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Synthesis of 9-(2-β-*C*-methyl-β-D-ribofuranosyl)-6-substituted purine derivatives as inhibitors of HCV RNA replication

Yili Ding,* Jean-Luc Girardet, Zhi Hong, Vicky C. H. Lai, Haoyun An,† Yung-hyo Koh, Stephanie Z. Shaw and Weidong Zhong

Valeant Pharmaceuticals International, 3300 Hyland Avenue Costa Mesa, CA 92626, USA

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Abstract—A series of 9-(2′-β-*C*-methyl-β-D-ribofuranosyl)-6-substituted purine derivatives were synthesized as potential inhibitors of HCV RNA replication. Their inhibitory activities in a cell based HCV replicon assay were reported. A prodrug approach was used to further improve the potency of these compounds by increasing the intracellular levels of 5′-monophosphate metabolites. These nucleotide prodrugs showed much improved inhibitory activities of HCV RNA replication.

Hepatitis C virus (HCV) infection is the leading cause of post-transfusion, non-A and non-B viral hepatitis.[1] About 170 million people worldwide are thought to be infected with this virus of which an estimated 4 million reside in the United States.[2] HCV infection causes significant liver diseases such as cirrhosis and may eventually lead to the development of hepatocellular carcinoma. Currently approved drugs for the treatment of hepatitis C are combinations of interferon-α and ribavirin, which result in a sustained virological response (SVR) in only ~50% of the treated patients and cause significant side effects in some cases.[3] Therefore, the development of more effective and HCV-specific antiviral agents is an urgent public health need.

Previous screening of available nucleoside collections in the HCV replicon assay has identified two nucleoside analogs, 2′-β-*C*-methyl adenosine and 2′-*O*-methyl cytidine, which inhibit HCV RNA replication without significant cytotoxicity.[4] Lower level of the intracellular 2′-*O*-methyl cytidine triphosphate was detected than that of the 2′-β-*C*-methyl adenosine triphosphate, suggesting that 2′-β-*C*-methyl adenosine was phosphorylated more efficient than 2′-*O*-methyl cytidine in the cells. Consistent with this, 2′-β-*C*-methyl adenosine showed a greater potency than 2′-*O*-methyl cytidine in inhibiting HCV RNA replication. However, 2′-β-*C*-methyl adenosine is a good substrate of adenosine deaminase, which has prevented this compound from further development. These interesting results prompted us to synthesize different types of 9-(2-β-*C*-methyl-β-D-ribofuranosyl)-6-substituted purine derivatives and evaluated their activities in the HCV subgenomic replicon assay.

In this letter, we report the synthesis of 9-(2-β-*C*-methyl-β-D-ribofuranosyl)-6-substituted purine derivatives including 6-aromatic substituted, sulfur substituted, *N*-substituted and *O*-substituted nucleosides. These compounds were tested as inhibitors of HCV RNA replication in a cell-based replicon assay.

Our synthetic strategy is summarized in Scheme 1. By using an established method,[5] we were able to obtain the key intermediate **2** from the coupling of 1,2,3,5-tetra-*O*-benzoyl 2-β-*C*-methyl-D-ribofuranose (**1**) and 6-chloropurine in the presence of TMSOTf. Starting from compound **2**, we were able to synthesize 9-(2-β-*C*-methyl-β-D-ribofuranosyl)-6-substituted purine derivatives.

Maintaining the amino group at position 6 of the purine base in the nucleoside is likely required for interaction with the viral polymerase via hydrogen bonding. Thus, compound **2** was reacted with 25 different nucleophilic amino derivatives in ethanol followed by deprotection

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* Corresponding author. Tel.: +1 714 5450100; fax: +1 714 6417222; e-mail: yding@icnpharm.com
† Present address: Acon laboratories, Inc., 4108 Sorrento Valley Boulevard, San Diego, CA 92121, USA.

