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Research paper

Design, synthesis, and biological evaluation of new N^4-Substituted 2′-deoxy-2′-fluoro-4′-azido cytidine derivatives as potent anti-HBV agents

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ABSTRACT

A series of new 2′-deoxy-2′-β-fluoro-4′-azido-β-D-arabinofuranosyl cytidine derivatives bearing heteroatom-containing N^4-substituents were designed and synthesized. Antiviral screening in HepG2.2.15 cells identified three analogs (**1a**, **1d** & **1g**) with good anti-HBV activity and low cytotoxicty. Of them, compound **1g** exhibited significant inhibitory activity on both HBV antigens secretion ($EC_{50,\ HBsAg}$ = 9 nM, $EC_{50,\ HBeAg}$ = 0.25 μM) and viral DNA replication (intracellular, EC_{50} = 0.099 μM; extracellular, EC_{50} < 0.01 μM).

1. Introduction

Globally, hepatitis B remains a major infectious disease, which affects more than 240 million individuals chronically worldwide and causes as many as 780,000 deaths a year [1]. Currently, there are only seven antiviral drugs in clinical use against hepatitis B virus (HBV) infection: two interferon-α (IFN-α) products as immune system modulators and five nucleoside/nucleotide analogs as viral polymerase inhibitors. However, due to limited efficacy, side effects and/or emergence of resistance, the current therapeutic options still cannot meet the clinic need for the treatment of chronic hepatitis B. Thus, it is urgent to develop new and effective anti-HBV drugs whether for monotherapy or combination therapy. Although a number of non-nucleoside compounds have been discovered with good anti-HBV activity [2–8], so far, none of them has got the FDA approval for clinical use. Compared with interferon-α, nucleosides/nucleotides are orally available and effective in almost all the patients, while causing fewer side effects, which is still one of the most promising areas for developing new potential anti-HBV therapeutics.

Our research group has been working on the design and discovery of novel nucleosides against human immunodeficiency virus (HIV) [9–12], HBV [13–16], and hepatitis C virus (HCV) [17]. Previously, we reported the discovery of 2′-deoxy-2′-β-fluoro-4′-azido-β-D-arabinofuranosyl cytidine (FNC, **6**) with good anti-HBV activity both in vitro [16] and in vivo [15]. In a recent research work, it has been demonstrated that N^4-alkylation (*e.g.* compound **7**) of nucleoside **6** could significantly reduce the in vivo toxicity of the compound [14]. However, these N^4-alkylated analogs are less

Abbreviations used: HBV, hepatitis B virus; IFN, interferon; FDA, food and drug administration; HIV, human immunodeficiency virus; HCV, hepatitis C virus; TPSCl, 2,4,6-triisopropylbenzenesulfonyl chloride; DIPEA, disopropylethylamine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CC_{50}, 50% cytotoxic concentration; EC_{50}, 50% effective concentration; 3 TC, lamivudine; ETV, entecavir; PCR, polymerase chain reaction; FQ-PCR, fluorescence quantitative PCR; HBsAg, HBV surface antigen; HBeAg, HBV e antigen; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; TLC, thin layer chromatography; MEM, minimum essential medium.

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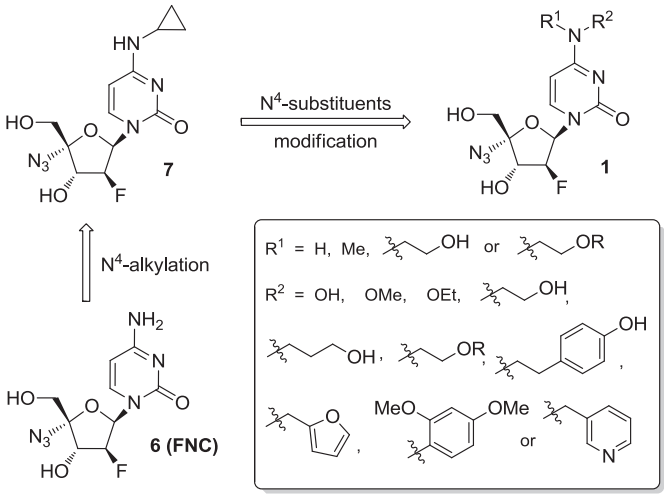


Scheme 1. Structure Exploration of the N^4-Alkylated FNC Derivatives.

potent than the parent compound. Herein, we carried out further structural exploration of the N^4-substituents by incorporating oxygen atoms as hydroxyl groups or ether bonds (Scheme 1), aiming at further increasing the antiviral potency. The biological activity of all the new cytidine derivatives was evaluated in HepG2.2.15 cell lines.

