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Synthesis and biological evaluation of new potent and selective HCV NS5A inhibitors

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ABSTRACT

NS5A inhibitors are a new class of direct-acting antiviral agents which display very potent anti-HCV activity in vitro and in humans. Rationally designed modifications to the central biphenyl linkage of a known NS5A series led to selection of several compounds that were synthesized and evaluated in a HCV genotype 1b replicon. The straight triphenyl linked compound **11a** showed similar anti-HCV activity to the clinical compound BMS-790052 and a superior cytotoxicity profile in three different cell lines, with an EC_{50} value of 26 pM and a therapeutic index of over four million in an HCV replicon assay. This triphenyl analog warrants further preclinical evaluation as an anti-HCV agent.

Hepatitis C virus (HCV) is an important pathogen affecting nearly 170 million people worldwide.[1] HCV infections become chronic in about 50% of cases,[2] and about 20% of these chronic persons develop liver cirrhosis that can lead to hepatocellular carcinoma.[3] Current therapies, interferon-alpha (IFN-α) and ribavirin, even when used with the newly approved HCV protease inhibitors Incivek and Victrelis, have limited efficacy and serious side-effects.[4] Therefore, there is a need for more effective and safer small molecule anti-HCV agents.

In our continuing efforts to identify more effective direct-acting antiviral agents (DAA), a relatively new target, the non-structural protein NS5A, has emerged as an attractive objective.[5] Currently in the clinic, the two most common targets for DAA with HCV are the non-structural proteins NS3 and NS5B.[6] Among the recently discovered NS5A inhibitors, BMS-790052 showed a median effective antiviral concentration (EC_{50}) in vitro, in the picomolar range and demonstrated in clinical trials, a reduction in HCV RNA of over 3 $\log_{10}$ IU/mL at 24 h following a single dose of 10 mg (Fig. 1).[7]

We carefully studied the SAR of some known NS5A inhibitors,[7,8] including BMS-790052 and earlier hits BMS-858, BMS-824, and BMS-665. For the BMS-790052 and BMS-665 series of compounds we found that many of the compounds were symmetrical or almost symmetrical around a central core, this core had only/mainly pi electron interaction capabilities with the NS5A protein, and the length of these molecules is quite different, leaving room for us to modify and optimize these molecules. We hypothesized that the two phenyl rings of BMS-790052 act only as a core linker between the two substituted imidazolylpyrrolidine regions. Thus, we envisaged that changing the length and/or the geometry of this central biphenyl linkage might lead to a more potent compound showing, perhaps, less cytotoxicity. Therefore, a series of bis-imidazolylpyrrolidine compounds with phenyl, phenoxyphenyl, triphenyl, pyridinyldiphenyl, triazole containing, and tetraphenyl linkages have been synthesized and evaluated for their anti-HCV activity and cytotoxicity in different cell lines.

Phenyl, phenoxyphenyl, phenylthiopheneyl, and phenylbenzenesulfonamide linked compounds **5a–d** were prepared in four steps by adapting a reported procedure[9] as depicted in Scheme 1.

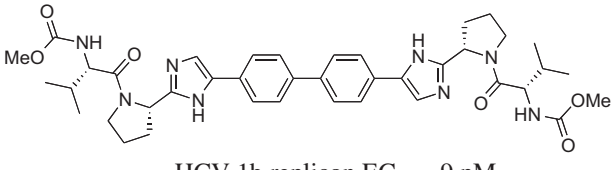

Figure 1. Chemical structure of BMS-790052.

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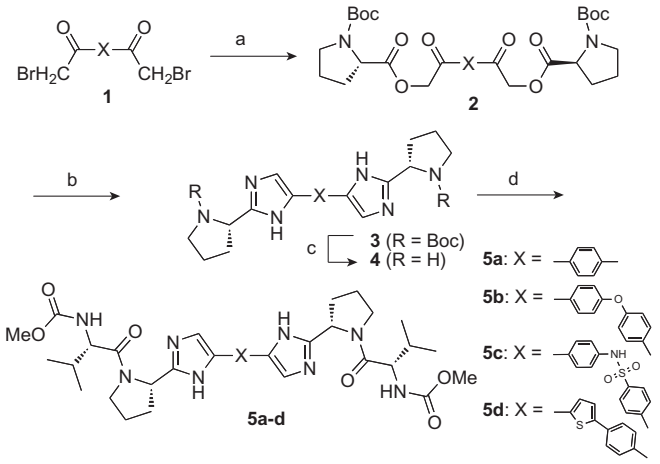
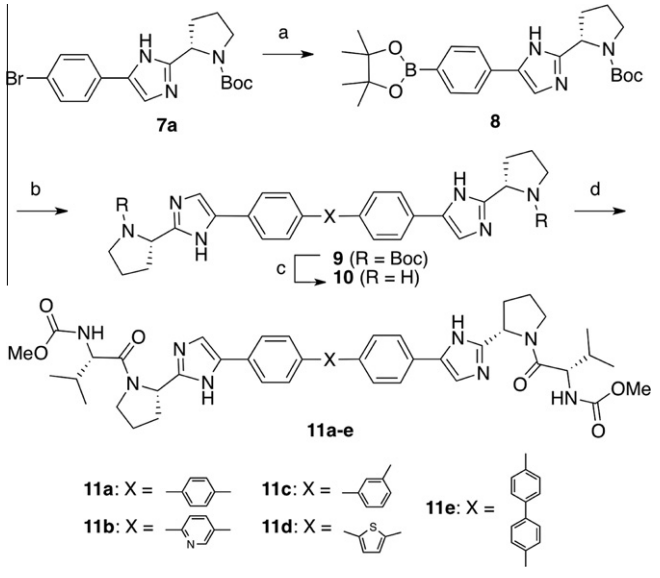


