*FABAD J. Pharm. Sci., 26, 129-136, 2001*

*BİLİMSEL TARAMALAR / SCIENTIFIC REVIEWS*

# Nucleosides as Anti-Hepatitis B Virus Agents

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***Nucleosides as Anti-Hepatitis B Virus Agents***

***Summary :*** *The nucleoside analogs, such as 3TC, FTC and L-FMAU appear to be the only promising class of compounds for the management of chronic HBV infection. In this paper, anti-HBV effects of these compounds and some other new derivatives, which were synthesized recently, were discussed.*

***Key Words:*** *Nucleosides, Anti-hepatitis Agents, HBV infection*

***Anti-Hepatit B Virüs Ajanı Nükleositler***

***Özet :*** *Yalnızca 3TC, FTC ve L-FMAU gibi bir sınıf nükleosit analoğu, kronik HBV infeksiyonlarının tedavisi için ümit verici bileşikler olarak ortaya çıkmışlardır. Bu derlemede, bu bileşiklerin ve son zamanlarda sentezlenen diğer bazı yeni bileşiklerin anti-HBV etkileri tartışılmıştır.*

***Anahtar kelimeler :*** *Nukleositler, Anti-Hepatit bileşikler, HBV enfeksiyonu*

*Received : 15.5.2001*
*Revised : 27.6.2001*
*Accepted : 28.6.2001*

Pandemic morbidity and mortality due to the hepatitis B virus (HBV) have been responsible for the intensive efforts in discovering more effective and less toxic antiviral agents. Additionally, despite over 300 million HBV chronic carriers, no effective and safe chemotherapeutic agents are available today for the treatment of these patients.

In fact hepatitis is not only a disease, a term that means inflammation of the liver. Since the liver plays a central role in human metabolism, just about any virus is capable of affecting it.

However, there are a number of viruses that seem expressly intent on infecting and damaging liver cells, the so-called hepatitis virus that scientist have given separate initials: hepatitis Virus A, B, C, D, E and G. Each of these alphabetical microbes belongs to a separate virus family.

Hepatitis B virus (HBV) can cause a serious form of hepatitis. HBV infection is responsible for both acute and chronic hepatitis. Acute HBV infection can be variable with most individuals showing no obvious clinical sign of the disease. Generally, at the end of incubation period, a flue-like illness with fever, fatigue, malaise and in some cases jaundice occurs.

Hepatitis B may develop into a chronic disease (lasting more than 6 months) in up to 10% of the 200,000 newly infected people annually. If left untreated, the risk of developing cirrhosis (scarring of the liver) and liver cancer is increased in patients with chronic hepatitis B.

Hepatitis B virus is spread through contact with infected blood. There are many ways of coming into contact with blood, such as from cuts, nosebleeds and menstrual blood. Even the tiniest amount of blood on common objects such as a toothbrush, razor or a man-

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icure instrument can carry enough of the virus to infect someone. Hepatitis B is also frequently spread through sexual contact and from mother to baby at birth. Infants born to HBV-infected mothers can contract the virus in up to 90% of cases. Approximately one-third or more of the hepatitis B cases result from unknown sources. A vaccine is available for individuals who are routinely exposed to blood in their work or who live with a person infected with hepatitis B.

Hepatitis B virus belongs to the family of *hepadnaviridae*, which is composed of several animal viruses, including human hepatitis B virus (HBV), woodchuck hepatitis (WHV), ground squirrel hepatitis virus (GSHV) and duck hepatitis B virus (DHBV). These viruses share common features such as genome organization, mode of replication and similar tropism for hepatocytes[1]. HBV has a small genome, which is composed of circular, partially double-stranded DNA[2].

The diagram of the HBV lifecycle is shown in Fig.1. The initial events (attachment, entry) remain poorly understood, and the cellular receptor for HBV is unknown. After initial virus entry, the viral core particle is translocated into the nucleus of the host cell, and the viral DNA is then repaired and matured, giving rise to a covalently closed circular DNA (cccDNA or supercoiled DNA). The cccDNA remains episomal and serves as a template for cellular RNA polymerase II, giving rise to several viral RNA transcripts. The largest of these RNA's serve as both an mRNA for the viral polymerase and the pregenomic RNA (pregRNA), which is slightly larger than the genomic size, and is packaged into viral particles. At the same time, the smaller RNA transcripts are translated into the viral structural proteins. The synthesis of viral DNA is accomplished with the reverse transcription of the pregRNA to the minus strand DNA by viral polymerase, followed by the synthesis of a shorter plus strand DNA to give a partially double stranded viral DNA. This newly synthesized viral DNA can be utilized as a resource for the cccDNA or functions as the viral nucleic acids in the matured virions budding out from the host cells.

