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Substituted tetracyclic indole core derivatives of HCV NS5A inhibitor MK-8742

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ABSTRACT

As part of an ongoing effort in NS5A inhibition at Merck we now describe our efforts for introducing substitution around the tetracyclic indole core of MK-8742. Fluoro substitution on the core combined with the fluoro substitutions on the proline ring improved the potency against GT1a Y93H significantly. However, no improvement on GT2b potency was achieved. Limiting the fluoro substitution to C-1 of the tetracyclic indole core had a positive impact on the potency against the resistance associated variants, such as GT1a Y93H and GT2b, and the PK profile as well. Compounds, such as **62**, with reduced potency shifts between wild type GT1a to GT2b, GT1a Y93H, and GT1a L31V were identified.

Hepatitis C virus (HCV) is the leading cause of chronic liver disease and liver transplants in the developed world. It was estimated that 130–170 million people worldwide are infected with HCV.[1] There are seven major genotypes (GT1-7) with a majority of them having multiple subtypes. However, genotypes 1, 2, and 3 account for 80–90% of all HCV infections.[2] Current HCV therapy has improved significantly over early approaches and utilizes interferon-free, direct-acting antiviral agents (DAA), with each targeting a different step in the HCV life cycle. Toward this strategy, three major categories of drugs have been developed including: NS3/4A protease inhibitors, non-structural protein 5A (NS5A) replication complex inhibitors, and NS5B polymerase nucleotide-like inhibitors.[3]

The HCV NS5A protein is a multifunctional RNA binding phosphoprotein essential for HCV replication.[4] Early clinical experience with NS5A inhibitors indicated that they suffered from a low genetic barrier to resistance and demonstrated weak activity against non-GT1 and GT1a variants.[5] Thus, the need for identification of NS5A inhibitors with increased potency against a wider variety of genotypes as well as NS5A resistance-associated polymorphisms became a focus for many groups. To date, five NS5A inhibitors have been approved for HCV treatment, either alone or in combination with other DAA's: daclatasvir (BMS-790052),[5,6] ledipasvir (GS-5885),[7] ombitasvir (ABT-267),[8] elbasvir (MK-8742),[9,10] and velpatasvir (GS-5816).[11]

Research efforts at Merck identified the tetracyclic indole-based inhibitor, MK-8742 (elbasvir) shown in Figure 1.[9,10] Continued effort sought to explore the impact of structural changes to the tetracyclic indole-based NS5A inhibitors, with the aim of determining if an inhibitor with a 'flat' profile could be obtained.

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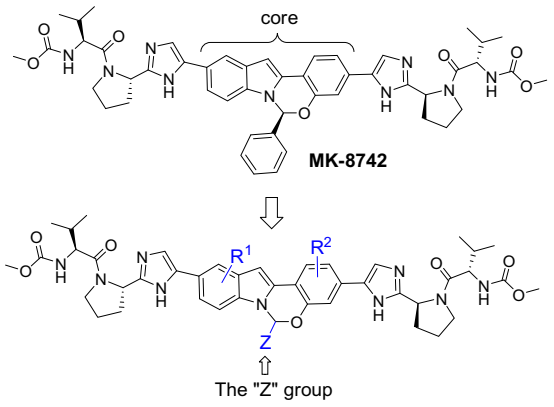


Figure 1. Strategy for core substitution of MK-8742 derivatives.

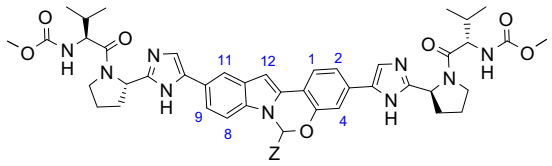
In particular, a "flat" potency profile is defined by a minimal potency shift (~10×) among genotypes and resistance-associated variants (RAV's), such as GT2b (bearing the resistance-associated methionine at position 31), GT1a Y93H, and GT1a L31V, which were used as model replicons in our SAR studies. Toward this end, we recently reported SAR studies directed toward the impact of variation on the Z-group (Fig.1) with either alkyl, substituted aryl, or a heteroaryl Z groups.[12,13] In addition, efforts to explore the L-proline-L-valine methyl carbamate region of this inhibitor class has also been recently reported.[14] These early SAR studies yielded NS5A inhibitors[13] with reduced potency shifts from GT1a to GT2b, GT1a Y93H, and GT1a L31V which had not been observed in our previous efforts on the core developments.[15–17] However, compounds with "flat" potency profiles have not been identified yet. SAR efforts were continued to work toward this goal. Herein, we describe our results on substitutions around the tetracyclic indole core (Fig. 1), and the impact of the changes on the potency against GT2b, GT1a Y93H, and GT1a L31V.