Scheme 1. Synthesis of **5a–d**. Reagents and conditions: (a) *N*-Boc-L-proline, MeCN, Et_3N, rt, 2 h, 51–100%; (b) NH_4OAc, toluene, 95–100 °C, 14 h, 51–73%; (c) 6 N HCl, MeOH, 50 °C, 4 h, 85–99%; (d) HOBt, EDAC, *N*-(methoxycarbonyl)-L-valine, MeCN, DIPEA, rt, 14 h, 64–95%.

Di bromoketones **1a** and **1c**,**d** were prepared by bromination of the corresponding diketone while **1b** was prepared by Friedel–Crafts acylation of the corresponding diphenylether with bromoacetyl chloride. Reaction of **1a–d** with *N*-Boc-L-proline to give the diesters **2a–d** in good to excellent yield. The esters **2a–d** were then refluxed in toluene with ammonium acetate to form imidazoles **3a–d**. Following Boc deprotection with 6 N HCl and coupling with *N*-(methoxycarbonyl)-L-valine in presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC), the target compounds **5a–d** were obtained in fair to excellent overall yields.

The tricyclic and tetracyclic linked analogs were prepared as described in Scheme 2. The key intermediate boronate **8** was prepared from the bromo derivative **7a** by a palladium catalyzed cross-coupling reaction with bis(pinacolato)diboron,[10] while the bromide **7a** was prepared from commercially available 2,4′-dibromoacetophenone, **6a** (Fig. 2) via esterification and cyclization, analogous to the preparation of compounds **3** in Scheme 1. Suzuki coupling of the boronate **8** with various dihalogenated arenes (1,4-diiodobenzene, 2,5-dibromopyridine, 1,3-dibromobenzene, 2,5-dibromothiophene, or 4,4′-dibromobiphenyl) resulted in the formation of tricyclic and tetracyclic linked compounds **9a–e** in excellent yields. After Boc deprotection with HCl and coupling with *N*-methoxycarbonyl-L-valine, compounds **11a–e** were obtained in fair overall yields (Scheme 2). Tricyclic analog **13** (Table 1) with the pyridine ring shifted to the terminal core position relative to **11b** was prepared by the sequence outlined in Scheme 2 utilizing boronate **7d** with pyridyl bromide **12** (Fig. 2) in place of the dibromide starting at step b.[11]



Scheme 2. Synthesis of **11a–e**. Reagents and conditions: (a) $Pd(PPh_3)_4$, bis(pinacolato) diboron, KOAc, 1,4-dioxane, 80 °C, 16 h, 84%; (b) $Pd(PPh_3)_4$, $NaHCO_3$, 1,2-dimethoxyethane, H_2O, dihalide (1,4-diiodobenzene, 2,5-dibromopyridine, 1,3-dibromobenzene, 2,5-dibromothiophene, or 4,4′-diiodobiphenyl), 80 °C, 14 h, 80–99%; (c) 6 N HCl, MeOH, 50 °C, 4 h, 86–95%; (d) HOBt, EDAC, *N*-(methoxycarbonyl)-L-valine, MeCN, DIPEA, rt, 14 h, 28–59%.