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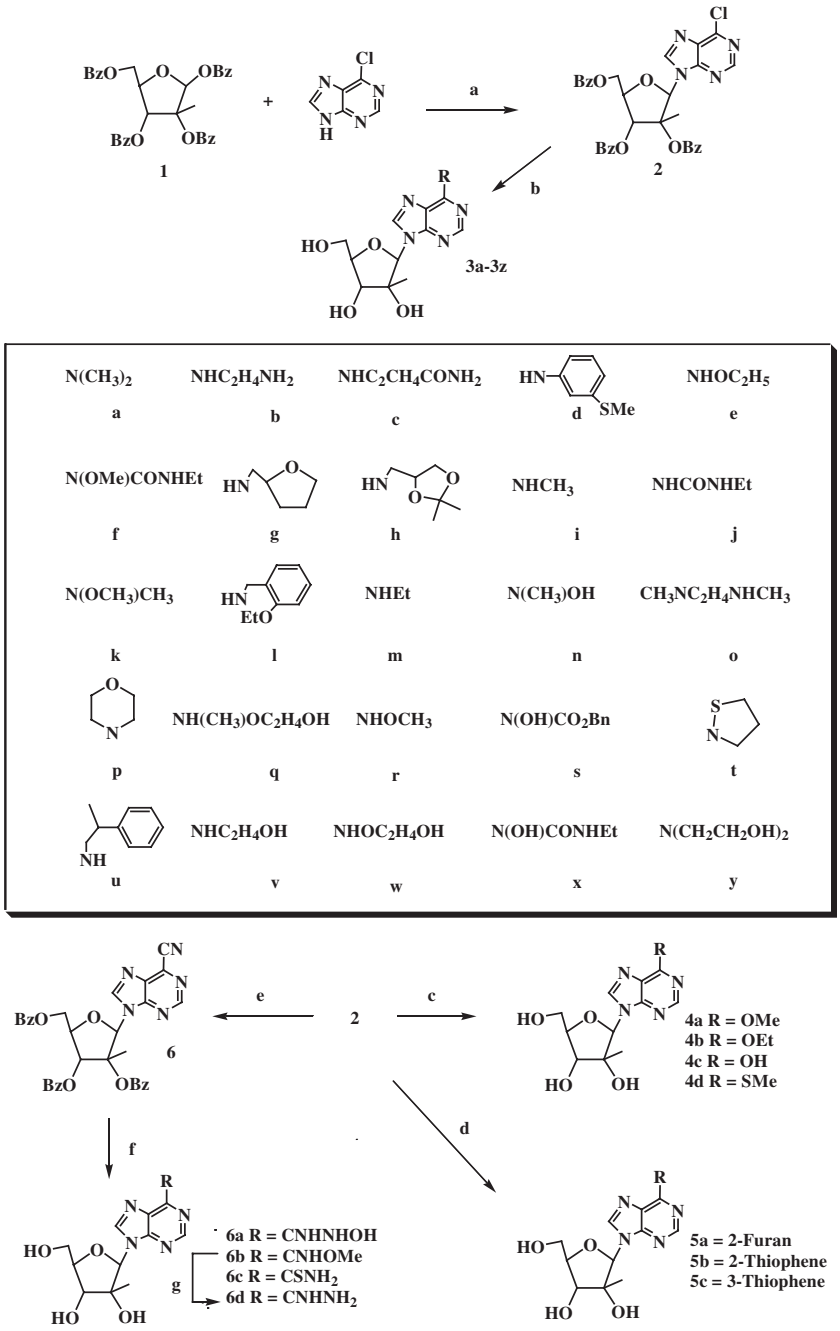


Scheme 1. (a) TMSOTf, BSA, CH_3CN, 60 °C, 3 h; (b) (i) amine, ethanol, 50 °C, 10 h; (ii) NH_3/MeOH or NaCN/MeOH, room temperature, 12 h; (c) NaOMe or NaOEt or NaOH or NaSMe, ethanol, 50 °C, 4 h; (d) (i) $Pd(PPh_3)_4$, K_2CO_3, toluene, $RB(OH)_2$, 100 °C, 12 h; (ii) NH_3/MeOH, room temperature, 10 h; (e) $Pd(PPh_3)_4$, $Zn(CN)_2$, toluene, 80 °C, 10 h; (f) (i) NH_2OH or NaCN/MeOH or H_2S; (ii) NH_3/MeOH, room temperature, 10 h; (g) NH_4Cl, MeOH, 70 °C, 15 h.

with NH_3/MeOH or NaCN/MeOH, to yield products **3a**–**y**. When aryl amines were used, the reactions were performed at 75 °C in ethanol for 12 h. In the reaction with 1,2-ethylene diamine, the disubstituted product **3z** was isolated as a minor product. Treatment of compound **2** with NaOMe, NaOEt, and NaOH in methanol at 50 °C for 4 h yielded compounds **4a**–**b** and 2′-β-*C*-methyl-β-D-ribofuranosyl hypoxanthine (**4c**), respectively, with concomitant deprotection.

