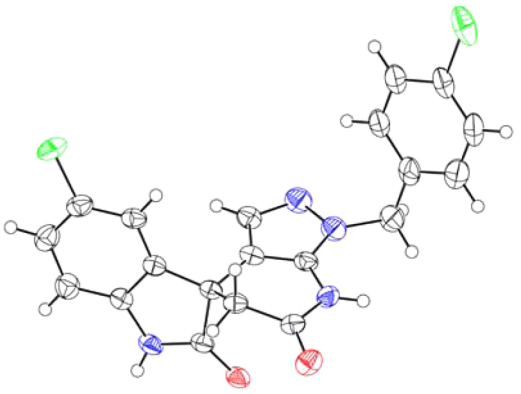
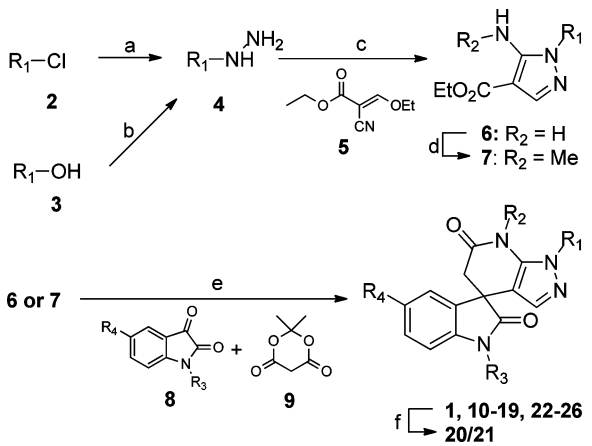


Figure 2. X-ray structure of the *R* enantiomer **1a**.

Scheme 1. Synthetic Route to the Spiropyrazolopyridones[a]



[a]Reagents and conditions: (a) $N_2H_4{\cdot}H_2O$, 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) $BH_3{\cdot}Me_2S$, THF, rt.

drum's acid, provides the access to a series of spiropyrazolopyridone 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 spiropyrazolopyridones **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 spiropyrazolopyridone 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

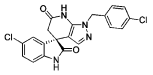
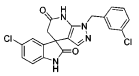
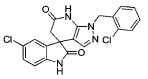
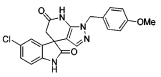
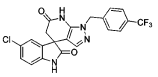
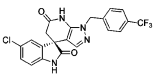
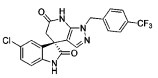
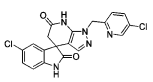
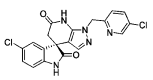
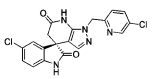
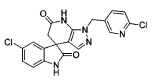
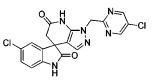
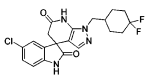
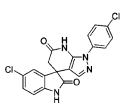
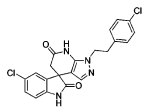
cmpd	Structure	DENV-2 EC_{50} (μM)[a]	HepG2 CC_{50} (μM)	Chirality
1a		0.012 (0.012)[b]	>10	*R*
10		3.9	>10	*racemate*
11		1.7	>10	*racemate*
12		6.2	>10	*racemate*
13		0.025	>10	*racemate*
13a		0.021	>10	*R*
13b		3.9	>10	*S*
14		0.67	33	*racemate*
14a		0.23 (0.042)[b]	33	*R*
14b		18.4	>10	*S*
15		32.8	>10	*racemate*
16		85.3	>10	*racemate*
17		22.5	>10	*racemate*
18		32.5	>10	*racemate*
19		7.2	>10	*racemate*

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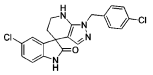
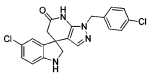
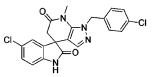
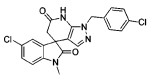
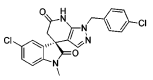
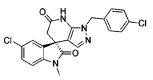
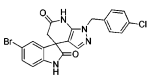
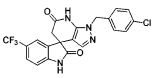
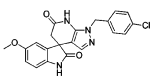
Table 1. continued

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*

[a]EC_{50} values are determined in the DENV-2 CFI assay (see Supporting Information). [b]Determined in the DENV-2 viral-titer reduction assay.[7]

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

			mouse microsomal stability	
cmpd	solubility (μM, pH 6.8)	LogP	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

[a]Hepatic extraction ratio.

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

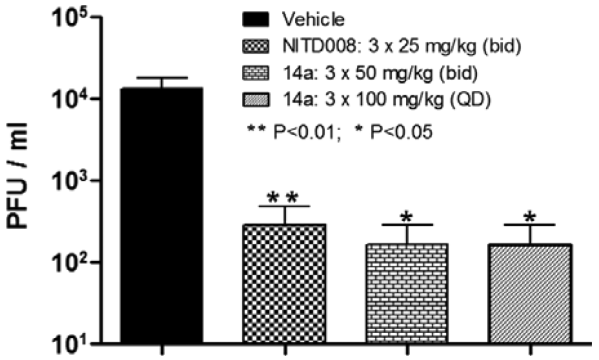


Figure 3. DENV-2 *in vivo* mouse efficacy of compound **14a**.

So we believe that this phenomenon is a general trend within this class of compounds.[23] As we explored the SAR around the R_1 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 assay[7] (EC_{50} = 42 nM) with lower lipophilicity (measured LogP = 2.8). However, the other R_1 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 R_1-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 R_4 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

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

	oral PK[b] parameters				intravenous PK[c] parameters		
cmpd	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

[a]C_{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. [b]Formulation for oral dose: suspension of 0.5% Tween80 and 0.5% methylcellulose in phosphate buffer, pH 6.8. [c]Formulation for intravenous dose: 10% NMP and 90% rat plasma.

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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 administrated 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 spiropyrazolopyridone 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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