As part of an ongoing SAR studies based on MK-8742, we wanted to explore the substitutions around the tetracyclic indole core and examine their impact on the potency against RAV's (Fig. 1). The initial efforts explored the impact of introducing fluorine substitution onto various positions of the tetracyclic indole core of either MK-8742 or compound **1** (Table 1). The preparation of the 1-F substituted analog **12** is outlined in Scheme 1. 3-Bromo-5-fluorophenyl acetate **3** was prepared from 3-bromo-5-fluorophenol **2** followed by an $AlCl_3$-promoted rearrangement to give 1-(4-bromo-2-fluoro-6-hydroxyphenyl)ethan-1-one **4**. Fisher indole synthesis of compound **4** afforded indole **6** in good yield. The aminal carbon was introduced by the reaction of **6** with (dibromomethyl)benzene under basic condition to produce tetracyclic indole **7**. Compound **7** was converted to bis-pinacol boronate ester **8** which upon treatment under Suzuki coupling conditions with *tert*-butyl (*S*)-2-(5-bromo-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate[10] gave the bis-Boc intermediate **9**. Treatment of the intermediate **9** under acidic conditions followed by HATU-mediated amide coupling with methoxycarbonyl-L-valine produced a mixture of two diastereomers at the aminal position. The mixture of diastereomers was subjected to chiral SFC separation to yield compound **12** as well as the less active isomer which was not reported here. The other fluoro-substituted analogs **13**–**17** and chloro-substituted analog **19** were prepared by using the same chemistry outlined in Scheme 1 starting from the appropriate fluoro or chloro starting material. The in vitro virologic profiles of the active isomers for the final compounds are reported unless otherwise noted.

The 12-F analog **18** was prepared from dibromo-3-F-indole **11** by following the chemistry outlined in Scheme 1. Compound **11** was prepared from the corresponding dibromo indole analog **10** under Select-Fluor mediated fluorination (Scheme 2). Compound **10** was prepared based on the chemistry outlined in Scheme 1. The 1-F-12-Cl disubstituted analog **20** was prepared based on the chemistry outlined in Schemes 1 and 2.

The in vitro antiviral profiles of compounds bearing either a fluoro or chloro substitution on the tetracyclic indole core are summarized in Table 1. Introduction of a fluoro to the C-1 position produced compound **12** which showed good potency against all the genotypes tested. However, the corresponding 2-F analog **13** showed reduced potency against GT2b and GT3a, as compared to compound **12**. The 4-F analog **14** showed diminished potency against multiple replicons tested, including genotype 2b, 3a, 4a, and 1a Y93H. The weaker potency of compounds **13** and **14** suggests that fluoro substitution on C-2 or C-4 positions were not very well-tolerated. SAR studies on the fluoro substitution at C-8, C-9, and C-11 positions were done by utilizing compound **1** for the sake

Table 1
In vitro potency profiles of MK-8742, compounds **1**, **12**–**20**[18,19]



ID[a]	Substitution on the core	Z	Replicon EC_{90} (nM)							
			GT1a	GT1b	GT2a	GT2b	GT3a	GT4a	GT1a Y93H	GT1a L31V
MK-8742	None	Ph	0.006	0.006	0.019	11	0.12	0.016	28	1
1	None	H	0.02	0.02	0.02	76	1	0.2	100	7
12	1-F	Ph	0.005	0.003	0.15	18	0.4	0.04	34	0.4
13	2-F	Ph	0.003	0.003	0.003	>100	1	0.02	62	1
14	4-F	Ph	0.04	0.003	0.4	>100	>10	1	>100	36
15	8-F	H	0.1	0.01	0.1	>100	>10	0.2	139	3
16	9-F	H	0.007	0.007	0.02	150	5	0.2	77	68
17	11-F	H	>0.8	>0.5	—	—	—	—	—	—
18	12-F	Ph	0.007	0.007	0.004	15	0.3	0.06	25	0.7
19	1-Cl	H	0.05	0.03	—	125	—	—	94	—
20	1-F-12-Cl	Ph	0.02	0.006	0.03	42	2	0.04	51	0.4

A hyphen "—" in the table means not tested.
[a] Active isomer at the aminal carbon if apply.

