Synthesis and Anti-HCV Activity of a Novel 2',3'-Dideoxy-2'- α -fluoro-2'- β -C-methyl Guanosine Phosphoramidate ProdrugWenquan Yu,[†] Ertong Li,[†] Zhigang Lv,[†] Ke Liu,[†] Xiaohe Guo,[‡] Yuan Liu,[†] and Junbiao Chang^{*,†}[†]College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou, Henan 450001, P. R. China[‡]High & New Technology Research Center of Henan Academy of Sciences, Zhengzhou, Henan 450002, P. R. China

Supporting Information

ABSTRACT: A novel 2',3'-dideoxy-2'- α -fluoro-2'- β -C-methyl-6-methoxy guanosine (**8**) and its phosphoramidate prodrug (**1**) have been designed and synthesized. Their biological activity was evaluated in both cytotoxicity and cell-based HCV replicon assays. Neither compounds exhibited cytotoxicity up to the highest concentration tested (100 μ M) in the Huh-7 cell line. The prodrug (**1**) displayed nanomolar level antiviral activity (EC_{50} = 0.39–1.1 μ M) against the HCV genotype (GT) 1a, 1b, 2a, and 1b S282T replicons.

KEYWORDS: 2',3'-Dideoxy guanosine, 2'- α -fluoro-2'- β -C-methyl, phosphoramidate prodrug, anti-HCV activity, NSSB RdRp inhibitor

Hepatitis C virus (HCV), a member of the Flaviviridae family, is a positive-sense single-stranded RNA virus. HCV infection affects over 170 million people worldwide¹ and is a major cause of hepatocellular carcinoma (HCC). So far, a series of potential molecular targets have been identified for anti-HCV drug discovery, including NS2-NS3 autoprotease, the NS3 protease, the NS3 helicase, and the NSSB polymerase. The NSSB, one of nonstructural proteins serving as the HCV RNA-dependent RNA polymerase (RdRp), is essential for the replication of the viral RNA genome and has attracted significant interest among medicinal chemists.^{2–5} Nucleosides active against HCV are NSSB RdRp inhibitors, and they need to be converted to the corresponding triphosphates that can incorporate into viral RNA at the 3'-terminal so as to stop viral RNA elongation as chain terminators. Some nucleosides are weakly active because they cannot be efficiently phosphorylated by specific nucleoside kinases or are not substrates of the kinases at all. However, nucleoside phosphates (nucleotides) per se cannot be used as drugs very often because they are chemically less stable and/or too polar to enter the cells.^{6,7} In order to bypass the rate-limiting first-step phosphorylation as well as to improve the chemical stability and biological activity, various nucleoside phosphate prodrugs have been designed and synthesized.^{7–10} Recently, the phosphoramidate prodrug strategy has been demonstrated as an effective approach for intracellular delivery of the monophosphorylated nucleosides. The monophosphate can be further phosphorylated to di-, and then the biologically active triphosphate.

Among various antiviral nucleos(t)ide compounds, the 2'-C-methyl substituted analogues^{11–17} display potent anti-HCV activity in vitro, in vivo, and in clinic (Figure 1). These nucleotides act as nonobligate chain terminators of HCV RdRp as they all possess a 3'-OH moiety. 2',3'-Dideoxy nucleosides, such as Zidovudine and Emtricitabine, exhibit good antiviral

Figure 1. Representative 2'-C-methyl substituted nucleotide analogues with potent anti-HCV activity.

activity against HIV and HBV because they cannot support the elongation of the newly synthesized viral polynucleotide due to the lack of the 3'-hydroxy group. However, such analogues were rarely reported in the literature with good anti-HCV activity, a major reason for which could be *ribo*-nucleosides with 3'-OH may not be good substrates of phosphorylation kinases.¹⁸ Herein, we designed and synthesized such a novel 2',3'-dideoxy-2'- α -fluoro-2'- β -C-methyl nucleoside analogue (**8**) and its phosphoramidate prodrug (**1**) for the treatment of HCV infection.^{19,20}

The synthesis of the target 2',3'-dideoxy-2'- α -fluoro-2'- β -C-methyl-6-methoxy guanosine (**8**) is described in Scheme 1. The starting material **4** was readily obtained according to a previously

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Scheme 1. Synthesis of 2',3'-Dideoxy-2'- α -fluoro-2'- β -C-methyl-6-methoxy Guanosine 8^a

