Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis, and biological evaluation of novel 2′-deoxy-2′-fluoro-2′-*C*-methyl 8-azanebularine derivatives as potent anti-HBV agents

Wu Yang[a,1], Youmei Peng[b,1], Jingwen Wang[a], Chuanjun Song[a], Wenquan Yu[a], Yubing Zhou[d], Jinhua Jiang[b], Qingduan Wang[b], Jie Wu[a,*], Junbiao Chang[a,c,*]

[a] College of Chemistry and Molecular Engineering, Zhengzhou University, Henan 450001, PR China
[b] Institute of Medical and Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450052, PR China
[c] Collaborative Innovation Center of New Drug Research and Safety Evaluation, Henan Province, Zhengzhou 450001, PR China
[d] Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, PR China

ARTICLE INFO

Keywords:
2′-Deoxy-2′-fluoro-2′-*C*-methyl 8-azanebularine analogues
Anti-HBV activity
Nucleoside reverse transcriptase inhibitor (NRTI)
Lamivudine-resistant HBV mutants
Viral DNA replication

ABSTRACT

Hepatitis B virus (HBV) is a global health problem requiring more efficient and better tolerated anti-HBV agent. In this paper, a series of novel 2′-deoxy-2′-fluoro-2′-*C*-methyl-β-D-arabinofuranosyl 8-azanebularine analogues (**1** and **2a)** and N^4-substituted 8-azaadenosine derivatives **(2b-g)** were designed, synthesized and screened for *in vitro* anti-HBV activity. Two concise and practical synthetic routes were developed toward the structural motif construction of 2′-deoxy-2′-fluoro-2′-*C*-methyl-β-D-arabinofuranosyl 8-azainosine from the ribonolactone **3** under mild conditions. The *in vitro* anti-HBV screening results showed that these 8-azanebularine analogues had a significant inhibitory effect on the expression of HBV antigens and HBV DNA at a concentration of 20 μM. Among them, halogen-substituted 8-azaadenosine derivative **2g** displayed activities comparable to that of 3TC. In particular, **2g** retained excellent activity against lamivudine-resistant HBV mutants.

Hepatitis B virus (HBV) infection presents a public health problem worldwide, with about 260 million people infected and nearly 887,000 deaths per year worldwide[1–3]. For many years, scientists have been committed to design new drugs against HBV. Among them, the modified nucleosides are effective in almost all the patients and have represented one of the most promising classes of polymerase inhibitors of HBV viruses. Six nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) have been approved by Food and Drug Administration (FDA) for the treatment of HBV[4–7]. However, on account of the poor toleration, frequent side effects, as well as the development of drug-resistant viruses, the current therapy for HBV treatment still cannot meet the clinical need[8–10]. New and effective drugs are still urgently needed to combat HBV infection, especially for nucleoside analogues with unreported scaffolds that do not select resistance-associated mutations.

Among derivatives developed in the nucleoside chemistry, some unnatural heterocycles can be introduced as nucleobases in the design of novel nucleoside analogues (Fig. 1). They can not only enhance *in vivo* stability but also endow interesting biological activities, e.g. ribavirin, a designed guanosine analogue bearing a 1,2,4-triazole[11–17]. In addition, the 8-azanebularine analogues, mimicking the natural purine nucleosides with 8-azapurine derivatives as nucleobases, have also received considerable attention as purine antagonists because of their excellent antiviral and antitumour activities, as well as their interesting biochemical and chemotherapeutic properties[18–20]. Our laboratory has been devoted to continuing interest in the investigation of novel new nucleoside analogues against the human immunodeficiency virus (HIV), HBV, and the hepatitis C virus (HCV)[21–29]. As part of our research projects, we demonstrated that a 2′-deoxy-2′-β-fluoro-4′-azido-β-D-arabinofuranosyl 1,2,3-triazole nucleoside analogue (**TAA-1a**) showed potential antiviral effects and retained excellent activity against lamivudine-resistant HBV mutants[30]. Based on 1,2,3-triazole nucleosides, an efficient linear strategy for the stereospecific formation of some 8-azanebularine analogues had been developed by our group[31].

On the other hand, ribonucleoside analogues with a 2′-deoxy-2′-fluoro-2′-*C*-methyl substituent have inspired a wide range of research over the past few years due to the high efficacy of sofosbuvir against the HCV NS5B polymerase[32]. In view of the potential biological activity of 8-azaguanosines and 2′-deoxy-2′-fluoro-2′-*C*-methylribofuranosyl moieties, we provided a full account of the synthesis and biological evaluation of 2′-deoxy-2′-fluoro-2′-*C*-methyl-β-D-arabinofuranosyl 8-azainosine (**1**), 8-azaadenosine (**2a)** and N^4-substituted 8-azaadenosine derivatives **(2b-g)** in this article. Two efficient linear protocols for the

* Corresponding authors at: College of Chemistry and Molecular Engineering, Zhengzhou University, Henan 450001, PR China (J. Chang).
E-mail addresses: wujie@zzu.edu.cn (J. Wu), changjunbiao@zzu.edu.cn (J. Chang).
[1] These authors contributed equally.

https://doi.org/10.1016/j.bmcl.2019.04.005
Received 21 January 2019; Received in revised form 29 March 2019; Accepted 3 April 2019

