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Discovery of ITX 4520: A highly potent orally bioavailable hepatitis C virus entry inhibitor

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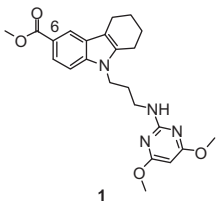
ABSTRACT

The manuscript reports an identification of a highly potent, orally bioavailable hepatitis C virus entry inhibitor through optimization of a previously reported class of molecules (**1**) that were not stable in the rat plasma. Compound **39** (ITX 4520) exhibited an excellent PK profile in both rats and dogs with good oral exposure, half-life and oral bioavailability. The compound is also well-tolerated in the preliminary in vivo toxicity studies and has been selected as a pre-clinical candidate for our HCV clinical pipeline.

Hepatitis C virus is a leading cause of chronic liver disease, cirrhosis, liver failure and primary liver cancer that affects almost 3% of the world's population or 170 million people worldwide.[1,2] There is currently no vaccine available to prevent hepatitis C virus infection due to its high degree of strain variation. The standard of care (SOC), until recently, has been a combination therapy with pegylated α-interferon and ribavirin and effective only in less than 50% of patients infected with most common genotype-1.[3] Most of the efforts are focused on the development of viral protease and polymerase inhibitors that are crucial for viral replication. Very recently, the FDA approved two protease inhibitors Incivek™ and Victrelis™ to use in combination with pegylated α-interferon and ribavirin. However, the occurrence of side effects and rapidly emerging resistant mutants suggest the need for new, more effective and safer antivirals targeting multiple steps of the HCV life cycle for the treatment of HCV.[4–6]

Virus entry into the host cell is a crucial step in the HCV life cycle, requiring at least five cellular co-factors, CD-81,[7] claudin,[8,9] occludin,[10] scavenger receptor-B1[11] and the more recently discovered epidermal growth factor receptors 1 and 2,[12] and represents an alternative potential target for therapeutic intervention. The entry inhibitors can prevent the re-infection of cured cells and can be most effective in combination with other antivirals targeting different stages of the HCV life cycle. We have recently reported[13] a series of highly potent small molecule HCV entry inhibitors as exemplified by compound **1** (Fig. 1) that showed an IC_{50} of 1.5 nM in the HCV2aCH-Rluc entry assay. Based on binding competition and resistance profiles,[14] the class of compounds appears to be mechanistically similar to ITX 5061[15] and targets the scavenger receptor B-1 (SR-B1), one of the cellular co-factors required for HCV entry. The series of compounds, though potent, is rapidly metabolized in plasma due to the presence of the labile methyl ester group. The corresponding amide derivatives were found to be stable in plasma; however, the compounds are not only less potent than compound **1** but also have poor pharmacokinetic properties such as low exposure, shorter half-life and no oral bioavailability. Here, we report the identification of methyl ester bioisosteric groups and an orally bioavailable HCV entry inhibitor



1
HCVcc IC_{50} = 1.5 nM

Figure 1. Previously reported HCV entry inhibitor.

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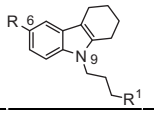
39 (ITX 4520) with a good pharmacokinetic profile that has been selected as a pre-clinical candidate.

We have investigated various groups at the 6-position to improve the pharmacokinetics and to retain high antiviral potency. We previously identified[13] that the 4,6-disubstituted-2-oxo/amino-pyrimidine or *N*,*N*-dimethyl sulfonamide groups with a three carbon linker at the 9-position were optimal for the antiviral activity and utilized these best side chains to explore the groups at the 6-position. We have primarily explored a variety of hydrogen-bond acceptor groups at the 6-position such as methyl sulfone, cyano, nitro, as well as known bioisosteric groups of the methyl ester[16a] such as oxadiazole,[16b] oxazole, triazole and tetrazoles since a hydrogen-bond acceptor at this position seems to be essential for the activity. The synthesis of target compounds **16–36** (Table 1) is outlined in Schemes 1–3.

