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

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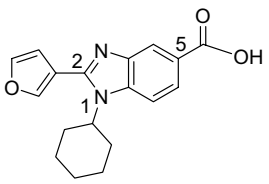
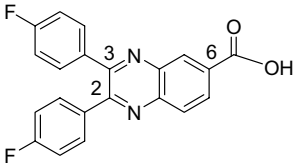
Abstract—From chemical compound library screening using an HCV NS5B RNA-dependent RNA polymerase enzymatic assay, we identified a substituted quinoxaline hit with an IC_{50} of 5.5 μM. A series of substituted quinoxaline amide derivatives were synthesized based on the hit's pharmacophore, and a good 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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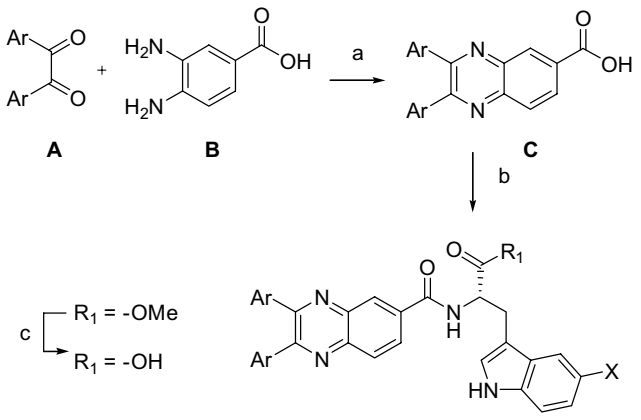
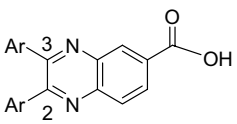


Scheme 1. Reagents and conditions: (a) NaOAc, AcOH, 118 °C, 4 h; (b) amino acid methyl ester, TBTU, DIPEA, DMF, 0 °C–rt, 24 h; (c) NaOH, DMF, H_2O, rt, 5–16 h.

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.

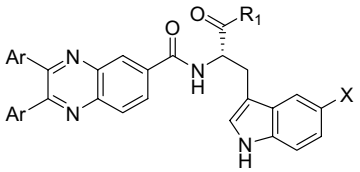
We first set out to explore SAR of the aromatic substituents of the initial hit **2** by synthesizing a small focused library of compounds **2**–**7** (Table 1). Compound **2** with bis 4-F-phenyl substitutions was the most potent among them with an IC_{50} of 5.5 μM. By comparison, compounds with unsubstituted 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**

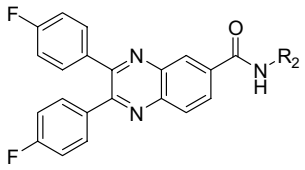


Compound	Ar	R_1	X	IC_{50} (μM)
8	2-Furyl–	OCH_3	H	>100
8a		OH	H	33
9	Ph–	OCH_3	H	>100
9a		OH	H	15
10	4-F–Ph–	OCH_3	H	>100
10a		OH	H	1.9
11	4-Me–Ph–	OCH_3	H	>100
11a		OH	ND	ND
12	3-OMe–Ph–	OCH_3	H	>100
12a		OH	H	>100
13	2-Pyridyl–	OCH_3	H	>100
13a		OH	H	>100
14	2-Furyl–	OCH_3	OH	26
14a		OH	OH	16
15	Ph–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

