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Structure–activity relationships of proline modifications around the tetracyclic-indole class of NS5A inhibitors

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ABSTRACT

We describe the impact of proline modifications, in our tetracyclic-indole based series of nonstructural protein 5A (NS5A) inhibitors, to their replicon profiles. This work identified NS5A inhibitors with an improved and flattened resistance profiles.

Hepatitis C virus (HCV) is currently the leading cause of chronic liver disease and has become the most common blood-borne infection in developed countries.[1] Because HCV infection is silent in its early stages, most individuals are not aware they are carrying the virus, unless they undergo screening for it. If left untreated HCV can progress to the point where it results in debilitating liver diseases such as cirrhosis or liver cancer[1] and ultimately liver transplantation.[2] HCV displays a high degree of genetic diversity and can be classified into seven genotypes (GT1–7) and more than 50 subtypes.[3] Additionally, recombinant forms of the virus have been identified, however their importance has yet to be established.[3] Early therapy for HCV involved the use of pegylated-interferon and ribavirin, which was limited by both efficacy and tolerability.[4] A detailed characterization of the HCV lifecycle was important to advancing treatments for HCV as it allowed researchers to directly target specific steps for intervention in HCV replication. These agents are now known as direct-acting antiviral agents (DAAs).[5,6] In particular, it is the combination of several DAAs, each targeting a different mechanism, into one regimen that has radically changed the outcomes for HCV patients and allowed for interferon-free regimens, with significantly improved rates of sustained virologic response (SVR).[7]

Nonstructural protein 5A (NS5A) is a viral phosphoprotein that is essential for the replication of HCV. NS5A interacts with a number of host proteins involved in numerous cellular processes including apoptosis, cell-cycling, innate immunity, and membrane biogenesis that can be subverted to favor viral replication. NS5A plays a critical role in viral ribonucleic acid (RNA) synthesis and virus assembly,[8] though its exact mechanistic role in the HCV life cycle remains enigmatic.[9] The potential utility[7,10] of an NS5A inhibitor in the treatment of HCV has been demonstrated clinically[11] and currently four NS5A inhibitors are in use for the treatment of HCV including daclatasvir (DCV),[12] ledipasvir (LDV),[13] ombitasvir (OBV),[14] and elbasvir[15](EBR), Figure 1.

We sought to determine if it is possible to identify an NS5A inhibitor, with potent inhibitory activity against all genotypes and clinically-relevant resistance-associated variants (RAVs). In particular, our goal was to identify a 'flat' compound, with flat as defined by a minimal potency shift (~10×) between the wild-type virus and clinically relevant variants. Our previous efforts[16–19] indicated that structural variations could influence the relative

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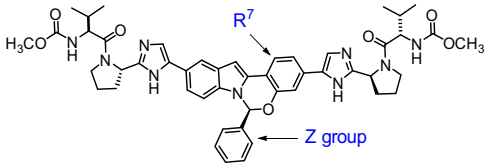
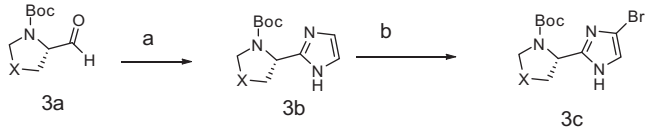


Figure 1. Elbasvir (**MK-8742**).



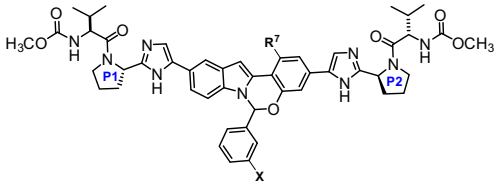
Scheme 1. Reagents and conditions: (a) Glyoxal, 7 N NH_3 in MeOH, rt; (b) NBS, THF then Na_2SO_3, $EtOH/H_2O$, reflux.