Fisher indole condensation of appropriate phenylhydrazines with cyclohexanone provided the tetrahydrocarbazoles **2** and **4**–**6**. The esterification of compound **2** gave intermediate **3**. Compounds **3–6** were then alkylated with different alkylating agents to obtain the intermediates **7–14**. The chloro displacement of compounds **7–10** with 2-oxo pyrimidines afforded the target compounds **15**, **17**, **18**, **21**, and **23**. The intermediates **11** and **12**, after tdeprotection of the Boc group, were reacted with 2-chloro-4,6-dimethoxy pyrimidine to obtain the desired compounds **16** and **22**. The deprotection of the diethyl acetal of **13** and **14** followed by coupling with 1,2-diaminobenzene yielded the compounds **20** and **25**. Oxadiazole analogs **26–31** were prepared by introducing different side-chains at the 9-position of the intermediates **3a**, **3b** and **5a** following the similar methods used for the preparation of compounds **17–19** from **4**. The 2-methyl-1,3,4-oxadiazole intermediate **3b** was prepared by the reaction of intermediate **3** with hydrazine and subsequent coupling with triethyl orthoacetate. The 5-methyl-1,2,4-oxadiazole intermediate **5a** was synthesized by the addition of hydroxylamine to **5**, followed by the condensation with methyl acetate. The intermediate 3-methyl-1,2, 4-oxaxdiazole (**3a**) was prepared by the condensation of methyl 2,3,4,9-tetrahydro-1*H*-carbazole-6-carboxylate (**3**) with the acetamide oxime. The compound **15**, after ester hydrolysis, was coupled with ethanolamine to obtain the corresponding amide analog **15a**, which was subsequently converted to oxazoline analog **32** with thionyl chloride and $NaHCO_3$. The oxidation of oxazoline **32** using bromotrichloromethane and DBU afforded the oxazole analog **33** (Scheme 2). The nitro group of **21** was reduced with Zn/AcOH in MeOH and the resulting amine, after diazotization, was subsequently treated with NaN_3 to afford the intermediate **21a** (Scheme 3). The azide functionality of the intermediate **21a** was then converted to triazoles **34** and **35** via click chemistry using appropriate alkynes. The tetrazole analogue **36** was prepared by reaction of the azide group of **21a** with triethyl orthoformate. All compounds were characterized by ^1H NMR, mass spectra[17] and were tested in the HCV2aCH-Rluc (HCVcc-2a) infectious assay.[18–20] Since the first tissue culture infectious clone is of genotype 2a,[18] the original, native HCV2a clone has long been the gold standard for in vitro assays. Genotype 1 clones are chimeras, but are used in some experiments to demonstrate genotype specificity, or lack of. We did not run duplicate assays for both genotype 2a and 1 at all times, as the series continues to demonstrate the nearly equal potency for both genotypes, as demonstrated in Table 6. The antiviral activity data is presented in Table 1.

The replacement of the methyl ester group of **1** by the methyl sulfone group as exemplified by **16–20** resulted in 6- to 300-fold reduction of the potency depending on the type of side chain present at the 9-position. While the nitrile analogs **22–25** were 10- to 30-fold less active, irrespective of the side chain present at the 9-position, the nitro analog **21** was equally potent to the

Table 1
Exploration of groups at the 6-position



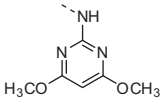
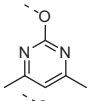
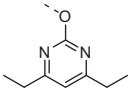
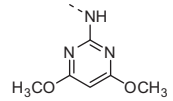
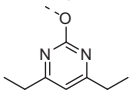
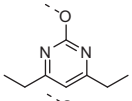
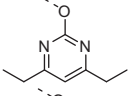
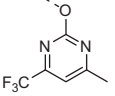
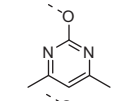
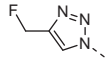
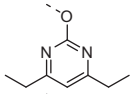
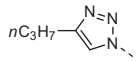
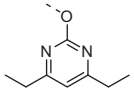
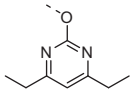
Compound	R	R^1	HCVcc IC_{50} (nM)
15	CO_2CH_3		1.5
16			10
17			50
18			30
19			500
20			50
21			1.5
22			15
23			15
24			50
25			50
26			5
27			15
28			1.5
29			1.5
30			1.5
31			1.5
32			50