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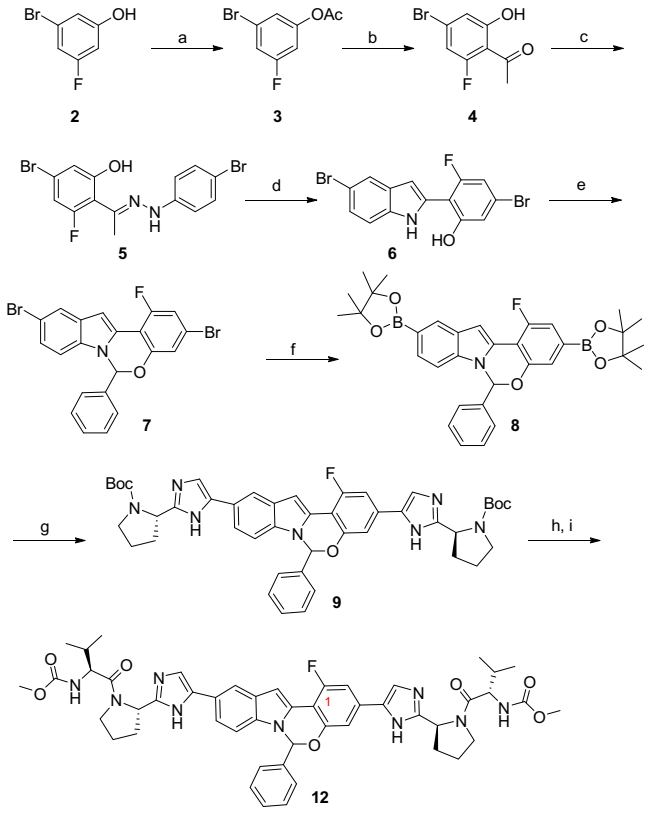
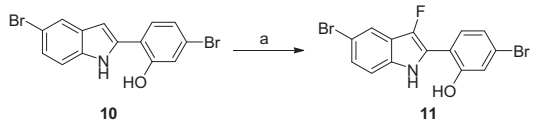


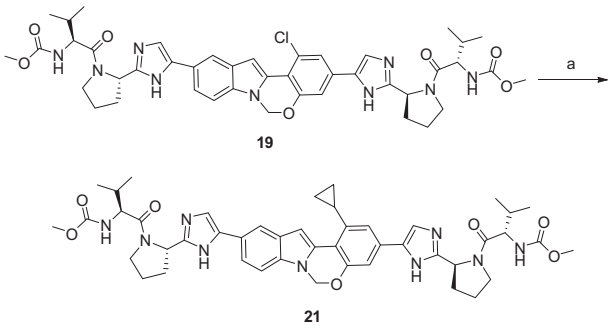
Scheme 1. Synthesis of **12.** Reagents and conditions: (a) AcCl, DIPEA, DCM, rt, 16 h; (b) $AlCl_3$, DCM, 140 °C, 3 h; (c) 4-bromophenyl hydrazine HCl, AcOH/EtOH (1:10), reflux, 6 h; (d) polyphosphoric acid, xylenes, 110 °C, 3 h; (e) $PhCHBr_2$, K_2CO_3, DMF, 100 °C, 16 h; (f) bis(pinacolato)diboron, Pd(dppf)Cl_2, KOAc, dioxane, 90 °C, 16 h; (g) *tert*-butyl (*S*)-2-(5-bromo-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate, Pd(dppf)Cl_2, K_2CO_3, dioxane/H_2O (10:1), 90 °C, 16 h; (h) HCl, MeOH, 0.5 h; (i) (methoxycarbonyl)-L-valine, BOP, DIPEA, DMF, 10 h; then chiral SFC separation.

