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Discovery of highly potent small molecule Hepatitis C Virus entry inhibitors

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ABSTRACT

Novel, highly potent small molecule HCV entry inhibitors are reported. The SAR exploration of a hit molecule identified from screening of a compound library led to the identification of highly potent compounds with IC_{50} values of <5 nM in the tissue culture HCV infectious assay.

Hepatitis C Virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma and is a leading indication for liver transplantation.[1] The U.S. Centers for Disease Control and Prevention (CDC) estimates the world wide prevalence of Hepatitis C Virus infection at ~3%, or 170 million individuals, and in the United States it estimates 3.2 million infected individuals.[2] The current standard of care is a combination therapy with pegylated interferon and the nucleoside analog ribavirin. However, the treatment has adverse side effects and moderate efficacy.[3] The anti-HCV drugs currently in development mostly target the viral enzymes, protease and polymerase that are essential for viral replication. More recently, two protease inhibitors, Incivek™ and Victrelis™ were approved by the FDA to be used in combination with pegylated interferon and ribavirin. The single agent studies have shown the rapid emergence of resistant mutants to these new inhibitors, suggesting cocktails of HCV specific antivirals targeting multiple steps in the infection cycle will likely be required for rapid viral clearance and to overcome resistant mutants.[4–6]

We have taken a different approach by focusing our efforts on the discovery and development of small molecule HCV entry inhibitors[7,8] that prevent entry of HCV into the host cell. The viral entry is a critical step in the life cycle of HCV, and inhibitors of viral entry can add significantly to a drug cocktail targeting multi-steps of the viral life cycle. The entry inhibitors can protect cured cells from re-infection and preventing viral spread could help stop amplification of resistant mutants. The HCV entry into hepatocytes requires at least five cellular co-factors, CD-81,[9] claudin,[10,11] occludin,[12] scavenger receptor B1[13] and the more recently discovered epidermal growth factor receptor 1 and 2.[14] We have recently reported[15] a highly potent HCV inhibitor ITX 5061 (Fig. 1), which blocks viral entry by binding to the scavenger receptor B1 (SR-B1). This first-in-class drug is currently in Phase Ib clinical trials (Fig. 1).

Here, we report the synthesis and SAR of novel small molecule HCV entry inhibitors that belong to a distinct chemical class from ITX 5061. A library of compounds, comprising small molecules and natural products, was screened using the HCV pseudo particle (HCVpp) entry assay[16–18] to identify compounds that were nearly equally effective against genotype 1a and 2b. The identified hits were further validated in a HCV2aCH-Rluc infectious assay (HCVcc-2a).[19–21] One of the hits identified is the compound Rimcazole[22] (**1**) that showed an IC_{50} of 1.2 μM in HCVcc-2a entry assay (Fig. 2).

The initial SAR exploration at the 2- and 3-positions of the carbazole (not shown) to improve the potency and drug-like properties, suggested that the methyl ester at the 3-position improved the potency by several fold. The compound **2** with a methyl ester at the 3-position of the carbazole showed an IC_{50} of 230 nM in HCVcc-2a entry assay, whereas its corresponding ethyl ester (**3**) and isopropyl ester (**4**) were less potent (Fig. 2). It was also observed that the tetrahydrocarbazoles are generally more potent than their corresponding carbazoles. This led us to focus our efforts on 6,9-disubstituted tetrahydrocarbazole series.

The synthesis of 6,9-disubstituted tetrahydrocarbazoles is shown in Scheme 1. The Fischer indole condensation of 4-hydrazinobenzoic acid with cyclohexanone gave tetrahydrocarbazole-6-carboxylic acid which was subsequently esterified to obtain the

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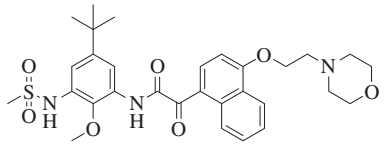
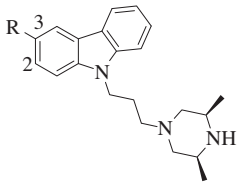


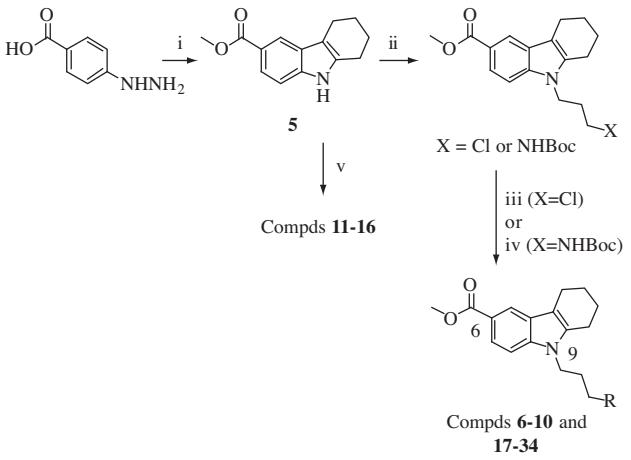
ITX 5061 (Phase Ib)

Figure 1. Stucture of HCV entry inhibitor ITX 5061.



