Development of Integrase Inhibitors

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There are two main types of HIV viruses, HIV-1 HIV-2. HIV-2 tends to progress slower than HIV-1 and it results in fewer deaths. This project was focused on testing a new category of novel Allosteric integrase inhibitors (ALLINIs) that block the function of integrase. ALLINIs bind to integrase outside of the active site and in return hinders the integrase which is important as some of the current integrase inhibitors are less effective due to a rise of resistance against it. HIV uses integrase to insert its viral DNA into the CD4 cell. To measure integrase inhibition, a fluorescence resonance energy transfer (FRET) assay was used. Integrase cuts and inserts its viral DNA into the CD4's DNA. Afterwards, DON3 and ACE become covalently linked. Attached at the end of DON3 is Biotin, and when the acceptor (ACE) is in close proximity of the added EU-SA reagent that is attached to the Biotin, they emit a signal. If the tested compound is effective however, the ACE and the DON3 would not become attached and would be too far away to emit a signal. The procedure begins by creating a reaction buffer. Integrase is then diluted into the reaction buffer. A substrate mix was prepared by adding DON3, and a dilution of ACE and LEDGF. In each micro-tube, 25 µl of substrate mix was added and then incubated. After the incubation, a detection buffer with the addition of the EU-SA was added into the reaction tubes. Then the samples were loaded onto a 384- well white plate and incubated overnight. The sample was read the next morning with a PerkinElmer plate reader using Lance settings. This procedure was used to compare four new compounds (NGJ- 9095, NGJ-9083, NGJ-9085, NGJ- 9092) to two previous compounds (NGJ-9022, NGJ-9021) created prior that was very effective at integration inhibition.