of simplified chemistry (**15**–**17**). Comparing with the potency profile of compound **1**, the 8-F analog **15** was weaker against GT2b and GT3a as was the case for the 9-F analog **16**. In addition, the 11-F analog **17** was weaker against both GT1a and GT1b. These results indicated that fluoro substitution at the C-8, C-9, or C-11 positions of the tetracyclic indole core was not tolerated as well. Gratifyingly, the 12-F substituted analog, compound **18**, demonstrated good potency against all genotypes tested, as illustrated in Table 1. Having explored fluorine substitution around the tetracyclic core, efforts shifted toward the introduction of chlorine at specific positions of the core. The 1-Cl substituted analog (**19**) showed a potency profile similar to the corresponding 1-H compound **1**, indicating that C-1 chloro substitution was tolerated. In addition, 1-F-12-Cl di-substituted analog (**20**) was also prepared and it showed good potency against all the genotypes tested.

Having established that both fluoro- and chloro-substitutions were tolerated at either the C-1 or C-12 position, we wondered if additional substitution such as an alkyl or an aryl substitution could be tolerated at these two positions. Introduction of a cyclopropyl group to the C-1 position of compound **1** was accomplished



Scheme 2. Synthesis of **11.** Reagents and conditions: (a) Select-Fluor, DMSO/CH_3CN (1:1), 25 °C, 1 h.



Scheme 3. Synthesis of **21.** Reagents and conditions: (a) cyclopropyl boronic acid, $Pd_2(dba)_3$, X-Phos, dioxane, 110 °C, 16 h.

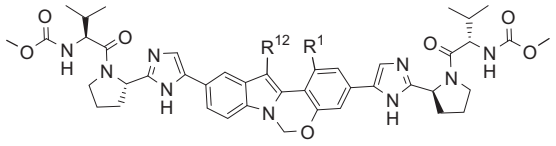
according to the synthetic route shown in Scheme 3. Compound **21** was prepared from the corresponding Cl analog **19** under standard Suzuki coupling conditions with cyclopropyl boronic acid. Compounds **22**–**24** were prepared from their corresponding chloro analog in an analogous manner to compound **21**. Compound **25** was prepared from compound **1** and benzyl alcohol under Pd/C and potassium carbonate conditions.

The in vitro antiviral profiles of compounds **21**–**25** are summarized in Table 2. Introduction of a cyclopropyl group to C-1 of compound **1** (compound **21**, Table 2) reduced the potency against GT1a, GT2b, and GT3a, and a similar result was observed for the corresponding 1-phenyl substituted analog **22**. The C-12 cyclopropyl analog **23** showed similar potency profile to compound **1** (Table 1), except for a ~10× decrease in potency against GT3a. The corresponding 12-phenyl substituted analog **24** was much weaker against GT1a Y93H than **23**. Finally, a 12-benzyl analog of compound **1** (compound **25**, Table 2) was prepared and found to be more potent than the corresponding phenyl analog **24** against GT 1a Y93H and GT2b, but still weaker against some of the genotypes compared to compound **1**. These results indicated that a cyclopropyl, phenyl, or benzyl substitution at either the C-1 or C-12 position was not preferred.

With the SAR data summarized in Tables 1 and 2 in hand, efforts were undertaken to combine the 1- or 12-fluorinated tetracyclic cores with modifications identified in the other regions of the molecule. The aim of this effort was to identify compounds with improved potency against the RAV's, especially GT1a Y93H and GT2b, which proved to be among the least susceptible RAV's based on our previous SAR observations.[12–17] As reported previously, replacement of L-proline with the (4*R*)-F-L-proline group demonstrated improved potency against GT1a Y93H.[15] To follow up on this finding, several analogs with mix and matched 1- or 12-fluorinated core combined with the (4*R*)-F-L-proline on either left- or right-hand side were prepared. The preparation of one such analog **32** is outlined in Scheme 4 which relies upon selective manipulation of the chloro bromo tetracyclic intermediate **27**. The chemistry is analogous to the route shown in Scheme 1 and the other fluorinated analogs **33**–**38** were prepared in a similar manner using the correct fluorinated starting materials according to Scheme 4.