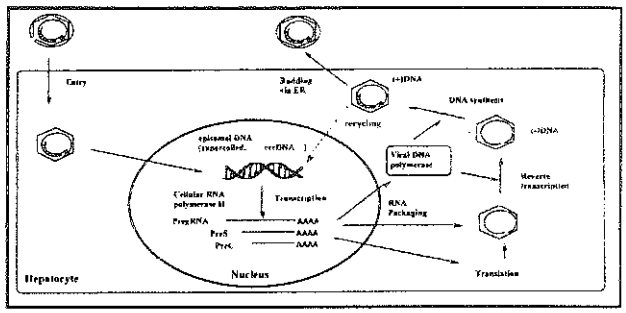


Figure 1. HBV Lifecycle[3]

The lack of an *in vitro* tissue culture system to propagate the hepatitis B virus has hampered the molecular biology studies as well as the screening of antiviral compounds. In 1987, Sells et al.[4] reported the establishment of an *in vitro* cell system, in which a human hepatoblastoma cell line (HepG2) is transfected with a plasmid carrying the hepatitis B virus genome. The cell line was designated as HepG2 2.2.15 cells, and it can constantly produce the HBV specific components, including the infectious Dane particles, HBsAg and HBeAg[5]. The validity of this system has been demonstrated since the virions produced by these cells can cause HBV infections in chimpanzees[6]. Generally, the HBV specific proteins (HBsAg or HBeAg) can be assayed by solid phase radioimmunoassay (RIA) and the viral DNA by southern-blot hybridization. Recently, polymerase chain reaction (PCR) technology has also been applied in the quantitation of viral DNA level[7]. Several other *in vitro* cell cultures have also been reported, including HB611[8,9], and duck hepatocytes[10]. It was found that antiviral compounds display different antiviral potency in different cell lines, probably due to the different metabolic rate[11]. However, the HepG2 2.2.15 cell system is the most commonly used cell line for *in vitro* screening of anti-HBV compounds. The establishment of experimental animal models (ducks, woodchucks) has also greatly facilitated the *in vivo* drug studies. Many of the nucleoside analogues have been studied and are currently under investigation as anti-HBV agents.

## Carbocyclic nucleosides

Carbohydrate-modified nucleosides are one of the major classes of compounds used as antiviral agents (Figure 2). The carbocyclic analogue of 2'-

deoxyguanosine (2'-CDG) was the first carbocyclic nucleoside reported to have potent antiviral activity exhibiting an inhibitory effect on HBV replication[12].

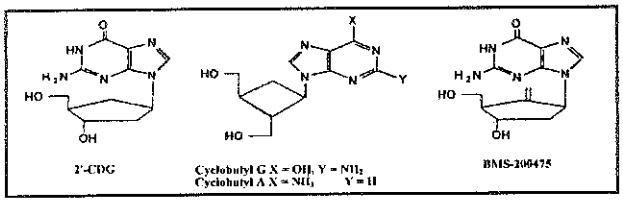


Figure 2. Carbohydrate-modified nucleosides

Several other carbocyclic nucleosides have also been reported to exhibit anti-HBV activity. For example, cyclobutyl G (Lobucavir) and cyclobutyl A are important compounds of this class[13]. Most recently, a novel carbocyclic nucleoside, BMS-200475, has been reported to exhibit potent anti-HBV activity[14]. BMS-200475 is currently undergoing phase I clinical trials as an anti-HBV agent.

Recently, cyclopropyl carbocyclic nucleosides have been synthesized and were evaluated for antiviral activity (Figure 3). The guanine analog showed moderate anti-HBV activity in 2.2.15 cells[15].

