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Anti-HIV activities of new PETT compounds in cell cultures

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The PETT series of non-nucleoside RT (NNRT) inhibitors has been expanded through the design and synthesis of several hundred new compounds. It has been possible to design PETT compounds which give a tenfold slower rate of HIV resistance development in cell cultures than NNRT inhibitors such as TIBO, nevirapine and L697661.
Some of the new PETT compounds inhibit wt HIV-1 in MT-4 cells at 1-10 ng/ml and HIV-1 with 100, 181 and 188 mutations at 10-100 ng/ml. These mutant HIV-1 strains are not inhibited at 10 µg/ml by TIBO, nevirapine or L697661. Some PETT compounds inhibit HIV-2 and SIV at 10-100 ng/ml in cell cultures.
Combinations of PETT compounds with AZT, ddI, ddC, PFA show synergistic effects against HIV-1 in cell cultures and HIV-1 mutants resistant to nucleoside analogues are sensitive to PETT compounds.
The large number of very potent PETT compounds available with different structures and causing a slow rate of resistance development makes it possible to select compounds with optimal properties for both oral use and vaginal use in microbicides.

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Novel Mutations in the Reverse Transcriptase of HIV-1 Reduce Susceptibility to Phosphonoformate in Laboratory and Clinical Isolates. J. Mellors[1], H. Bazmi[1], R. Schinazi[2], B. Roy[3], Y. Hsiov[3], E. Arnold[3], J. Weir[4], D. Mayers[4]. [1]University of Pittsburgh/VAMC, Pittsburgh, PA, [2]Emory University/VAMC, Decatur, GA, [3]Rutgers University/CAMB, Piscataway, NJ, [4]Military Medicine Consortium for Applied Retroviral Research, Rockville, MD, USA.

We investigated whether HIV-1 variants resistant to phosphonoformate (PFA) could be selected in cell culture or isolated from HIV-infected patients after prolonged PFA therapy (>3 months) for CMV retinitis. HIV-1 exhibiting ~8-fold resistance to PFA was isolated *in vitro* after 13 passages of virus in 400 µM PFA. Resistant virus exhibited hypersusceptibility to AZT (100-fold), TIBO R82150 (30-fold), and nevirapine (20-fold). DNA sequence analysis of reverse transcriptase (RT) cloned from resistant virus identified two mutations in all clones: Q161L and H208Y. Eight HIV-1 isolates from patients receiving prolonged PFA were tested for PFA susceptibility. Six of 8 isolates showed reduced PFA susceptibility (2 to 5-fold). Sequence analysis of these isolates revealed the H208Y substitution in two, the Q161L in one, and a W88S/G substitution in four. Site-directed mutagenesis demonstrated that the Q161L, W88S, and H208Y substitutions reduced viral susceptibility to PFA 10-fold, 5-fold, and 2-5 fold, respectively. Viruses encoding the Q161L or Q161L+H208Y mutations were hypersusceptible to AZT, TIBO R82150, and nevirapine. In the crystal structure of HIV-1 RT, the Q161 residues lies just beneath the dNTP binding site. The Q161L substitution may affect the structure of the dNTP binding site and its affinity for PFA. In contrast, the W88S substitution probably affects template-primer binding to the fingers domain of RT, with secondary effects on PFA susceptibility. HIV-1 resistance to PFA can develop *in vitro* and in treated patients, and is caused by substitutions that affect template-primer and dNTP binding sites in RT. Since PFA resistant variants are hypersusceptible to AZT and non-nucleoside RT inhibitors, combination therapy with these agents should be considered.