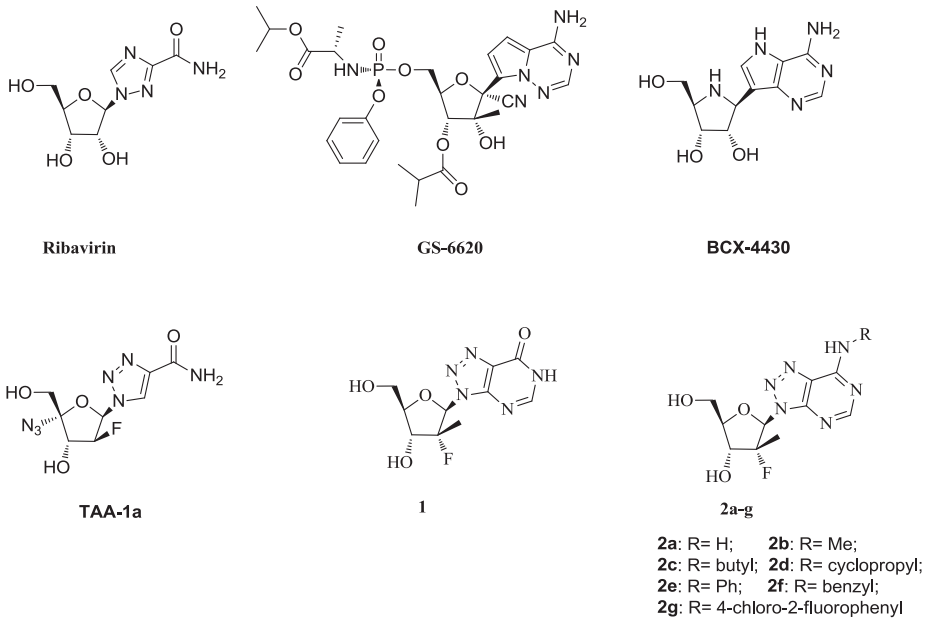
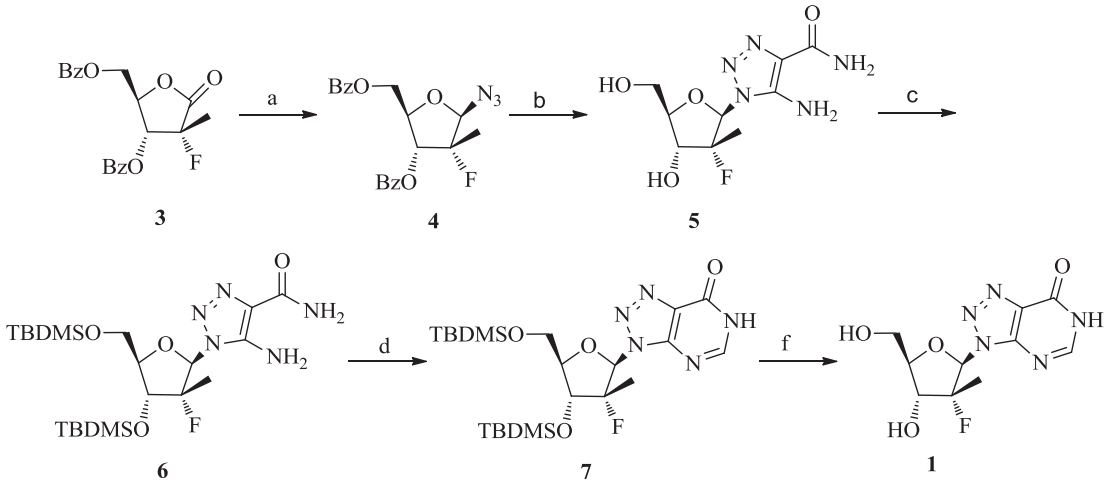
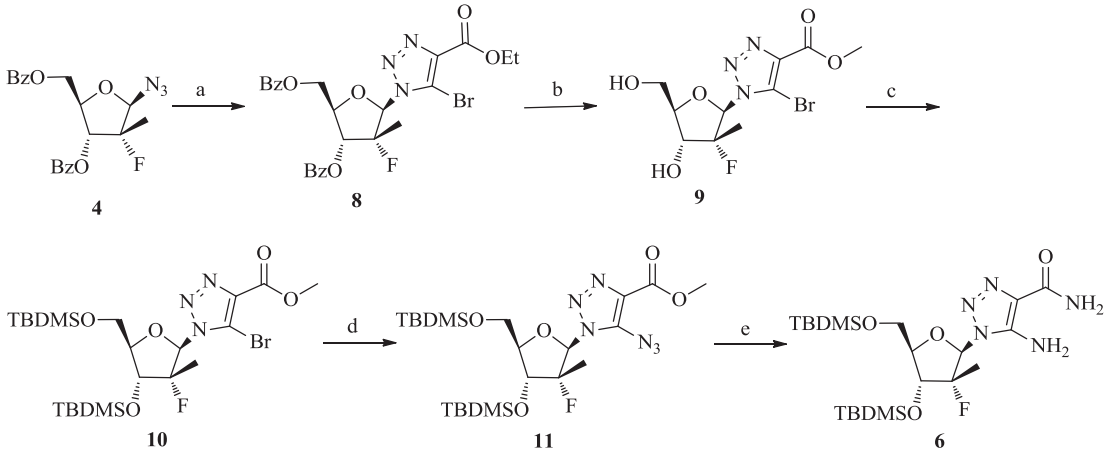


Fig. 1. Nucleoside analogues with unnatural heterocycles.