1	R = H	IC_{50} = 1.2 μM
2	R = $COOCH_3$	IC_{50} = 230 nM
3	R = $COOC_2H_5$	IC_{50} = 600 nM
4	R = $COO^iC_3H_7$	IC_{50} = 630 nM

Figure 2. The structure of hit compound (**1**) identified from screening of a library.



Scheme 1. Reagents and conditions: (i) (a) cyclohexanone, AcOH, reflux, overnight; (b) MeOH, H_2SO_4, reflux, overnight; (ii) NaH, 1-bromo-3-chloro propane, DMF, 3 h; or NaH, DMF, BocNH-$(CH_2)_3$-OTs, rt, 18 h; (iii) *n*-$Bu_4NH_4^+I^-$, 2° amine, DMF, rt, 2–3 days; (or) K_2CO_3, DMF, imidazole or 2-methyl imidazole or purine, *n*-$Bu_4NH_4^+I$, rt, 18 h; (or) 2-oxo pyrimidines, K_2CO_3, 80 °C, overnight; (or) phenyl sulfinate, DMF, 80 °C, overnight; (iv) (a) TFA, CH_2Cl_2, 0 °C-rt, 3 h; (b) 2-chloro pyrimidines, DMF, DIEA, 80 °C, overnight; (or) aryl sulfonyl chloride or isopropyl sulfonyl chloride, CH_2Cl_2, Et_3N, rt, overnight; (v) NaH, DMF, 3-chloropropyl sulfonamides, rt, 18 h; (or) NaH, DMF, 3-chloropropyl-*N*,*N*-dimethylamide, rt, 18 h.

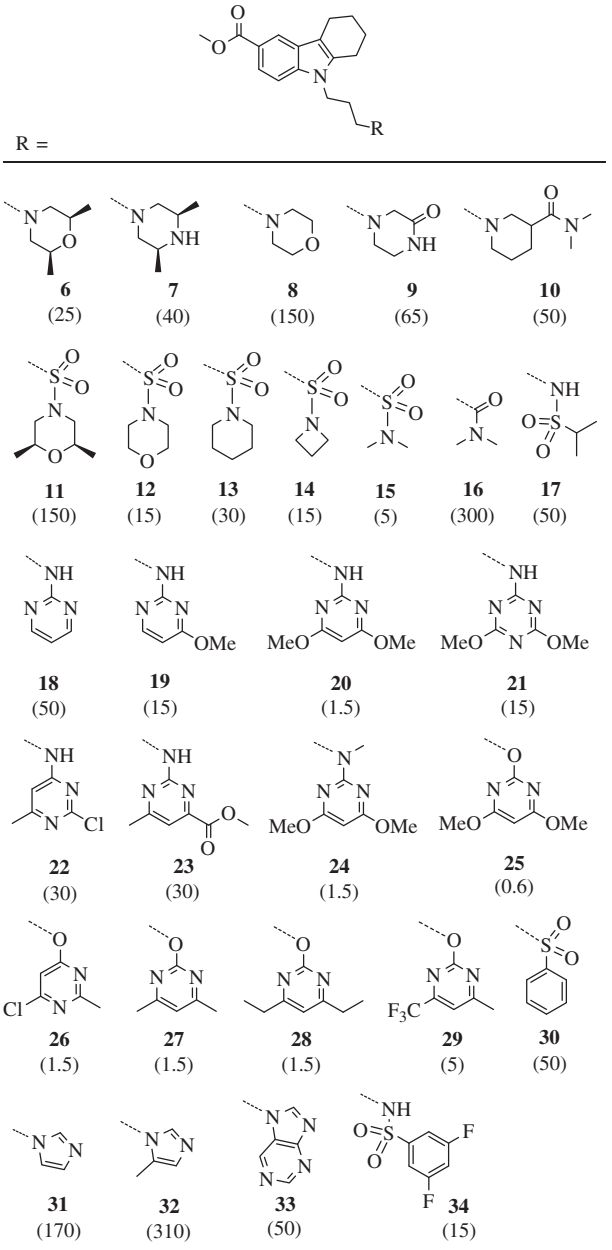


Figure 3. SAR exploration of groups at 9-position. The values within parenthesis indicate IC_{50} in nM are from HCV2aCH-Rluc assay. The IC_{50} values are mean of three experiments.

