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DIOXOLANE CYTOSINE NUCLEOSIDES AS ANTI-HEPATITIS B AGENTS

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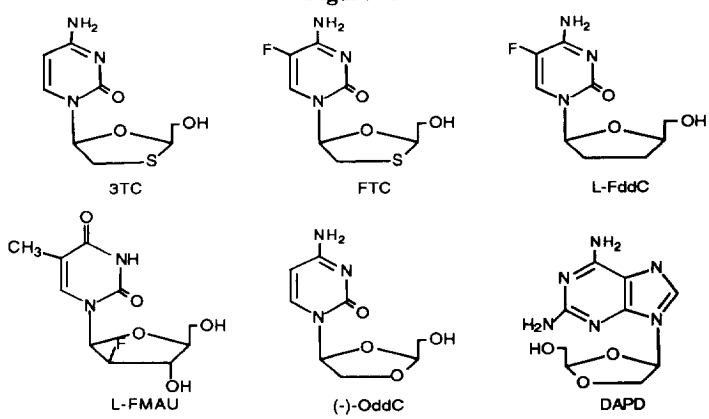
Abstract. In order to study the structure-activity relationships, several dioxolane pyrimidine nucleosides have been synthesized and their anti-HBV activities have been evaluated in 2.2.15 cells. From the study it was found that 5-fluoro-cytosine derivatives exhibited the most potent anti-HBV activity.

Recently, several nucleosides have been reported as potent antiviral agents against human hepatitis B virus (HBV). These include β-*L*-(2-hydroxymethyl-1,3-oxathiolan-4-yl)cytosine (3TC),[1] β-*L*-(2-hydroxymethyl-1,3-oxathiolan-4-yl)-5-fluorocytosine (FTC),[2] β-*L*-2',3'-dideoxy-5-fluoro-cytidine (*L*-FddC),[3,4] 2'-fluoro-5-methyl-β-*L*-arabinofuranosyluracil (*L*-FMAU),[5] and β-*D*-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2,6-diaminopurine (DAPD).[6] These nucleosides are currently undergoing preclinical and clinical studies as anti-HBV agents (Figure 1).

Figure 1



Previously, we have found that (-)-β-*L*-dioxolane-cytosine [(-)-OddC] exhibited extremely potent anti-HBV activity in 2.2.15 cells.[7] However, its *in vitro* cellular cytoxicity precluded its use as an useful anti-HBV agent. We have also synthesized and evaluated the corresponding *D*-isomer as a potential anti-HBV agent.[7] The *D*-isomer was found to be significantly less toxic than the *L*-isomer, however, its anti-HBV potency was also significantly reduced, comparing to the potency of the *L*-isomer. Thus, it was of interest to study the structure-activity relationships (SAR) of the *D*-dioxolane nucleosides to search for more potent anti-HBV agents while maintaining low cellular toxicity. Particularly, of interest was the study of the SAR of cytosine derivatives due to

Scheme 1

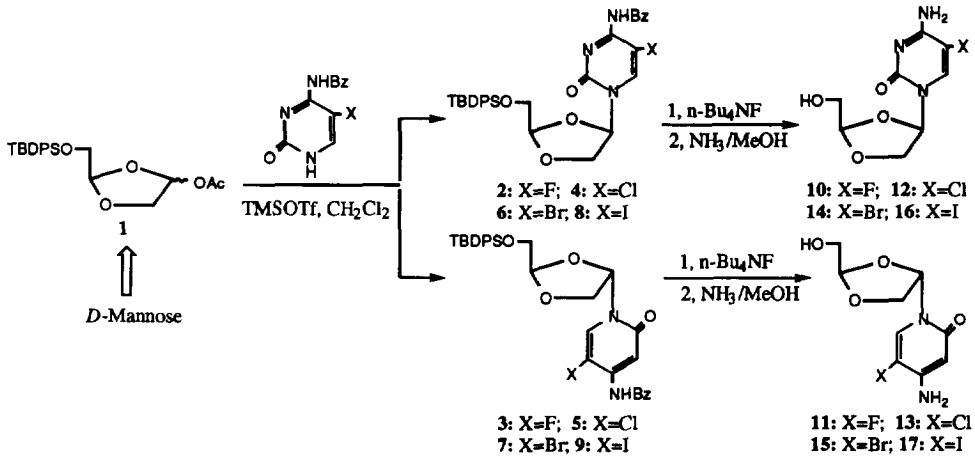
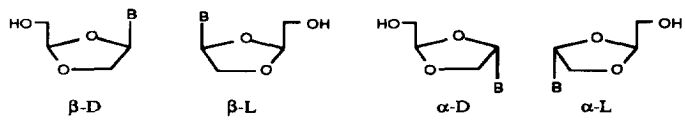


Table 1. Antiviral Activity Against Hepatitis B Virus in human hepatoma cell line (2.2.15 cells), Cytotoxicity in H1 CEM cells (including inhibition of Mitochondrial DNA Synthesis).