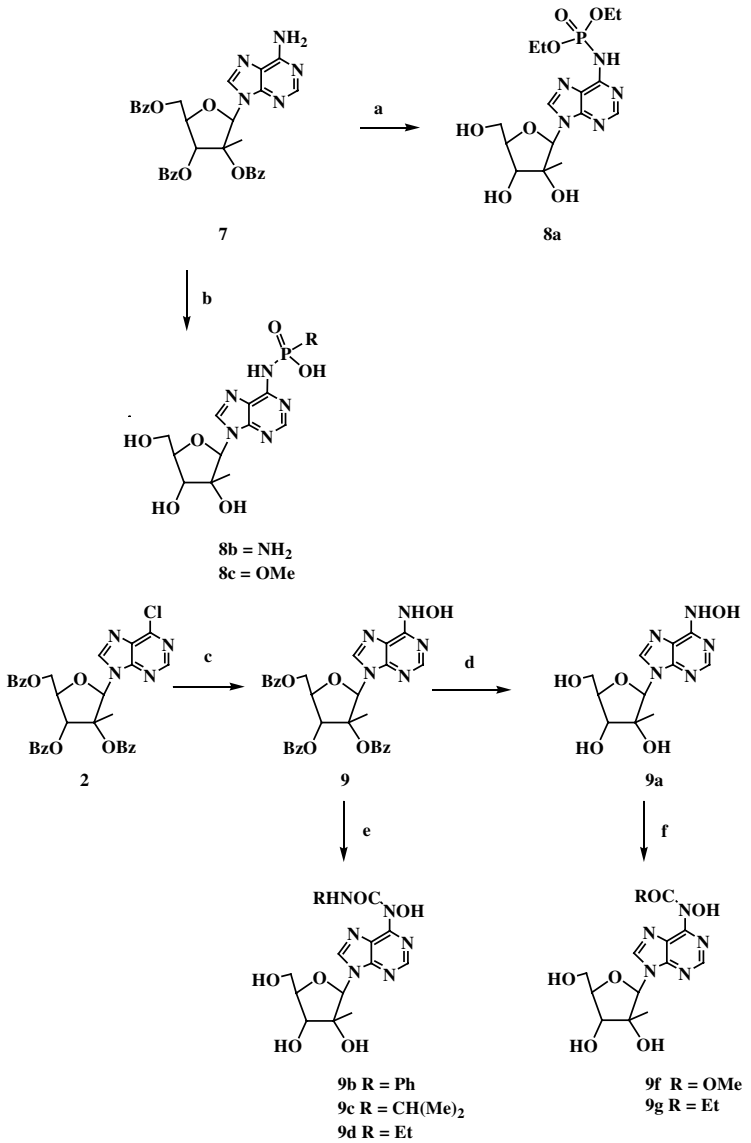
Replacement of the oxygen atom at the 6-position of guanine by a sulfur atom generated clinically widely used anticancer drugs thioguanine and 6-mercaptopurine.[6] Therefore, we decided to investigate this type of modification with our 2′-β-*C*-methyl nucleosides. Reaction of compound **2** with NaSMe at 50 °C produced 9-(2-β-*C*-methyl-β-D-ribofuranosyl)-6-thiomethoxyl purine (**4d**).

Recently, a class of *C*-6 aryl purine nucleosides has been shown to possess cytostatic activity toward T-lymphoblastoid, He–La, and L 1210 cells, as well as L929 cell lines.[7] In the light of this result, we prepared three 9-(2-β-*C*-methyl-β-D-ribofuranosyl) 6-aryl-purine deriva-

tives **5a**–**c**, through the Suzuki–Miyaura cross-coupling reaction[8] of compound **2** with three aromatic boronic acid derivatives, followed by deprotection with NH_3/MeOH.