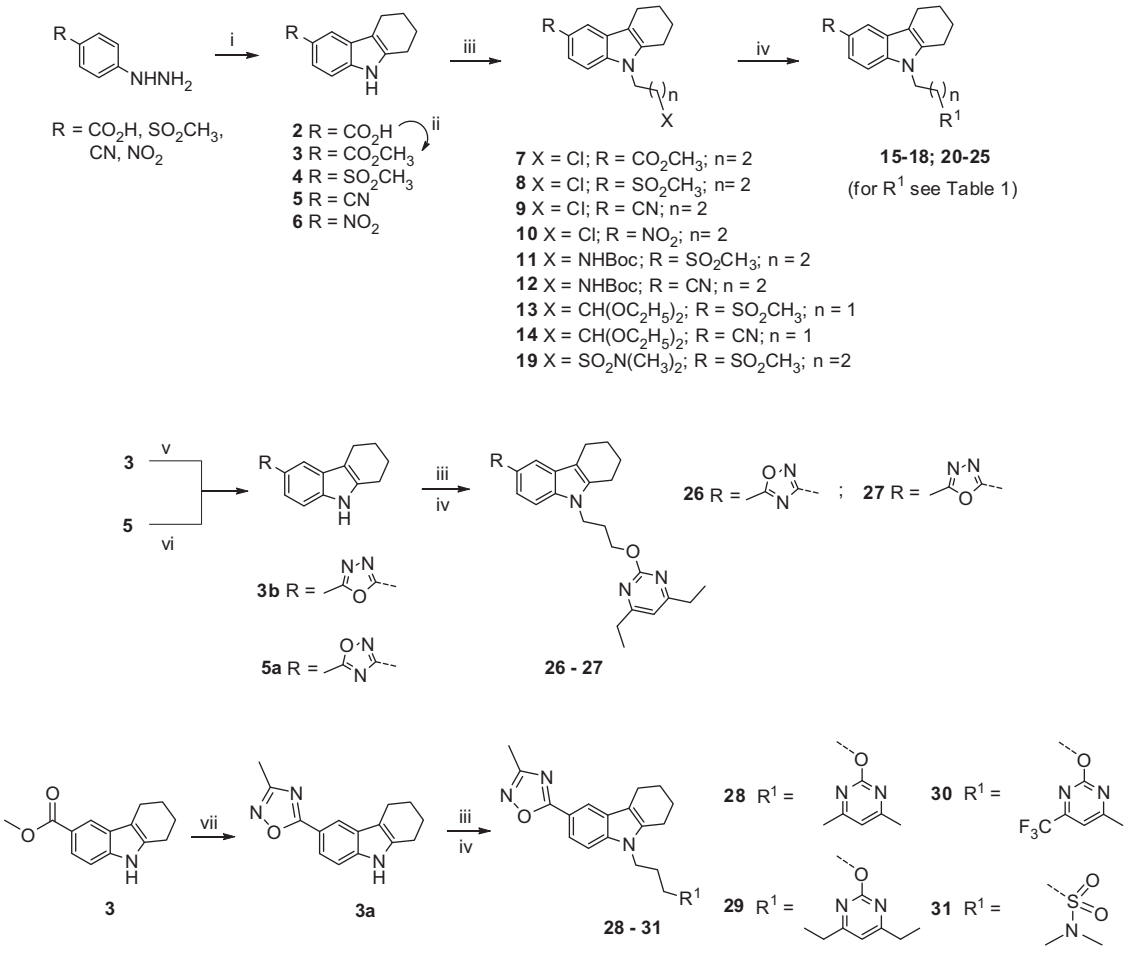
Table 1 (*continued*)