Compounds (B)	Anti-HBV Activity $EC_{50}(\mu M)$	Cytotoxicity CEM $IC_{50}(\mu M)$	Cytotoxicity Mitochondria $IC_{50}(\mu M)$	Selectivity
β-D-cytosine	0.01	2.4	>10	240
α-D-cytosine	5.0			
β-D-5-fluorocytosine (**10**)	0.02	>30	>10	>1,500
α-D-5-fluorocytosine (**11**)	2.0	>10	ND	> 5
β-D-5-chlorocytosine (**12**)	>5	>100	ND	
α-D-5-chlorocytosine (**13**)	ND			
β-D-5-bromocytosine (**14**)	>5	>100	ND	
α-D-5-bromocytosine (**15**)	ND			
β-D-5-iodocytosine (**16**)	>5	>100	ND	
α-D-5-iodocytosine (**17**)	ND	ND	ND	
β-L-cytosine	0.0005	0.056	>1μM	
α-L-cytosine	>5.0	>50	>50	
β-L-5-fluorocytosine	0.0005	0.4	>2	800
α-L-5-fluorocytosine	0.4	31.7	>68.0	79
β-D-uracil	>20.0			
β-D-5-fluorouracil	>5.0			
β-D-5-bromouracil	>5.0			
β-D-5-methylcytosine	>5.0			5,000
β-L-oxathiolanecytosine (3TC)	0.01	50	50	
β-D-oxathiolanecytosine				

the finding that the cytosine derivatives exhibit, in general, more potent anti-HBV activity than the other analogues.[1-4,7] Therefore, herein we report the synthesis and anti-HBV activity of 5-substituted *D*-dioxolane - cytosine nucleosides (5-F, Cl, -Br, and -I) along with other previously unreported pyrimidine derivatives.

The dioxolane intermediate **1**, which was prepared from *D*-mannose according to the method previously reported by our laboratory,[8] was condensed with silylated 5-substituted N-benzoylcytosine derivatives (5-F, Cl, - Br, and -I) to anomeric mixtures of the 5-substituted cytosine-nucleosides **2-9** (Scheme 1). The individual isomers (β, **2, 4, 6 and 8** and α:,**3, 5, 7 and 9**) were separated by silica gel column chromatography, and were subsequently treated with n-Bu4NF to remove the 5'-silyl protecting group followed by the treatment with methanolic ammonia to give the final products **10-17**.[9-16] Some uracil derivatives have been also prepared by condensation of the appropriate silylated heterocyclic bases with the dioxolane acetate **1** followed by a routine work-up procedure. The structural assignments of the synthesized derivatives were made on the basis of the ^1H NMR studies. *Cis* - and *rans* - arrangements of the 5'-CH_2 group with the cytosine ring were established by the nuclear Overhauser effect.

The anti-HBV activity of the newly synthesized *D*-dioxolane cytosine nucleosides indicated that the 5-fluoro-cytosine derivative exhibited the most potent anti-HBV activity while the 5-chloro and 5-bromo derivatives exhibited significantly less potent anti-HBVactivity in a human hepatoma cell line carrying the HBV (2.2.15 cells). The 5-iodo derivative was also found to be less potent than the 5-fluoro derivative. Previously, we have found that the β-*D*-cytosine derivative (EC_{50} 0.01 μM) exhibited potent anti-HBV activity.[7] Several uracil (uracil, 5-bromo, and 5-fluoro) and cytosine (5-methyl-cytosine) derivatives have also been evaluated as potential anti-HBV agents. However, these compounds did not exhibit any significant antiviral activity. In order to compare the anti-HBV potency, several other cytosine nucleosides are also included in Table 1.

In summary, we have studied the structure-activity relationships of *D*-dioxolane-cytosine nucleosides as anti-HBV agents and have discovered that the cytosine and 5-fluoro-cytosine derivatives are the most potent anti-HBV agents with less cellular toxicity than the β-*L*-5-fluoro-cytosine derivative. Therefore, any modification other than the 5-fluoro group at C5 position of the pyrimidine ring significantly reduces the anti-HBV activity. In view of the high selectivity (>1500) exhibited by the cytosine and 5-fluoro-cytosine derivative, further virological and biochemical studies are warranted.

