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Structure–activity relationship (SAR) studies of quinoxalines as novel HCV NS5B RNA-dependent RNA polymerase inhibitors☆

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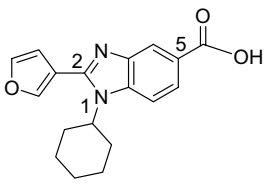
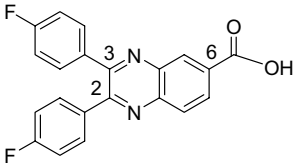
Abstract—From chemical compound library screening using an HCV NS5B RNA-dependent RNA polymerase enzymatic assay, we identified a substituted quinoxaline hit with an IC_{50} of 5.5 μM. A series of substituted quinoxaline amide derivatives were synthesized based on the hit's pharmacophore, and a good structure–activity relationship was observed. Computer modeling analysis was employed to help comprehend the SAR.

Chronic hepatitis C virus (HCV) infection is the leading cause of liver diseases and hepatocarcinoma.[1] An estimation suggests that 170 million people worldwide are infected with the virus with 4 million of them residing in the United States.[2] The HCV viral genome exhibits a high degree of heterogeneity, which presents a formidable hurdle to develop effective prophylactic vaccines. Current standard therapies consist of combinations of pegylated IFN-α and ribavirin, and are inadequate to cure the viral infection in a significant proportion of patients, especially those infected with genotype 1 HCV.[3] Progresses have been made in the discovery of direct anti-HCV inhibitors, especially the HCV NS3 protease inhibitors. However, they are presently still in various stages of clinical development. Thus, it is important to continue the effort to discover and develop novel and potent anti-HCV therapeutics.

HCV is a small positive-sense single-stranded RNA virus that belongs to the *Flaviviridae* family.[4] Its RNA genome encodes a few enzymes essential for viral replication, including the NS3 protease and NS5B RNA-dependent RNA polymerase (RdRp).[5] Naturally they have become popular drug targets. NS5B RdRp constitutes the key component of the viral replication machinery. It plays a central role in the viral RNA synthesis, and has been proven to be a validated drug target.[6] We have devised an HCV NS5B RdRp enzymatic assay to screen our small molecular compound library for NS5B inhibitors. From the high throughput screening, an interesting hit **2** was identified. Although this compound is not particularly potent with an IC_{50} of 5.5 μM, its quinoxaline 6-carboxylate pharmacophore bears good resemblance to a known benzoimidazole 5-carboxylate NS5B RdRp inhibitor **1** reported by Boehringer Ingelheim.[7] The hit shares the same structural feature of a bicyclic aromatic core substituted with two single cyclic groups at the left side and a carboxylate group at the right. Since **1** has been successfully optimized to achieve nanomolar potency, we attempted to optimize compound **2** through the similar SAR studies. In this communication, we report the chemical synthesis and structure–activity relationship (SAR) of these quinoxaline compounds.



1, IC_{50} = 1.6 uM
(Boehringer Ingelheim)



Hit: 2, IC_{50} = 5.5 uM

Keywords: HCV; NS5B polymerase; Quinoxaline; Inhibitor.
☆ This paper is dedicated to Professor Albert H. Soloway of The Ohio State University at the occasion of his 82th birthday.
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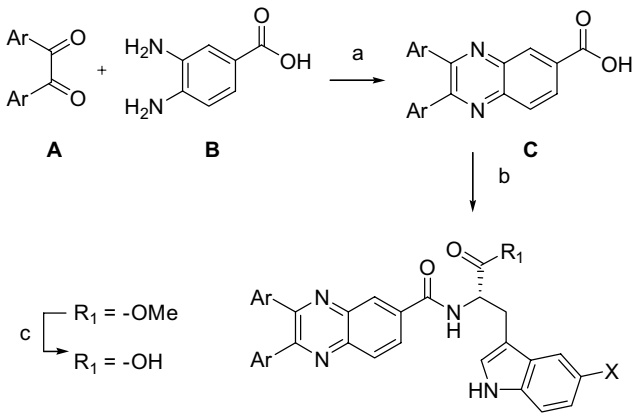
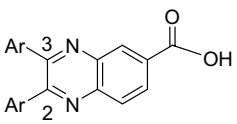


Scheme 1. Reagents and conditions: (a) NaOAc, AcOH, 118 °C, 4 h; (b) amino acid methyl ester, TBTU, DIPEA, DMF, 0 °C–rt, 24 h; (c) NaOH, DMF, H_2O, rt, 5–16 h.