^aReagents and conditions: (a) TBDMSCl, imidazole, DMF, rt, 81%; (b) 1,1-thiocarbonyldiimidazole, MeCN, rt, 69%; (c) *n*Bu₃SnH, AIBN, toluene, 80 °C, 75%; (d) TBAF, THF, rt, 64%.

reported synthetic route.¹³ Selective protection of the 5'-OH group in nucleoside 4 with *tert*-butyldimethylsilyl chloride in the presence of imidazole resulted in compound 5, which was further converted into intermediate 6 by the treatment with 1,1-thiocarbonyldiimidazole in acetonitrile. Bu₃SnH/AIBN-mediated 3'-deoxylation of compound 6 and subsequent removal of the silyl protecting group with TBAF afforded the 2',3'-dideoxy guanosine 8.

To synthesize the phosphoramidate prodrug 1, the corresponding perfluorophenyl phosphoramidate (12) was prepared (Scheme 2). Treatment of phenyl dichlorophosphate (10) with

Scheme 2. Synthesis of the Phosphoramidate Prodrug 1^a

^aReagents and conditions: (a) PO(OPh)Cl₂ (10), NEt₃, CH₂Cl₂, -78 °C ~ rt; (b) pentafluorophenol, NEt₃, CH₂Cl₂, rt; then recrystallized in EtOAc/hexanes, 39% (over two steps); (c) guanosine 8, *t*BuMgCl, THF, rt, 83%.

L-alanyl cyclopentyl ester hydrochloride (9) and triethylamine at -78 °C followed by reaction of the resulting monochlorophosphate 11 with pentafluorophenol and triethylamine gave the S_p-cyclopentyl ester 12 after the recrystallization from a mixture of ethyl acetate and hexanes. Reaction of nucleoside 8 with compound 12 in the presence of *tert*-butyl magnesium chloride afforded the desired prodrug 1 as the pure S_p-enantiomer.

The biological evaluation was carried out in cytotoxicity and cell-based HCV replicon assays, respectively. As summarized in Table 1, neither guanosine 8 nor its phosphoramidate prodrug (1) exhibited cytotoxicity at the highest concentration tested (100 μ M) in Huh-7 cells. In the same cell line containing

Table 1. Cytotoxicity and Anti-HCV Activity of Guanosine 8 and Its Phosphoramidate Prodrug 1^a

compd	CC ₅₀ (μ M)	HCV 1b EC ₅₀ (μ M)	HCV 1a EC ₅₀ (μ M)	HCV 2a EC ₅₀ (μ M)	HCV 1b S282T EC ₅₀ (μ M)
8	>100	>100	ND	ND	ND
1	>100	0.58	1.1	0.39	0.81

^aEC₅₀: 50% effective concentration. CC₅₀: 50% cytotoxic concentration.

replicating HCV genotype (GT) 1b replicon, although the nucleoside 8 was inactive, its prodrug 1 inhibited the replication of the replicon with an EC₅₀ of 0.58 μ M. In light of these encouraging results, the antiviral activity of compound 1 was further profiled using different HCV replicon cells. This prodrug also displayed nanomolar antiviral activity (EC₅₀ = 0.81 μ M) against the HCV GT 1a, 2a, and 1b S282T replicons.

In summary, we have designed and synthesized a novel 2',3'-dideoxy-2'- α -fluoro-2'- β -C-methyl-6-methoxy guanosine (8) and its phosphoramidate prodrug (1). Neither compound is cytotoxic in the Huh-7 cell line at the highest tested concentration up to 100 μ M. Although guanosine 8 is inactive, the corresponding prodrug (1) displayed potent anti-HCV activity against the genotype (GT) 1a, 1b, 2a, and 1b S282T replicons with similar EC₅₀s (between 0.39 and 1.1 μ M). For the first time, we have discovered a 2',3'-dideoxy nucleotide analogue possessing potent antiviral activity against HCV infection. These encouraging results warrant further investigation toward the development of compound 1 as a potential anti-HCV agent.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00174.

Experimental procedures, characterization data, and biological assays (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

HCV, hepatitis C virus; HCC, hepatocellular carcinoma; RdRp, RNA-dependent RNA polymerase; TBDMSCl, *tert*-butyldimethylsilyl chloride; DMF, *N,N*-dimethylformamide; AIBN, azobisisobutyronitrile; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran; GT, genotype; EC₅₀, 50% effective concen-

tration; CC₅₀, 50% cytotoxic concentration; PE, petroleum ether; LTR, long terminal repeat; DMEM, Dulbecco's minimum essential medium; FBS, fetal bovine serum

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