The cyano group was then introduced at position 6 of the purine ring by reacting compound **2** with palladium tetrakis(triphenylphosphine) and zinc cyanide to yield compound **6**. Compound **6** was subsequently converted into 6-hydroxyaminoamidine, methoxylmino, thioamidine derivatives **6a**–**c** by reactions with NH_2OH/H_2O, NaCN/MeOH, and H_2S, respectively, followed by deprotection with NH_3/MeOH. The 6-amidine substituted purine derivative **6d** was obtained from the reaction of compound **6b** with NH_4Cl in methanol.

Several phosphoramidate and hydroxylamino derivatives of 6-*N*-substituted 2′-β-*C*-methyl adenosine were also prepared. The synthetic strategy is summarized in Scheme 2. Treatment of compound **2** with a 2 M solution of NH_3/MeOH at room temperature, gave the 2′,3′,5′-tri-*O*-benzoyl 2′-β-*C*-methyl adenosine (**7**), which was dissolved into dry pyridine and added to the mixture of 1,2,4-triazole, $POCl_3$, and triethylamine in acetonitrile. This mixture was stirred at room temperature for 1 h. When the reaction mixture was treated with ethanol, after work up and deprotection with NaCN/MeOH, diethyloxyl phosphoramidate derivative **8a** was obtained in 15% yield. When the reaction mixture was treated with triethylammonia carbonate, after work up and deprotection with NH_3/MeOH,



Scheme 2. (a) (i) $POCl_3$, 1,2,4-triazole, triethylamine, CH_3CN, 0 °C, 30 min; (ii) **7**, pyridine, room temperature; 1 h; (iii) ethanol, room temperature, 1 h; (iv) NaCN, MeOH, 15% overall yield; (b) (i) $POCl_3$, 1,2,4-triazole, triethylamine, CH_3CN, 0 °C, 30 min; (ii) **7**, pyridine, room temperature; 1 h; (iii) triethylammonia carbonate, room temperature, 1 h; (iv) NH_3/MeOH; (c) NH_2OH, room temperature, 10 h; (d) NaCN/MeOH, room temperature, 12 h; (e) (i) $(Me)_2CHNCO$ or EtNCO or PhNCO, $CHCl_3$, 40 °C, 10 h; (ii) NaCN/MeOH, room temperature, 12 h; (f) (i) TMSI, 1,2,4-triazole, pyridine, room temperature, 4 h; (ii) C_2H_5COCl or $CH(OMe)_3$, pyridine, room temperature, 10 h; (iii) NH_4OH.

2-hydroxyamino phosphoramidate derivative **8b** was isolated in 18% yield, and 2-hydroxymethoxyl phosphoramidate derivative **8c** was isolated in 5% yield.

Treatment of compound **2** with hydroxylamine at room temperature for 10 h produced compound **9**, and after deprotection with NaCN/MeOH, 6-*N*-hydroxylamine derivative **9a** was obtained. When compound **9** was treated with isopropyl isocyanate, ethyl isocyanate and phenyl isocyanate in $CHCl_3$ at 40 °C for 10 h, followed by deprotection with NaCN/MeOH and purification on silica gel column, only monosubstituted hydroxylamine derivatives **9b**,**c**, and **9d** were obtained. Treatment of compound **9a** with propionyl chloride and methyl formate in pyridine in the presence of 1,2,4-triazole and TMSI yielded compounds **9f** and **9g**.[9]

Compounds **3a–z**, **4a–d**, **5a–c**, **6a–d**, **8a–c**, and **9a–g** were assayed for their ability to inhibit HCV RNA replication in a subgenomic replicon cell line.[10] The potency of these compounds against HCV replicon is expressed as EC_{50}. The MTS assay was utilized to determine the associated cytotoxicity (CC_{50}).[11] Table 1 summarize the HCV replicon activity and cytotoxicity of these compounds. As shown, several of these 2′-*C*-methyl nucleosides showed low micromolar inhibitory activities in the HCV replicon assay.

To exert antiviral activity in cell culture, nucleoside analogs need to be phosphorylated intracellularly into corresponding triphosphates. These triphosphates are the active forms of the nucleoside inhibitors. The inhibitory potency of the nucleoside inhibitors should thus correlate with the levels of the triphosphates formed in the cells.

Therefore, the observed low activity for some of these compounds in HCV replicon assay may be due to their poor conversion to the active triphosphate forms by cellular kinases. Therefore, a prodrug approach that can deliver nucleoside monophosphates to the cells may overcome the rate-limiting nucleoside phosphorylation step and improve antiviral activity of the compounds.[12] As in most cases, monophosphates are usually converted efficiently into the corresponding triphosphates.