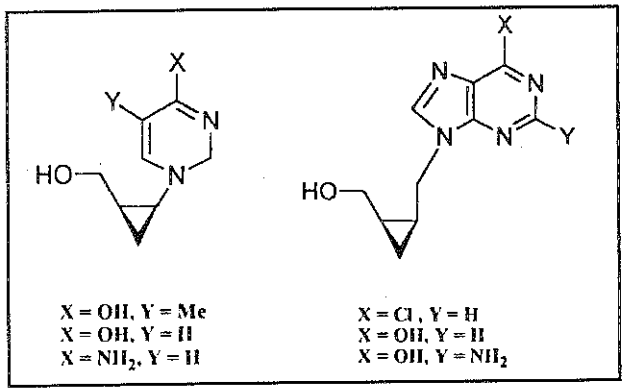


Figure 3. 1-[2-(Hydroxymethyl) cyclopropyl] methyl nucleosides

## 2', 3'-Dideoxy nucleosides

Since the replication strategy of HBV resembles that of the retroviruses, in particular the reverse transcription, many 2',3'-dideoxy nucleoside RT (Reverse Transcriptase) inhibitors have been studied as potential anti-HBV agents (Figure 4). AZT (3'-azido-3'-deoxythymidine), the first approved anti-HIV agent, was found to be ineffective against HBV in the cell culture assays (2.2.15 cells). Although ddA (2',3'-dideoxyadenosine) showed a significant inhibitory effect on DHBV replication[16], the clinical trial with the parent drug ddI (2',3'-dideoxyinosine) indicated that it was not effective against chronic hepatitis B virus in humans[17]. In a cell culture study, the anti-HBV activities of several 2',3'-dideoxynucleosides were compared[18]. It was found that ddC (2',3'-dideoxycytosine) exhibits a significant inhibitory effect on the replication of HBV DNA. Another analogue, ddG (2',3'-dideoxyguanine), also shows significant antiviral effect at the same level, although the ddDAP (2',3'-dideoxy, 2,6-diaminopurine) derivative is less potent in vitro[18].

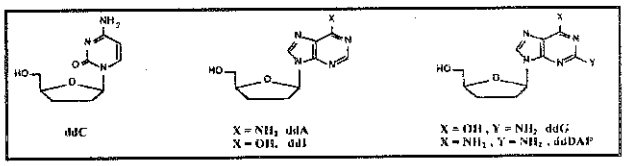


Figure 4. 2',3'-dideoxy nucleosides

## 2'-Fluoro-arabinofuranosylpyrimidine nucleosides

The 2'-fluoro-substituted arabinofuranosylpyrimidine nucleosides (Figure 5) have shown potent in vitro and in vivo activity against medically important herpes viruses[19-22].

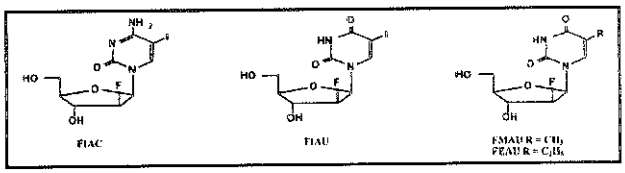


Figure 5. 2'-fluoro substituted arabinofuranosyl nucleosides

Analogues, FIAC (2'-fluoro, 5-iodo arabinofuranosyl cytosine), FMAU (2'-fluoro, 5-methyl arabinofuranosyl uracil) and FEAU (2'-fluoro, 5-ethyl arabinofuranosyl uracil) have been shown to inhibit WHV replication in chronically infected woodchucks[23]. To a lesser extent, FMAU and FIAC (Figure 5) demonstrated activities against DHBV in the duck models[24]. Another analogue, FIAU, has been shown to reduce the level of HBV DNA in patients with chronic hepatitis B virus[25]. In a cell culture study, FIAC and FIAU (Figure 5) were reported to inhibit HBV replication by 90% at concentrations of 34.7 and 24.4 μM, respectively. Although the mechanism of action underlying the anti-HBV activities of these

compounds is not well characterized, FIACTP (2'-fluoro, 5-iodo arabinofuranosyl cytosine triphosphate) has been shown to inhibit endogenous HBV DNA polymerase activity, which suggests that the anti-HBV activity of this class of compounds may be mediated, in part, at the level of viral DNA polymerase[26].