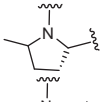
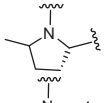
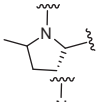
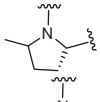
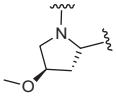
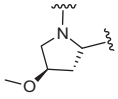
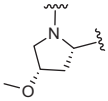
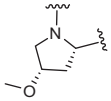
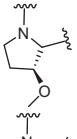
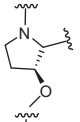
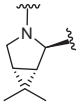
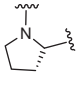
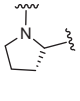
activity of various genotype and mutant activities and thus the 'flatness' of the in vitro profile could also vary. We had previously identified compound **1** (Table 1), and thought it was relevant to achieving the goal outlined above as it showed surprisingly good, low nanomolar, potency on the key RAVs GT1a_L31V and GT1a_Y93H,[18] and effectively flattened the GT1 mutant ratio. We therefore used compound **1** as a starting point for further optimization and as a baseline from which to explore the impact of variations to the proline rings on the flatness of the in vitro profiles.

The preparation of the requisite bromoimidazole fragments **3c** is illustrated in Scheme 1.[20–22] Condensation of aldehydes **3a**, which were prepared according to literature procedures,[20] with glyoxal in the presence of 7 M ammonia in MeOH afforded the imidazole **3b**. Bromination with NBS yielded the intermediate dibromo adduct which was treated under reductive conditions employing Na_2SO_3 to obtain bromide **3c** which was ready for coupling.

A general preparation of symmetric proline analogs **2**–**6** and **9**–**15** is shown in Scheme 2. Utilizing a Fischer indole synthesis protocol,[20–22] 5-bromoacetophenone **3d** was condensed with *p*-bromophenylhydrazine under acidic conditions to form the intermediate hydrazone which was treated at high temperature

Table 1
In vitro potency for compounds **1**–**8** in replicons



No.	P1	P2	X	R^7	Replicon, EC_{90}[23] (nM) GT1a	GT1a L31V	GT1a Y93H	GT2b[†]	GT3a
1			OMe	F	0.01	0.20	5.76	5.72	0.07
2[a]			H	H	ND	1.72	64	75	6.14
3[b]			H	H	ND	5.74	158	129	51.5
4			OMe	F	0.015	0.19	0.79	90.7	5.52
5			OMe	F	0.017	0.04	8.87	5.74	0.71
6			OMe	F	2.67	24.5	3.86	322	30.0
7[c]			H	F	0.020	21.0	>100	337	>10
8			OMe	F	0.002	0.90	26.1	51.8	0.92

ND: not determined.
[†] The replicon bears methionine at position 31.
[a] Single diastereomer A.
[b] Single diastereomer B.
[c] Mixture of diastereomers at aminal carbon.

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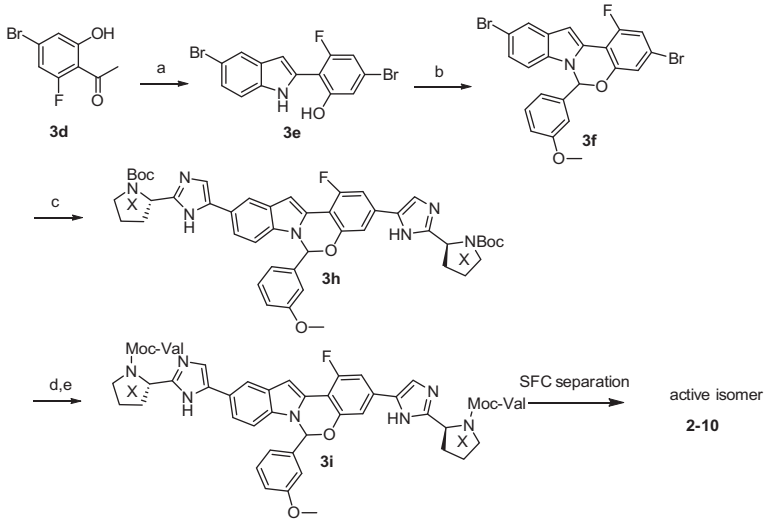


Scheme 2. Reagents and conditions: (a) (1) *p*-Bromophenylhydrazine, AcOH, EtOH, reflux; 65% then (2) PPA, 110 °C; (b) 3-methoxybenzaldehyde, *p*-toluenesulfonic acid, *o*-xylene, 170 °C, 15 h.; (c) bis(pinacolato)diboron, KOAc, $PdCl_2$(dppf), dioxane, 110 °C then bromoimidazole **3c**, 1 M Na_2CO_3, Pd(dppf)Cl_2, THF, 90 °C; (d) 4 N HCl, MeOH; (e) Moc-L-valine, HATU, DIPEA, DMF.

