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Synthesis and evaluation of non-dimeric HCV NS5A inhibitors

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Abstract

Based on the symmetrical bidentate structure of the NS5A inhibitor BMS-790052, a series of new monodentate molecules were designed. The synthesis of 36 new non-dimeric NS5A inhibitors is reported along with their ability to block HCV replication in an HCV 1b replicon system. Among them compound **5a** showed picomolar range activity along with an excellent selectivity index (SI - 90,000).

Keywords

NS5A; Antiviral agent; HCV

According to the World Health Organization, HCV has infected an estimated 170 million individuals worldwide and more than three million people contract HCV each year.¹ Of the six major HCV genotypes known, type 1a/b accounts for approximately 70% of all infections in the US, Europe, China and Japan.² Although often asymptomatic, it can progress to chronic hepatitis leading to liver cirrhosis and in many cases hepatocellular carcinoma. Until recently, HCV therapy, utilizing interferon/ribavirin (IFN/RBV), was effective in only 40-60% of patients and was often associated with significant side effects. In May 2011, two HCV protease inhibitors, Incivek and Victrelis, were approved by the US FDA for use in combination with the standard of care (IFN/RBV) and provided a sustained virologic response (SVR) of about 70 to 80% in genotype 1 HCV-infected subjects.³ However, these drugs have limitations that include the need of response-guided therapy and the management of severe skin rashes and anemia.

In the search for new therapeutic agents interfering with HCV replication, non-structural (NS) viral proteins have been primarily targeted including NS2, NS3 (protease), NS4A, NS4B, NS5B (polymerase) and NS5A. NS5A is a large phosphoprotein (49kDa) required for HCV replication and particle assembly. Although its ultimate role in the HCV replication cycle is not fully understood,⁴ it has been shown to be a required component of the HCV RNA replication and several molecules have been recently identified as potent inhibitors. For example, BMS-790052 has subnanomolar EC₅₀ values against genotype 1 replicons and

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a phase I clinical trial, in patients chronically infected with HCV, produced a 3.3 log₁₀ reduction in mean viral load 24 h after administration of a single 100 mg dose.⁵ Unfortunately, for both subtypes 1a and 1b, treatment with BMS-790052 quickly lead to the emergence of several drug-induced mutations.

We hypothesized that lower molecular weight unsymmetrical compounds could be developed to have greater activity toward known NS5A mutations and therefore, a higher barrier to resistance (Figure 1). As part of our SAR study, modifications were combined on various positions of the monodentate NS5A scaffold (**A**, **B**, **C** and **D**) and served as probes for 3D-exploration of the NS5A binding area (Figure 1). Based on this strategy, we have developed a second generation of unsymmetrical, still highly potent, NS5A inhibitors.

Modifications on the various targeted positions have been achieved through general Scheme 1 by adapting chemistry previously described.⁶ Commercially available phenyl methyl ketones **1** were brominated in moderate to excellent yields then condensed with *N*-Boc protected proline in presence of DIPEA to afford compounds **2**. Further, cyclization of the keto-ester of **2** to the corresponding imidazole was accomplished in presence of ammonium acetate followed by Boc deprotection under acidic conditions gave compounds **3**. Modification of **Part C** and **D** was achieved by reaction of **3** with various protected amino acids under traditional peptidic coupling conditions and generated the analogs **4a-o**. Secondary modifications of **Part A** were explored using Pd-catalyzed cross coupling between the bromo compound **4b** and various substituted phenyl boronic acids (Scheme 2, compounds **6a-l**) but also by alkylation of amino derivative **8** obtained by hydrogenation of azido compound **4c** (Scheme 3, compounds **9a-b**). Because we recently determined that halogenation of BMS-790052' imidazole ring using NBS, NCS or NIS lead to highly potent compounds exhibiting excellent overall profiles,⁷ we restricted our efforts on **Part B** to the preparation of compounds **5a-c** and **7** (Scheme 1 and 2).

Overall, 36 monodentate compounds were evaluated in an *in vitro* HCV replicon assay and their potential toxic effects assessed in multiple cell lines (Table 1), including: Huh-7 (a human hepatocellular carcinoma cell line), primary human hepatocytes, Vero (African green monkey kidney epithelial cells), and PBM (primary human peripheral blood mononuclear cells). While the first simple truncated compound **4a** and modified derivatives **4b** and **4d-o** did not show any activity against HCV in the replicon system when tested up to 10 µM (Table 1), functionalization of the phenyl ring with an azido group lead to compound **4c**, exhibiting a median effective concentration (EC₅₀) of 8 nM against HCV with no apparent toxicity in four different cell lines. Interestingly, halogenation of **4c** on the imidazole moiety afforded even more potent compounds, including bromo derivative **5a** that displayed an EC₅₀ of 0.7 nM. Elongation of compound **4a** by addition of a second phenyl ring lead to compound **4j** that displayed an EC₅₀ of 300 nM. Interestingly, substitution of this second ring with a 4-CF₃- or 4-OH-group lead to compounds **6c** and **6e** displaying EC₅₀ values of 4.6 and 5 nM, respectively. However, unlike for compound **4c** bearing only one phenyl ring, halogenation of the imidazole ring of compound **6c** led to loss of anti-HCV activity (compound **7**).