As shown in Scheme 3, the triazolodiphenyl linked compound **17** was prepared from azido **7c** and acetylene **14** by using Cu(I)-catalyzed Huisgen azide–alkyne 1,3-dipolar cycloaddition (CuAAC).[12] The iodo derivative **7b**, prepared from the corresponding bromoketone **6b** (Fig. 2), was reacted with trimethylsilylacetylene under Sonogashira coupling conditions[13] then desilylated under basic conditions to give the acetylene derivative **14** (Scheme 3). The cycloaddition reaction of azido compound **7c** (prepared analogously to Scheme 1) and acetylene **14** resulted in the formation of triazole intermediate **15** in 67% yield. After *N*-Boc deprotection with 6 N HCl and coupling with *N*-methoxycarbonyl-L-valine, the triazolo-diphenyl compound **17** was obtained in good overall yield (Scheme 3). The related triazole **18** (Table 1) was prepared by Scheme 3 and started from the Boc'ed azide **7c** and involved Boc removal (70%) and acylation with *N*-(methoxycarbonyl)-L-valine (95%). A CuAAC reaction of 1,4-diethynylbenzene with the resulting azide gave **18** in a 40% isolated yield.

All synthesized NS5A inhibitors were evaluated for inhibition of HCV RNA replication in Huh7 cells containing a subgenomic HCV 1b replicon. Cytotoxicity in Huh7 cells was determined simultaneously with anti-HCV activity by extraction and amplification of both HCV RNA and ribosomal RNA (rRNA).[14] Cytotoxicity was determined in peripheral blood mononuclear (PBM), CEM (a human-T-cell-derived cell line), and Vero (kidney epithelial cells from the African green monkey) cells.[15] The results are summarized in Table 1.

First, it is noteworthy that compound **5a** which possess only one phenyl ring as a linker showed a complete loss of cytotoxicity, but also significantly less potency against HCV replication with a EC_{50} >1 nM. This suggested that the length of the linker could have a marked effect on both anti-HCV activity as well as cytotoxicity. Furthermore, increase of the linker's size to three straight aromatic rings (compounds **11a** and **11b**) did not severely impact the anti-HCV activity, while for compound **11a**, no cytotoxicity was observed up to 100 μM in PBM, CEM and Vero cells. It is interesting that **13** which has the pyridine ring shifted to the outside aryl ring of the core has similar cytotoxicity but 10-fold less anti-HCV activity when compared to its similar pyridyl analog **11b**. However, the four straight aromatic ring (**11e**) seems to be problematic with a 37-fold loss of activity at the EC_{50} level when compared to the biphenyl derivative BMS-790052.

Introduction of torsion angle by incorporation of an oxygen atom (**5b**), a sulfonamide (**5c**), a 1,3-substituted phenyl (**11c**), a 1,3-triazole (**17**) or a thiophene ring (**11d**) between the two phenyl ring of the linker led to a substantial decrease of activity (16- to 15,000-fold). With a 2-phenylthiophene core (**5d**) a torsion angle was also introduced and accompanied by an almost complete loss of anti-HCV activity. Compound **18** which contains five aromatic rings in its core was less potent but gave a similar profile to its shorter triazole analog **17** but with significantly reduced cytotoxicity.

From this SAR study we can conclude that: (i) the length of the straight central core linkage is optimal at 2–3 phenyl or pyridyl

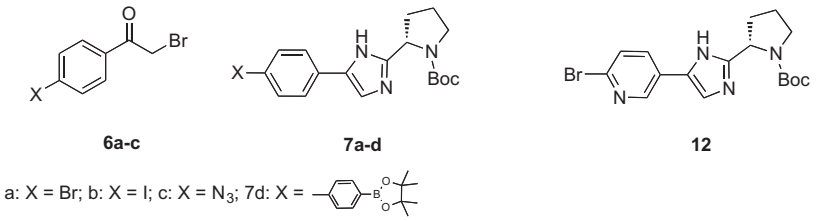
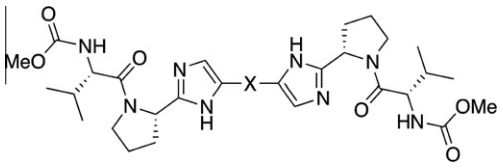
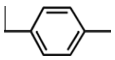
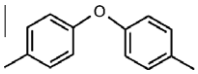
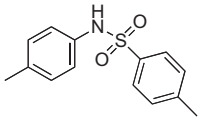
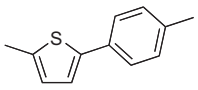
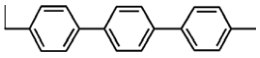
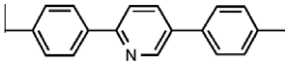
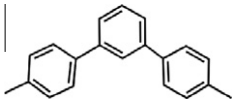
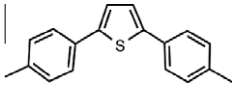
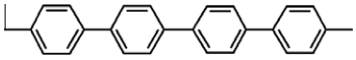
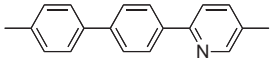
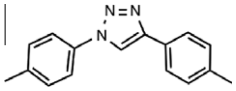
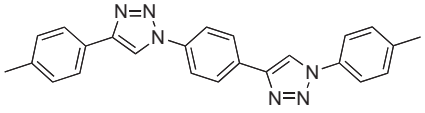
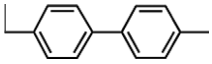