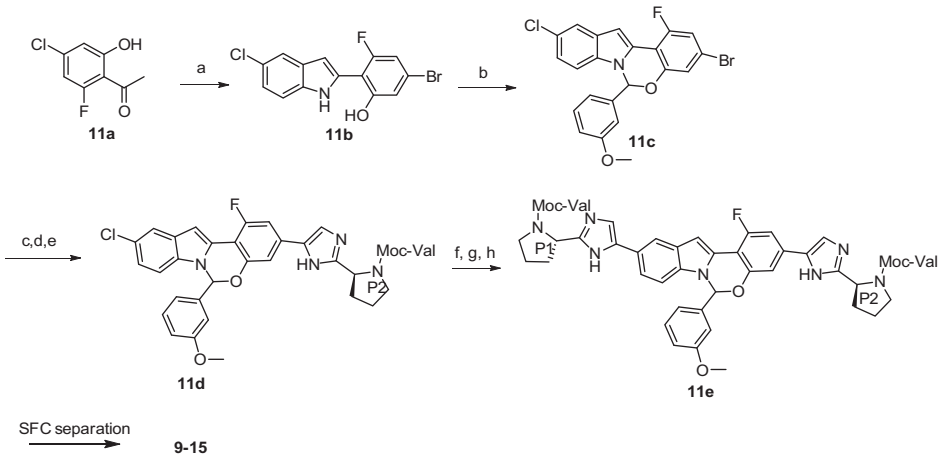
with polyphosphoric acid (PPA) to afford indole **3e**. Treatment of **3e** with 3-methoxybenzaldehyde, *p*-toluenesulfonic acid in *o*-xylene at 170 °C gave **3f**. Treatment of dibromide intermediate **3f** with two equivalents of bis(pinacolato)diboron in the presence of KOAc and Pd(dppf)Cl_2 afforded the intermediate bis-pinacol boronic ester, the key coupling partner. Reaction of this core under palladium catalysis with two equivalents of bromoimidazole intermediates **3c** afforded the bis Boc adducts **3h**. Global deprotection with HCl followed by HATU promoted double amide coupling reactions with Moc-L-valine afforded the final compounds as mixtures of two epimeric aminal compounds.[22] The mixtures were subjected to SFC chiral separation to afford compounds as the single, more potent diastereomer of the pair. Unless otherwise noted, the more active diastereomer is reported in the SAR tables.

The synthesis of mixed proline analogs begins as in Scheme 3. Using a Fisher indole synthesis, the 5-bromoacetophenone **11a** was converted to indole **11b**. This material was cyclized under the standard conditions to afford **11c** which was ready for selective functionalization of the bromide. Intermediate **11c** was subjected to the borylation/Suzuki coupling protocol used previously utilizing Pd(dppf)Cl_2 as catalyst to afford the mono-coupled material which after HCl deprotection and capping yielded chloride **11d**. With compound **11d** in hand, treatment with $Pd_2(dba)_3$ in the presence of bis(pinacolato)diboron and KOAc afforded the intermediate boronate which was treated directly with bromoimidazole **3c** to afford the Boc-protected intermediate. Treatment with HCl followed by capping afforded compound **11e** which underwent SFC chiral separation to afford final compounds **7**–**8**, **16**–**21**, **22**–**25** and **27** shown in Tables 1 and 3–6. Compounds in present communication were synthesized according to previously published patent proccedures.[21,22]

