Kun Silprasit 2010: Recombinant HIV-1 Reverse Transcriptase and Its Mutants Study; Cloning, Expression, Purification and Preliminary Crystallization for X-Ray Crystallography. Doctor of Philosophy (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Kiattawee Choowongkomon, Ph.D. 138 pages.

The serious epidemic disease, HIV can resistance to these drug substances in some patients and transmission of drug resistant HIV has been described for many years. We propose to test novel NNRTIs form computational drugs design, dipyridodiazepinone derivatives. Therefore, we cloned, expressed and purified recombinant HIV-1 RT. The protocol was developed to get highly recovery of heterodimeric enzyme. We developed simple and fast method for determine RT activity, PicoGreen-fluorometric assay. PicoGreen dye interact with HIV-RT reaction products like, RNA/DNA heteroduplex, the highly sensitive dye can detect even a small amount of the reaction product. We report the efficiency of the fluorometric method using PicoGreen dye to measure enzyme activity and anti-HIV. A small amount of PicoGreen dye is enough to test a minimal quantity of substrate to rapidly determine the enzyme kinetic properties Km and Vmax. The enzyme kinetic study and kinetic parameters, K_m and V_{max}, were rapidly determined. In addition, we applied this method to screen HIV-RT inhibitors. The five inhibitors showed higher inhibition activity than the commercial drug nevirapine. The fifty percent inhibitions were determined by using inhibitor dose response curve. Two inhibitors showed higher inhibition efficiency with their IC_{50} values. Our purposed fluorometric method using PicoGreen dye showed easy, rapid, and sensitive detection protocol to determine HIV-RT activity and enzyme kinetic study. Moreover, this PicoGreen-fluorometric assay can be used for high-throughput inhibitor screening application. In addition, we crystallized HIV-1 RT/novel complex. The single crystal can be grown from our condition and molecular structure will be further studied.

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