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Research paper

Novel benzidine and diaminofluorene prolinamide derivatives as potent hepatitis C virus NS5A inhibitors

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ABSTRACT

Our study describes the discovery of a series of highly potent hepatitis C virus (HCV) NS5A inhibitors based on symmetrical prolinamide derivatives of benzidine and diaminofluorene. Through modification of benzidine, L-proline, and diaminofluorene derivatives, we developed novel inhibitor structures, which allowed us to establish a library of potent HCV NS5A inhibitors. After optimizing the benzidine prolinamide backbone, we identified inhibitors embedding meta-substituted benzidine core structures that exhibited the most potent anti-HCV activities. Furthermore, through a battery of studies including hERG ligand binding assay, CYP_{450} binding assay, rat plasma stability test, human liver microsomal stability test, and pharmacokinetic studies, the identified compounds **24**, **26**, **27**, **42**, and **43** are found to be nontoxic, and are expected to be effective therapeutic anti-HCV agents.

1. Introduction

Hepatitis C virus (HCV) infection is reported in almost 170 million individuals worldwide (~2.4% of the population), including approximately 5 million people in the United States with additional 3 to 4 million global population every year [1–5]. It has been estimated that around 70–80% of those infested with HCV will progress to chronic hepatitis, which, if left untreated, may result in liver cirrhosis and eventually liver cancer, with ultimately lethal consequences (1–5%) [6,7]. It is well accepted that the HCV infection is one of the main reasons for liver transplantation in patients [8]. Although vaccines are available for infection with other widespread liver viruses such as hepatitis A virus (HAV) and hepatitis B virus (HBV), no vaccines are available for HCV infection [8]. Recently, telaprevir (VX-950, Vertex Pharmaceuticals and Johnson & Johnson) and boceprevir (Victrelis, Merck) were approved as anti-HCV NS3-4A protease inhibitors by the US Food and Drug Administration (FDA). The traditional therapy for patients with HCV infection consists of oral doses of ribavirin (RBV) in combination with subcutaneous injections of PEG–IFN–α and protease inhibitor for a total duration of 24–28 weeks [9–11]. However, this interferon-centered therapy not only has serious side effects, including anemia, rash and depression, but is also associated with a restricted sustained virologic response (SVR), notably in those infected with HCV genotype 1 (G-1) [12,13]. In G-1-infected patients, favorable results have been reported after the addition of

Abbreviations: HCV, hepatitis C virus; Peg-IFNα, pegylated interferon α; SVR, sustained virological response; GT1a, hepatitis C virus genotype 1a, a subtype of hepatitis C virus genotype 1; GT1b, hepatitis C virus genotype 1b, a subtype of hepatitis C virus genotype 1; DAA, direct-acting antiviral; NS5A, hepatitis C virus nonstructural protein 5A; pM, picomolar; RNA, ribonucleic acid; Boc, *tert*-butoxycarbonyl; TFA, trifluoroacetic acid; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; NFSI, N-fluorobenzenesulfonimide; LiHMDS, lithium hexamethyldisilazide; EC_{50}, concentration at which inhibition is half-maximal; μM, micromolar; nM, nanomolar; SAR, structure–activity relationship; hERG, the human ether-a -go-go-related gene; CYP_{450}, cytochrome P450; PK, pharmacokinetics; HPBCD, (2-hydroxypropyl)-β-cyclodextrin; IV, intravenous; p.o., orally; WSTs, water-soluble tetrazolium salts; DNA, deoxyribonucleic acid.

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either boceprevir or telaprevir to the standard of care [14]. Although promising, advantageous outcome is expected from the launching of direct acting antivirals (DAAs) into the therapeutic regimen, their conceivable limitations include a limited genetic barrier, which may be attributable to drug-resistant mutants developed through long term medical care [10,15–20]. Therefore, the discovery of safe and effective antiviral candidates aimed at diverse HCV gene targets is greatly needed [21–28].

A polyprotein of almost 3200 amino acids is encoded from the HCV genome, which contains three structural proteins (E1, E2, and core) and six nonstructural proteins (NS2, NS3, NS4A-4B, and NS5A-5B) [29–32]. Among the nonstructural proteins, NS5A has been shown to have a direct role in viral replication, virus assembly, virion production, virus persistence, and pathogenesis [33,34]. There are three domains in NS5A: domain 1 (37–213 residues), containing the zinc-binding shape essential for HCV RNA replication; domain 2 (250–342 residues), which cooperates with cellular proteins and NS5B; and domain 3 (356–447 residues), which has a role in infectious HCV assembly, however not HCV RNA replication [35–43].