compound **5**. The alkylation of compound **5** with 1-bromo-3-chloro-propane followed by chloro displacement with various nucleophiles afforded compounds **6**–**10** and **25**–**33**. The alkylation with 3-chloropropyl sulfonamides or 3-chloropropyl-*N*,*N*-dimethylamide yielded the compounds **11**–**16**. The 2-amino pyrimidine analogs (**18**–**24**) were prepared by the alkylation of compound **5** with BocNH-$(CH_2)_3$-OTs, deprotection of the Boc group and subsequent reaction with 2-chloro pyrimidines. The compounds **17** and **34** were prepared by the reaction of methyl 9-(3-aminopropyl)-2,3,4,9-tetrahydro-1*H*-carbazole-6-carboxylate (intermediate of the 2-amino pyrimidine compounds) with isopropylsulfonyl chloride or 3,5-difluorophenylsulfonyl chloride in the presence of Et_3N. The synthesized compounds were characterized by ^1H NMR and mass spectra[23] and were tested in the HCV2aCH-Rluc infectious assay.[15]

To understand the SAR, we explored alternatives to the 2,6-dimethyl piperazine with a methyl ester group at 6-position of tetrahydrocarbazole (Fig. 3). The replacement of 2,6-dimethyl piperazine with 2,6-dimethyl morpholine as exemplified by **6** resulted in modest increase in the potency. The morpholine (**8**), 2-oxo-piperiazine (**9**) and piperidine analogues (**10**) are less potent than the 2,6-dimethyl morpholine analogue (**6**). The sulfonamide and carboxamide analogues (**11**–**16**) were prepared to investigate the impact of basic amine group on potency. The smaller *N*,*N*-dialkyl sulfonamides showed higher potency than larger *N*,*N*-dialkyl sulfonamides. Of these, the dimethyl sulfonamide compound **15** showed excellent potency with an IC_{50} of 5 nM. The corresponding dimethyl carboxamide (**16**) and the reverse sulfonamide (**17**) analogs were prepared and found to be less potent than compound **15**. It was observed that aromatic heterocycles were also active at this position. The compound with a 4,6-dimethoxy-2-amino pyrimidine (**20**) is the most potent with an IC_{50} of 1.5 nM, whereas its corresponding mono methoxy (**19**) or unsubstituted pyrimidine (**18**) are less potent. The dimethoxy triazine (**21**) analog is less potent than its corresponding pyrimidine **20**. The *N*-methylated and 2-oxo analogues of

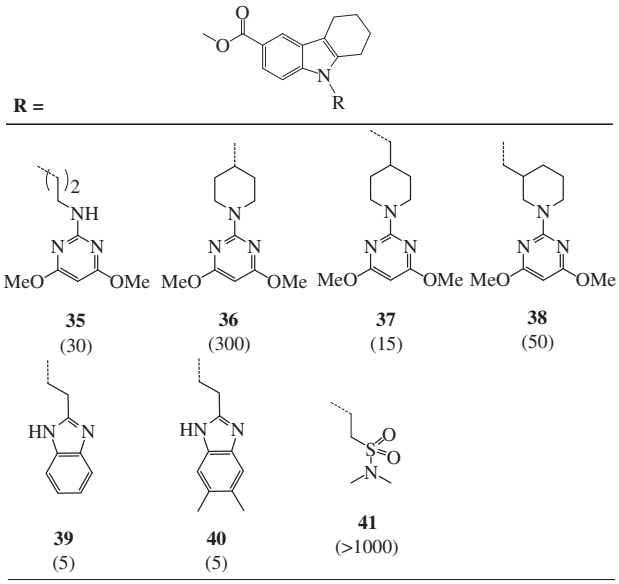
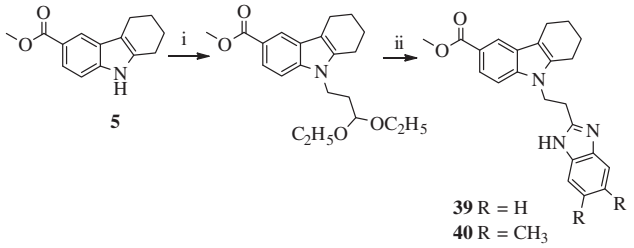


Figure 4. Exploration of chain length and conformationally-constrained linkers. The values within parenthesis indicate IC_{50} in nM are from the HCV2aCH-Rluc assay.



