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6-[1-(4-Fluorophenyl)methyl-1*H*-pyrrol-2-yl)]-2,4-dioxo-5-hexenoic acid ethyl ester a novel diketo acid derivative which selectively inhibits the HIV-1 viral replication in cell culture and the ribonuclease H activity in vitro

Enzo Tramontano[a,*], Francesca Esposito[a], Roberta Badas[a], Roberto Di Santo[b], Roberta Costi[b], Paolo La Colla[a]

[a] *Department of Sciences and Biomedical Technologies, University of Cagliari, Cittadella Universitaria SS554, 09142 Monserrato (Cagliari), Italy*
[b] *Department of Pharmaceutical Studies, University of Rome "La Sapienza", P.le Aldo Moro 5, I-00185, Rome, Italy*

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Abstract

The human immunodeficiency virus-type 1 (HIV-1) reverse transcriptase (RT) is a multifunctional enzyme which displays DNA polymerase activity, which recognizes RNA and DNA templates, and a degradative ribonuclease H (RNase H) activity. While both RT functions are required for retroviral replication, until now only the polymerase function has been widely explored as drug target. We have identified a novel diketo acid derivative, 6-[1-(4-fluorophenyl)methyl-1*H*-pyrrol-2-yl)]-2,4-dioxo-5-hexenoic acid ethyl ester (RDS 1643), which inhibits in enzyme assays the HIV-1 RT-associated polymerase-independent RNase H activity but has no effect on the HIV-1 RT-associated RNA-dependent DNA polymerase (RDDP) activity and on the RNase H activities displayed by the Avian Myeloblastosis Virus and *E. coli*. Time-dependence studies revealed that the compound is active independently on the order of its addition to the reaction mixture, and inhibition kinetics studies demonstrated that RDS 1643 inhibits the RNase H activity noncompetitively, with a K_I value of 17 μM. When RDS 1643 was combined with non-nucleoside RT inhibitors (NNRTI), such as efavirenz and nevirapine, results indicated that RDS 1643 does not affect the NNRTIs anti-RDDP activity and that, vice versa, the NNRTIs do not alter the RNase H inhibition by RDS 1643. When assayed on the viral replication in cell-based assays, RDS 1643 inhibited the HIV-1_{IIIB} strain with an EC_{50} of 14 μM. Similar results were obtained against the Y181C and Y181C/K103N HIV-1 NNRTI resistant mutant strains. RDS 1643 may be the first HIV-1 inhibitor selectively targeted to the viral RT-associated RNase-H function.
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Keywords: HIV; AIDS; Reverse transcriptase; Ribonuclease H; Antiviral agents; Diketoacid

1. Introduction

Conversion of the single-stranded RNA retroviral genome into double-stranded proviral DNA is an obligatory event in the human immunodeficiency virus (HIV) replication cycle which is accomplished, in the host cell cytoplasm, by the virus-coded enzyme reverse transcriptase (RT). This process requires a combination of an RNA- and DNA-dependent DNA polymerase activity together with a degradative function which hydrolyzes the RNA component of the RNA-DNA replication hybrid intermediate and is termed, therefore, ribonuclease H (RNase H). The two RT-associated enzymatic functions are carried out by two distinct catalytic sites which, albeit the HIV-1 RT is composed by two polypeptide subunits (p66 and p51), reside in the p66 subunit at a distance of approximately 18 base pairs from each other (Arts and Le Grice, 1998; Hughes et al., 1998; Klarmann et al., 2002). The N terminus of p66 accommodates the active site which catalyzes the RNA- and DNA-dependent DNA polymerase

* Corresponding author. Tel.: +39 070 6754205; fax: +39 070 6754210.
E-mail address: tramon@unica.it (E. Tramontano).

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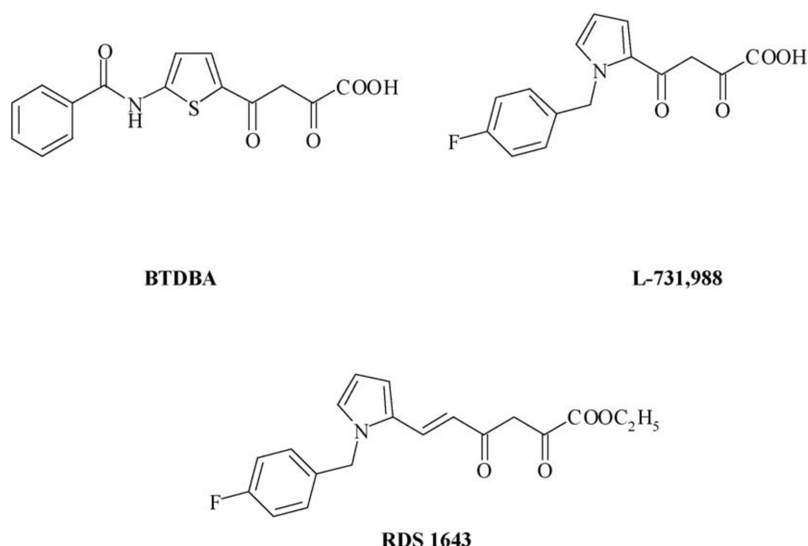