Compound	R	R^1	HCVcc IC_{50} (nM)
33			1.5
34			50
35			15
36			1.5

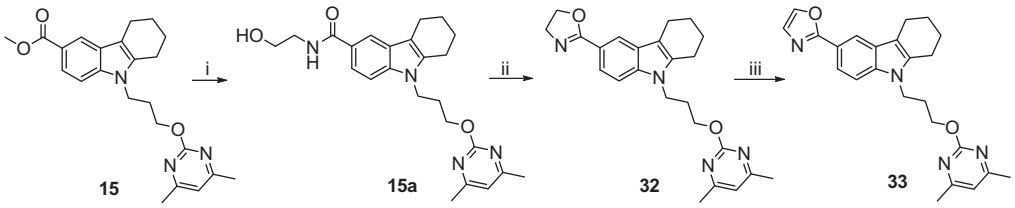
compound **1**. Notably, 3-methyl-1,2,4-oxadiazole analogs **28–31** were as potent as the compound **1**. Moreover, the 3-methyl-1,2,4-oxadiazole group at 6-position was tolerable with different side chains at 9-position. The regio-isomeric analogs of 3-methyl-1,2,4-oxadiazole **26** and **27** were 3- and 10-fold less potent compared to the corresponding compound **29**. The oxazole analog **33** was equally potent as the 3-methyl-1,2,4-oxadiazole analog **29**, whereas the oxazoline analog **32** displayed 33-fold less activity than the corresponding oxazole analog **29**. We also explored triazoles at 6-position as shown by **34** and **35**, but observed inferior activity compared to the corresponding 3-methyl-1,2,4-oxadiazole analogs. The tetrazole analog **36** was found to be as potent as 3-methyl-1,2,4-oxadiazole analog **28**.

With identification of the bioisosteric groups of methyl ester, we have evaluated the in vivo pharmacokinetic properties of the compounds **16**, **29** and **31** in rats at 2 mg/kg via i.v. route (Table 2). The methylsulfone analog **16** showed better exposure and reduced clearance than the corresponding methyl ester analog **1**, but had shorter half-life. Surprisingly, the oxadiazole analogs **29** and **31** have higher clearance than the methyl ester analogue **1**. The MS analysis of microsomal samples of **29** revealed the oxidative metabolism of the molecule (addition of 32 mass units to the parent molecule), which probably occurs at the side chain and on the tetrahydrocarbazole ring.

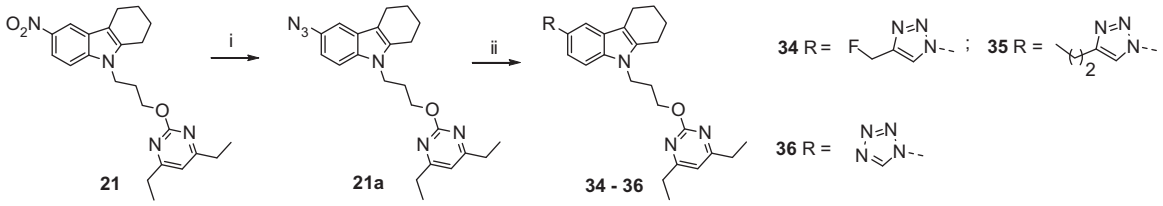
Consequently, we focused efforts on modifying the side chain at the 9-position and tetrahydro ring to improve the metabolic stability and pharmacokinetics (Table 3). We have found that the replacement of 2-oxopyrimidines with difluorosubstituted phenols or thiophenols, as exemplified by **37–40**, considerably improved the metabolic stability and was also tolerable in terms of the