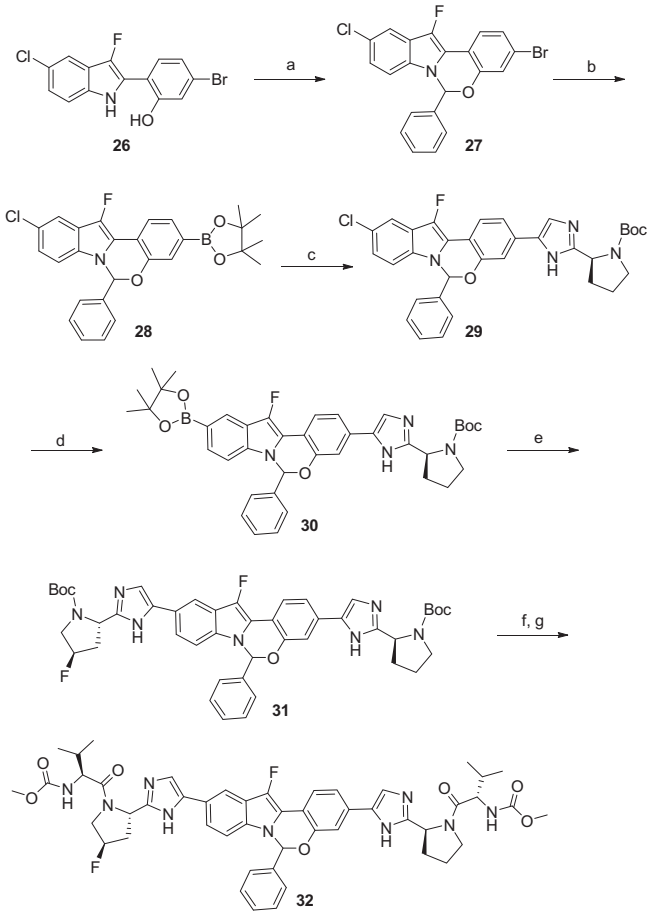
The in vitro antiviral profiles for compounds **32**–**38** are summarized in Table 3. Combination of the 12-F core with (4'*R*)-F-L-proline (**32**) demonstrated good potency against all genotypes tested with 10× improvement on GT1a Y93H potency compared to the corresponding L-proline analog **18** (Table 1). A similar result was obtained for analog which combined the 12-F core and (4″*R*)-F-L-proline (**33**) with a 10x improvement against GT1a Y93H observed. Combination of both (4′*R*)-F-L-proline and (4″*R*)-F-L-proline with the 12-F core afforded compound **34** which showed similar potency profile as compounds **32** and **33**. Combination of the 1-F core with either (4′*R*)-F-L-proline or (4″*R*)-F-L-proline afforded

Table 2
In vitro potency profiles of compounds **21**–**25**[18,19]



ID	R^{12}	R^1	Replicon EC_{90} (nM)							
			GT1a	GT1b	GT2a	GT2b	GT3a	GT4a	GT1a Y93H	GT1a L31V
21	H	*c*-Pr	0.7	—	—	454	74	—	890	0.7
22	H	Ph	1	—	—	108	47	—	250	1
23	*c*-Pr	H	0.03	0.004	0.005	122	10	0.3	100	8
24	Ph	H	0.09	0.004	0.01	204	3	0.09	5000	4
25	Benzyl	H	0.1	0.01	—	28	—	—	187	9

A hyphen "—" in the table means not tested.



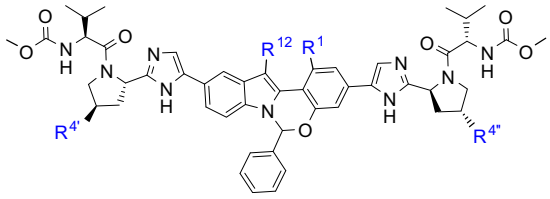
Scheme 4. Synthesis of **32.** Reagents and conditions: (a) $PhCHBr_2$, K_2CO_3, DMF, 100 °C, 16 h; (b) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, dioxane, 90 °C, 16 h; (c) *tert*-butyl (*S*)-2-(5-bromo-1H-imidazol-2-yl)pyrrolidine-1-carboxylate (prepared based on procedures described in reference 10), $Pd(dppf)Cl_2$, K_2CO_3, dioxane/H_2O (10:1), 90 °C, 16 h; (d) bis(pinacolato)diboron, $Pd_2(dba)_3$, X-Phos, dioxane, 110 °C, 2 h; (e) *tert*-butyl (2*S*,4*R*)-2-(5-bromo-1H-imidazol-2-yl)-4-fluoropyrrolidine-1-carboxylate (prepared based on procedures described in reference 10), K_2CO_3, dioxane/H_2O (10:1), 90 °C, 16 h; (f) HCl, MeOH, 0.5 h; (g) (methoxycarbonyl)-L-valine, BOP, DIPEA, DMF, 10 h; then chiral SFC separation.