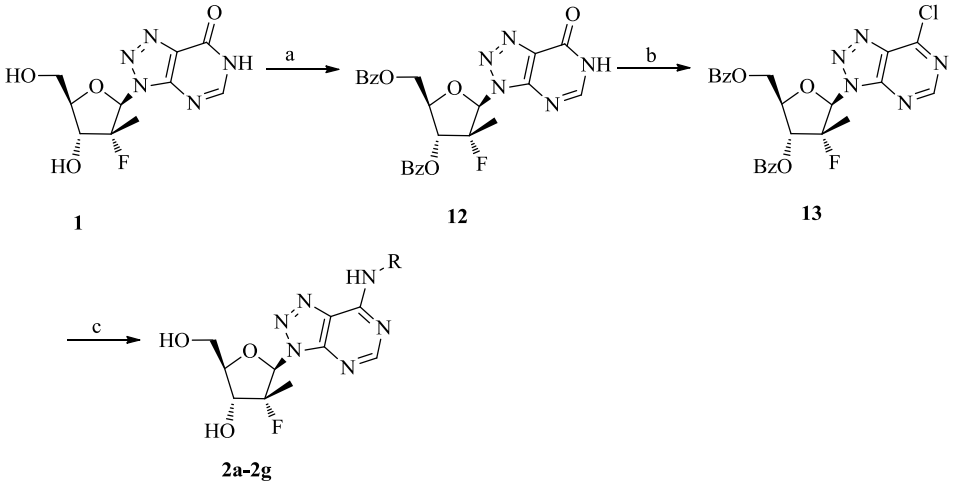
Scheme 1. *Reagents and conditions*: (a) (i) LTTBA, Ac_2O, DMAP, THF, −20 °C, 5 h, 90%; (ii) $SnCl_4$, $TMSN_3$, PhCl, 65°C, 6 h, 30%; (b) Cyanoacetamide, EtONa, EtOH, 50 °C, 20 h, 80%; (c) TBDMSCl, Imidazole, DMF, rt, 12 h, 95%; (d) $HC(OEt)_3$, 145 °C, 48 h, 63%; (f) NH_4F, MeOH, 60 °C, 13 h, 89%.



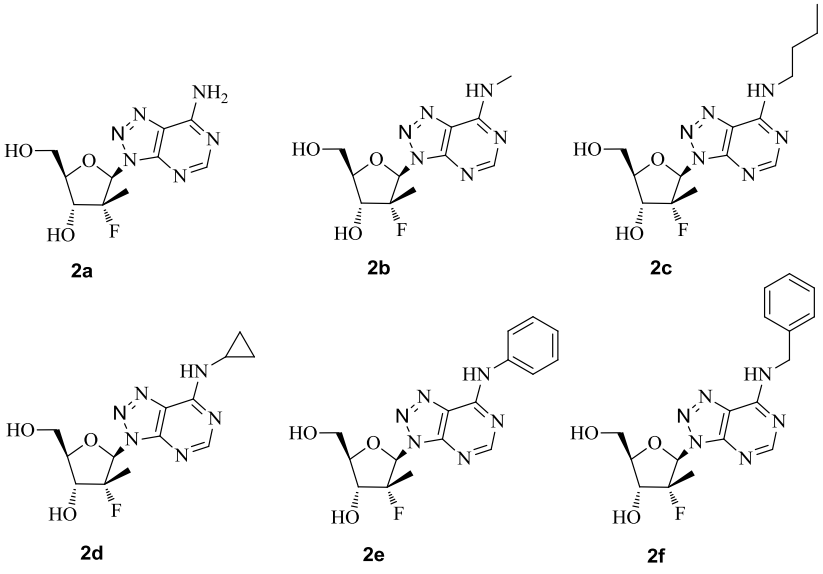
Scheme 2. *Reagents and conditions*: (a) Ethyl 3-bromopropiolate, $Cu(OAc)_2$, CuBr, THF, 80 °C, 48 h, 97%; (b) *t*-Butylamine, MeOH, rt, 48 h, 70%; (c) TBDMSCl, Imidazole, DMF, rt, 12 h, 84%; (d) NaN_3, DMF, 40 °C, 15 h, 85%; (e) (i) NH_3/MeOH, rt, 24 h; (ii) H_2, Pd/C, MeOH, rt, 6 h, 83% over the two steps.

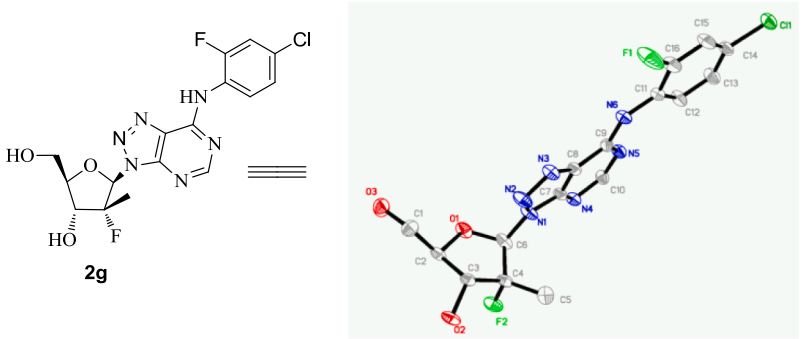
stereospecific formation of 8-azainosine structural motif were developed. By screening, **2g** has potential antiviral effects against wild-type HBV and lamivudine-resistant HBV mutants.