Scheme 1. Reagents and conditions: (i) (a) cyclohexanone, AcOH, reflux, overnight; (ii) MeOH, H_2SO_4, reflux, overnight; (iii) for compounds **7–10**: NaH, 1-bromo-3-chloro propane, DMF, rt, 3 h; for compounds **11** and **12**: NaH, DMF, BocNH-$(CH_2)_3$-OTs, rt, 18 h; for compounds **13** and **14**: NaH, 3-chloropropionaldehyde diethylacetal, *n*-$Bu_4NH_4^+I^-$, DMF, 50 °C, 24 h; for compound **19** and **31**: NaH, 3-chloro-*N*,*N*-dimethylpropane-1-sulfonamide, *n*-$Bu_4NH_4^+I^-$, DMF, rt, 16 h; (iv) for compounds **16** and **22**: (a) TFA, CH_2Cl_2, 0 °C to rt, 3 h; (b) 2-chloro-4,6-dimethoxy pyrimidine, DMF, DIEA, 80 °C, overnight; for compound **20** and **25**: (a) AcOH, aq HCl, 0 °C to rt, 3 h; (b) *o*-phenylene diamine, DMF, 150 °C, overnight; for compounds **17**, **18**, **21**, **23**, **26–30**: 2-oxo pyrimidines, K_2CO_3, 80 °C, overnight; (v) (a) NH_2NH_2, MeOH, reflux, 16 h; (b) triethyl orthoacetate, MeOH, reflux, 16 h; (vi) (a) NaOMe, $NH_2OH.HCl$, MeOH, reflux, 2 days; (b) MeOAc, K_2CO_3, reflux, 16 h; (vii) acetamide oxime, KO^{t}Bu, THF, reflux, 16 h.



Scheme 2. Reagents and conditions: (i) (a) 2 N aq NaOH, MeOH, 60 °C, 3 h; (b) ethanolamine, HATU, Et_3N, CH_2Cl_2, rt, 6 h; (ii) $SOCl_2$, CH_2Cl_2, 10 min, then $NaHCO_3$, 30 min, rt; (iii) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C to rt, 16 h.



Scheme 3. Reagents and conditions: (i) (a) Zn, MeOH/AcOH, 3 h; (b) $NaNO_2$, AcOH/H_2O, 0 °C, 20 min, then aq.NaN_3, rt, 16 h; (ii) for compounds **34** and **35**: 1-alkynes, 1 M sodium ascorbate, $^tBuOH/H_2O$, 0.3 M $CuSO_4$, rt, 16 h; for compound **36**: triethyl orthoformate, 2-methoxy ethanol, NaN_3, $Yb(OTf)_3 \cdot H_2O$, 100 °C, 16 h.

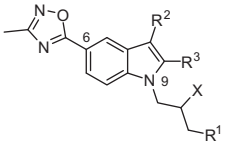
Table 2
Intravenous pharmacokinetic properties of selected compounds in rats[a]

Compd	AUCall (μG/L h)	Cmax (μg/L)	$t_{1/2}$ (h)	Vz (L/kg)	Cl (L/h/kg)
1	521	182	2.16	11.28	3.75
16	902	1076	0.63	3.51	2.21
29	497	1051	3.22	18.94	4.09
31	520	1240	0.71	3.96	3.97

[a] iv 2 mg/kg.

antiviral activity (Table 3). Compound **43** with a 5-fluoro benzimidazole side chain also displayed good microsomal stability in rats, but was not stable in human microsomes. The difluorophenyl sulfone and difluorophenyl sulfoxide side chain analogs **41** and **42** were found to have moderate stability in rats and poor stability in human microsomes. We have introduced the fluorine atom into the carbon-linker of the side chain as exemplified by **46**, which not only had good microsomal stability in both rat and human