Table 3. SAR of 2, 3-difluorophenyl quinoxalines



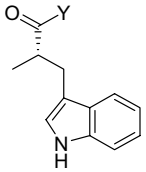
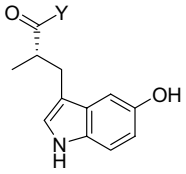
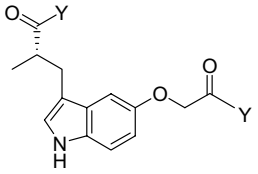
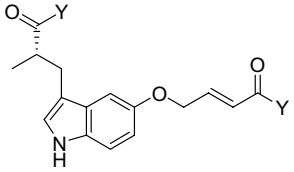
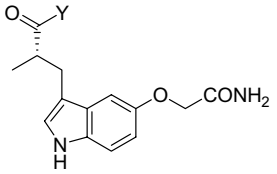
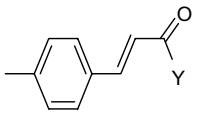
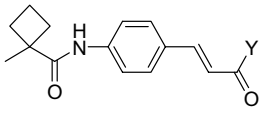
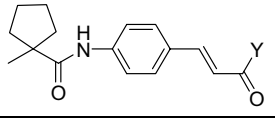
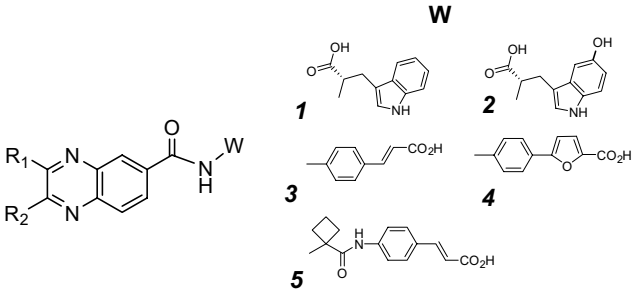
Compound	R_2	Y	IC_{50} (μM)
10		OMe	>100
10a		OH	1.9
16		OMe	5.5
16a		OH	1.3
19		OMe	100
19a		OH	1.7
20		OMe	53
20a		OH	2.2
21		OMe	8.6
21a		OH	3.2
22		OEt	>100
22a		OH	17
23		OEt	37
23a		OH	0.69
24		OEt	>100
24a		OH	1.2

Table 4. SAR of 2,3-asymmetric quinoxalines



Compound	R_1	R_2	W	IC_{50} (μM)
25	Ph–	Cyclohexyl–	1	3.7
25a	Cyclohexyl–	Ph–		1.8
26	Ph–	Cyclohexyl–	2	5.0
26a	Cyclohexyl–	Ph–		0.6
27	4-F–Ph–	Cyclohexyl–	1	3.4
27a	Cyclohexyl–	4-F–Ph–		1.6
28	4-F–Ph–	Cyclohexyl–	2	4.1
28a	Cyclohexyl–	4-F–Ph–		4.6
29	Ph–	Cyclohexyl–	3	1.3
30	4-F–Ph–	Cyclohexyl–	3	4.1
30a	Cyclohexyl–	4-F–Ph–		4.6
31	Ph–	Cyclohexyl–	4	1.8
31a	Cyclohexyl–	Ph–		1.6
32	4-F–Ph–	Cyclohexyl–	5	5.1
32a	Cyclohexyl–	4-F–Ph–		1.3

compounds. As illustrated in Table 3, many of the compounds from this series showed similar activity as **16a**. Compound **23a** is the most potent one, showing an IC_{50} of 0.69 μM, which is a 2-fold improvement over **16a**. A closely related analog (**24a**) carrying a cyclopentyl ring is slightly weaker than **23a**. Other L-tryptophan containing carboxylic acid derivatives, **10a**–**21a**, are almost equally potent as **16a**. Similar to the results in Table 2, all of the ester derivatives (**10**, **16**, and **19**–**24**) are either significantly weaker or inactive (IC_{50} > 100 μM) in the NS5B RdRp assay, underlining the importance of a carboxylic acid group for inhibitory activity.

All the compounds discussed above have symmetric bis Ar groups on the quinoxaline ring. From an early publication of inhibitor **1**, it seems that compounds with asymmetric substituents, especially the presence of a cyclohexyl group, possess optimal activity.[7] This postulation prompted us to synthesize a focused set of asymmetric quinoxalines with R_1 and R_2 being cyclohexyl, 4-F-phenyl or phenyl. Different amino acids were used to form the quinoxaline amide derivatives. As demonstrated in Table 4, all of the compounds possessed low micromolar potency with **26a** being the most potent (0.6 μM). In contrary to scaffold **1**, it appears that cyclohexyl in the R_1 position yielded more potency than in the R_2 position. This is the trend for the pairs of compounds **25a/25**, **26a/26**, **27a/27**, and **32a/32**. The rest two pairs of compounds in Table 4 have very similar IC_{50} values. Unlike in scaffold **1**, a cyclohexyl group in the quinoxaline scaffold does not significantly enhance IC_{50} values.

