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Design and synthesis of imidazole N–H substituted amide prodrugs as inhibitors of hepatitis C virus replication

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ABSTRACT

Twenty-five novel imidazole N–H substituted **Daclatasvir** (**BMS-790052**, **DCV**) analogues (**8a–8y**) were designed and synthesized as potential prodrugs. Structure modifications were performed in order to improve potency and pharmacokinetic (PK) properties. All target compounds were evaluated in a hepatitis C virus (HCV) genotype 1b replicon, and the 2-oxoethyl acetate substituted compound **8t** showed similar anti-HCV activity (EC_{50} = 0.08 nM) to that of the lead compound **Daclatasvir**. Moreover, the utility of prodrug **8t** was demonstrated through similar exposure of the parent compound when the prodrugs were dosed in vivo. PK studies showed that prodrug **8t** was an ideal candidate for a slower and sustained release form of **Daclatasvir**.

Hepatitis C virus (HCV) infection is a global epidemic with an associated high risk for serious liver disease. An estimated 200 million people have been infected by HCV worldwide.[1] HCV usually progresses to a chronic state that persists for decades, but eventually becomes responsible for the development of chronic liver diseases, such as liver cirrhosis and hepatocellular carcinoma.[2,3] The traditional standard of care for HCV infection through treatment with pegylated-interferon and ribavirin has significant toxicity, and its sustained virological response rate is less than 50% in those infected by the genotype-1 (GT-1) virus.[4,5]

HCV was identified as a positive strand RNA virus with highly genetically diverse species that were classified into six genotypes (1–6) with more than 90 different subtypes.[6] The viral proteins can be divided into structural and nonstructural (NS) precursor regions. Previous studies have reported that the NS proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B are important for the replication of HCV virus; thus, the development of direct-acting antiviral agents against HCV infection has focused predominantly on inhibitors of the viral NS proteins.[7,8] Recently, several NS3/4A, NS5A, and NS5B inhibitors have been approved for the treatment of HCV GT-1 infection and have attracted considerable interest.

The NS5A protein was speculated to play a critical role in the replication of HCV RNA, the modulation of the host cell responses, and assembly of viral particles.[9–11] Several NS5A inhibitors exhibit robust potency profiles in HCV replicon assays in vitro and suppression of HCV replication in clinical trials (Fig. 1). This family of compounds includes some of the most active antiviral compounds known, with low picomolar median effective concentrations (EC_{50}) in HCV replicon assays.

Daclatasvir (**BMS-790052**, **DCV**) is an oral, once-daily, highly selective NS5A inhibitor developed by Bristol–Myers Squibb. As the first-in-class inhibitor targeting NS5A, the EC_{50} values of **DCV** are 9 and 28 pM against GT-1b and GT-2a replicons, respectively.[12] **DCV** is active against multiple HCV genotypes. Lemm et al. reported that **DCV** shows the highest in vitro potency among all known anti-HCV compounds, with a picomolar range of EC_{50} values against HCV replicons from various genotypes. In addition, **DCV** has been marketed and launched successfully to treat GT-1 HCV-infected patients.[13]

Preparation of a prodrug is an efficient approach to enhance the biopharmaceutical, physicochemical, or pharmacokinetic (PK) properties of pharmacologically potent compounds, thereby improving the development and utility of a potential drug.[14] Previous reports on **DCV** have demonstrated that the imidazole N–H is essential to the anti-HCV activity, as internal hydrogen bonding interactions may exist between the valine C=O and imidazole N–H on both sides of the dimer to control the configurations of **DCV**.[15] Amides and esters are two of the most frequently used moieties in designing small-molecule prodrugs because hydrolases