Table 3
Exploration of side chains at 6-position



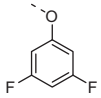
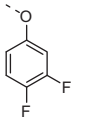
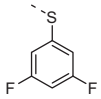
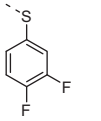
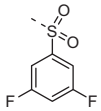
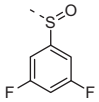
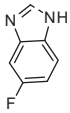
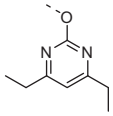
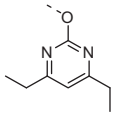
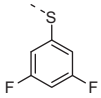
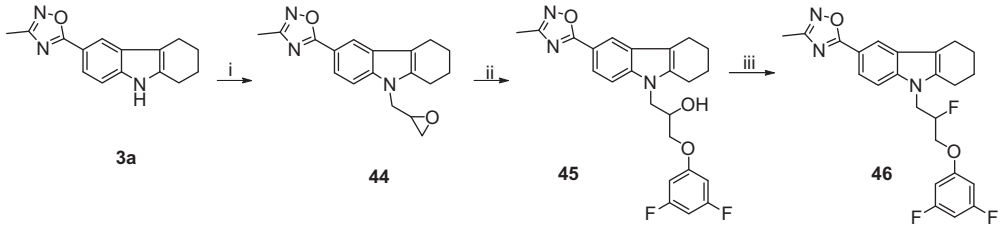
Compd	R^1	R^2 and R^3	X	HCVcc[a] IC_{50} (nM)	HLM[b] ($t_{1/2}$)	RLM[b] ($t_{1/2}$)
37			H	1.5	55	53
38			H	1.5	22	34
39			H	1.5	43	59
40			H	1.5	27	25
41			H	5	9	19

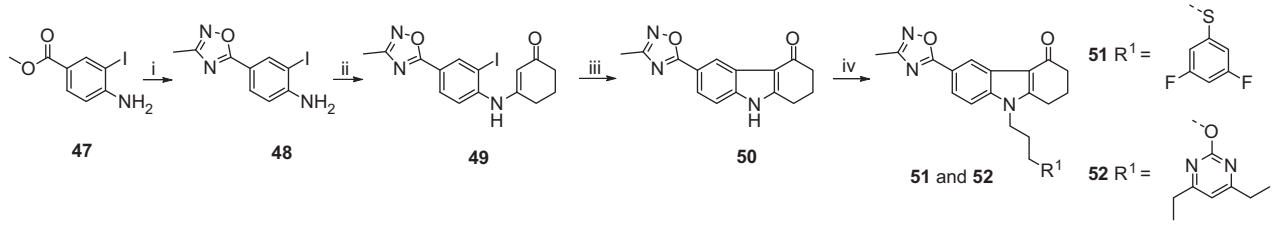
Table 3 (*continued*)

Compd	R^1	R^2 and R^3	X	HCVcc[a] IC_{50} (nM)	HLM[b] ($t_{1/2}$)	RLM[b] ($t_{1/2}$)
42			H	15	4	18
43			H	1.5	8	110
46			F	1.5	65	49
51			H	5	8	22
52			H	1.5	8	28
55			H	15	8	8
56			H	15	18	18

[a] HCV2aCH-Rluc entry assay.
[b] 1 μM of compound was incubated with 0.5 mg/mL of human or rat hepatic microsomes; $t_{1/2}$ in minutes; HLM: Human liver microsomes; RLM: Rat liver microsomes.



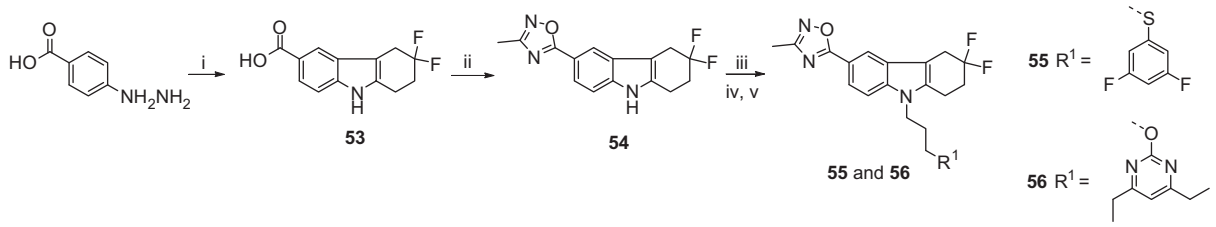
Scheme 4. Reagents and conditions: (i) NaH, (±)-epichlorohydrin, DMF, n-$Bu_4NH_4^+I^-$, 2 h; (ii) 3,5-difluorophenol, Cs_2CO_3, THF, 60 °C, 16 h; (iii) DAST, CH_2Cl_2, 0 °C to rt, 1 h.