In 2010, a landmark NS5A inhibitor, daclatasvir (**1**), was reported to present excellent anti-HCV activity, especially in patients with HCV G-1 infection. This new class of inhibitor was approved by the US FDA in 2014 [44–48]. The effective concentration (EC_{50}) value of daclatasvir was two-digit picomolar (pM) range in *in vitro* assay, and treatment with a single 100 mg dose in clinical trials reduced HCV RNA levels by an average of $3.3\log_{10}$ without apparent toxicity [49]. This result stimulated numerous research groups and pharmaceutical companies to focus on the development of new inhibitors targeting NS5A [50–59]. Currently, there are many candidate compounds in this series: ABT-267, ACH-2928, ACH-3102, AZD-7295, BMS-346, BMS-665, BMS-824393, EDP-239, GS-5885, GSK-2336805, IDX-719, MK-4882, MK-8742, PPI-461, and PPI-1301 (Fig. 1) [60–67]. Most recently, interferon-free multi-class drug combinations (daclatasvir and asunaprevier, Viekira Pak®, and Harvoni®) have been reported as the most optimal therapy [68]. The structure of daclatasvir is characterized by a central biaryl core unit linked to an imidazole and proline moiety, and lastly, a methyl carbamate L-valine moiety as a capping group (Fig. 2) [69]. In 2012, Schinazi et al. reported nascent NS5A inhibitors containing a part of biaryl core and some modifications on other parts through "Click" and C–C bond cross coupling reactions [70,71]. We recently developed a new class of NS5A inhibitors represented by BMK-20113, which has benzidine (I) and L-proline (II) connected as an amide functionality and a variety of capping groups (III) [72]. In this paper, we report improvements in antiviral potency through the introduction of new modifications to the backbone of BMK-20113: L-proline to other proline isosteres (area I in Fig. 2), and benzidine to substituted benzidine derivatives (area II in Fig. 2).

2. Chemistry

The symmetry in the benzidine-proline scaffold greatly streamlined our strategy of synthesizing BMK-20113. Initially, we wanted to evaluate the antiviral activity of the proline variation;

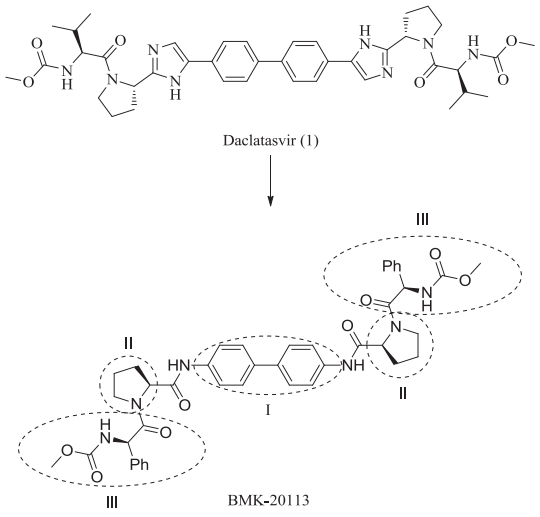

Fig. 2. Strategy for designing HCV NS5A inhibitors.

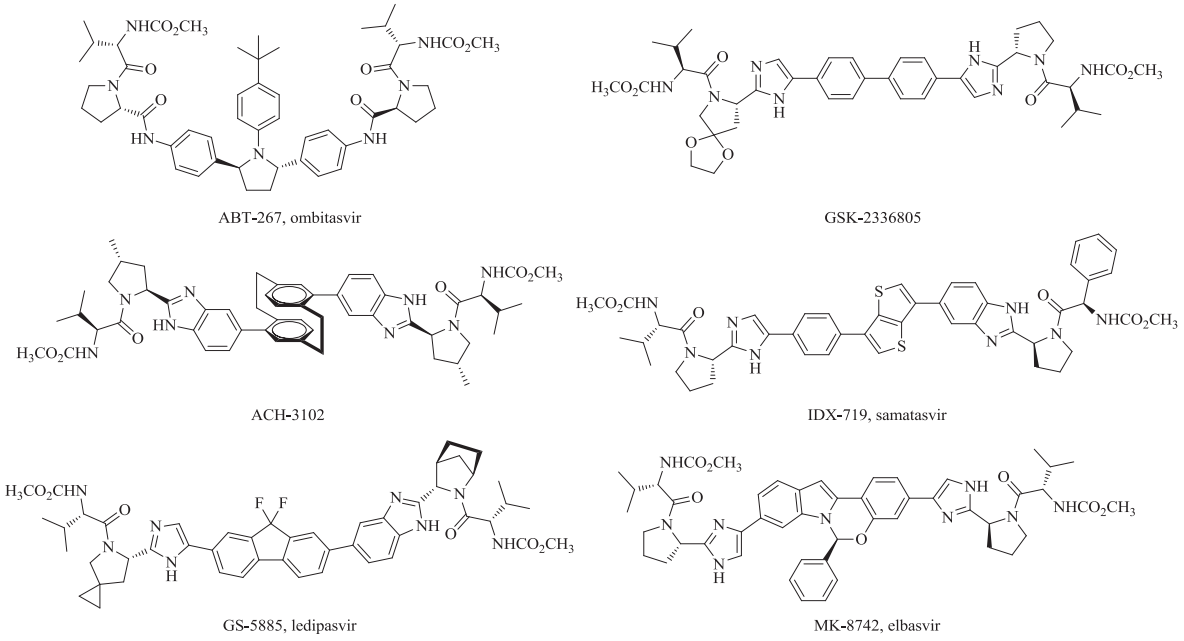

Fig. 1. Structure of NS5A inhibitors.