The synthesis of 8-azainosine derivative **1** was illustrated in Scheme 1. The key starting material 1-β-azido sugar **4** was prepared from the lactone **3** through reduction, esterification, and followed by substitution with $TMSN_3$. The 1,3-dipolar cycloaddition reaction of **4** with 2-cyanoacetamide furnished triazole **5** in a regiospecific manner, which further reacted with tert-Butyldimethylsilyl chloride (TBDMSCl) to provide intermediate **6** for the following hypoxanthine formation reaction [33]. Notably, only



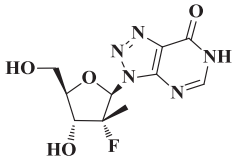
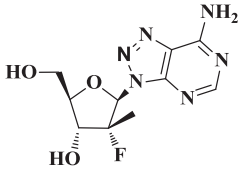
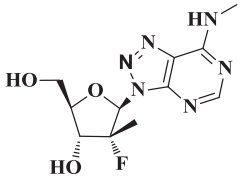
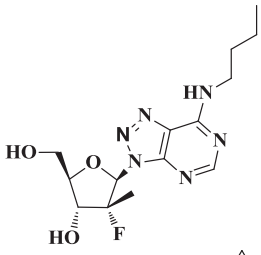
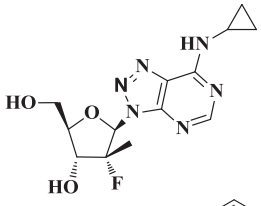
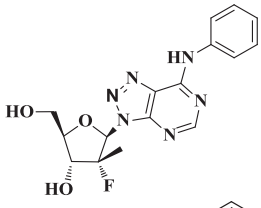
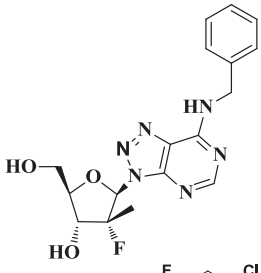
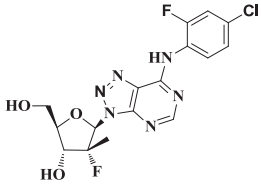
2a: R= H; **2b**: R= Me;
2c: R= butyl; **2d**: R= cyclopropyl;
2e: R= Ph; **2f**: R= Benzyl;
2g: R= 4-chloro-2-fluorophenyl





Scheme 3. *Reagents and conditions*: (a) BzCl, Et_3N, acetone, rt, 12 h, 98%; (b) $SOCl_2$, DMF, DCM, reflux, 24 h, 71%.(c) NH_3, CH_3OH, 60 °C, 19 h, 97% for **2a** (i) RNH_2, DIPEA, CH_2Cl_2, 60 °C, 3–5 h; (ii) NH_3/CH_3OH, rt, 24 h, 91% for **2b**, 90% for **2c**, 87% for **2d**, 94% for **2e**, 80% for **2f**, 36% for **2g**, all over the two steps.

Table 1
The inhibitory effect of **1** and **2a-g** on HBsAg and HBeAg secretion and cytotoxicity.

Compounds	Structure	CC_{50}(μM)[b]	Inhibition percentages (%)[a]	
			HBeAg	HBsAg
3TC		–	39.6 ± 4.2	42.4 ± 3.9
1		> 600	7.4 ± 1.0	8.5 ± 1.7
2a		> 600	10.0 ± 3.3	9.9 ± 2.6
2b		> 600	24.4 ± 6.1	20.1 ± 4.2
2c		> 600	11.6 ± 3.1	17.0 ± 2.5
2d		> 600	16.7 ± 4.3	11.4 ± 3.8
2e		501 ± 19	17.7 ± 2.7	14.8 ± 3.6
2f		> 600	11.9 ± 2.9	8.5 ± 1.6
2g		245 ± 11	31.6 ± 6.6	39.7 ± 7.3

[a] HepG2.2.15 cells were treated with **1**, **2a-g** and lamivudine at the concentration of 20 μM for 9 days. The levels of HBsAg and HBeAg in the supernatant were detected by ELISA assays.

[b] CC_{50}: 50% cytotoxic concentration, measured by MTT assay in the HepG2 cell line treated with **1** and **2a-g** for 9 days.

Table 2
The inhibitory Effect of **2g** on Intracellular HBV DNA in HepG2.2.15 Cell.