Drug-drug interactions have become an important issue in modern health care. It is now apparent that many drug-drug interactions can be explained by alterations in the metabolic enzymes that are present in the liver and other extra-hepatic tissues. Many of the major pharmacokinetic interactions between drugs are due to hepatic cytochrome P450 (CYP450) enzymes being affected by one or more of the drugs present in a given individual. Furthermore, drug interactions can be a result of inhibition or induction of CYP450 enzymes. The potential drug-drug interaction liabilities of the most active compounds were investigated by a CYP450 reversible inhibition assay. Compounds **4c**, **5a**, **6c** and **6j** did not

appear to inhibit the major CYP450 enzymes at the IC₉₀ level and had a favorable CYP profile, which is suggestive of no drug-drug interactions (Table 2). Due to the large therapeutic window, the CYP inhibitions observed in the micromolar ranges are expected not to be clinically relevant at therapeutic doses.

In conclusion, extremely potent non-dimeric NS5A inhibitors (picomolar activity) with a wide therapeutic window ($> 10^4$) have been discovered. Out of all compounds prepared, compound **5a** appeared to be the most promising with an EC₅₀ of 0.7 nM and no toxicity or inhibition of major human CYP enzymes at therapeutically relevant concentrations. *In vitro* resistance profile of compound **5a** was established by mutation selection in HCV subgenomic replicon containing Huh-7 cells. After 2 months exposure, Y93H and Q30E were among the selected resistant virus, similar to those observed with BMS-790052 treatment, which confirmed that this monodentate compound acts as an NS5A inhibitor. Through this work, we demonstrated for the first time that, a bidentate structure (i.e. BMS-790052) was not a *sine qua non* condition for a molecule to inhibit HCV NS5A.

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Figure 1.
Structures of BMS-790052 and targeted monodentate compounds.

Scheme 1.

Reagents and conditions: (a) Br₂, CH₂Cl₂, rt, overnight, 70-95%; (b) Boc-Pro-OH, DIPEA, CH₃CN, rt, 5 h; (c) NH₄OAc, toluene, 95 °C, 15-20 h, 62-72% 2 steps; (d) 6N HCl, CH₃OH, 50 °C; (e) R⁴-(CO)NH-CHR³-COOH, DIPEA, HOBt, EDC, CH₃CN, 0 °C to rt, 15 h, 45-65% 2 steps; (f) NXS, CH₂Cl₂, rt, 0.5-15 h, 42-65% (X = Br, Cl, I).

Scheme 2.

Reagents and conditions: a) $R^5\text{-Ph-B(OH)}_2$, $\text{PdCl}_2(\text{PPh}_3)_2$, Na_2CO_3 , THF, rt, 50-77%; (b) NCS, CH_2Cl_2 , rt, 15 h, 58%.

Scheme 3.

Reagents and conditions: (a) H₂, Pd/C, MeOH, rt, 15 h, 91%; (b) MeOC(O)Cl, pyridine, rt or MeSO₂Cl, pyridine, rt, 15 h, 60-75%.

Table 1

Structures, Anti-HCV Activity and Cytotoxicity of BMS-790052 and compounds **4a-o**, **5a-c**, **6a-l**, **7**, **8**, **9a-b**.

Cmpd	R ¹	R ²	R ³	R ⁴	HCV Replicon in Huh-7 cells			Cytotoxicity, CC ₅₀ (μM) ^a		
					EC ₅₀ (nM)	EC ₉₀ (nM)	PBM	CEM	Vero	
BMS	N/A ^b	N/A	N/A	N/A	0.01	0.03	19	9.6	21	
4a	H	H	<i>i</i> Pr	OMe	>1000	N/A	>100	>100	>100	
4b	4-Br	H	<i>i</i> Pr	OMe	>100	N/A	>100	48	50	
4c	4-N ₃	H	<i>i</i> Pr	OMe	8	52	>100	>100	>100	
4d	4-CN	H	<i>i</i> Pr	OMe	>100	N/A	>100	33	98	
4e	4-NO ₂	H	<i>i</i> Pr	OMe	>100	N/A	>100	>100	>100	
4f	4-CF ₃ O	H	<i>i</i> Pr	OMe	>100	N/A	49	30	>100	
4g	4-CF ₃	H	<i>i</i> Pr	OMe	>100	N/A	>100	26	>100	
4h	4-OMe	H	<i>i</i> Pr	OMe	>1000	N/A	>100	>100	>100	
4i	4-Me	H	<i>i</i> Pr	OMe	>1000	N/A	>100	>100	>100	
4j	4-Ph	H	<i>i</i> Pr	OMe	300	900	14	18	70	
4k	4-Ph	H	<i>i</i> Bu	OMe	>100	>1000	16	25	61	
4l	4-Ph	H	Me	<i>i</i> Bu	>1000	N/A	16	13	11	
4m	4-Ph	H	allyl	Me	>1000	N/A	100	21	73	
4n	2,4-diCl	H	<i>i</i> Pr	OMe	1000	N/A	56	15	>100	
4o	4-PhSO ₂ -	H	<i>i</i> Pr	OMe	>100	N/A	>100	>100	>100	
5a	4-N ₃	Br	<i>i</i> Pr	OMe	0.7	2.9	35	16	52	
5b	4-N ₃	Cl	<i>i</i> Pr	OMe	4	14	94	12	>100	
5c	4-N ₃	I	<i>i</i> Pr	OMe	2	3	64.2	14.2	>100	
6a	4-CN-Ph	H	<i>i</i> Pr	OMe	>100	N/A	>100	33	98	
6b	4-Me-Ph	H	<i>i</i> Pr	OMe	>100	N/A	90	14	16	