Our first intent was to have a general scan for tolerability of groups and preferred substitution patterns, on the proline rings. Table 1 summarizes the data for this investigation. For this purpose, prolines bearing a methyl at the 5 position were examined first. Compounds **2** and **3**, which were diastereomers of methyl group, both showed lower potency across the board. Interestingly, differences in the relative activity of the individual diastereomers were observed, with compound **2** exhibiting a moderately better profile than compound **3**. Activity at the 4 position of the proline ring was examined with a methoxy substitution. The (*R*)-methoxy isomer **4** was superior in GT1a_Y93H potency compared to the corresponding (*S*)-isomer **5**, but was weaker versus GT2b and GT3a. While neither compound **4** or **5** displayed an overall profile that was improved as compared to **1**, it did demonstrate that both genotype and mutant potencies were sensitive to changes at the 4-position. The preference of 4-proline to 3-proline was evident in comparison between compounds **4** and **6**. We also investigated the impact of fusing the 3,4 positions with a cyclopropyl group. This modification proved to be detrimental to potency as exemplified by compound **7**. Bridging the 3 and 5 positions on the proline, with a second ring, as shown by compound **8** also failed to demonstrate meaningful improvement. Overall, the data in Table 1 suggested the methoxy proline at the 4-position was preferred, but in comparison to **1**, we did not see an improvement in the overall profile.

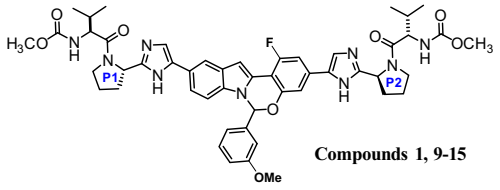
We next expanded the diversity of proline variants at the 4-position of the proline ring, with a focus on improving the GT1a_Y93H activity. We were encouraged by the improvement that compound **4** showed versus this mutant and wanted to see if we could broaden its activity profile to also cover GT1a_L31V, GT2b and GT3a. With this objective in mind we maintained the beta-configuration for the substituents described in Table 2, analogs **9**–**15**, with the variations made simultaneously to both proline rings (P1 = P2). The 4-fluoro analog **9** improved the GT1a_Y93H potency into the picomolar range, but it also showed a detrimental effect in GT2b potency. The difluoromethoxy derivative **10** was less potent in GT1a_Y93H, when compared with its methoxy counterpart **4** and was also weaker in GT2b and GT3a (**10** vs **4**). Spiro-fused rings were also examined, as exemplified by **11** and **12**. Compound **11** exhibited potency roughly comparable to **1**, while **12** demonstrated an improvement in GT1a_Y93H (0.44 nM for **12** and 5.76 nM for **1**). The fused-cyclopropyl proline analog **13** demonstrated improved activity for GT1a_Y93H, but exhibited weaker potency in GT1a_L31V, when compared to **1**. Increasing the size of the ring fused to the proline had a detrimental effect on the in vitro profiles as illustrated by **14** and **15**.

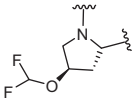
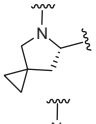
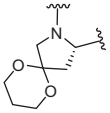
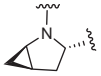
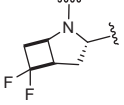
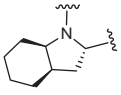
Although the proline analogs **9** and **13** (Table 2) demonstrated improved GT1a_Y93H potency, their GT2b potency was slightly lower. Therefore, we sought to investigate the impact of independent substitution of either the left or the right proline, on potency. Table 3 summarizes data for this investigation. The combination of a 4-fluoro-proline on the left with an unsubstituted proline on the right, compound **16**, showed a profile similar to compound **17**, which possessed the reversed combination of prolines. Both



Scheme 3. Reagents and conditions: (a) (1). *p*-Bromophenylhydrazine, AcOH, EtOH, reflux; then (2) PPA, 110 °C; (b) 3-methoxybenzaldehyde, *p*-toluenesulfonic acid, *o*-xylene, 170 °C, 15 h.; (c) bis(pinacolato)diboron, KOAc, $PdCl_2(dppf)_2$, dioxane, 110 °C then bromoimidazole **3c**, 1 M Na_2CO_3, $Pd(dppf)Cl_2$, THF, 90 °C; (d) 4 N HCl, MeOH; (e) Moc-L-valine, HATU, DIPEA, DMF; (f) bis(pinacolato)diboron, KOAc, $Pd_2(dba)_3$, dioxane, 120 °C, bromoimidazole **3c**, 1 M Na_2CO_3, THF, 90 °C; (g) 4 N HCl, MeOH; (h) Moc-L-valine, HATU, DIPEA, DMF.