Scheme 5. Reagents and conditions: (i) acetamide oxime, K_2CO_3, C_2H_5OH, reflux, 2 days; (ii) 1,3-cyclohexane dione, p-TsOH, toluene, reflux, 16 h; (iii) $Pd(OAc)_2$, tri(o-tolyl)phosphine, NaOAc, DMF, microwave, 160 °C, 30 min; (iv) for compound **52**: a) NaH, 1-bromo-3-chloro propane, DMF, rt, 3 h; (b) 4,6-diethyl-2-oxopyrimidine, K_2CO_3, 80 °C, overnight; for compound **51**: NaH, (3-chloropropyl)-(3,5-difluorophenyl)sulfane, n-$Bu_4NH_4^+I^-$, DMF, 80 °C, overnight.

microsomes but also exhibited high antiviral activity (IC_{50} = 1.5 nM). Parallel to side chain modifications, we have also modified the tetrahydro ring of tetrahydrocarbazole by introducing electron-withdrawing groups to minimize the oxidative metabolism. The 4-oxo analogs **51** and **52** were potent but had poor metabolic stability in human microsomes and moderate stability in rats. The 3,3′-difluoro analogs **55** and **56** were less potent and had poor microsomal stability.

The synthesis of compound **46** is outlined in Scheme 4. The alkylation of intermediate **3a** with racemic-epichlorohydrin provided the intermediate **44**, which was then reacted with 3,5-difluorophenol to obtain the **45**. The hydroxyl group of the **45** was



Scheme 6. Reagents and conditions: (i) 4,4-difluoro cyclohexanone, AcOH, reflux, overnight. (ii) a) MeOH, H_2SO_4, reflux, overnight; (b) acetamide oxime, K_2CO_3, C_2H_5OH, reflux, 2–4 days; (iii) for compound **56**: a) NaH, 1-bromo-3-chloro propane, DMF, rt, 3 h; (b) 4,6-diethyl-2-oxopyrimidine, K_2CO_3, 80 °C, overnight; for compound **55**: (3-chloropropyl)-(3,5-difluorophenyl)sulfane, *n*-$Bu_4NH_4^+I^-$, DMF, 80 °C, overnight.

Table 4
Intravenous pharmacokinetic properties of selected compounds in rats and dogs[a]

Compd	AUCall (μG/L h)	C_{max} (μg/L)	$t_{1/2}$ (h)	Vz (L/kg)	Cl (L/h/kg)
37	404	821	3.76	22.09	3.64
46	691	1708	1.42	5.52	2.72
39	1184	2989	1.5	3.64	1.68
39[b]	1746	3876	1.1	1.8	1.14

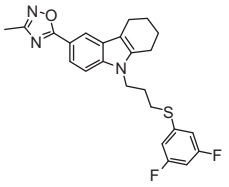
[a] iv 2 mg/kg.
[b] PK data in dog.

Table 5
Oral pharmacokinetic properties of selected compounds in rat and dog

Compd	AUCall (μG/L h)	C_{max} (μg/L)	T_{max} (h)	$t_{1/2}$ (h)	%*F*[a]
46[b]	3138	747	2.67	1.46	38
39[b]	4226	955	2.33	6.01	42[c]
39[d]	2010	625	2.0	4.07	34

[a] Formulation: 5% dimethylacetamide + 10% preconcentrate[21] + 85% saline.
[b] PK data in rats; dose 20 mg/kg.
[c] Oral bioavailability of the compound varies from 40% to 100% depending on the formulation.
[d] PK data in dogs; dose:10 mg/kg.