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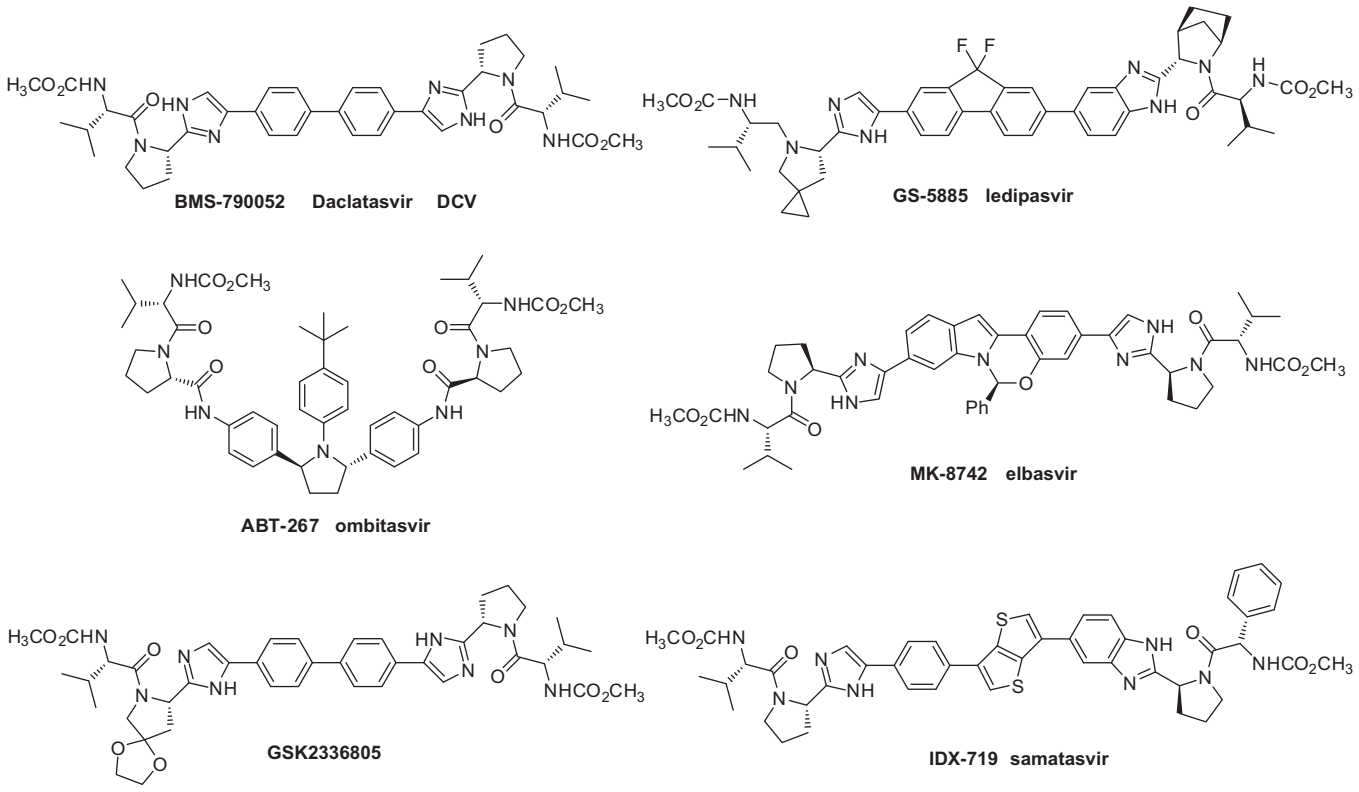


Figure 1. Chemical structure of NS5A inhibitors.

are abundant in the body.[16] To discover novel prodrugs of NS5A inhibitors with improved cellular potency, PK properties, and minimized side effects, we designed and synthesized 25 prodrugs of **DCV** (Fig. 2) and tested their anti-HCV activities.