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References and notes

1. Doong, S. L.; Tsai, C. H.; Schinazi, R. F.; Liotta, D. C.; Cheng, Y.-C. *Proc. Natl. Acad. Sci.* **1992**,*88*, 8495.
2. Furman, P. A.; Davis, M.; Liottta, D. C.; Paff, M.; Frick, L. W.; Nelson, D. J.; Dornsife, R. E.; Wurster, J. A.; Wilson, L. J.; Fyfe, J. A.; Tuttle, J. V.; Miller, W. H.; Condreay, L.; Averett, D. R.; Schinazi, R. F.; Painter, G. R. *Antimicrob. Agents and Chemother.* **1992**, *36*, 2686.
3. Gosselin, G.; Schinazi, R.F.;Sommadossi, J.-P.; Mathe, C.; Bergone, M.-C.; Aubertin, A.-M.; Kirn A.; Imbach, J.-L. Antimicrob. Agents Chemother. **1994**, *38*, 1292.
4. Lin, T. S.; Luo, M. Z.; Pai, S. B.; Dutschuman, G. E.; Cheng, Y.-C. *J. Med. Chem.* **1994**, *37*, 798.

5. Chu, C. K.; Ma, T. W.; Shanmuganathan, K.; Wang, C. G.; Xiang, Y. J.; Pai, S. B.; Yao, G. Q.; Sommadossi, J.-P.; Cheng, Y. C. *Antimicrob. Agents Chemother.* **1995**, *39*, 979.
6. Schinazi, R. F.; Chu, C. K.; Korba, B. E.; Sommadossi, J.-P. *Abstract 33rd ICAAC, New Orleans,* **1993**, 222427.
7. Kim, H. O.; Shanmuganathan, K.; Alves, A. J.; Jeong, L. S.; Beach, J. W.; Chang, C. N.; Chu, C. K. *Tetrahedron Lett* . **1992**, *33*, 6899.
8. Kim, H. O.; Ahn, S. K.; Alves, A. J.; Beach, J. W.; Jeong, L. S.; Choi, B. G.; Van Roey, P.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **1992**, *35*, 1987.
9. Compound **10**: m.p.181-182°C (MeOH-ether); $[\alpha]_D^{25}$ 54.6 (c 0.85, MeOH); UV (H_2O) λ_{max} 280 nm (ε 9,000) (pH7), 286 nm (ε 12000) (pH 2), 279 nm (ε 9400) (pH 11); NMR ^1H (DMSO-d_6) δ 8.17 (d, 1H, H_6), 7.8-7.5 (br s, NH_2), 6.13 (m, 1H, $H_{1'}$), 4.92 (br s, OH), 4.14 (dd, 1H, $H_{2'a}$), 4.07 (dd, 1H, $H_{2'b}$), 5.32 (t, 1H, $H_{4'}$), 3.68 (m, 1H, $H_{5'}$); Anal Calcd for ($C_8H_{10}FN_3O_4$) C: 41.56, H: 4.36, N: 18.18. Found; C: 41.66, H: 4.40, N: 18.08.
10. Compound **11**: m.p.153-154°C (MeOH-ether); $[\alpha]_D^{25}$ -76.3 (c 0.50, MeOH); UV (H_2O) λ_{max} 280 nm (ε 9100) (pH 7), 286 nm (ε 11,400) (pH 2), 280 nm (ε 9100) (pH 11); NMR ^1H (DMSO-d_6) δ 7.80 (d, 1H, H_6), 7.57 (br s, NH_2), 6.04 (m, 1H, $H_{1'}$), 5.47 (t, 1H, $H_{4'}$), 5.01 (t, OH)4.29 (dd, 1H, $H_{2'a}$), 3.94 (dd, 1H, $H_{2'b}$), 3.43 (m, 1H, $H_{5'}$); Anal Calcd for ($C_8H_{10}FN_3O_4$) C: 41.56, H: 4.36, N: 18.18. Found; C: 41.49, H: 4.37, N: 18.08.
11. Compound **12**: m.p.194-195°C (MeOH-ether); $[\alpha]_D^{25}$ 41.9 (c 0.4, MeOH); UV (H_2O) λ_{max} 281 nm (ε 7500) (pH 7), 291 nm (ε 10500) (pH 2), 281 nm (ε 7800) (pH 11); NMR ^1H (DMSO-d_6) δ 8.32 (s, 1H, H_6), 7.27-7.85 (br d, NH_2), 6.13 (dd, 1H, $H_{1'}$), 5.31 (t, OH), 4.15 (dd,1H, $H_{2'a}$), 4.09 (dd, 1H, $H_{2'b}$), 4.94 (t, 1H, $H_{4'}$); 3.68 (dd, 1H, $H_{5'}$); Anal calcd for ($C_8H_{10}ClN_3O_4.1H_2O$) C: 36.14, H: 4.55, N: 15.82, Cl: 13.34. Found; C: 36.44, H: 4.47, N: 15.67, Cl: 13.43.