compounds **35** and **36** both of which showed good potency against all genotypes tested, with ~10× improvement on GT1a Y93H compared to the corresponding L-proline analog **12** (Table 1). Very interestingly, analog **37** which combined the 1-F core with both (4′*R*)-F-L-proline and (4″*R*)-F-L-proline showed a significantly improved potency against GT1a Y93H. Its GT1a Y93H EC_{90} was determined to be in the double digit picomolar range, and is ~10× more potent than either compounds **35** or **36** and ~100× more potent than the corresponding L-proline analog **12** (Table 1). The calculated potency shift of **37** from GT1a Y93H to GT1a is only 16-fold, which was unprecedented in our previous effort. For comparison, the corresponding analog without the 1-F substitution (**38**) was prepared. Compound **38** showed potency similar to either **35** or **36** (against GT1a Y93H) while being ~20× less potent than compound **37** (GT1a Y93H). This result clearly demonstrated the importance of the 1-F substitution of compound **37** for potency improvement against GT1a Y93H.

The significant potency improvement of compound **37** against GT1a Y93H was encouraging. However, the GT2b EC_{90} value of compound **37** did not meet the goal of a 10x shift requirement. Efforts were undertaken to modify the other regions of the molecule to improve GT2b potency while retaining the excellent potency against GT1a Y93H. In a previous report,[13] alkoxy substituted phenyl "Z" groups on the tetracyclic indole core demonstrated good potency on all genotypes tested including GT2b. Analogs (**39**–**45**) incorporating these alkoxy substituted phenyl "Z" groups coupled the 1,4′,4″-tri-fluoro substitution were prepared according to the chemistry outlined in Scheme 4. The in vitro antiviral profiles for these compounds are summarized in Table 4. The 3-MeO-Ph analog **39** showed a similar potency profile to phenyl analog **37** (Table 3) with EC_{90} value against GT1a Y93H in the double digit picomolar range. This result confirmed the synergistic effect of the 1,4′,4″-tri-fluoro substitution against GT1a Y93H. Introduction of other alkoxy groups, such as EtO (**40**), *i*-PrO (**41**), *n*-BuO (**42**), or CF_3O (**43**) group to the C-3 position of the phenyl all demonstrated similar profiles as compound **39** however with no improvement in potency against GT2b. The introduction of a tetrahydrofuran-(3*R*)-oxy group to the C-3 of the phenyl "Z" group (**44**) offered a good balance between GT1a Y93H and GT2b potency, however, at the expense of the potency against GT1a Y93H, which was >10× less potent than compound **39**. Introducing a trifluoromethoxy group to the 4-position to the phenyl "Z" group, **45**, showed a similar result to the corresponding 3-substituted analog **43**.

Having been unable to improve the potency against GT2b with the 1,4′,4″-tri-fluoro substitution analogs (**39**–**45**) shown in Table 4, efforts were redirected toward exploration of analogs bearing 1,4″-di-fluoro substitution since compound **36** in Table 3 showed a more balanced GT1a Y93H and GT2b potency profile. 1,4″-Di-fluoro substituted analogs **46**–**51** were prepared based upon the chemistry outlined in Scheme 3 and the in vitro antiviral profiles for these derivatives are summarized in Table 5. Not surprisingly, the 3-MeO-Ph analog **46** showed ~ 10-fold loss in potency against

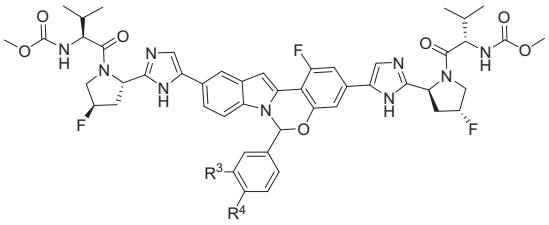
Table 3
In vitro potency profiles of compounds **32**–**38**[18,19]

