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Development and optimization of anti-HIV nucleoside analogs and prodrugs: A review of their cellular pharmacology, structure-activity relationships and pharmacokinetics

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Abstract

Significant improvements in antiviral therapy have been realized over the past 10 years. Numerous nucleoside analogs, as well as prodrugs of active compounds, have been synthesized and tested for anti-HIV activity. In addition to the five nucleoside analogs currently used clinically for the treatment of HIV infection, a broad spectrum of anti-HIV nucleoside analogs (including 2′,3′-dideoxynucleoside analogs, oxathiolanyl 2′,3′-dideoxynucleoside analogs, dioxolanyl 2′,3′-dideoxynucleoside analogs, carbocyclic 2′,3′-dideoxynucleoside analogs and acyclic nucleoside analogs) and their prodrugs (including ester prodrugs, phospholipid prodrugs, dihydropyridine prodrugs, pronucleotides and dinucleotide analogs), targeted at HIV reverse transcriptase, are reviewed with focus on structure-activity relationships, cellular pharmacology and pharmacokinetics. Several of these anti-viral agents show promise in the treatment of AIDS.

Keywords: Anti-HIV; Reverse transcriptase; 2′,3′-Dideoxynucleoside analog; Nucleotide analog; Prodrug; Cellular pharmacology; Pharmacokinetics

Contents

1. Introduction 118
2. 2′,3′-Dideoxynucleoside analogs 120
   2.1. Structure-activity relationships 120
   2.2. Thymidine (T) analogs 121
      2.2.1. Cellular pharmacology 121
      2.2.2. Pharmacokinetics 122
   2.3. 2′-Deoxyuridine (dU) analogs 123
      2.3.1. Cellular pharmacology 123
      2.3.2. Pharmacokinetics 124
   2.4. 2′-Deoxycytidine (dC) analogs 124

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2.4.1. Cellular pharmacology 125
2.4.2. Pharmacokinetics 125
2.5. 2′-Deoxyadenosine (dA) and 2′-deoxyinosine (dI) analogs 125
2.5.1. Cellular pharmacology 125
2.5.2. Pharmacokinetics 127
3. Oxathiolanyl 2′,3′-dideoxynucleoside analogs 127
3.1. Structure-activity relationships 127
3.2. Cellular pharmacology 128
3.3. Pharmacokinetics 128
4. Dioxolanyl 2′,3′-dideoxynucleoside analogs 129
4.1. Structure-activity relationships 129
4.2. Cellular pharmacology 130
4.3. Pharmacokinetics 130
5. Carbocyclic 2′,3′-dideoxynucleoside analogs 131
5.1. Structure-activity relationships 131
5.2. Cellular pharmacology 131
5.3. Pharmacokinetics 132
6. Acyclic nucleoside analogs 132
6.1. Structure-activity relationships 132
6.2. Cellular pharmacology 134
6.3. Pharmacokinetics 135
7. Ester prodrugs of 2′,3′-dideoxynucleoside analogs 135
8. Phospholipid prodrugs of 2′,3′-dideoxynucleoside analogs 135
8.1. Rationale for prodrug design 135
8.2. Cellular pharmacology 136
8.3. Pharmacokinetics 136
9. Dihydropyridine prodrugs of 2′,3′-dideoxynucleoside analogs 136
9.1. Rationale for prodrug design 136
9.2. Cellular pharmacology 137
9.3. Pharmacokinetics 137
10. Pronucleotides: phosphatidyl 2′,3′-dideoxynucleoside analogs 137
10.1. Rationale for prodrug design 137
10.2. Structure-activity relationships 138
10.3. Cellular pharmacology 138
10.4. Pharmacokinetics 139
11. Dinucleotide analogs 140
11.1. Rationale for prodrug design 140
11.2. Structure-activity relationships 140
12. Other pronucleotide approaches 141
13. Conclusions 141
References 141

1. Introduction

In combating acquired immunodeficiency syndrome (AIDS) and AIDS related complex (ARC), the search for therapeutic agents possessing activity against human immunodeficiency virus (HIV) has yielded a number of compounds demonstrating potent and selective antiviral activity. Human immunodeficiency virus reverse transcriptase (HIV-RT) remains a primary target for the treatment of HIV infection [1]. As HIV-RT inhibitors, five anti-HIV nucleoside analogs have been approved by Food and Drug Administration (FDA) and are currently used clinically. These anti-HIV nucleoside analogs include zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), and lamivudine (3TC). Except for 3TC, which has a β-L-configuration, these nucleoside analogs have β-D-configurations similar to natural nucleosides.