12. Compound **13**: m.p.175-176 °C (MeOH-ether); $[\alpha]_D^{25}$ -51.4 (c 0.80, MeOH); UV (MeOH) λ_{max} 282 nm (ε 8600) (pH 7), 291 nm (ε 12300) (pH 2), 282 nm (ε 9200) (pH 11); NMR ^1H (DMSO-d_6) δ 7.82 (s, 1H, H_6); 7.3-7.9 (br d, NH_2); 6.00 (dd, 1H, $H_{1'}$); 4.98 (t, 1H, $H_{4'}$); 4.31 (dd, 1H, $H_{2'a}$); 3.97 (dd, 1H, $H_{2'b}$), 3.43 (m, 1H, $H_{5'}$); Anal calcd for ($C_8H_{10}ClN_3O_4 \cdot 0.12$ EtOAC) C: 40.01, H: 4.14, N: 16.27, Cl: 13.73. Found; C: 39.89, H: 4.40, N: 16.41, Cl: 14.07.
13. Compound **14**: m.p. 199-200°C(MeOH-ether); $[\alpha]_D^{25}$ 35.1 (c 0.7, MeOH); UV (H_2O) λ_{max} 282 nm (ε 7200) (pH 7), 293 nm (ε 9200) (pH 2), 283 nm (ε 7300) (pH 11); NMR ^1H (DMSO-d_6) δ 8.39 (s, 1H, H_6), 7.6-7.9 (br d, NH_2), 6.17 (d, 1H, $H_{1'}$), 5.37 (t, OH); 4.95 (t, 1H, $H_{4'}$), 4.16 (d, 1H, $H_{2'a}$); 4.07 (dd, 1H, $H_{2'b}$), 3.68 (m, 1H, $H_{5'}$); Anal Calcd for ($C_8H_{10}BrN_3O_4$) C: 32.90, H: 3.45, N: 14.39, Br: 27.36. Found; C: 32.71, H: 3.40, N: 14.22 , Br: 27.21.
14. Compound **15**: m.p. 203-205°C (MeOH-ether); $[\alpha]_D^{25}$ -39.9 (c 0.92, MeOH); UV (H_2O) λ_{max} 282 nm (ε 7900) (pH 7), 293 nm (ε 11600) (pH 2), 283 nm (ε 8900) (pH 11); NMR ^1H (DMSO-d_6) δ 7.8 (s, 1H, H_6); 7.50 (br s, NH_2), 6.00 (dd, 1H, $H_{1'}$), 5.48 (t, 1H, $H_{4'}$);, 5.01(t, OH), 4.30 (dd, 1H, $H_{2'a}$), 3.97 (dd, 1H, $H_{2'b}$), 3.43 (m, 1H, $H_{5'}$); Anal Calcd for ($C_8H_{10}N_3BrO_4$) C: 32.90, H: 3.45, N: 14.39, Br: 27.36. Found C: 33.04, H: 3.48, N: 14.29, Br: 27.28
15. Compound **16**: m.p. 176 dec MeOH-ether); $[\alpha]_D^{25}$ 17.4 (c 0.72, MeOH); UV (H_2O) λ_{max} 289.4 nm (ε 6100) (pH 7), 303.7 nm (ε 8800) (pH 2), 207.5 nm (ε 6200) (pH 11); NMR ^1H (DMSO-d_6) δ 8.4(s, 1H, H_6), 7.82 (br s, NH_2), 6.11 (dd, 1H, $H_{1'}$), 4.95 (s, 1H, $H_{4'}$), 4.08 (d, 2H, H_2), 3.64 (dd, 1H, $H_{5'}$); Anal calcd for ($C_8H_{10}IN_3O_4$. 0.15 EtOAC) C: 29.32, H: 3.20, N: 11.93, I: 35.78. Found; C: 29.54, H: 3.17, N: 12.04.
16. Compound **17**: m.p. 184 dec MeOH-ether); $[\alpha]_D^{25}$ -21.1 (c 0.8, MeOH); UV (H_2O) λ_{max} 288.9 nm (ε 6500) (pH 7), 303.2 nm (ε 8200) (pH 2), 290.0 nm (ε 6500) (pH 11); NMR ^1H (DMSO-d_6) δ 7.85 (s, 1H, H_6), 7.52 (br s, NH_2), 6.02 (dd, 1H, $H_{1'}$), 5.46 (t, 1H, $H_{4'}$), 4.30 (dd, 1H, $H_{2'a}$), 3.95 (dd, 1H, $H_{2'b}$), 3.45 (dd, 1H, $H_{5'}$); Anal Calcd for ($C_8H_{10}IN_3O_4$) C: 28.34, H: 2.97, N: 12.39, I: 37.43. Found; C: 28.61, H: 2.99, N: 12.2, I: 37.28.

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