Compounds		day 3		day 6		day 9	
	Concentration (μM)	HBV DNA2 × 10^4 copies/mL	Inhibition rates (%)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)
control		5.7 ± 1.4		6.6 ± 1.7		8.6 ± 2.7	
3TC	20	3.0 ± 0.7**	47.4	2.0 ± 0.5**	69.7	1.9 ± 0.5**	77.9
2g	10	4.9 ± 1.2*	14.0	4.5 ± 1.4*	31.8	4.8 ± 1.0**	44.2
2g	20	4.1 ± 0.7*	28.1	3.5 ± 1.3**	47.0	2.8 ± 0.5**	67.4
2g	40	3.6 ± 0.8**	36.8	2.3 ± 0.9**	65.2	1.7 ± 0.6**	80.2

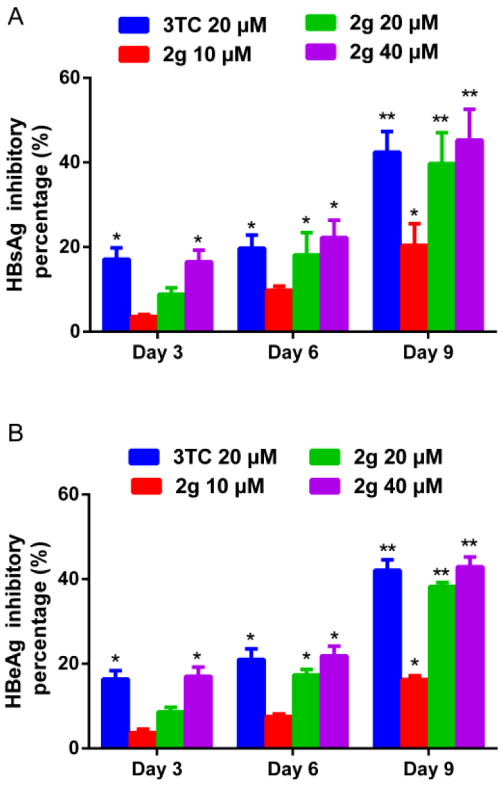


Fig. 2. The inhibitory effect of **2g** against HBsAg (A) and HBeAg (B) in the HepG2.2.15 cell. The HepG2.2.15 cell was treated with **2g** (10, 20 and 40 μM) and 3TC (20 μM) for 3, 6 or 9 days. The HBsAg (A) and HBeAg (B) in the supernatants were quantified using ELISA method. Data were presented as mean ± SD of three experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the no-drug control group.

stoichiometric TBDMSCl could be added in the protection reaction and the reaction time should be limited within 12 h. Or else, the amino group of **5** could further react with surplus TBDMSCl. Condensation of amino amide **6** with triethyl orthoformate resulted in the formation of 8-azainosine derivative **7** as a single key product in 63% isolated yield. Further desilylation with ammonium fluoride furnished 2′-deoxy-2′-fluoro-2′-*C*-methyl-β-D-arabinofuranosyl 8-azainosine **1** in a high yield.

To achieve the key intermediate **6**, we also could make use of another synthetic protocol (Scheme 2)[31]. Copper-catalyzed [3 + 2] cycloaddition of **4** with ethyl 3-bromopropiolate resulted in the formation of triazole **8**. Transesterification and protection with TBDMS group yielded **10**. The following nucleophilic aromatic substitution of **10** with sodium azide provided **11** in an excellent isolated yield. Ammonolysis of the ester followed by hydrogenation of the azide group afforded the key intermediate **6** in 83% isolated yield in two steps.

Starting from **1**, 6-chloro-8-azapurine **13** was prepared by benzoylation and then chlorination the carbonyl group with $SOCl_2$. Debenzoylation and amination of **13** with NH_3/CH_3OH at 60 °C afforded 2′-deoxy-2′-fluoro-2′-*C*-methyl-β-D-arabinofuranosyl 8-azaadenosine **2a** in 98% isolated yield (Scheme 3). Likewise, reactions of **13** with different amines in the presence of disopropylethylamine (DIPEA) followed by deprotection provided **2b-2g** in a medium or high yield. The structure of **2g** was further confirmed by X-ray crystallography.

To investigate the inhibitory effect of **1** and **2a-g** on the production of the hepatitis B surface antigen (HBsAg) and hepatitis Be antigen (HBeAg), the HepG2.2.15 cells were treated with the test compounds at a concentration of 20 μM for 9 days and lamivudine (3TC, 20 μM) used as a positive control. The supernatant was collected and the titers of HBsAg and HBeAg were determined by ELISA kits. As shown in Tables 1 and 2a-g have a significant inhibitory effect on the expression of HBV antigens at a concentration of 20 μM and exhibit low cytotoxicity. Replacing the carbonyl in **1** with primary amine leads to the formation of **2a-2g** with better inhibitory activity. Incorporation of substituents (alkyl, cyclopropane and phenyl) into the primary amine brings better inhibitory activity, especially **2b** has a more obvious inhibitory activity. If the hydrogen on the benzene ring of **2e** is replaced by halogen, the resulting compound (**2g**) presents significantly increased inhibitory activity and better inhibitory activity than other compounds. Specifically, **2g** significantly reduces the production of HBsAg and HBeAg with inhibitory rates of 39.7% and 31.6% on day 9, respectively, while 3TC-treated (20 μM) groups show 42.4% and 39.6% inhibition on day 9. The cytotoxicity (CC_{50s}) of **2g** were 409 ± 16 μM and 245 ± 11 μM in the HepG2 cell treated for 3 and 9 days, respectively, with inhibitory rate of 7.7% at the concentration of 40 μM for 9 days. These results indicated that **2g** at 20 μM is equally effective compared to 3TC at 20 μM in inhibition of both HBsAg and HBeAg secretion with a favorable cytotoxicity profile.