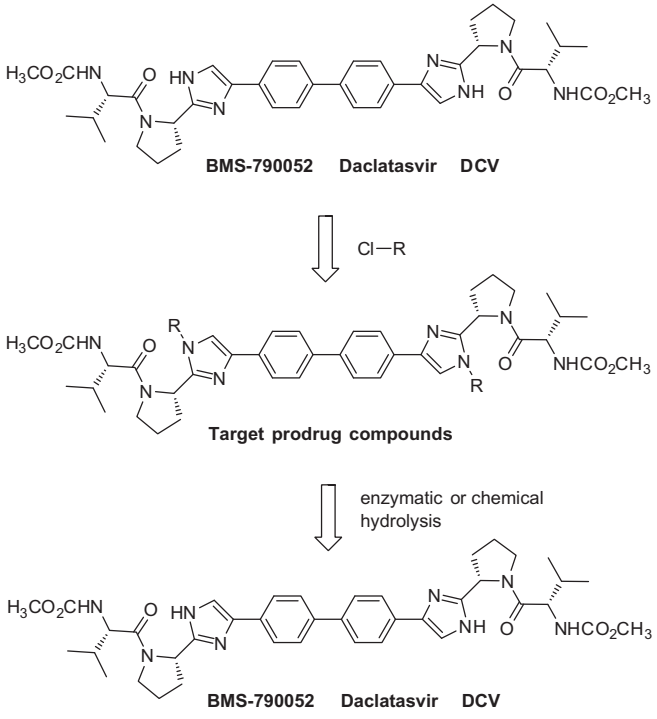
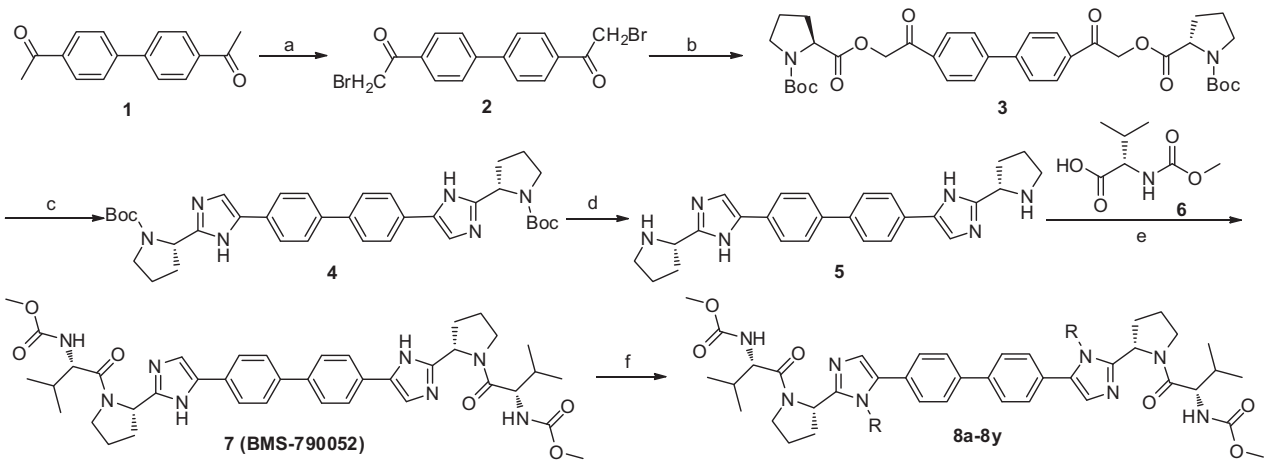


Figure 2. A design for DCV prodrugs.

The symmetrical structure of the target compounds was synthesized according to general Scheme 1. 1,1′-([1,1′-Biphenyl]-4,4′-diyl)diethanone **1** was first converted to 1,1′-([1,1′-biphenyl]-4,4′-diyl)bis(2-bromoethanone) **2** with Br_2 in CH_2Cl_2, which was then coupled with *N*-Boc protected proline in the presence of *N*,*N*-diisopropylethylamine (DIPEA). Refluxing the compound **3** with ammonium acetate in toluene formed the imidazole ring and led to the versatile Boc-protected intermediate **4**. After the removal of Boc protection with 6 N HCl, the resulting amine was coupled with *N*-(methoxycarbonyl)-L-valine **6** by using *o*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate in the presence of DIPEA to provide **7** (**DCV**). Acylation of **7** with corresponding acyl chlorides gave **8a**–**8y** in fair to excellent overall yields. All the target compounds were new chemical entities and are shown in Table 1.