Scheme 2. Reagents and conditions: (i) NaH, 3-chloropropionaldehyde diethylacetal, *n*-$Bu_4NH_4^+I^-$, DMF, 50 °C, 24 h; (ii) (a) AcOH, aq HCl, 0 °C to rt, 3 h; (b) *o*-phenylene diamine, DMF, 150 °C, overnight.

compound **20** were prepared to examine the necessity of an H-bond donor. The *N*-methylated analogue (**24**) is as potent as compound **20**. The 4,6-disubstituted-2-oxo pyrimidine analogs (**25**–**29**) showed equal or better potency when compared to the corresponding 4,6-disubstituted-2-amino pyrimidines. The other aromatic heterocycles/aromatics such as imidazoles (**31**, **32**), purine (**33**), phenyl sulfone (**30**) or difluoro phenyl sulfonamides (**34**) are also active but less potent than pyrimidine analogues. The SAR suggests that the position has wide latitude for a variety of groups and in general disubstituted 2-amino/2-oxo pyrimidines and dimethyl sulfonamide groups are best for high potency.

The length of side chain was then investigated by preparing 2-carbon and 4-carbon linker analogues as exemplified by **35** and **41**, which were found to be less potent than the 3-carbon linker analogues. The introduction of conformational constraint (**36**–**40**) into the linker also resulted in decreased potency, suggesting both flexibility and a 3-carbon linker are optimal for the potency (Fig. 4). The potency of benzimdazole analogues (**39** and **40**) was only reduced by 3-folds. Compounds **35**–**38** and **41** were prepared similarly to compounds **20** and **15**, respectively. The benzimidazole analogues **39** and **40** were prepared by the alkylation of compound **5** with 3-bromo-1,1-diethoxypropane and subsequent hydrolysis followed by the reaction of resulting aldehyde with 1,2-diamino benzene (Fig. 4 and Scheme 2).

We subsequently examined plasma stability of one of the most potent compounds **20** due to the presence of methyl ester group in

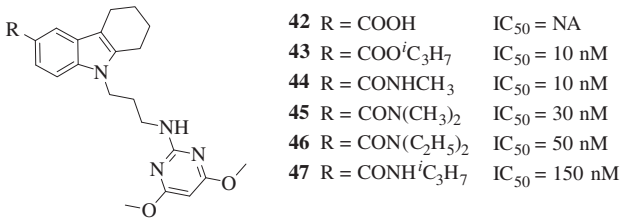


Figure 5. Isopropyl and amide derivatives of compound **20**. NA: not active; the IC_{50} values are from the HCV2aCH-Rluc assay.

Table 1
PK profile of compounds **43**, **44** and **45** in rat[a]

Compd	AUCall (uG/L h)	Cmax (uG/L)	$T_{1/2}$ (h)	Vz (L/Kg)	Cl (L/h/Kg)	%F
43	896	547	1.24	17.56	4.31	19
44	45	51	0.47	1.33	3.49	0.8
45	41	51	0.55	2.05	2.7	0.6

[a] iv Dose 2 mg/kg; oral dose 20 mg/kg.

the series of compounds. Compound **20** is rapidly degraded in rat plasma likely by plasma esterases and exclusively forms the metabolite **42**, which is confirmed by LC–MS and by preparation of an authentic sample. The metabolite, carboxylic acid (**42**), was tested and found to be completely inactive in HCVcc-2a entry assay (Fig. 5). The corresponding isopropyl ester (**43**) and amide derivates (**44**–**47**) were prepared to improve plasma stability and were tested for both the potency and plasma stability. The amide derivatives are very stable in rat plasma, whereas the isopropyl ester analogue is slightly more stable than the methyl ester. In the HCVcc-2a entry assay, although both the isopropyl ester (**43**) and amide analogues (**44** and **45**) are less potent than the methyl ester analogue (**20**), they are reasonably active (Fig. 5).

We consequently investigated the in vivo PK for compounds **43**, **44** and **45**. All the compounds showed poor PK properties (Table 1). Of these, compound **44** showed 19% oral bioavailability. However, the compound also has high clearance, shorter half-life and poor exposure in rats, which signify that the class of compounds has to be further investigated to improve the PK properties and to find bioisosteres of the methyl ester group (Table 1).

We have also investigated the mechanism of action of the series with one of the more advanced compounds.[24] Based on binding competition and resistance profiles, this compound class appears to be mechanistically similar to ITX 5061[15] and targets one of the cellular co-factors required for virus entry, the scavenger receptor B-1 (SR-B1).

In conclusion, we have identified highly potent small molecule HCV entry inhibitors through SAR exploration of a hit molecule identified from screening of a library. Further identification of bioisosteric groups of the methyl ester, SAR exploration on tetrahydro ring and optimization of PK properties of this series will be the subject of future publications.

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24. Unpublished results.