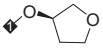


ID[a]	$R^{4'}$	R^{12}	R^1	$R^{4''}$	Replicon EC_{90} (nM)							
					GT1a	GT1b	GT2a	GT2b	GT3a	GT4a	GT1a Y93H	GT1a L31V
32[b]	F	F	H	H	0.005	0.003	0.03	—	2	0.1	2	2
33	H	F	H	F	0.002	0.001	0.003	33	0.2	0.04	2	1
34[b]	F	F	H	F	0.02	0.005	0.3	58	8	0.3	2	7
35[b]	F	H	F	H	0.003	0.003	0.01	32	0.3	0.05	4	2
36[b]	H	H	F	F	0.004	0.003	0.04	16	0.1	0.07	1	2
37[b]	F	H	F	F	0.005	0.003	0.2	72	0.8	0.1	0.08	9
38[b]	F	H	H	F	0.01	0.004	0.7	63	2	0.2	2	10

A hyphen "—" in the table means not tested.
[a] Active isomer at the aminal carbon.
[b] Mixture of two diastereomers at the aminal carbon.

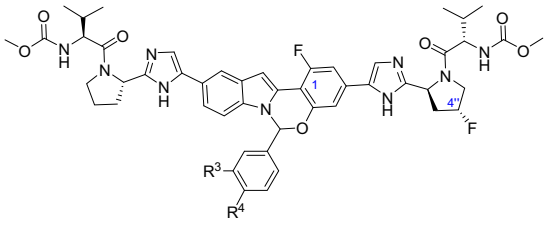
Table 4
In vitro potency profiles of compounds **39**–**45**[18,19]



ID[a]	R^3	R^4	Replicon EC_{90} (nM)					
			GT1a	GT1b	GT2b	GT3a	GT1a Y93H	GT1a L31V
39	MeO	H	0.006	0.007	39	—	0.07	2
40	EtO	H	0.01	—	69	0.6	0.2	3
41	*i*-PrO	H	0.01	—	122	1	0.1	4
42	*n*-BuO	H		0.008	126	—	0.1	4
43	CF_3O	H	0.006	0.005	52	—	0.2	7
44[b]		H	0.04	—	2	2	1	7
45	H	CF_3O	0.01	—	35	1	0.2	—

A hyphen "—" in the table means not tested.
[a] Active isomer at the aminal carbon.
[b] Mixture of two diastereomers at the aminal carbon.

Table 5
In vitro potency profiles of compounds **46**–**51**[18,19]



ID[a]	R^3	R^4	Replicon EC_{90} (nM)					
			GT1a	GT1b	GT2b	GT3a	GT1a Y93H	GT1a L31V
46	MeO	H	0.002	0.007	19	0.1	0.6	0.3
47	EtO	H	0.01	—	17	0.1	0.4	—
48	*i*-PrO	H	0.01	—	28	0.07	0.9	0.6
49	CF_3O	H	0.02	—	48	—	1.7	1.8
50	CHF_2O	H	0.002	—	22	0.2	0.3	0.7
51	H	4-CF_3O	0.03	0.008	13	—	0.3	—

A hyphen "—" in the table means not tested.
[a] Active isomer at the aminal carbon.

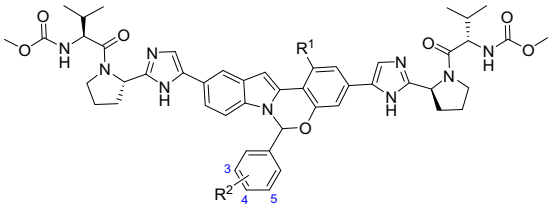
GT1a Y93H than **39**; however, no meaningful improvement on GT2b was observed. Similar SAR trends were observed for compounds **47**–**51** as was shown for compound **46**.