Compounds were generally prepared according to Scheme 1. Basically, condensation of α-diketones (**A**) with 3,4-diaminobenzoic acid (**B**) in the presence of sodium acetate in acetic acid afforded 2,3-biaryl substituted quinoxaline-6-carboxylic acid derivatives (**C**) with a typical yield of 80–100%.[8] The compounds (**C**) were precipitated out of the solution upon addition of cold water and were isolated by vacuum filtration. Coupling of **C** with L-tryptophan methyl or ethyl ester hydrochloride using TBTU and *N*,*N*-diisopropyl-ethylamine followed by saponification at the room temperature provided the desired products.[7] The resulting compounds were separated by reverse phase HPLC, and their corresponding structures were confirmed by ^1H and ^{13}C NMR and high resolution MS analysis. In some cases, the structures were assigned using 2-D NMR. These resultant compounds were evaluated for inhibitory activity against HCV NS5B RdRp using a published procedure.[9] Their IC_{50}s were determined, which are summarized in Tables 1–4.

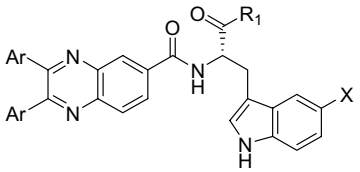
We first set out to explore SAR of the aromatic substituents of the initial hit **2** by synthesizing a small focused library of compounds **2**–**7** (Table 1). Compound **2** with bis 4-F-phenyl substitutions was the most potent among them with an IC_{50} of 5.5 μM. By comparison, compounds with unsubstituted phenyl (**4**) or 4-Me-phenyl (**5**) reduced the inhibitory potency by 8- and 14-fold, respectively. This highlights the sensitivity of the size and electronic property of the substituents on the phenyl ring. The addition of –OMe at the meta position of phenyl ring in compound **6** rendered a total loss of activity with an $IC_{50} > 100$ μM. Replacement of the phenyl ring with a furyl or 2-pyridyl group in compounds **3** and **7** also led to diminishing activity.

Table 1. Initial SAR studies on **2**



Compound	Ar	IC_{50} (μM)
2	4-F–Ph–	5.5
3	2-Furyl–	17
4	Ph–	79
5	4-Me–Ph	40
6	3-OMe–Ph	>100
7	2-Pyridyl–	>100

Table 2. IC_{50} values of compounds **8**–**29**



Compound	Ar	R_1	X	IC_{50} (μM)
8	2-Furyl–	OCH_3	H	>100
8a		OH	H	33
9	Ph–	OCH_3	H	>100
9a		OH	H	15
10	4-F–Ph–	OCH_3	H	>100
10a		OH	H	1.9
11	4-Me–Ph–	OCH_3	H	>100
11a		OH	ND	ND
12	3-OMe–Ph–	OCH_3	H	>100
12a		OH	H	>100
13	2-Pyridyl–	OCH_3	H	>100
13a		OH	H	>100
14	2-Furyl–	OCH_3	OH	26
14a		OH	OH	16
15	Ph–	OCH_3	OH	72
15a		OH	OH	11
16	4-F–Ph–	OCH_3	OH	5.5
16a		OH	OH	1.3
17	4-Me–Ph	OCH_3	OH	49
17a		OH	OH	6.1
18	3-OMe–Ph–	OCH_3	OH	>100
18a		OH	OH	15

It was reported that the modification of the carboxylate group in **1** by coupling it with L-tryptophan derivatives afforded compounds with nanomolar inhibitory potency.[7] We applied the same strategy to the hit **2** and made a series of compounds. As illustrated in Table 2, the most potent compound in this series, **16a**, had an IC_{50} of 1.3 μM, which is 4-fold lower than that of **2**. Interestingly, compound **16a** carries 4-F-phenyl substituents as **2**. Other compounds with different Ar groups, such as **8a**–**15a**, **17a**, **18a**, all exhibited weaker activities (Table 2). It is also worth to note that the ester derivative **16,** in comparison to **16a**, is 4-fold weaker, which applies to all the ester derivatives of the carboxylic acid counterparts listed in Table 2 (**8**–**18**). This result suggests the potential importance of –COOH group in forming NS5B-inhibitor interactions.

The improved potency of **16a** over the initial hit **2** prompted us to further probe a more diversified set of amino-acid derivatives. As the 4-F-phenyl had been shown to be the optimized among the substitutions evaluated, we kept it unchanged in the new series of