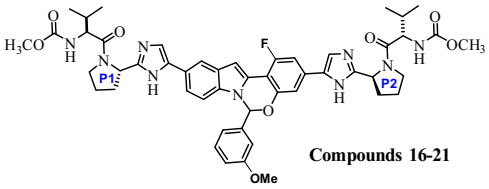
Table 2
In vitro profile of compounds **9**–**15** in replicons



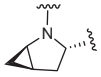
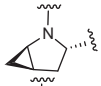
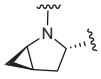
No.	Proline P1 = P2	Genotype, EC_{90}[23] (nM)				
		GT1a	GT1a L31V	GT1a Y93H	GT2b[†]	GT3a
9		0.006	1.59	0.065	39.0	ND
10		0.021	ND	5.27	55.6	12.9
11		0.011	0.050	23.7	38.8	0.16
12[a]		0.018	0.20	0.44	114	15.0
13		0.005	1.72	0.51	22.5	0.17
14		0.017	0.078	52.6	58	0.29
15		0.18	0.51	37.6	21	ND

ND: not determined.
[†] The replicon bears methionine at position 31.
[a] Mixture of diastereomers at aminal carbon.

Table 3
In vitro profile of compounds with mixed prolines **16**–**21** in replicons

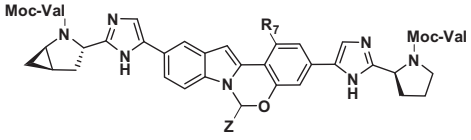


Compounds 16-21

No.	P1	P2	Replicon, EC_{90}[23] nM				
			GT1a	GT1a L31V	GT1a Y93H	GT2b[†]	GT3a
1			0.01	0.20	5.76	5.72	0.07
16			0.010	0.29	1.81	33.6	ND
17			<0.002	0.34	0.55	18.8	0.13
18			0.007	0.084	1.64	19.4	0.15
19			0.026	0.36	10.9	49.3	ND
20			0.032	0.92	5.15	52.2	ND
21			0.30	13.6	91.4	391	ND

ND: not determined.
[†] The replicon bears methionine at position 31.

Table 4
In vitro profile of compounds **22** and **23**



Compounds 22 and 23

No.	Z	R^7	Replicon, EC_{90}[23] (nM)			
			GT1a	GT1a L31V	GT1a Y93H	GT2b[†]
1		F	0.01	0.20 (20×)	5.76 (230×)	5.72 (570×)
22		F	0.016	0.42 (26×)	3.17 (200×)	16.3 (1000)
23		H	0.013	0.13 (10×)	6.45 (500×)	4.66 (360×)

[†] The replicon bears methionine at position 31; numbers in parentheses are fold-shift relative to the GT1a potency, set = 1.

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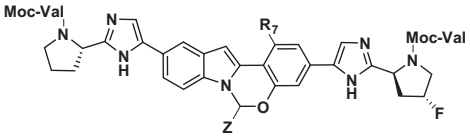
Table 5
In vitro profile of compounds **24**–**25** in replicons

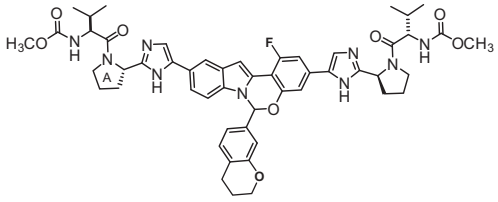


Compounds 24 and 25

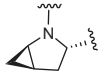
No.	Z	R^7	Replicon, EC_{90}[23] (nM)			
			GT1a	GT1a L31V	GT1a Y93H	GT2b[†]
1		F	0.01	0.20 (20×)	5.76 (230×)	5.72 (570×)
24		F	0.006	2.17 (362×)	1.40 (233×)	63.6 (10600×)
25		H	<0.02	0.81 (>41×)	19.8 (>990×)	60.8 (>3040×)

[†] The replicon bears methionine at position 31; numbers in parentheses are fold-shift relative to the GT1a potency, set = 1.