Cmpd	R ¹	R ²	R ³	R ⁴	HCV Replicon in Huh-7 cells		Cytotoxicity, CC ₅₀ (μM) ^a		
					EC ₅₀ (nM)	EC ₉₀ (nM)	PBM	CEM	Vero
6c	4-CF ₃ -Ph	H	<i>i</i> Pr	OMe	4.6	9.6	>100	14	12
6d	2-CF ₃ -Ph	H	<i>i</i> Pr	OMe	>100	N/A	24	13	75
6e	3-CF ₃ -Ph	H	<i>i</i> Pr	OMe	>100	N/A	44	12	32
6d	4-CF ₃ O-Ph	H	<i>i</i> Pr	OMe	>100	N/A	11	10	10
6e	4-HO-Ph	H	<i>i</i> Pr	OMe	5	17	70	13	55
6f	3-OH-Ph	H	<i>i</i> Pr	OMe	>1000	N/A	>100	50	>100
6g	4-MeO-Ph	H	<i>i</i> Pr	OMe	>100	N/A	85	13	30
6h	4-NH ₂ C(O)-Ph	H	<i>i</i> Pr	OMe	21	76	>100	>100	>100
6i	4-MeOOC-Ph	H	<i>i</i> Pr	OMe	>100	N/A	14	17	61
6j	4-HO-CH ₂ -Ph	H	<i>i</i> Pr	OMe	2	9	>100	59	67
6k	4-MeSO ₂ -Ph	H	<i>i</i> Pr	OMe	>100	N/A	>100	6	54
6l	4-N ₃ -Ph	H	<i>i</i> Pr	OMe	>100	N/A	37	11	11
7	4-CF ₃ -Ph	Cl	<i>i</i> Pr	OMe	>100	N/A	11	10	10
8	4-NH ₂	H	<i>i</i> Pr	OMe	200	300	>100	>100	>100
9a	4-NHCO ₂ Me	H	<i>i</i> Pr	OMe	>100	N/A	>100	>100	>100
9b	4-NHSO ₂ Me	H	<i>i</i> Pr	OMe	>100	N/A	>100	13	>100

^a All compounds had a CC₅₀ of > 1 μM in Huh-7 cells

^b N/A, not applied.

Table 2

Cytochrome P450 inhibition data for compounds **4c**, **5a**, **6c**, **6j** and BMS-790052^a

Cmpd	CYP1A2 (μM)		CYP3A4 (μM)		CYP2D6 (μM)		CYP2C9 (μM)	
	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀
4c	> 100	> 100	5.1 ± 0.6	96.1	> 100	> 100	> 100	> 100
5a	80.9 ± 21.1	> 100	1.5 ± 1.6	46.1 ± 23.8	50.5 ± 6.8	> 100	50.5 ± 19.7	> 100
6c	82.9 ± 13.4	> 100	6.5 ± 0.6	> 100	62.1 ± 32.9	> 100	9.1 ± 1.9	> 100
6j	56.7 ± 12.4	> 100	6.9 ± 2.1	> 100	> 100	> 100	31.2 ± 26.0	> 100
BMS-790052	> 100	> 100	7.2 ± 1.7	> 100	> 100	> 100	59.5 ± 13.6	> 100

^aMean ± SD of at least two independent assays; IC₅₀ = 50% inhibitory concentration. The following positive controls were used (IC₅₀, μM): α-naphthoflavone for 1A2 (> 93% at 3 μM); ketoconazole for 3A4 (> 93% at 10 μM); PH-053 (proprietary) for 2D6 (> 70% at 100 μM); sulfaphenazole for 2C9 (> 90% at 10 μM).