To further confirm the antiviral activity of **2g** in HepG2.2.15 cells, the secretion of HBsAg, HBeAg and HBV DNA levels were evaluated after treatment with different concentrations of **2g** (10, 20 and 40 μM) for 3, 6 and 9 days. The significant reductions of HBsAg and HBeAg secretion were observed in a time- and dose-dependent manner (Fig. 2). Consistent with the inhibitory effects on HBeAg and HBsAg secretion, treatment with **2g** at concentrations of 10, 20, and 40 μM for 3, 6 and 9 days results in the reduction of both the intracellular and extracellular HBV DNA levels in a time- and dose-dependent manner. The mean inhibition percentages of HBV DNA level with **2g** at the dosages of 10, 20 and 40 μM are 44.2%, 67.4% and 80.2%, respectively, intracellularly (Table 2) and 36.9%, 65.0% and 82.5%, respectively, extracellularly (Table 3), on day 9. The inhibition rate of 3TC (20 μM) on intracellular and extracellular HBV DNA level is 77.9% and 78.6%, on day 9. The results show that **2g** has a significant inhibitory effect on HBsAg, HBeAg and HBV DNA in the HepG2.2.15 cell line. The inhibitory effect of **2g** at the concentration of 40 μM on HBV DNA is equivalent to 3TC at the concentration of 20 μM. Although the inhibitory effect of **2g** (20 μM) on HBV DNA is lower than that of 3TC (20 μM), **2g** retains activity against the lamivudine-resistant HBV mutant.

To determine the inhibitory effect of **2g** against lamivudine-resistant HBV, the HBeAg in the supernatants and HBV DNA were detected in L180M/M204V mutant cell lines after **2g** treatment at 10, 20

Table 3
The inhibitory Effect of **2g** on Extracellular HBV DNA in HepG2.2.15 Cell.

Compounds		day 3		day 6		day 9	
	Concentration (μM)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)
control		4.9 ± 1.2		6.1 ± 1.5		10.3 ± 3.0	
3TC	20	3.3 ± 0.9*	32.7	2.8 ± 0.7**	54.1	2.2 ± 0.6**	78.6
2g	10	4.0 ± 1.1*	18.4	4.2 ± 0.9*	31.1	6.5 ± 2.0*	36.9
2g	20	3.7 ± 0.9*	24.5	3.5 ± 1.2**	42.6	3.6 ± 1.3**	65.0
2g	40	3.2 ± 1.3*	34.7	1.9 ± 0.5**	68.9	1.8 ± 0.4**	82.5

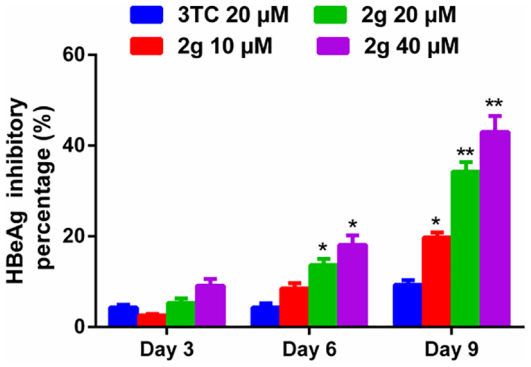


Fig. 3. The inhibitory effect of **2g** against HBeAg in the L180M/M204V mutant cell line. The cell line was treated with **2g** (10, 20 and 40 μM) and 3TC (20 μM) for 3, 6 and 9 days. The HBeAg in the supernatants were quantified using ELISA method. Data were presented as mean ± SD of three experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the no drug control group.

and 40 μM for 3, 6 and 9 days. The level of HBsAg from lamivudine-resistant cell line transfected with the L180M/M204V mutant is lower limit of detection, and then it was excluded from the antigen criterion[34]. The antigen inhibitory efficiency of **2g** was assessed only based on HBeAg. As shown in Fig. 3, lamivudine showed lower inhibitory effect on HBeAg production from lamivudine-resistant HBV than that from wild-type HBV. However, **2g** significantly reduces the secretion of HBeAg of lamivudine-resistant HBV in a time- and dose-dependent manner. The inhibitory effects on HBeAg secretion of wild-type and lamivudine-resistant HBV have no obvious difference.

Consistent with its inhibitory effects on HBeAg secretion, the HBV DNA level decreased in a time-and dose-dependent manner after 3, 6 or 9 days treatment with **2g** at the concentrations of 10, 20 and 40 μM. The mean inhibition percentage of HBV DNA level with **2g** at the dosages of 10, 20, and 40 μM is 36.6%, 65.2% and 76.4%, respectively, intracellularly (Table 4) and 32.1%, 61.4% and 83.6%, respectively, extracellularly (Table 5), on day 9. In contrast, the mutant rtL180M/M204V is less susceptible to 3TC than wild-type HBV, with an inhibition rate of only 11.2% and 19.3%, intracellularly and extracellularly, on day 9. The results show that **2g** is effective against both wild-type and lamivudine-resistant HBV.

In summary, we have developed an efficient route for the synthesis of 2′-deoxy-2′-fluoro-2′-*C*-methyl-β-D-arabinofuranosyl 8-azanebularine analogues, including 8-azainosine (**1**) and 8-azaadenosine derivatives (**2a–g**), in a highly stereo- and regioselective manner. The synthetic compounds were tested for anti wild-type and lamivudine-resistant HBV activity. The results showed that these compounds had a significant inhibitory effect on the expression of HBV antigens and HBV DNA at a concentration of 20 μM. Among them, halogen-substituted 8-azaadenosine derivative (**2g**) displays activities comparable to that of 3TC in inhibition of wild-type HBV antigens and HBV DNA. In particular, **2g** is also effective against lamivudine-resistant HBV. These results provide strong support for the development of **2g** and its 8-azaadenosine derivatives as a potential alternative or complementary therapy for the treatment of HBV infection.