With the knowledge of the SAR from Tables 4 and 5, efforts were undertaken to combine the fluorine substitution on the C-1 position of the tetracyclic indole core with various "Z" groups, without any substitutions on the L-prolines. For comparison sake, their corresponding C-1 unsubstituted analogs were also prepared. Compounds **52**–**63** were prepared according to the chemistry outlined in Scheme 3 and their in vitro antiviral profiles are summarized in Table 6. While the 3-MeO-Ph analog **52** showed a similar potency profile to its corresponding 1-H analog **53**, we observed a trend that compounds bearing the 1-F substitution demonstrated better potency on GT1a Y93H and GT2b than the corresponding 1-H analogs. For example, the 3-EtO-Ph analog with 1-F core (**54**) is 8× and 5× more potent against GT1a Y93H and GT2b, respectively, than its corresponding 1-H analog **55**. 3-*i*-PrO-Ph analog with 1-F core (**56**) is 6× and 8× more potent against GT1a Y93H and GT2b, respectively, than the 1-H analog **57**. A similar trend was observed among the rest of the compounds listed in Table 6 (**58**–**63**). From the survey summarized in Table 6, compounds, such as **62**, were identified which possessed potent activity against both GT2b and GT1a Y93H. Using the EC_{90} values in Table 6, the calculated potency shifts for genotypes 2b, 1a Y93H, and 1a L31V from GT1a were 70×, 300×, and 10× for compound **62**, which was progress toward our 10-fold shift goal.

Several compounds bearing 1-F substitution on the core were tested in rat pharmacokinetic (PK) studies and the data are summarized in Table 7. Compound **12** showed good bioavailability upon oral administration. The PK profile of compound **12** was improved over similar analogs bearing an unsubstituted tetracyclic indole core, as reported previously.[13] Compound **52** showed a better PK profile than the corresponding 1-H analog **53**. Compound **62** demonstrated reduced bioavailability relative to **12** and **52**.

In conclusion, a systematic SAR study of substitutions on the tetracyclic core led to the identification of well-tolerated fluorine substitutions at both the C-1 and C-12 positions. C-1 fluoro

Table 6
In vitro potency profiles of compounds **52**–**63**[18,19]



ID[a]	R^1	R^2	Replicon EC_{90} (nM)				
			GT1a	GT2b	GT3a	GT1a Y93H	GT1a L31V
52	F	3-MeO	0.01	6	0.07	6	0.2
53	H	3-MeO	0.005	2	0.1	8	0.2
54	F	3-EtO	0.01	8	0.02	2	0.2
55	H	3-EtO	0.02	22	0.2	16	0.9
56	F	3-*i*-PrO	0.01	18	0.05	6	0.2
57	H	3-*i*-PrO	0.01	145	0.08	34	0.5
58	F	4-MeO	0.01	5	—	3	0.2
59	H	4-MeO	0.04	15	—	82	—
60	F	3,5-Di-MeO	0.004	0.6	0.06	3	0.1
61	H	3,5-Di-MeO	0.004	11	0.08	8	0.3
62	F	3-Cl-5-MeO	0.01	0.7	0.1	3	0.1
63[b]	H	3-Cl-5-MeO	0.03	202	0.8	15	0.2

A hyphen "—" in the table means not tested.
[a] Active isomer at the aminal carbon.
[b] Mixture of two diastereomers at the aminal carbon.

Table 7
Rat PK profiles of compounds **12**, **52**, **53**, and **62**[20]

	12	**52**	**53**	**62**
Dose (mpk)	5	10	5	10
PO AUC (μM h)	2.8	2.1	0.51	0.75
PO Cmax (μM)	0.33	0.24	0.1	0.1
$T_{1/2}$ (h)	3.6	5.9	4.3	5.2
Cl (ml/min/kg)	11	10	11	8
F (%)	33%	12	6	3

Male Sprague–Dawley rats were used. Compound was dosed at 2 mg/kg, IV in 60% PEG200 as well as at indicated doses in P.O. in 0.5% MC (**12**) or 10% TWEEN (**52**, **53**, **62**).

substitution on the tetracyclic core was shown to improve the potency against both GT1a Y93H and GT2b (bearing the naturally occurring 31 M RAV), and also showed comparable or improved rat PK profiles over the corresponding 1-H analogs. Combination of the C-1 fluoro substituted tetracyclic core, with (4*R*)-F-L-proline motifs, offered significant potency improvement against GT1a Y93H, but led to weaker GT2b potency. Compounds with reduced potency shifts from GT1a to GT2b, GT1a Y93H, and GT1a L31V, such as compound **62**, have been identified. However, these compounds still have not achieved a "flat" profile, i.e. a potency shift of ~10-fold. Combinations of these SAR findings with other SAR observations from other regions of the molecule are under investigation and the results will be reported in separate publications.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.08.002. These data include MOL files and InChiKeys of the most important compounds described in this article.

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