In view of findings about these nucleosides, Olgen, et al.[27], synthesized 2'-deoxy-2'-fluoro D- and L- arabinofuranosyl 1,2,3-triazole and imidazole derivatives as anti-HBV agents (Figure 6). The fluoroarabinofuranosyl glycoside was combined with heterocyclic triazole and imidazole structures. Unfortunately, no significant activities against HBV were found for the synthesized nucleosides. All compounds have shown 50% inhibition activity at (100 μM against HBV.

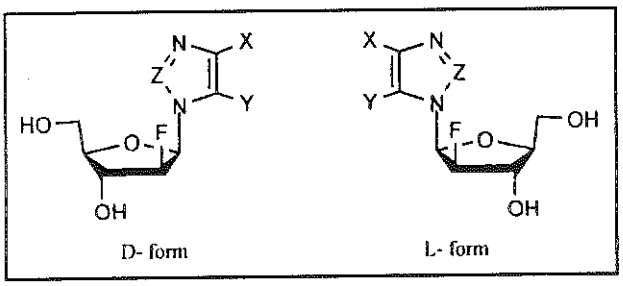


Figure 6. 2'-deoxy-2'-fluoro - D and L- arabinofuranosyl 1,2,3-triazole and imidazoles

**Oxathiolane, dioxolane nucleosides and L-nucleosides**

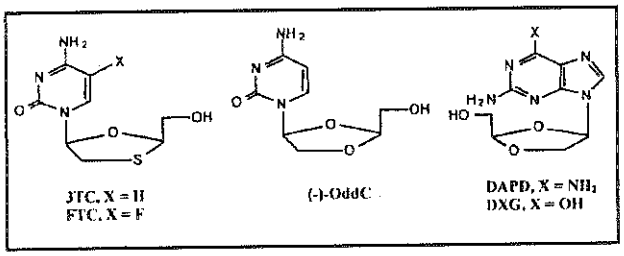


Figure 7. Oxathiolane and dioxolane nucleosides

In addition to its *in vitro* anti-HBV activity, 3TC [(-)-β-L-2',3'-dideoxy-3'-thiacyctidine] has also been shown to effectively suppress DHBV in ducks and HBV in chimpanzees (Figure 7)[28]. In patients with HIV infection and chronic hepatitis B, 3TC rapidly reduced HBV-DNA with no serious adverse effects[29]. In a recent phase II clinical trial, the HBV-DNA levels became undetectable in 70% of the patients who received 25 mg dose[30]. 3TC has recently been approved by FDA for the treatment of AIDS patients in combination with AZT and is undergoing phase-III clinical trials as an anti-HBV agent.

FTC, (cis-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan - 5-yl]-cytosine) (Figure 7), has been shown to exhibit potent anti-HBV activity *in vitro* ($EC_{50}$ 0.01 μM) in the hepatoma cell lines[31,32]. Additionally, FTC also showed strong inhibition to the replication of DHBV in vivo in chronically infected ducks[33]. The D-enantiomer was not as potent as the L-isomer and it did not show significant cytotoxicity, either. Biological studies suggested that the β-L-isomer can be efficiently phosphorylated by dCK (deoxy Cytidine Kinase). However, the β-D-isomer is not a good substrate for the cytidine deaminase, which degrades the D-isomer at a much higher rate than the L-isomer. This difference between the D and L-isomers towards anabolic and catabolic enzymes results in the enhanced antiviral potency of the L-isomer. Recently, it was reported that neither the D-, L-isomer nor the racemate of (±)-FTC show any dose-dependent adverse effects on the mitochondrial function. FTC is currently undergoing phase II clinical trials as an anti-HBV agent. Extensive structure-activity relationship studies led to the discovery of L-(-)-OddC β-L-dioxolane cytosine) and DAPD (β-2,6-diaminopurine dioxolanes) (Figure 7). L-(-)-OddC exhibits extremely potent anti-HIV activity in cell cultures [$EC_{50}$ 2 and 5 nM in PBM (Peripheral Blood Mononuclear) and CEM (Human T-cells Lymphoblastic Leukemia cells) cells, respectively] and anti-HBV activity ($EC_{50}$ 0.5 nM in 2.2.15 cells)[34]. Since L-(-)-OddC inhibits the growth of hepatocellular and prostate tumors that are generally difficult to treat, it is currently being developed as an anti-cancer agent[35]. DAPD is the 2,6-diaminopurine dioxolane analogue with the sugar moiety in the natural D-configuration. DAPD exhibits potent activity against both HIV[36] ($EC_{50}$ 0.03 μM in PBM cells) and HBV[37] ($EC_{50}$ 0.09 μM in 2.2.15 cells) with a favorable toxicity profile. Animal studies indicated that DAPD is the prodrug of dioxolane-guanine (DXG), and it can be converted to DXG *in vivo* by adenosine deaminase (ADA)[38,39]. Currently, DAPD is under-

