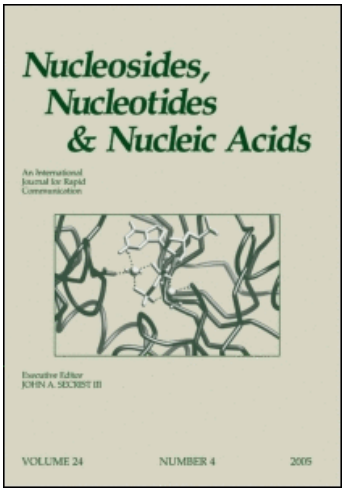

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Synthesis and Anti-Hepatitis C Virus Activity of Nucleoside Derivatives of *N* 3 ,5'-Anhydro-4-(β-d-Ribofuranosyl)-8-Azapurin-2-Ones

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SYNTHESIS AND ANTI-HEPATITIS C VIRUS ACTIVITY OF NUCLEOSIDE DERIVATIVES OF N^3,5′-ANHYDRO-4-(β-D-RIBOFURANOSYL)-8-AZA-PURIN-2-ONES

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▫ *A series of N^3,5′-Anhydro-4-(β-D-ribofuranosyl)-8-azapurin-2-ones were prepared in multistep reactions from uridine as potential anti-hepatitis C virus (HCV) agents. The synthetic details as well as biological evaluations are discussed.*

INTRODUCTION

Hepatitis C virus (HCV) has chronically infected an estimated 170 million people around the world. HCV infection is the major cause of chronic liver disease, often leading to hepatic failure and hepatocellular carcinoma.[1] The currently approved therapy for chronic hepatitis C is long term treatment with interferon-α (INF-α), either alone or in combination with ribavirin.[2] However, the low rate of response and the associated adverse effects of such treatments, along with the lack

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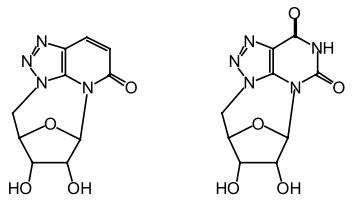


FIGURE 1

of preventive vaccine necessitates the search for new therapies.[2,3] During the course of our studies to find small molecules that selectively inhibit HCV, we discovered that N^9,5′-anhydro-4-(β-D-ribofuranosyl)-*v*-triazolo[4,5-*b*]pyridin-2-one (**1**) inhibits the production of HCV-RNA, in the HCV-subgenomic replicon cell line (Huh7 cells) EC_{50} = 19.7 μM and EC_{90} = 79.8 μM.[4,5] Using **1** as a lead, we synthesized the hypoxanthine analogue **2** (Figure 1) and several 8-azapurine derivatives (**7a–d, 10**) as a part of our efforts in search of superior agents. Four compounds (**2, 7a, 7b** and **10**) emerged from this investigation as more potent anti-HCV agents (Table 1). However, this increase in antiviral activity was accompanied by increased cytotoxicity.

RESULTS AND DISCUSSION

Chemistry

Uridine was converted to 5-bromo-2′,3′-*O*-isopropylidene-5′-*O*-mesyl derivative **3a**,[6] followed by NaN_3 treatment in DMF affording the 5′-azido-5′-deoxy derivative **3b**. Upon heating **3b** in DMF at 110–120°C for 30 h, the [2,3]-dipolar addition of the 5′-azido group to the 5,6-double bond occurred with concomitant elimination of HBr from the adduct resulting in the formation of 9,5′-cyclonucleoside **4** (Scheme 1).

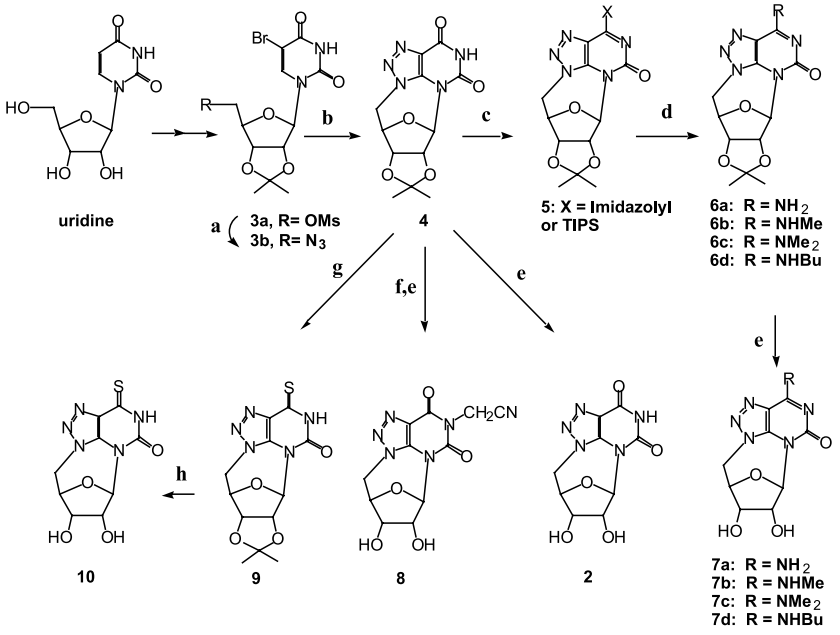
TABLE 1 Anti-HCV Activity of Synthesized Compounds in the Huh 7 Replicon Cells