Table 4
Inhibitory Effect of **2g** on Intracellular HBV DNA Levels in lamivudine-resistant cell.

compounds		day 3		day 6		day 9	
	Concentration (μM)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)
control		8.0 ± 2.1		10.1 ± 3.5		16.1 ± 5.8	
3TC	20	7.5 ± 1.8	6.3	9.3 ± 3.8	7.9	14.3 ± 5.0*	11.2
2g	10	7.6 ± 2.2	5.0	7.8 ± 2.0*	22.8	10.2 ± 2.1*	36.6
2g	20	6.6 ± 1.6*	17.5	5.9 ± 1.3**	41.6	5.6 ± 1.5**	65.2
2g	40	5.5 ± 1.8*	31.3	3.4 ± 1.0**	66.3	3.8 ± 0.9**	76.4

Table 5
Inhibitory Effect of **2g** on Extracellular HBV DNA Levels in lamivudine-resistant cell.

compounds		day 3		day 6		day 9	
	Concentration (μM)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)	HBV DNA × 10^4 copies/mL	Inhibition rates (%)
control		7.3 ± 2.1		9.5 ± 3.3		14.0 ± 4.5	
3TC	20	7.0 ± 1.6	4.1	8.7 ± 2.6	8.4	11.3 ± 3.2*	19.3
2g	10	6.5 ± 1.9	11.0	6.9 ± 2.0*	27.4	9.5 ± 2.7*	32.1
2g	20	4.7 ± 0.9*	35.6	4.8 ± 1.3**	49.5	5.4 ± 1.4**	61.4
2g	40	4.0 ± 2.2**	45.2	3.7 ± 0.9**	61.1	2.3 ± 0.5**	83.6

Acknowledgements

This work was funded by the National Natural Science Foundation (Grand No. 21771163, 81330075, and U1804283), the Science Foundation of Henan Provience (Grand No. 162300410243) and Project for the Leading Young Teachers in Henan Provincial Institutions of Higher Education of China.

Appendix A. Supplementary data

Experimental details, ^{1}H and ^{13}C NMR spectra of compounds **1–13**, X-ray structure and data of **2g** (PDF). Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.04.005.

