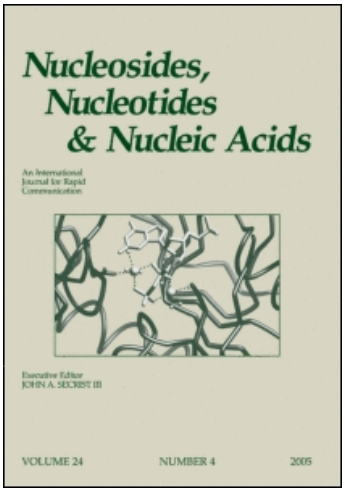

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2'-Deoxy-4'-*C*-Ethynyl-2-Fluoroadenosine: A Nucleoside Reverse Transcriptase Inhibitor with Highly Potent Activity Against Wide Spectrum of HIV-1 Strains, Favorable Toxic Profiles, and Stability in Plasma

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2′-DEOXY-4′-*C*-ETHYNYL-2-FLUOROADENOSINE: A NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR WITH HIGHLY POTENT ACTIVITY AGAINST WIDE SPECTRUM OF HIV-1 STRAINS, FAVORABLE TOXIC PROFILES, AND STABILITY IN PLASMA

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□ *Working hypotheses to solve the critical problems of the existing highly active anti-retroviral therapy were proposed. The study based on the hypotheses proved the validity of the hypotheses and resulted in the development of 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, with highly potent activity against all HIV-1, very favorable toxic profiles, and stability in plasma. The nucleoside will prevent or delay the emergence of drug-resistant HIV-1 variants and be an ideal therapeutic agent for both HIV-1 and HBV infections.*

Keywords 2′-Deoxy-4′-*C*-ethynyl nucleoside; 2,′3′-dideoxynucleoside; nucleoside reverse transcriptase inhibitors; drug resistant HIV; HAART

INTRODUCTION

Highly active antiretroviral therapy (HAART) has dramatically improved the quality of life and prognosis of patients infected with HIV-1. However, the existing HAART has the following critical problems,[1] emergence of drug-resistant HIV-1 variants,[2] requirement of frequent and large dose of drugs, and[3] side effects of drugs.

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One of the authors (H.O) proposed working hypotheses to solve the problems on the basis of the fundamentals of both organic chemistry and biochemistry, and his past findings of relationships between the structures of nucleoside derivatives and biological activity. They are composed of the following three ways.

1) The Way to Prevent the Emergence of Drug-Resistant HIV-1 Strains

All clinical nucleoside reverse transcriptase inhibitors (**NRTI**s) belong to the family of 2′,3′-dideoxynucleoside (**ddN**). The **ddN** structure has been assumed essential for nucleoside derivatives to be anti-HIV active, i.e., to be the chain terminator of proviral DNA biosynthesis. However, HIV-1 variants resistant to all these clinical **NRTI**s emerged.

Resistance to these **ddN**s means that HIV-1 can acquire the ability to discriminate between **ddN** and physiologic 2′-deoxynucleoside (**dN**) and does not accept **ddN** into the active center of its reverse transcriptase (RT) and/or selectively cut off the incorporated **ddN** from proviral DNA terminus. Therefore, the nucleoside that could prevent the emergence of drug-resistant HIV-1 variants must satisfy the following conditions.[1] To prevent the discrimination from **dN** by HIV, the nucleoside must have the structure very much like **dN**. Therefore, the nucleoside must have 3′-OH.[2] In spite of having 3′-OH, the nucleoside must be the chain terminator of proviral DNA biosynthesis. 4′-*C*-Substituted-2′-deoxy nucleoside (**4′SdN**) was designed as the nucleoside that could satisfy these conditions on the basis of the following two hypotheses: a) It will be difficult for HIV to discriminate between **dN** and **4′SdN** because **4′SdN** is very much like **dN**. b) The neopentyl-type secondary 3′-OH of **4′SdN** would be too unreactive to be used for elongation of proviral DNA biosynthesis. Thus, **4′SdN** could be the chain terminator of proviral DNA biosynthesis.

2) The Way to Decrease the Toxicity of Nucleosides

Most of nucleoside antibiotics have the structures of one position modified physiologic nucleosides. In 1960s and 1970s many organic chemists synthesized the hybrids of nucleoside antibiotics, and nucleoside derivatives modified at two or more positions of physiologic nucleosides expecting to obtain nucleoside derivatives having more potent biological activity. However, none of them showed notable biological activity. These results suggested that the intracellular important enzymes do not recognize these modified nucleosides as their substrates. Therefore, it is expected that the toxicity of **4′SdN**s could be decreased by additional modification.