Compounds	EC_{90} (μM)	CC_{50} (μM)
1	79.8	30.6
2	23.7	24.4
7a	10.9	11.0
7b	<6.25	6.7
7c	>100	>100
7d	>100	>100
8	>100	>100
10	43.2	32

Treatment of **4** with Ph_3P, imidazole, iPr_2NEt and I_2 in toluene at 95–100°C gave 6-imidazolyl derivative **5** in 81% yield. When **5** was treated with NH_4OH for 24 h at room temperature, the 8-aza-isoguanine derivative **6a** was obtained. Various 6-(substituted amino) derivatives **6b–d** were prepared directly from **4** by treatment with triisopropylbenzenesulfonyl chloride and 4-(dimethylamino)pyridine, followed by reaction with the corresponding amines. Compounds **6a–d** were deblocked to the diols (**7a–d**) by acid hydrolysis. Acid hydrolysis of **4** yielded the 8-aza-xanthine analogue **2**. Cyanomethylation of **4** with $BrCH_2CN$, NaH and LiBr in DMF, followed by acid hydrolysis afforded **8**. Thiation of **4** with Lawesson's reagent in $ClCH_2CH_2Cl$ at reflux gave **9**, which was hydrolyzed to give 9,5′-anhydro-(3-β-D-ribofuranosyl)-8-aza-6-thioxanthine **10**.

Anti-HCV Subgenomic Replicon Assay

HCV-replicon RNA-containing Huh7 cells (Clone A cells; Apath, LLC, St. Louis, MO) were kept in exponential growth in DMEM media (high glucose, no pyruvate) containing 10% fetal bovine serum, 1X non-essential amino acids, penicillin-streptomycin-glutamine (100 units/mL, 100 μg/mL, and 2.92 mg/L, respectively), and G418 (500 to 1000 μg/mL).[7] Antiviral assays were performed in the same media without G418. Cells were seeded in a 96-well plate at 1000 cells per well and test compounds were added immediately after seeding. At the end of



SCHEME 1 Reagent: (a) NaN_3/DMF, 80°C; (b) DMF, 110–120°C, 30 h; (c) Ph_3P/imidazole/iPr_2NEt/I_2/toluene; (d) Et_3N/DMAP/TIPSCl/amine; (e) 1NHCl/THF, 90°C; (f) 60% NaH/LiBr/$BrCH_2CN$/DMF; (g) Lawesson's reagent/DCE, reflux; (h) CF_3CO_2H/H_2O, 50–55°C.

the 4-day incubation, total cellular RNA was isolated (RNeasy 96 kit, Qiagen, CA). Replicon RNA and the internal control (TaqMan Ribosomal RNA control Reagents, Applied Biosystems, CA) were amplified in a single-step multiplex RT-PCR protocol, as recommended by the manufacturer. The HCV primers and probe were designed using the Primer Express software (Applied Biosystems, CA) and cover highly conserved 5′ untranslated region sequences. The results are summarized in Table 1.

CONCLUSIONS

It is apparent from the biological results that a substituent on C-6 appears to contribute to the toxicity of these compounds. Among C-6-amino substituent, the most active one was the methylamino analogue, but it was also toxic. It seems that the activity would be reduced as the size of substituent on the amino group grows larger. Cyanomethyl substitution on C-7 did not show significant anti-HCV activity. The 6-thio analogue exhibited moderate anti-HCV activity and toxicity.

When tested in cell-free systems, this class of compounds did not inhibit purified NS5B RNA-dependent RNA polymerase in vitro. Studies on the mechanism(s) of action of these and related compounds are underway. Since the mode of action this class of compounds is different from the known NS5B inhibitors, identification of compounds demonstrating a better anti-HCV activity to cytotoxicity profile would be of interest.

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