Fig. 1. Chemical structures of RNase H and IN inhibitors.

activity, while the C terminus accommodates the active site which catalyzes the RNase H activity. Since both activities are absolutely required for DNA provirus formation (Hughes et al., 1998; Klarmann et al., 2002), the RT-associated RNase H function is an attractive target for the development of new antiretroviral agents (Klarmann et al., 2002; Tarrago-Litvak et al., 2002).

In spite of these considerations, all RT inhibitors currently approved for the treatment of HIV infection, or presently under investigation in clinical trials, inhibit its polymerase activity, while none of them blocks its RNase H activity (Parniak and Sluis-Cremer, 2000; Gulick, 2003). Until now in fact, only a few compounds have been described to inhibit the HIV-1 RNase H function in vitro (Tarrago-Litvak et al., 2002). Among them are sulfated polyanions (Moelling et al., 1989), the natural marine product illimaquinone (Loya et al., 1990; Loya and Hizi, 1993), the azidothymidylate (AZTMP) (Tan et al., 1991), the *N*-(4-*tert*-butylbenzoyl)-2-hydroxyl-1-naphthaldehydfe hydrazone (BBNH) (Borkow et al., 1997), the 4-chlorophenylhydrazone of mesoxalic acid (CPHM) (Davis et al., 2000), the *N*-hydroxyimides (Klumpp et al., 2003) and few others (Klarmann et al., 2002; Tarrago-Litvak et al., 2002). In reality, with the exception of the *N*-hydroxyimides which do not inhibit the *E. coli* RNase H and block the HIV-1 RT-associated polymerase activity at a concentration 40-fold higher than the concentration required to inhibit the RNase H function (Klumpp et al., 2003), none of them is truly selective in vitro for the HIV-1 RNase H either because they inhibit also other viral or cellular recombinant enzymes (Moelling et al., 1989; Loya et al., 1990; Loya and Hizi, 1993; Davis et al., 2000), or because they are active on both HIV-1 RT-associated polymerase and RNase H functions (Borkow et al., 1997). Moreover, with the exception of BBNH (Borkow et al., 1997), none of them inhibits the HIV-1 replication in cell-based assays (Klarmann et al., 2002; Tarrago-Litvak et al., 2002; Moelling et al., 1989; Loya et al., 1990; Loya and Hizi, 1993; Tan et al., 1991; Davis et al., 2000; Klumpp et al., 2003). Recently, the diketo acid (DKA) derivative 4-[5-(benzo-ylamino)thien-2-yl]-2,4-dioxobutanoic acid (BTDBA, Fig. 1) has been reported to inhibit the HIV-1 RT RNase H function without affecting its polymerase activity (Shaw-Reid et al., 2003). Even though this DKA derivative was not highly selective for RNase H since (i) it inhibited in the same concentration range also the HIV-1 integrase (IN) in enzyme assays and (ii) it did not block the viral replication in cell-based assays, it provided the proof of concept for direct inhibition of the HIV-1 RT RNase H associated activity by DKAs (Shaw-Reid et al., 2003).

In this report we identified a novel DKA derivative, the 6-[1-(4-fluorophenyl)methyl-1*H*-pyrrol-2-yl)]-2,4-dioxo-5-hexenoic acid ethyl ester (RDS 1643, Fig. 1), which selectively inhibits the RNase H function of the HIV-1 RT, without affecting neither its DNA polymerase associated activity, nor the RNase H activity associated to the Avian Myeloblastosis Virus (AMV) RT or the *E. coli* RNase H, while it barely affected the HIV-1 IN activity. We also performed a partial characterization of the interaction between RDS 1643 and the HIV-1 RT by time of addition, kinetic and drug association studies. Finally, we demonstrated that RDS 1643 inhibits the replication of wild type (wt) and drug-resistant HIV-1 strains in cell-based assays.

2. Materials and methods

2.1. Materials

P6HRT-prot plasmid was kindly provided by Dr. S. Le Grice (NCI at Frederick). All buffer components were obtained from Sigma–Aldrich unless specified; [γ^{32}P]-ATP, [^{3}H]-dGTP, poly(rC)-oligo(dG)$_{12–18}$ and Hi-Trap desalting column were obtained from Amersham Biosciences; G-25

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