References

1. Burwitz BJ, Wettengel JM, Mück-Häusl MA, et al. Hepatocytic expression of human sodium-taurocholate cotransporting polypeptide enables hepatitis B virus infection of macaques. *Nat Commun.* 2017;8:2146.
2. Li X, Zhou TC, Wu CH, et al. Correlations between mitochondrial DNA haplogroup D5 and chronic hepatitis B virus infection in Yunnan, China. *Sci Rep UK.* 2018;8:869.
3. Tsai KN, Kuo CF, Ou JHJ. Mechanisms of hepatitis B virus persistence. *Trends Microbiol.* 2018;26:33–42.
4. Cuestas ML, Mathet VL, Oubiña JR. Drug delivery systems and liver targeting for improved pharmacotherapy of the hepatitis B virus (HBV) infection. *Pharm Res.* 2010;27:1184–1202.
5. Grossi G, Loglio A, Facchetti F, et al. Tenofovir alafenamide as a rescue therapy in a patient with HBV-cirrhosis with a history of Fanconi syndrome and multidrug resistance. *J Hepatol.* 2018;68:195–198.
6. Gioti EG, Koftis TV, Neokosmidis E, et al. A new scalable synthesis of entecavir. *Tetrahedron.* 2018;74:519–527.
7. Singh US, Mulamoottil VA, Chu CK. 2'-Fluoro-6'-methylene carbocyclic adenosine and its phosphoramidate prodrug: a novel anti-HBV agent, active against drug-resistant HBVmutants. *Med Res Rev.* 2018;38:977–1002.
8. Perrillo RP. Current treatment of chronic hepatitis B: benefits and limitations. *Semin Liver Dis.* 2005;25:20–28.
9. Taiwo BO, Marconi VC, Berzins B, et al. Dolutegravir plus lamivudine maintain HIV-1 suppression through week 48 in a pilot randomized trial. *Clin Infect Dis.* 2018;66:1794–1797.
10. Pulido F, Ribera E, Lagarde M, et al. Dual therapy with darunavir and ritonavir plus lamivudine vs triple therapy with darunavir and ritonavir plus tenofovir disoproxil fumarate and emtricitabine or abacavir and lamivudine for maintenance of human immunodeficiency virus type 1 viral suppression: randomized, open-label, non-inferiority DUAL-GESIDA 8014-RIS-EST45 trial. *Clin Infect Dis.* 2017;65:2112–2118.
11. Amblard F, Cho JH, Schinazi RF. Cu(I)-Catalyzed huisgen azide-alkyne 1,3-dipolar cycloaddition reaction in nucleoside, nucleotide, and oligonucleotide chemistry. *Chem Rev.* 2009;109:4207–4220.
12. Seio K, Kanamori T, Masaki Y. Solvent- and environment-dependent fluorescence of modified nucleobases. *Tetrahedron Lett.* 2018;59:1977–1985.
13. Firestine SM, Wu WD, Youn H, Davisson VJ. Interrogating the mechanism of a tight binding inhibitor of AIR carboxylase. *Bioorgan Med Chem.* 2009;17:794–803.
14. Štambaský J, Hocek M, Kočovský P. C-nucleosides: synthetic strategies and biological applications. *Chem Rev.* 2009;109:6729–6764.
15. Zhurilo NI, Chudinov MV, Matveev AV, et al. Isosteric ribavirin analogues: synthesis and antiviral activities. *Bioorg Med Chem Lett.* 2018;28:11–14.
16. Lopes AB, Wagner P, Souza ROMAd, et al. Functionalization of 2H–1,2,3-triazole *C*-nucleoside template via N^2 selective arylation. *J Org Chem.* 2016;81:4540–4549.
17. Solarte C, Santos MD, Gonzalez S, et al. Synthesis of *C*-ribosyl-1,2,3-triazolyl carboxamides. *Synthesis.* 2017;49:1993–2002.
18. Toti K, Renders M, Groaz E, Herdewijn P, Calenberghl SV. Nucleosides with transposed base or 4'-hydroxymethyl moieties and their corresponding oligonucleotides. *Chem Rev.* 2015;115:13484–13525.
19. Gillerman I, Fischer B. Investigations into the origin of the molecular recognition of several adenosine deaminase inhibitors. *J Med Chem.* 2011;54:107–121.
20. Seela F, Jiang D, Xu KY. 8-Aza-2'-deoxyguanosine: base pairing, mismatch discrimination and nucleobase anion fluorescence sensing in single-stranded and duplex DNA. *Org Biomol Chem.* 2009;7:3463–3473.
21. Yang QH, Zhao XJ, Zang LM, et al. Anti-hepatitis B virus activities of a-DDB-FNC, a novel nucleoside-biphenyld-icarboxylate compound in cells and ducks, and its anti-immunological liver injury effect in mice. *Antivir Res.* 2012;96:333–339.
22. Yang QH, Kang JF, Zheng LY, et al. Synthesis and biological evaluation of 4-substituted fluoronucleoside analogs for the treatment of hepatitis B virusinfection. *J Med Chem.* 2015;58:3693–3703.
23. Wang Q, Hu WD, Wang SY, et al. Synthesis of new 2'-deoxy-2'-fluoro-4'-azido nucleoside analogues as potentanti-HIV agents. *Eur J Med Chem.* 2011;46:4178–4183.
24. Wang Q, Li YF, Song CJ, et al. Synthesis and anti-HIV activity of 2'-deoxy-2'-fluoro-4'-*C*-ethynyl nucleoside. *Bioorg Med Chem Lett.* 2010;20:4053–4056.
25. Lv ZG, He W, Tian XH, et al. Design, synthesis, and biological evaluation of new N4-Substituted 2'-deoxy-2'-fluoro-4'-azido cytidine derivatives as potent anti-HBV agents. *Eur J Med Chem.* 2015;101:103–110.
26. Yang QH, Zhao XJ, Yu WQ, et al. Anti-hepatitis B virus activity of a-DDB-FNCG, a novel nucleoside-biphenyldicarboxylate compound in vitro and in vivo. *J Pharm Sci.* 2014;126:208–215.
27. Zheng LY, Wang Q, Yang XR, et al. Antiviral activity of FNC, 2′-deoxy-2′-β- fluoro-4′-azidocytidine, against human and duck HBV replication. *Antivir Ther.* 2012;17:679–687.
28. Hu WD, Yang QH, Wang SY, et al. A new route for the synthesis of the 4- amino-5-fluoro-7-(2'-deoxy-2'-fluoro-2'-C-methyl–d-ribofuranosyl)-1H-pyrrolo[2,3-d]pyrimidine. *Nucleos Nucleot Nucl.* 2013;32:389–395.
29. Peng YM, Yu WQ, Li ET, et al. Discovery of an orally active and liver-targeted prodrug of 5-fluoro-2'-deoxyuridine for the treatment of hepatocellular carcinoma. *J Med Chem.* 2016;59:3661–3670.
30. Liu Y, Peng YM, Lu JJ, et al. Design, synthesis, and biological evaluation of new 1,2,3-triazolo-2'-deoxy-2'-fluoro-4'-azido nucleoside derivatives as potent anti-HBV agents. *Eur J Med Chem.* 2018;143:137–149.
31. Yang W, Ma HR, Yang Q, et al. The first example of palladium-catalyzed cascade amidine arylation-intramolecular ester amidation for the synthesis of hypoxanthines: application to the synthesis of 8-azanebularine analogues. *Org Biomol Chem.* 2017;15:379–386.
32. Sofia MJ, Bao D, Chang W, et al. Discovery of a β-D-2'-Deoxy-2'-a-fluoro-2'-β- *C*-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J Med Chem.* 2010;53:7202–7218.
33. Joubert N, Schinazi RF, Agrofoglio LA. Efficient Pd(0)-catalyzed synthesis of 1,2,3-triazolo-3'-deoxycarbanucleosides and their analogues. *Tetrahedron.* 2005;61:11744–11750.
34. Zhou YB, Wang YF, Zhang Y, et al. In vitro activity of cepharanthine hydrochloride against clinical wild-type and lamivudine-resistant hepatitis B virus isolates. *Eur J Pharmacol.* 2012;683:10–15.