going preclinical studies as an anti-HIV and anti-HBV agent.

The interesting findings that some of the nucleosides with unnatural L-configuration show more potent antiviral activity with lower cytoxicity than corresponding D-counterparts led to the extensive screening of L-nucleosides (Figure 8) as potential antiviral agents. In this regard, several new anti-HBV agents have been discovered.

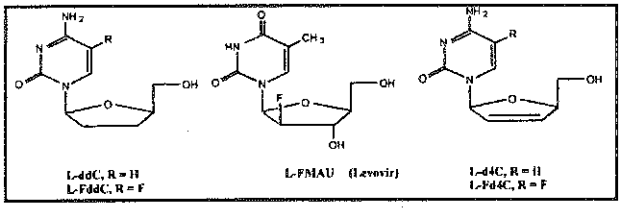


Figure 8. Structures of some active L-nucleosides

The L-enantiomer of the anti-HIV drug ddC was reported to exhibit potent anti-HBV activity ($EC_{50}$ 0.01 μM, 2.2.15 cells). Its 5-fluoro congener (L-FddC) (Figure 8) showed not only potent anti-HBV activity ($EC_{50}$ 0.01 μM) but also potent anti-HIV activity without significant toxicity in cell cultures[40,41]. Recently, L-FddC has also been shown to be a strong inhibitor of DHBV both in vitro and in vivo[42]. Two other L-nucleosides, namely L-Fd4C (L-2'-fluoro-2',3'-dideoxycytosine) and L-d4C (L-2',3'-didehydro-2',3'-dideoxycytosine) (Figure 8) have also been reported to have potent antiviral activities[43]. L-Fd4C shows extremely potent anti-HBV activity ($EC_{50}$ 2 nM in 2.2.15 cells) and anti-HIV activity ($EC_{50}$ 0.09 μM in CEM cells), whereas L-d4C is less potent against both viruses (8 nM and 1.0 μM for HBV and HIV, respectively).

Another L-nucleoside, L-FMAU [1-(2-fluoro-5-methyl-β-L-arabinofuranosyl)uracil], (Figure 8) was reported by Chu et al.[44], as a potent antiviral agent against HBV ($EC_{50}$ 0.1 μM in 2.2.15 cells). L-FMAU was found to have a low cytotoxicity while the D-counterpart, D-FMAU, was determined to be less active and significantly more toxic. In addition to its potent anti-HBV activity, L-FMAU also exhibits potent anti-EBV activity. The anti-HBV mechanism of L-FMAU has been reported by Pai et al.[45]. It was found that L-FMAU produced a dose-dependent inhibition to the viral DNA replication in 2.2.15 cells with a 50% inhibitory concentration at 0.1 μM. There was no inhibitory effect on HBV transcription or protein synthesis. L-FMAU was found to be metabolized in cells by the cellular thymidine kinase and deoxycitidine kinase to its monophosphate, and subsequently to the di- and triphosphate by some other unknown enzymes (Figure 9).

