

POSTER 27**DERIVATIVES OF MESOXALIC ACID INHIBIT TRANSLOCATION OF HIV-1 REVERSE TRANSCRIPTASE**

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Previous studies have shown that the 4-chlorophenylhydrazone of mesoxalic acid (CPHM) can inhibit the ribonuclease H (RNase H) activity of HIV-1 reverse transcriptase (RT). It was later demonstrated that this compound can also affect DNA synthesis. The D185N mutation in HIV-1 RT neutralizes the effect of the inhibitor on RNase H cleavage, suggesting that the compound binds in the vicinity of the polymerase active site. However, the mechanism of action remains elusive. Here we demonstrate that CPHM shows hot-spots for DNA synthesis inhibition. These hot spots correlate with sequences that show a bias to the pre-translocated conformation of the HIV-1 RT-DNA/DNA complex. In this complex, the 3'-end of the primer blocks the nucleotide binding site. Site-specific footprinting experiments and binding studies reveal that CPHM traps and stabilizes the pre-translocated complex, which provides a mechanism of action. The increased stability of the complex also diminishes the turnover of the reaction under steady-state conditions, which, in turn, helps to explain why RNase H cleavage appears to be inhibited. Most importantly, the proposed mechanism of action for DNA synthesis inhibition, i.e. inhibition of HIV-1 RT translocation, is identical to the mechanism of action of the pyrophosphate analogue foscarnet. However, in contrast to foscarnet, which shows a broad spectrum of activity against several viral polymerases, CPHM specifically inhibits HIV-1 RT. We show that foscarnet is able to inhibit both HIV-1 RT and the DNA polymerase of the human cytomegalovirus (HCMV), while CPHM does not affect the HCMV enzyme. The results of this study validate the pre-translocated complex of HIV-1 RT as a specific target. Our findings show that this complex can be targeted by chemically distinct classes of compounds.