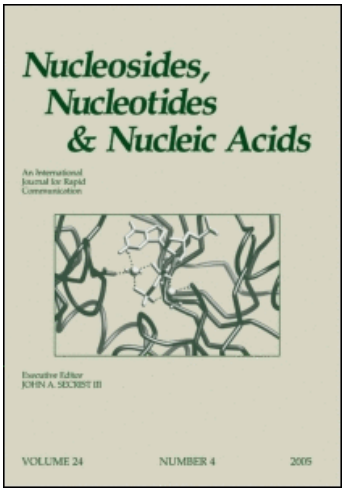


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Mechanism of Inhibition of HIV Reverse Transcriptase by 1-(2-Deoxy-β-$_d$-ribofuranosyl)-4-acetylimidazolin-2-one (Imidine)

T. I. Kalman[a]; K. Sen[a]; X-J. Jiang[a]
[a] Departments of Medicinal Chemistry and Biochemical Pharmacology, State University at New York, Buffalo, New York

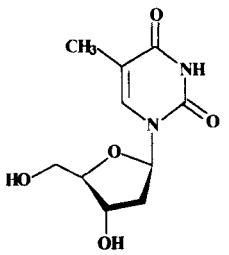
To cite this Article Kalman, T. I. , Sen, K. and Jiang, X-J.(1999) 'Mechanism of Inhibition of HIV Reverse Transcriptase by 1-(2-Deoxy-β-$_d$-ribofuranosyl)-4-acetylimidazolin-2-one (Imidine)', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 847 — 848
To link to this Article: DOI: 10.1080/15257779908041578
URL: http://dx.doi.org/10.1080/15257779908041578

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 847–848 (1999)

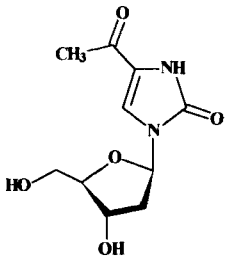
MECHANISM OF INHIBITION OF HIV REVERSE TRANSCRIPTASE BY 1-(2-DEOXY-β-D-RIBOFURANOSYL)-4-ACETYLIMIDAZOLIN-2-ONE (IMIDINE)

T. I. Kalman*, K. Sen and X.-J. Jiang
State University at New York, Departments of Medicinal Chemistry and Biochemical Pharmacology, 457 Cooke Hall, Buffalo, New York 14260

Imidine[1] (dImd), a structural isomer of thymidine, was designed as a potential anti-HIV agent based on the rationale that the lack of fidelity of HIV reverse transcriptase (HIV-RT) may result in misincorporation of the analogue into viral DNA leading to increased unrepaired mispairing and inhibition of viral replication.



dThd



dImd

The triphosphate of dImd (dITP), inhibited competitively the utilization of dTTP and dCTP by HIV-RT, using poly(rA):$(dT)_{12\text{-}18}$ and poly(rC):$(dG)_{12\text{-}18}$, as template/primer, respectively. $Oligo(dT)_{16}$ on a $poly(rA)_n$ template (>500 bases) was extended to full length products in the presence of 25 μM dTTP. In contrast, when dTTP was replaced by 100 μM dITP, elongation stopped when the product reached 32-mer length. Primer extension was also examined on a defined RNA template of 145 nucleotides, having a stretch of **AAAA** at sites 17-20 followed by a **U**. A 16-mer primer, complementary to the sequence before the first quartet, was extended to 20-mer products by both dITP and dTTP (at 100 μM), however, dITP produced additional strong stops at sites 17, 18 and 19, which appeared as dark pause bands on electrophoresis (PAGE).

An efficiency of 79% for insertion of dIMP vs. dTMP opposite to **A** was obtained using a natural 363-mer RNA template, and a 23-mer primer designed to perform a "running-start" primer extension assay[2] for insertion at site 26 (after 2 **C**'s) in the presence of saturating dCTP. Using an analogous 23-mer synthetic DNA template of the same sequence yielded an insertion efficiency of 75%.

5'-^{32}P ----------primer---------*CCN* N = T or I

3' ----------template------GGACUGGUU----- 5'

or

5'-^{32}P ----------primer---------*CCN* N = T or I

3' ----------template------GGACTGGTT----- 5'

Synthetic, 27-mer oligonucleotides containing either a **T** or an **I** at position 19 with an appropriate primer for running-start kinetics[3] were used for the determination of the fidelities for insertion opposite to either **T** or **I**.

5'-^{32}P ----------primer---------*CCX* X = A, G, C, T

3' ----------template------GGNGATATGAA----- 5' N = T or I

The misinsertion frequencies opposite to **T** or **I** were: 0.00043 and 0.0015 for **G**; 0.00012 and 0.0022 for **C**; and 0.00019 and 0.0011 for **T**, respectively. These data indicate that when dIMP is incorporated into viral DNA in place of dTMP, the resulting template will exhibit 3.5- to 18-fold lower fidelity at the substituted sites, validating the prediction of the original hypothesis.

REFERENCES

1. Jiang, X.-J., Kalman, T. I. *Nucleosides Nucleotides*, **1994**, *13*, 379-388.
2. Boosalis, M.S., Petruska, J., Goodman, M. F. *J. Biol. Chem.*, **1987**, *262*, 14689-14696.
3. Shy, W. E., Hertel, W. H., Kroin, J. S., Bloom, L. B., Goodman, M. F., Richardson, F. C. *Cancer Res.* **1993**, *53*, 4582-4587.