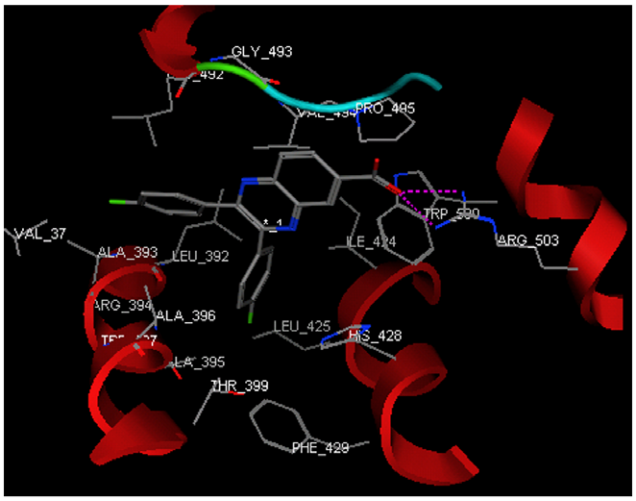


Figure 1. Binding mode of **2** in the allosteric site of NS5B (PDB code: 2BRL) predicted by GLIDE docking.

To further understand SAR and binding mode of quinoxaline derivatives to HCV NS5B RdRp, we performed a molecular modeling study using GLIDE docking software.[10] X-ray structure of a related analog of **1** was published and available in PDB database (PDB code: 2BRL).[11] We assumed the hit **2** binds to the same allosteric site as compound **1**. The extreme precision mode of GLIDE was employed for the docking, and up to 10 poses were saved for analysis. All of the saved poses were similar and therefore, the top scored pose is depicted in Figure 1.

The resultant binding mode displays numerous interactions between **2** and NS5B. These interactions are remarkably comparable with those for the analog of **1** reported in the literature.[11] Notably, the –COOH group of **2** engages in the ionic-ionic type H-bonding interactions with the basic guanidine side chain of Arg 503 of NS5B. The 4-F-phenyl group at the position 2 of quinoxaline extends into a narrow hydrophobic pocket defined by two α-helixes, and the same group specifically makes hydrophobic contacts with the pocket defined by the side chains of Leu 392, Ala 396, Ala 395, Thr 399, Leu 425, and Phe 429. The other 4-F-phenyl at the 3-position of quinoxaline forms hydrophobic interactions with side chains of Leu 392, Ala 393, and Leu 492. In addition, the quinoxaline ring makes hydrophobic interactions with Ile 424, Gly 493, Val 494, and Pro 495 in the binding site.

The predicted binding mode agrees well with the observed SAR. For example, when –COOH group was converted into –COOMe, the only ionic H-bond interaction between an inhibitor and NS5B disappears upon docking. This drastically reduced the inhibitory potency, resulting in higher IC_{50} values for ester derivatives of **8**–**18** (Table 2). The hydrophobic nature of the Ar binding sites in NS5B prefers a hydrophobic substituent, and therefore, a hydrophilic group such as 2-pyridyl (**7**) would be detrimental to the activity (Table 1). More interestingly, according to the predicted pose of **2** in NS5B, any extended groups stemming from the coupling of –COOH of **2** with an amino-acid derivative would extend into the surface of NS5B without any twell-defined interactions. This determines the insensitivity of the amino acid derivatives to the potency and explains the flat SAR in compounds **10**, **16a**–**24a** (Table 3), and **25a**–**32a** (Table 4).

In summary, we identified an interesting substituted quinoxaline hit from high throughput screening using an NS5B enzymatic assay. Based on the hit's pharmacophore and published similar inhibitors, many substituted quinoxaline amide derivatives were synthesized. A good structure–activity relationship has been observed for these compounds. One of the most potent compounds has an IC_{50} of 0.6 μM in the enzymatic assay. Further optimization would yield more potent compounds and explore NS5B RdRp as a potential drug target for anti-HCV drug discovery.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.103.

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