The active antiretroviral form of a nucleoside analog is its triphosphate anabolite. By sharing the anabolic pathway of the naturally occurring nu-

cleosides, antiviral nucleoside analogs are phosphorylated to the corresponding mono-, di-, and triphosphates. Phosphoryation of nucleosides is catalyzed by cellular kinases as illustrated in Fig. 1. Although the efficiency of the phosphorylation of nucleoside analogs is generally lower than that of the naturally occurring nucleoside, the potent and selective anti-HIV activity of the nucleoside analog resides in strong inhibition of HIV-RT with relatively little effect on cellular polymerases exerted by the nucleoside-triphosphate. Once incorporated into the growing proviral DNA chain, nucleoside analogs terminate the viral DNA elongation owing to a lack of the 3′-OH. While the mechanism of antiviral activity of nucleoside analogs may ultimately be the same, it should be noted that each compound has its own distinct metabolic and pharmacological properties. Besides the inhibition of HIV-RT, the effect of triphosphates of nucleoside analogs on cellular polymerases (pol α, β, δ, γ and ε) are also important to the understanding of the potential mechanisms involved in in vitro and in vivo activity and toxicity. Pol α, which is the main polymerase responsible for cellular DNA synthesis, is not inhibited by all FDA-approved nucleoside analogs.

A number of cell lines have been employed to investigate the in vitro anti-HIV activity and cytotoxicity of the nucleoside analogs, including human

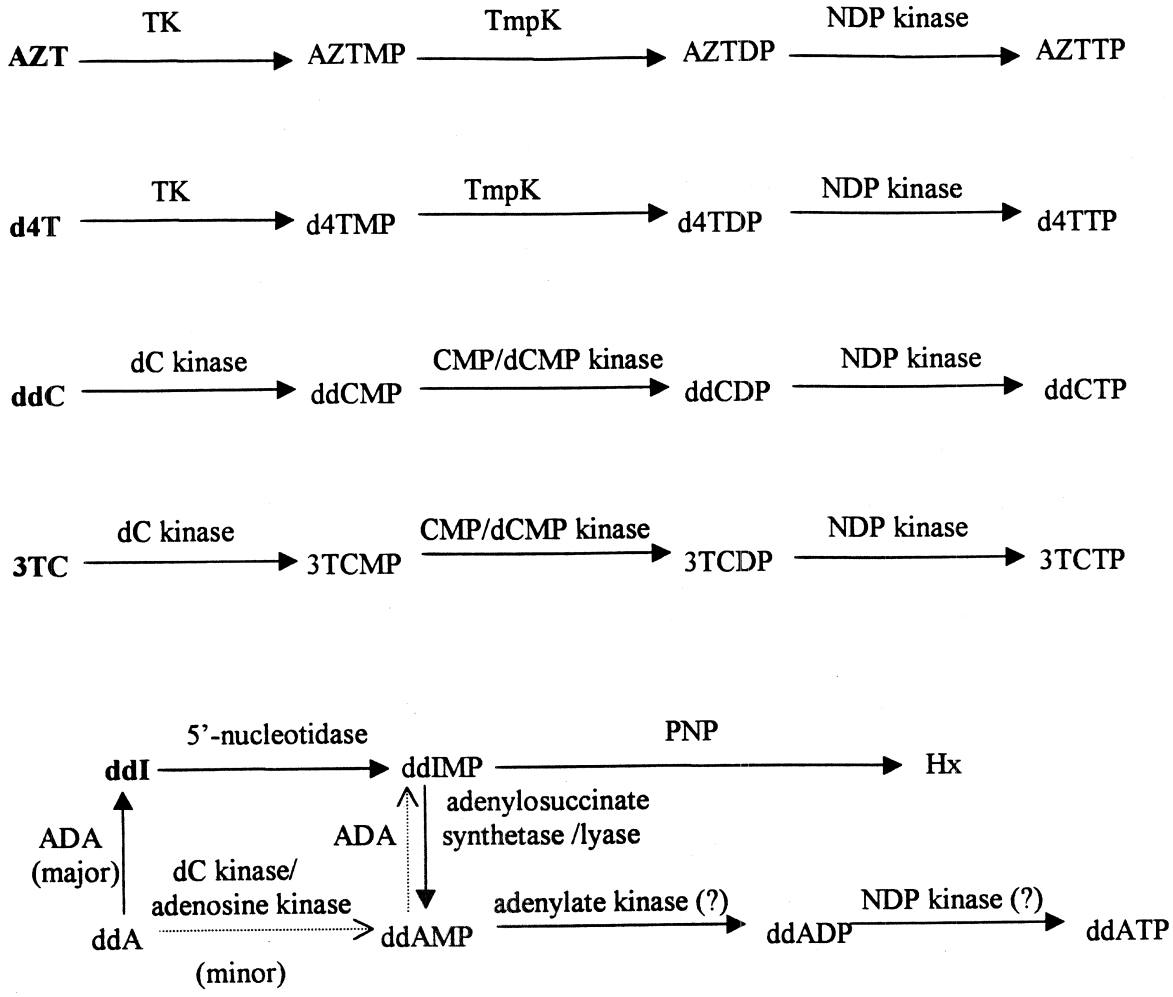

Fig. 1. The intracellular phosphorylation pathways of FDA-approved nucleoside analogs.