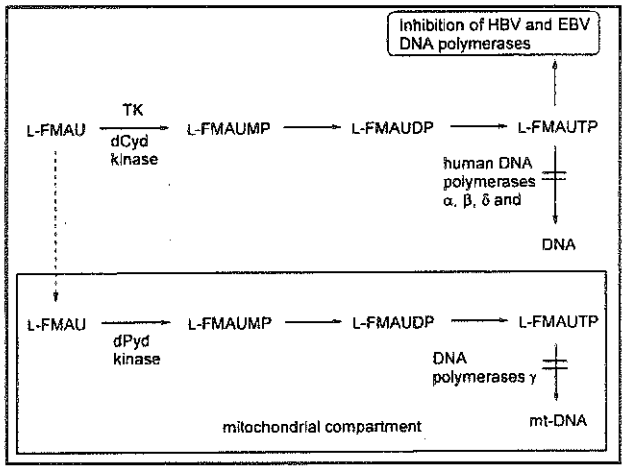


Figure 9. Proposed metabolism of L-FMAU[46]

Although the precise mechanism of action of L-FMAU is not clear, a dose-dependent inhibition of HBV DNA synthesis by L-FMAUTP (L-5-methyl-2-fluoro arabinofuranosyl uracil triphosphate) was observed in the DNA polymerase assays with isolated HBV particles, suggesting that inhibition of viral DNA polymerase may account for its anti-HBV activity.

In addition to its *in vitro* anti-HBV activity, L-FMAU also exhibits potent activity against DHBV in primary duck hepatocytes ($EC_{50}$ 0.1 μM)[47,48]. The pharmacokinetics of L-FMAU in rats have been reported by Wright et al.[49]. A linear disposition of L-FMAU, was observed over the dosage of 10 to 50 mg/kg after intravenous administration. L-FMAU with these outstanding features, is considered as a promising clinical agent for the treatment of chronic HBV infections[50].

Recently, a different class of nucleoside containing the imidazo [4, 5-e] [1, 3] diazepine heterocyclic ring system was also reported by Chen et al.[51]. Among the synthesized nucleosides, 6-amino-8-hydroxy-4H-1-β-D-ribofuranosylimidazo[4,5-e] [1,3] diazepine-4-one was found to be a potent anti-hepatitis B virus agent (Figure 10).

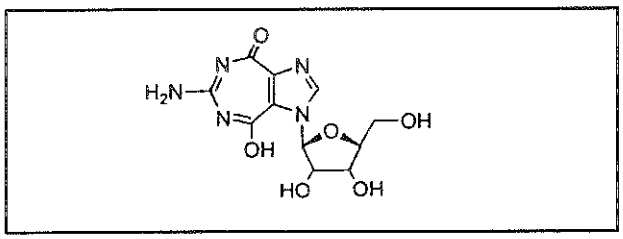

Figure 10. Structure of 6-amino-8-hydroxy-4H-1-β-D-ribofuranosylimidazo[4,5-e] [1,3] diazepine-4-one.

The other nucleosides based on oxaselenolane structure were synthesized and evaluated for anti-HBV activity[52]. Among the synthesized racemic nucleosides, cytosine ($EC_{50}$ 1.2 μM) and 5-fluorocytosine ($EC_{50}$ 1.2 μM) analogues exhibited potent anti-HBV activity (Figure 11).

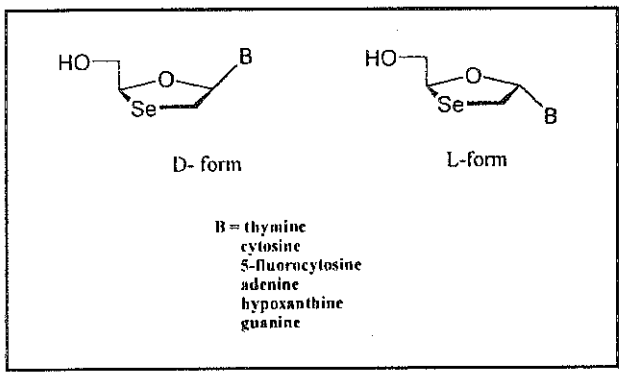


Figure 11. Structure of 1-[2-(hydroxymethyl)-1,3-oxaselenolane nucleosides

## Conclusion

In summary, the foregoing discussion indicates that, among a number of anti-HBV agents studied *in vitro* and *in vivo*, the nucleoside analogs, such as 3TC, FTC and L-FMAU appear to be the only promising class of compounds for the management of chronic HBV infection. Since there are still no effective and safe chemotherapeutics for the treatment of hepatitis, it is obvious that further studies are necessary for the development of new antiviral drugs.

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