3) The Way to Provide Nucleosides with Stability to Both Enzymatic and Acidic Glycolysias

The lone pair of the ring oxygen plays an important role in both enzymatic and acidic glycolysis of nucleosides by participating to form an oxocarbonium ion. The steric hindrance between the 4′-substituent and 3′-OH of **4′SdN** makes the ring conformation into 3′-endo (*N*-type). It will be difficult for the lone pair of the ring oxygen of **4′SdN** with 3′-endo conformation to form an oxocarbpnium ion because the three bonds, C4-O-C1-C2, are hard to be co-planar. Thus, the introduction of a substituent at the 4′-position of nucleosides provide them with stability to both enzymatic and acidic glycolysis.

RESULTS AND DISCUSSION

At first, the validity of the hypotheses was confirmed with 4′-*C*-methyl nucleosides (**4′MeN**s)[1,2] Structure-activity relationship study on **4′SdN**s revealed that the relative order of anti-HIV activity by the substituents was $C \equiv CH \geq CN > N_3 > CH = CH_2 > Me = Et > C \equiv CH\text{-}CH_3$ and that purine analogs were generally less toxic than pyrimidine analog. Finally we developed 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine (**4′Ed2FA**),[3–7] a nucleoside derivative modified at two positions (2 and 4′) of physiologic 2′-deoxyadenosine (**dA**), that is highly active against all existing HIV-1 strains, has low toxicity, and is stable to both enzymatic and acidic glycolysis.[7,8]

Biological Properties of 4′Ed2FA[7,8]

1) **Anti-HIV activity**. EC_{50}(Wild type) = 0.2 nM,[cf. **AZT** = 22 nM]; EC_{50}(MDR) = 0.14 nM, [cf. **AZT** = 15,300 nM]; EC_{50}(M184V) = 3.1 nM,[cf. **AZT** = 16.0 nM].
2) **Toxicity**. DNA polymerase α: $IC_{50} > 200$ μM, DNA polymerase β: $IC_{50} > 200$ μM. Human mitochondrial DNA polymerase γ: $IC_{50} = 10$ μM, [cf. **ddA**: $IC_{50} = 0.2$ μM.]. Mouse toxicity: No acute toxicity up to 100 mg/kg by both oral and intravenous administration.
3) **Stability to enzymatic and acidic glycolysis**.[7,8] Half-life of **4′Ed2FATP** in plasma: $T_{1/2} \sim 18$ hours. [cf. **AZTTP**: $T_{1/2} \sim 3$ hours]. About 50% of the cells were protected against the infection of HIV for 24 hours after removal of extracellular **4′Ed2FA** in both MT4 cells and MAGI cells cultured in the presence of 0.1 μM of **4′Ed2FA**. **4′Ed2FA** is completely stable to adenosine deaminase under the conditions where **4′EdA** was completely deaminated within 60 minutes. Only a small part (3%) was hydrolyzed under the acidic conditions of gastric juice (pH 1.06) at 24°C, while **ddA** was completely decomposed in 5 minutes.

SUMMARY

A study on the synthesis and biological evaluation of **4′SdN**s was conducted based on the hypotheses proposed based on the fundamentals of both organic chemistry and biochemistry, and old scientific findings. The study proved the validity of the hypotheses and resulted in the development of **4′Ed2FA**. **4′Ed2FA** is highly potent against all HIV-1s, is stable to intracellular catabolism and acidic degradation, has a very long intracellular $T_{1/2}$, does not greatly inhibit DNA polymerase γ and does not have acute mouse toxicity. These results strongly suggest that **4′Ed2FA** deserves further study for the development of a highly potent therapeutic agent for HIV infection, which will solve the problems of the existing HAART.

In addition, it should be noted that **4′Ed2FA** could be an ideal drug for both HIV and HBV infections. Hepatitis B virus (HBV) is a DNA virus, however, it also belongs to the family of retrovirus because it uses RT when it replicates. It was found that the **NRTI**s for HIV are also active against HBV, and **3TC** has been used for HBV infection.[9,10] However, HBV resistant to **3TC** has emerged. Since **4′Ed2FA** is highly active against **3TC**-resistant HIV and will prevent the emergence of drug-resistant HIV, it also is expected to be active against drug-resistant HBV. Thus, **4′Ed2FA** could be an ideal drug for use against both HIV and HBV.

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