T-cell lines such as MT-2, MT-4, H9, CEM, and ATH8, human peripheral blood mononuclear (PBM) cells, human peripheral blood lymphocytes (PBL), and murine C3H cells. The antiviral activity of nucleoside analogs depends critically on cellular kinases for the metabolic steps to produce the 5′-triphosphate. It is, therefore, not surprising that a compound's activity may vary to some extent with the type of cell line and the phase in the cell cycle, since the occurrence and the relative amounts of these kinases vary under different circumstances [2,3]. Based on the phosphorylation profiles, AZT and d4T are considered as cell-activation-dependent nucleoside analogs, while ddI, ddC and 3TC are classified as cell-activation-independent nucleoside analogs [4].

Myelosuppressive effects such as neutropenia and anemia are the main clinical adverse effects associated with AZT [5,6]. Nucleoside analogs are evaluated for hematological toxicity by inhibition of in vitro colony formation of human hematopoietic progenitors CFU-granulocyte (CFU-G) and burst-forming unit-erythroid (CFU-E) in bone marrow cells. Peripheral neuropathy is the primary adverse effect associated with ddI, ddC and d4T [7,8]. The decreased mitochondrial DNA (mtDNA) synthesis caused by nucleoside analogs is monitored as it is proposed to be related to delayed cytotoxicity in vitro and in vivo side effects of these compounds.

Unlike the natural nucleosides entering cells via facilitated transporters, anti-HIV nucleoside analogs permeate into cells by either simple diffusion or by a combination of simple diffusion and nucleoside/nucleobase carrier-mediated transportation [9,10]. Generally, in vivo nucleoside analogs are extensively distributed throughout the body but with limited penetration into the central nervous systems (CNS); they are rather rapidly cleared from the systemic circulation with apparent half-lives usually less than 3 h. Severe adverse effects and drug resistance are frequently observed clinically. Efforts have therefore been continuously made to develop and optimize nucleoside analogs. In addition to rigorous searches for new compounds with increased anti-HIV potency and selectivity, novel prodrug approaches have also been extensively investigated to improve the therapeutic characteristics of the drugs. Prodrugs are designed to increase exposure of anti-HIV nucleoside analogs to the CNS and to target delivery of the drugs to the lymphatic system. The lymphatic system and CNS serve as reservoirs for HIV.

This current review is intended to give an overview of the field of nucleoside analogs and their prodrugs, with special focuses on the structure-activity relationships, cellular pharmacology and pharmacokinetics of the representative compounds.

2. 2′,3′-Dideoxynucleoside analogs

2.1. Structure-activity relationships

2′,3′-Dideoxynucleoside analogs (dT, ddC, ddA, ddI and ddG) except for ddU have anti-HIV activity in ATH8 cell cultures to various extents [11]. In ATH8 cells, 3′-azido (AZT) or 3′-fluoro substitution (FLT) or 2′,3′-didehydro (d4T) modification greatly potentiates the anti-HIV activity of dT. On the other hand, 3′-fluoro substitution (FLC) or 2′,3′-didehydro (Fd4C) modification decreases the anti-HIV activity of ddC. Similarly, 3′-azido (AZdA) substitution or 2′,3′-didehydro (d4A) modification decreases the anti-HIV activity of ddA and increases its cytotoxicity [12]. In MT-4 and CH3 cells, 3′-azido (AZdU) or 3′-fluoro (FddU) substitution significantly increases the anti-HIV activity of ddU [13]. In MT-4 cells, 3′-azido (AZdG) or 3′-fluoro (FddG) substitution increases the anti-HIV activity of ddG. 2,6-Diaminopurine 2′,3′-dideoxyribose (ddDAPR), AZddDAPR and FddDAPR have higher anti-HIV activity than ddG. However, AZddDAPR is also one of the most cytotoxic nucleoside analogs [14]. 2′,3′-Didehydro (d4G) modification of ddG severely increases the cytotoxicity [15]. In H9 cells, 2′-'up' fluoro-substitution abolishes the anti-HIV activity of AZT, FLT and d4T, and decreases that of ddC [16,17]. 2′-Azido-modification of dideoxypyrimidine analogs do not possess any anti-HIV activity [18]. In MT-4 cells, 5′-isocyano-modification annihilates the anti-HIV activity of dT, AZT and AZdU [19]. In MT-4 cells, 5′-*O*-phosphonomethylation abolishes the anti-HIV activity of dT and ddC, and decreases that of FLT and AZT [20]. In CEM cells, selected 5-halo-6-alkoxy (or azido)-derivatives of AZT and FLT show equipotency against HIV [21,22].