To demonstrate this concept with the 2′-β-*C*-methyl nucleoside derivatives, we chose compounds **4a** and **4b** as the starting materials to synthesize their monophosphate SATE-prodrugs. The synthetic strategy is shown in Scheme 3. By using an established method,[13] compounds **4a** and **4b** were coupled with the phosphoramidites **11a** and **11b** in the presence of tetrazole, followed by oxidation of the resulting phosphites with *t*-butyl hydroperoxide in DMF, to produce the 5′-monophosphate prodrugs **10a** and **10b**.[14]

Compounds **10a** and **10b** were assayed for their ability to inhibit HCV RNA replication in the replicon assay. As shown in Table 1, both of them showed a much improved potency (90- and 180-fold over **4a** and **4b**, respectively). This result clearly demonstrated that by bypassing the rate-limiting phosphorylation step, the activity of this class of nucleoside inhibitors in suppressing HCV RNA replication can be significantly enhanced.

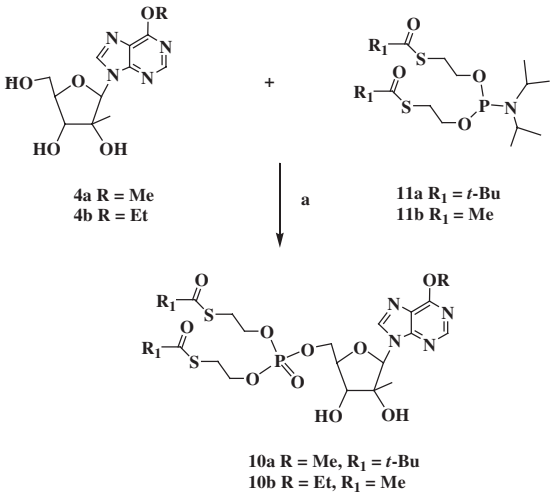
Table 1. Inhibitory potency and cytotoxicity of compounds **3a–z**, **4a–d**, **5a–c**, **6a–d**, **8a–c**, **9a–g**, and **10a–b** in HCV replicon assay

Compounds	EC_{50}, μM[a]	Cytotoxicity CC_{50}, μM[b]
3a	9	300
3b	25	300
3c	150	300
3d	300	300
3e	30	300
3f	80	300
3g	300	300
3h	300	300
3i	1	300
3j	80	300
3k	33	300
3l	50	250
3m	8	300
3n	4	300
3o	200	300
3p	100	300
3q	80	300
3r	4	300
3s	20	300
3t	15	200
3u	25	300
3v	60	300
3w	80	300
3x	200	300
3y	20	300
3z	80	300
4a	9	300
4b	13	300
4c	8	300
4d	11	300
5a	275	300
5b	80	200
5c	300	300
6a	100	300
6b	120	300
6c	120	300
6d	100	300
8a	25	300
8b	6	300
8c	33	300
9a	1.8	300
9b	1.8	300
9c	6	300
9d	2.2	200
9f	3	250
9g	3.3	300
10a	0.1	300
10b	0.08	300
2′-β-*C*-Methyl adenosine	0.3	300

[a] Compounds were incubated in cell culture for 48 h prior to determination of the relative amount of HCV replicon RNA using a luciferase reporter assay.

[b] Compound cytotoxicity was determined by MTS assay on parallel samples.

In summary, a series of 9-(2-β-*C*-methyl-β-D-ribofuranosyl)-6-substituted purine derivatives were synthe-



Scheme 3. (a) (i) Tetrazole; (ii) *t*-BuOOH, DMF.

sized and tested for their ability to inhibit HCV RNA replication in the replicon cells. Several of these adenosine analogs showed low micromolar activity in the replicon assay. In order to increase the intracellular levels of nucleoside triphosphates, which usually correlate with the antiviral potency of nucleoside inhibitors, two SATE-prodrugs of 5′-monophosphate nucleoside derivatives were synthesized. These prodrugs showed a much improved potency in inhibiting HCV RNA replication. This result indicated that the nucleotide prodrug approach could be applied to anti-HCV nucleosides, the same way as it was applied to anti-HIV and anti-HBV nucleosides.

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