All synthesized **DCV** prodrugs were evaluated for inhibition of HCV RNA replication in Huh7 cells containing a subgenomic HCV 1b replicon. When the R group was straight chain alkyl acyl, compounds showed moderate anti-HCV activities, whereas longer alkyl chains resulted in lowered potency, for example, **8a** (EC_{50} = 12 nM) > **8b** (EC_{50} = 39 nM) > **8c** (EC_{50} = 85 nM) > **8g** (EC_{50} = 231 nM) > **8h** (EC_{50} = 324 nM) > **8i** (EC_{50} = 542 nM). When the acyl alkyl terminal was tertiary butyl (such as **8e**, **8f**, and **8j**), anti-HCV activity was completely lost. When the R group was acly annular alkane, ring extension (**8l**) or contraction (**8m**) resulted in a slight decrease in activity when compared with **8k**. Furthermore, some carbamate prodrugs of **DCV** (**8n**–**8s**) were also synthesized. Surprisingly, the carbamate prodrugs had no activity against HCV, except compound **8t**, which demonstrated anti-HCV activity with an EC_{50} of 0.08 nM, comparable to the control compound **DCV** (EC_{50} = 0.009 nM). To discover the prodrug with improved PK properties, we also designed aromatic prodrugs of **DCV** (**8u**–**8y**). Almost all of them possessed anti-HCV activity with EC_{50} values ranging from 77 to 988 nM.



Scheme 1. Synthetic route for the preparation of the target compounds **8a**–**8y**. Reagents and conditions: (a) Br_2, CH_2Cl_2, rt, overnight, 86%; (b) *N*-Boc-L-proline, MeCN, Et_3N, rt, 2 h, 98%; (c) NH_4OAc, toulene, 130 °C, 15 h, 85%; (d) 6 N HCl, MeOH, 50 °C, 4 h, 87%; (e) HATU, *N*-(methoxycarbonyl)-L-valine, DIPEA, rt, 14 h, 83%; (f) RCOCl, TEA, CH_2Cl_2, rt, 3 h, 64–87%.

Table 1
Chemical structure and anti-HCV activity of the target compounds **8a**–**8y**

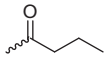
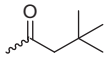
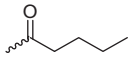
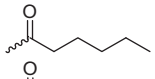
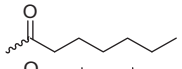
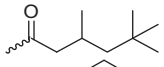
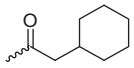
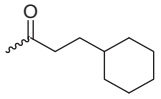
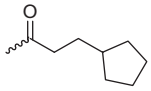
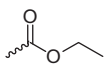
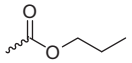
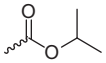
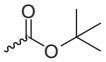
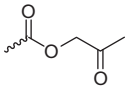
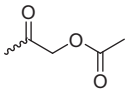
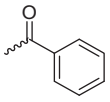
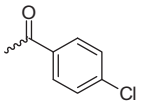
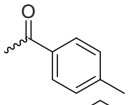
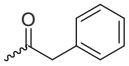
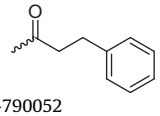
Compound	R	HCV replicon[a] EC_{50}(nM)
8a		12
8b		39
8c		85
8d		131
8e		>100,000
8f		>100,000
8g		231
8h		324
8i		542
8j		>100,000
8k		110
8l		124
8m		195
8n		>100,000
8o		>100,000
8p		>100,000

Table 1 (*continued*)

Compound	R	HCV replicon[a] EC_{50}(nM)
8q		>100,000
8r		>100,000
8s		>100,000
8t		0.08
8u		155
8v		77
8w		231
8x		687
8y		988
	BMS-790052	0.009

[a] Data represent mean values of at least two experiments.

Table 2
Conversion of Ester Prodrug **8t** to Parent DCV in vitro

Compound	pH 2	pH 7	Blood
DCV	Stable	Stable	Stable
8t	Stable	Stable	Unstable[a]

[a] $t_{1/2}$ < 30 min.