2-Chloro-modification substantially decreases the

anti-HIV activity of ddA and d4A [23]. In MT-4 cells, 5-chloro-derivative of FddU (FddClU) and AZdU (AZdClU) have significantly higher selectivity than that of FddU and AZdU [24,25]. 5-Chloro derivatives of FddC, AZdC, d4C and ddC result in reduced cytotoxicity with slightly reduced anti-HIV activity [26]. 6-Halo-2′,3′-dideoxypurine ribofuranosides have potent anti-HIV activity in various cells [27].

In CEM cells, β-L-ddC and β-L-FddC have potent anti-HIV and HBV activity with little inhibition of mtDNA synthesis, compared to β-D-ddC (natural configuration) [28]. Relative to d4C, β-L-Fd4C has more potent anti-HIV activity and less cytotoxicity, while β-L-d4C has comparable anti-HIV potency and cytotoxicity; β-L-Fd4C and β-L-d4C have little effect on mtDNA synthesis [29]. Among the isomeric dideoxynucleoside analogs, 4(*S*)-(6-amino-9H-purin-9-yl)tetrahydro-2(*S*)-furanmethanol (isoddA) and isoddG are potent anti-HIV activity in various cell cultures and low cytotoxicity [30].

2.2. Thymidine (T) analogs

The structures of thymidine and the antiviral thymidine analogs dT, AZT, FLT and d4T are shown in Fig. 2. The cellular kinases responsible for the phosphorylation of thymidine analogs are thymidine kinase (TK), thymidylate kinase (TmpK), and nucleoside diphosphate (NDP) kinase, which, respectively, mediate the metabolism to the mono-, di- and triphosphate anabolites. In this category, AZT and d4T are FDA approved anti-HIV drugs. FLT was tested in clinical trials without success owing to severe toxicity at doses required for anti-HIV efficacy.

2.2.1. Cellular pharmacology

Zidovudine has potent and selective anti-HIV activity in various human cell cultures [31,32]. AZT is phosphorylated to its monophosphate (AZTMP), diphosphate (AZTDP) and triphosphate (AZTTP) anabolites by TK, TmpK and NDP kinase, respec-

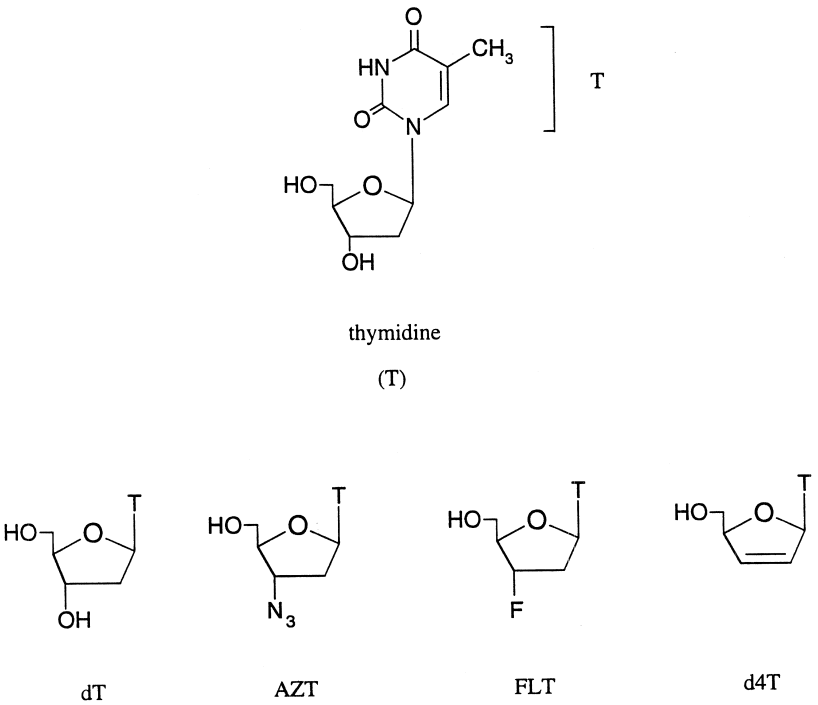

Fig. 2. Thymidine (T) and its analogs.

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