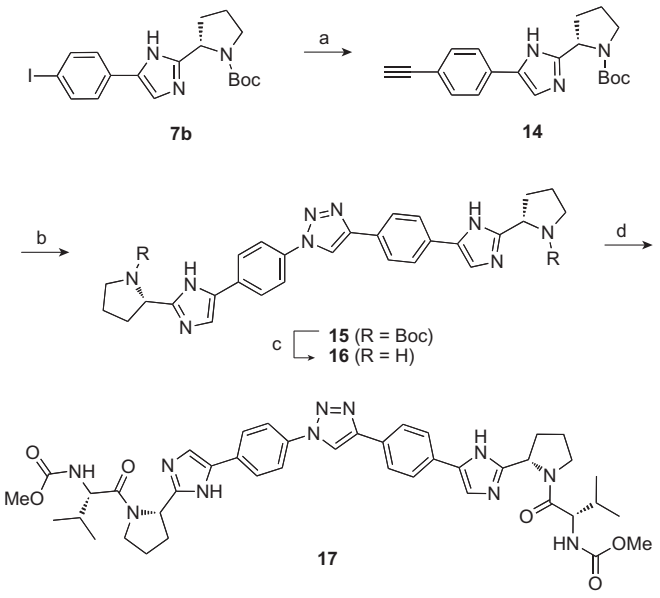
Figure 2. Chemical structures for **6a–c**, **7a–d** and **12**.

Table 1
In vitro anti-HCV activity and cytotoxicity data for compounds **5a–d**, **11a–e**, **13**, **17** and **18**



Compd	X	Anti-HCV[a] (pM)		Cytotoxicity CC_{50} (μM)		
		EC_{50}	EC_{90}	PBM	CEM	Vero
5a		>1000	>1000	>100	>100	>100
5b		30,000	90,000	16	16	19
5c		100,000	>100,000	15	13	>100
5d		90,600	>100,000	93	48	99
11a		26	90	>100	>100	>100
11b		26	68	3.9	2.3	6.5
11c		800	2900	18	3.0	>100
11d		100	300	26	12	>100
11e		240	1000	>100	>100	>100
13		200	700	5.5	31	29
17		200	900	18	5.0	35
18		600	1633	46	>100	>100
BMS-790052		6.6	23	19	9.6	21

[a] All dose–response determinations were calculated from at least four concentrations. All concentrations were run in triplicate and reported as mean values.



Scheme 3. Synthesis of triazole **17**. Reagents and conditions: (a) (i) trimethylsilylacetylene, CuI, $Pd(PPh_3)_4$, Et_3N, THF, rt, 2 h; (ii) K_2CO_3, THF, MeOH, rt, 3 h, 72%; (b) **7c**, $CuSO_4$, sodium ascorbate, *t*-BuOH, H_2O, 95–100 °C, 14 h, 67%; (c) 6 N HCl, MeOH, 50 °C, 4 h, 85%; (d) HOBt, EDAC, *N*-(methoxycarbonyl)-L-valine, MeCN, DIPEA, rt, 14 h, 80%.

rings (**11a**, **11b** and BMS-790052), shorter (**5a**) or longer (**11e**) than this range caused markedly reduced antiviral activity; (ii) for the three aryl core linkage, pyridyl is tolerated as the middle aryl ring (**11b**) while a ten fold loss of anti-HCV activity is observed with pyridyl at the outer aryl position (**13**); (iii) straight linked compounds (**11a**, **11b** and BMS-790052) have a superior anti-HCV effect relative to the non-linear linked compounds (**5b**–**d**, **11c**, **11d**, **17** and **18**); and (iv) for the three aryl core linkage, the linkage composed completely of phenyl rings rather than containing a pyridyl ring results in an improved cytotoxicity profile (**11a** vs **11b** and **13**).

In summary, 12 HCV inhibitors with different lengths and angles at the central aromatic linkage were synthesized and evaluated for anti-HCV activity and cytotoxicity in cell based assays. This study revealed that the straight tricyclic aromatic linked compounds **11a** and **11b** retain potent anti-HCV activity in the low picomolar range and, in the case of compound **11a**, have significantly less cytotoxicity in vitro versus BMS-790052, with a therapeutic index CC_{50}/EC_{50} over four million. Thus, this compound warrants further preclinical investigation as a potential anti-HCV agent.

Acknowledgments

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11. Scheme 2 yields for the synthesis of **13** step: b 59%; c ~100%; d 45%.
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