Table 6
Profile for compound **39** (ITX 4520)



Molecular weight	439.5
cLogP	6.5
tPSA	37.2
HCVcc 1a IC_{50}	1.5 nM
HCVcc 1a IC_{90}	150 nM
HCVcc 2a IC_{50}	1.5 nM
HCVcc 2a IC_{90}	50 nM
Solubility (formulation)	>1 mg/mL
in vitro toxicity (CC_{50}-Huh7)	100 μM
AMES	negative
hERG IC_{50}	>10 μM
$t_{1/2}$ (rat)	6.01 h
$t_{1/2}$ (dog)	4.07 h
%*F* (rat)	40–100%[a]
%*F* (dog)	34%

[a] Oral bioavailability of the compound varies from 40–100% depending on the formulation.

converted to flouro using the reagent (diethylamino)sulfur trifluoride (DAST) to yield the target compound **46**.

The synthesis of compounds **51** and **52** is shown in Scheme 5. The commercially available compound **47** was reacted with acetamide oxime to obtain the intermediate **48**, which was then condensed with cyclohexane-1,3-dione to yield the enaminone **49**. The cyclisation of enaminone **49** to tetrahydrocarbazolone **50** was achieved using intramolecular Heck reaction under microwave irradiation. The alkylation of tetrahydrocarbazolone **50** with 1-bromo-3-chloro propane and subsequent chloro displacement of the resulting intermediate furnished the target compound **52**. Compound **51** was obtained by the alkylation of tetrahydrocarbazolone **50** with 3-chloropropyl-3,5-difluorophenyl sulfane. Compounds **55** and **56** were prepared similarly to compound **51** and **52**, by alkylation of the intermediate **54** (Scheme 6).

The in vivo pharmacokinetics were subsequently studied in rats using both i.v. and p.o. routes for potent and metabolically stable compounds **37**, **39** and **46**, and the PK parameters are shown in Table 4 and 5. Of these, compound **39** (ITX 4520) displayed good plasma oral exposure (AUC = 9.61 μM h), moderate clearance and good oral bioavailability. The PK of compound **39** was also studied at higher doses orally (50, 200 and 500 mg/kg) in rats, which showed dose-dependent plasma oral exposure up to 200 mg/kg. Compound **39** displayed a similar PK profile when dosed orally at 10 mg/kg in dogs (Table 5). Compound **46** was also orally bioavailable, but had higher clearance and lower exposure compared to **39**. Since the sulfur atom in compound **39** is a potentially metabolically labile, we investigated formation of the sulfoxide metabolite (**42**) in vivo but did not observe the metabolite when administered via i.v. at 2 mg/kg. However, when administered orally at 20 mg/kg, we noticed minor formation of the sulfoxide metabolite (AUCall = 143 μG/L h).

We have selected compound **39** for further evaluation based on its potency (IC_{50} = 1.5 nM; IC_{90} = 50 nM) and pharmacokinetic properties. The compound was equally potent against both HCV genotype-1 and genotype-2 and did not inhibit the entry of the related virus bovine viral diarrhea (BVDV) and heterologous VSV-G pseudo particles. Compound **39** also did not inhibit HCV RNA replication as confirmed by testing the compound in replicon assay. We have screened compound **39** against 97 off-target assays (Cerep Diversity Profile)[22] and compound **39** displayed only 30–50% of binding at A3-adenosine and cannabinoid-1 (CB-1) receptors in radio-ligand binding assay at 10 μM. Additionally, compound **39** was also tested in hERG and AMES assay at 10 μM, which did not have activity in both the assays. The properties compound **39** are shown in Table 6.

We have performed preliminary toxicological studies by repeat dosing of compound **39** in rats orally at 20 mg/kg for 7 days and have examined the levels of liver enzymes aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALK) at 0, 1, 4 and 7 days since the site of action is liver. No elevation of enzymes was observed. We also noticed neither mortality nor abnormality in weight of the animals.

In summary, we have identified a highly potent hepatitis C virus entry inhibitor with excellent in vivo pharmacokinetics through optimization of previously reported series of compounds that were unstable in plasma. Compound **39** (ITX 4520) exhibited a good drug-like profile and has been selected as a pre-clinical candidate.

Acknowledgments

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