Table 6
In vitro profile of compounds **26** and **27** in replicons



Compounds 26 and 27

No.	A	Replicon, EC_{90}[23] nM			
		GT1a	GT1a L31V	GT1a Y93H	GT2b[†]
26		0.003	0.0025 (1×)	0.48 (160×)	0.31 (103×)
27		0.004	0.007 (2×)	0.12 (30×)	0.38 (95×)

[†] The replicon bears methionine at position 31; numbers in parentheses are fold-shift relative to the GT1a potency, set = 1.

derivatives showed moderate improvement in GT1a_Y93H potency when compared to **1** while both were weaker in comparison to the bis-fluoro proline **9**. By contrast, there seemed to be a preference for the fused cyclopropyl proline on the left side (**18**) over the right side (**19**), but interestingly **18** showed improved activity for GT1a_L31V potency, as compared with the bis-cyclopropyl proline **13** (0.084 nM for **18** vs 1.72 nM for **13**). However, compounds **1** and **18** were similar in overall mutant profile with **18** trending slightly better in GT1a_Y93H. The combination of cyclopropyl-proline (left) and fluoro-proline (right) yielded compound **20**. This combination did not show added benefit to the potency profile when compared with **1**. A silyl-proline had previously demonstrated advantages in PK properties in our earlier studies and thus was introduced, compound **21**.[24] However, incorporation of this motif coupled with the 4-fluoroproline in compound **21** showed reduced inhibitory activity across all genotypes.

Although the proline modifications in Table 3 showed unpredictable differences in mutant profile compared to compound **1**, we decided to further investigate the fused cyclopropyl proline variant on the left to see if it could provide a path toward a flatter compound. For this analysis we chose to look at the shift in potency versus the wild type virus as well as the absolute value of the replicon activity. The SAR work was conducted by exploring the combination of different Z-groups, with and without the R^7 fluorine substitution. The results are summarized in Table 4, with the values in parentheses being the potency shift of variants versus genotype 1a activity. In general, these modifications yielded compounds with in vitro profiles similar to each other.

Table 7
Pharmacokinetic parameters of compounds **23** and **27** in rat

No.	IV			PO			
	Dose (mpk)	$t_{1/2}$ (h)	Cl (ml/min/kg)	Dose (mpk)	AUC (μM.h)	Cmax (μM)	*F*%
23	2	4.7	6	10	3.64	0.63	13
27	2	4.6	11.4	10	0.63	0.073	4

Vehicle: IV (60% PEG200); PO (10% TWEEN).

When conducting a similar investigation, but this time using the fluoro-proline variant on the right, (Table 5), similar results were obtained, where **24** and **25** were comparable to **1** for GT1a_L31V and GT1a_Y93H, but weaker for GT2b.

From a previous SAR study, we observed that a chroman ring system for the Z group could result in improved mutant profiles, as illustrated by compound **26**.[18] When the cyclopropyl proline on the left side was combined with this Z group, compound **27**, we observed a more balanced profile (Table 6). More specifically, the potency shift for GT1a_Y93H was 30× versus the 160× for regular proline analog **26**. Also, the potency shifts for GT1a_L31V and GT2b were maintained in a range similar to **26**.

Owing to the most balanced mutant profiles, compounds **23** and **27** were selected for exploratory rat PK studies with the results summarized in Table 7. Compound **23** demonstrated acceptable oral exposure and bioavailability in the rat. However, compound **27** showed lower exposure and reduced bioavailability. Both compounds showed similar half-life measurements.

We found the replicon profile of compound **1** to be sensitive to proline ring substitutions, some of which produced analogs with improved in vitro profiles. More specifically, the incorporation of either a cyclopropyl-fused proline, or a fluoro-substituted proline provided increased potency versus the GT1a_Y93H RAV. When proline modifications were combined with other structural features, e.g., Z-group changes, inhibitors with improved resistance profiles were identified. These data indicate the unpredictable impact that various structural combinations can have on the in vitro profiles. Compounds **23** and **27** both demonstrate mutant profiles with smaller potency shifts against GT1a, potentially providing a path forward toward the identification of an NS5A inhibitor with a 'flat' profile. Additional efforts toward the optimization of activity against common resistance-associated polymorphisms will be reported in future publications.

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