2. Results and discussion

2.1. Chemistry

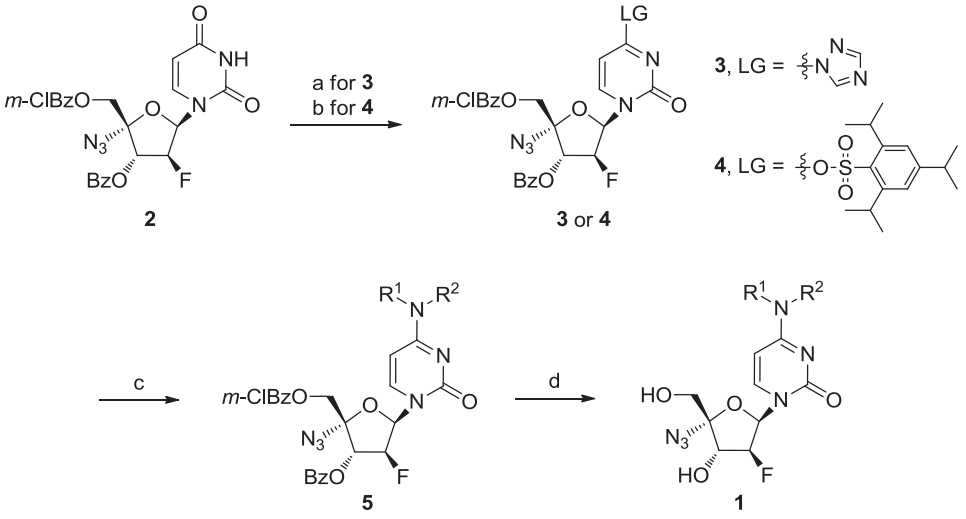
The target nucleosides **1** were synthesized as outlined in Scheme 2. The key starting material, bisbenzoyl uridine **2** was prepared from 1,3,5-*O*-tribenzoyl-2-deoxy-2-fluoro-D-arabinofuranoside using a previously reported synthetic route [10]. The 4-carbonyl group in compound **2** was selectively activated by reaction with 1,2,4-triazole [14] or 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) [18] to form intermediates **3** or **4**. Treatment of compound **3** (or **4**) with substituted amines in the presence of disopropylethylamine (DIPEA) followed by deprotection with ammonia in methanol afforded compound **1a**–**l** in 39–75% yield. All these new synthesized nucleoside analogs were fully characterized by ^{1}H NMR, ^{13}C NMR and HRMS. Analytical HPLC indicated that all the tested compounds possess a purity of at least 95%.

2.2. Cytotoxicity

In the HepG2.2.15 cell line, the cytotoxicity of these new cytidine derivatives (**1a**–**l**) was measured by an MTT assay. As shown in Table 1, most of these nucleoside analogs (**1a**–**c** and **1f**–**j**) exhibited further reduced toxicity compared to the parent compound **7**. Among them, two compounds (**1a** and **1g**) are less toxic than the positive control drug lamivudine (3 TC, CC_{50} = 859 μM). In particular, no cytotoxicity was observed for the bis(2-hydroxyethyl) amino analog **1g** up to the highest concentration tested (1250 μM).

2.3. Anti-HBV activity screening

The anti-HBV activity of these nucleosides (**1a**–**l**) was firstly screened in the HepG2.2.15 cell line using entecavir (ETV) as a positive control. After the treatment of the cells with the test compounds at the concentration of 1 μM for 9 days, their inhibitory activity against HBV DNA replication was measured by an FQ-PCR assay (Table 2). Compared to the parent compound **7**, direct N^4-hydroxylation greatly increased the anti-HBV activity of the compound (**1a**). O-Alkylation of **1a** did not improve the biological activity, with the ethoxy cytidine **1c** being more potent than its methoxy analog **1b**. Further *N*-methylation of **1b** significantly decreased the biological activity of the compound (**1d**). Analogs bearing N^4-hydroxyalkyl groups (**1e**–**f**) maintained the good antiviral activity. Incorporation of one more hydroxyethyl group to the N^4-position of **1e** led to compound **1g** with significant potency against HBV DNA replication (96.9%). However, the morpholine analog (**1h**) completely lost the anti-HBV activity. Introduction of aromatic rings (e.g. benzene, furan and pyridine rings) to the N^4-



(a) 1,2,4-triazole, Py., $POCl_3$, CH_2Cl_2, reflux; (b) 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl), DIPEA, DMAP CH_2Cl_2, rt; (c) R^1R^2NH, DIPEA, DMAP (when **4** was used), CH_2Cl_2, rt; (d) sat. NH_3 in methanol, rt.

Scheme 2. Synthesis of Compounds **1** from Bisbenzoyl Protected Uridine **2.**