Table 3
Pharmacokinetic parameters[a]

Compound administered	Compound measured	C_{max} (ug/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{(0-8)}$ (ug * h/mL)	$AUC_{(0-\infty)}$ (ug * h/mL)	MRT (h)
DCV	DCV	0.484 ± 0.163	3.01 ± 0.23	1.66 ± 0.24	3.21 ± 0.88	3.32 ± 0.98	3.43 ± 0.51
8t	DCV	0.341 ± 0.112	4.05 ± 0.21	2.93 ± 0.31	3.33 ± 0.93	3.44 ± 0.89	4.01 ± 0.22

[a] Each value represents the mean ± SD of 6 rats.

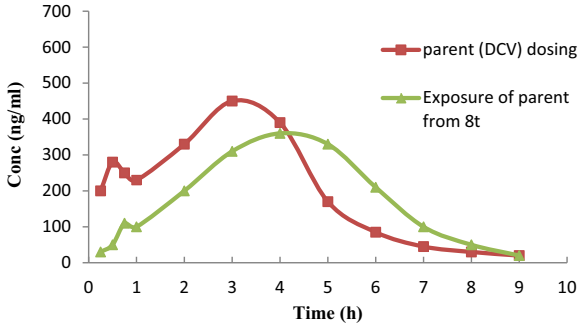


Figure 3. Comparison of in vivo exposure of parent (**DCV**) when rats are dosed with parent (red) or prodrug (**8t**, green) at 5 mg/kg parent or parent equivalent.

Compounds with electron-withdrawing group at the para position of benzene ring exhibited better potency than those with electron-donating group, for example, **8v** (IC_{50} = 77 nM) > **8u** (IC_{50} = 155 nM) > **8w** (IC_{50} = 231 nM). Among the 25 **DCV** derivatives tested in HCV replicon cells, 16 compounds showed modest anti-HCV activity. Moreover, the 2-oxoethyl acetate compound **8t** seemed to be the most effective.

Previous reports have demonstrated that the imidazole N–H is essential to the anti-HCV activity,[15] the substituent at the N would greatly decrease the activity. The synthesized prodrugs were hydrolyzed to release the corresponding free **DCV** that would then exert their anti-HCV activity (Fig. 2). We speculated that the most potent anti-HCV activity achieved by **8t** was due to its high-efficiency hydrolysis to the corresponding parent **DCV**. In the stability study, **8t** was initially stable in both acidic (pH 2/water) and neutral (pH 7.0/water) conditions (Table 2). Therefore, considering anti-HCV activity and hydrolysis stability, the ester prodrug **8t** was selected for further PK studies to observe whether it is a good candidate for a slower and sustained release form of **DCV**.

The selected compound **8t** was evaluated in a rat PK study. As shown in Figure 3, the systemic exposure of **8t** was converted to **DCV** after low oral doses (5 mg/kg). The drug exposure of **8t** was similar to that of the parent (**DCV**) itself at equivalent dose levels. Although plasma concentrations of the **DCV** released from **8t** were lower than those of **DCV** during the first 4.1 h, they became higher than that observed with **DCV** dosing and remained higher throughout the remainder of the experiment. Table 3 summarizes the PK parameters related to these curves. The areas under the curve from time 0 to 8 h ($AUC_{(0-8)}$) obtained from both curves were very similar. However, the PK behavior of the drug released from the **8t** and the **DCV** itself was different. This result suggests that the prodrug did not change the bioavailability of **DCV**, but was well-absorbed and efficiently converted to **DCV** in vivo, prolonging its activity. Therefore, **DCV** can be released from **8t** in mice, and **8t** and **DCV** have similar anti-HCV activities, demonstrating that **8t** can serve as a prodrug of **DCV**.

In the present study, we efficiently synthesized a series of prodrugs of **DCV** and evaluated their anti-HCV activity. Several target compounds exhibited modest anti-HCV activity. Among the tested molecules, compound **8t** demonstrated potent inhibition of HCV replication in a GT-1b replicon with EC_{50} = 80 pM. The utility of prodrug **8t** was demonstrated through similar exposure of the parent compound when the prodrugs were dosed in vivo. Our PK studies of **8t** demonstrated that this prodrug is an excellent candidate for a slower and sustained release form of **DCV**. Finally, further in vivo studies and structure optimizations are currently being investigated in our laboratory and will be the subject of future 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.2015.06.006.

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