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Activity of HIV-I Protease Inhibitors in Various Enzymatic Assays.

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Retroviral proteases have been identified as useful targets for antiviral interference. Their main function is to allow correct processing of viral structural proteins; thereby ensuring the maturation of infectious progeny virus. Based on its amino acid sequence and structural conformation, HIV-I protease has been identified as an aspartic protease. This enabled us to synthesize HIV-I protease specific inhibitors.These inhibitors were characterized in cleavage assays using various proteases, peptides and viral precursor protein.

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Subcellular Localization and Antiviral Activity of Cationic Dye/Poly r(A-U) Combinations.
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Experiments have been designed to examine the effects of 24 anthraquinone (AQ), xanthene (XAN) and ellipticine (EPC) dyes on the antiviral and interferon-inducing activities of poly r(A-U) using a human foreskin fibroblast - vesicular stomatitis virus bioassay in which 0.05 mM dye has been combined with poly r(A-U) to produce a dye/ribonucleotide ratio of 1/4. Poly r(A-U) and the dyes alone were not effective antiviral agents. When poly r(A-U) was combined with adriamycin (ADR), ametantrone (HAQ), carminic acid (CAR), daunomycin (DMN), mitoxantrone (DHAQ), N^2-methyl-9-hydroxy-ellipticine (NMHE), N^2,N^6-dimethyl-9-hydroxy-ellipticine (DMHE), rhodamine 123 (R123), rhodamine B (RB), rhodamine 6G (R6G) and sulforhodamine B (SB), antiviral activity of poly r(A-U) was enhanced 8- to 20-fold, while 50% effective doses of the poly r(A-U), ADR, CAR, DMN, NMHE, HAQ, R6G, RB, DHAQ, DMHE, SB and R123 decreased 18-, 60-, 60-, 61-, 113-, 125-, 156-, 250-, 251-, 274-, 313- and 347-fold, respectively. Additional bioassays demonstrated that the enhanced antiviral activity of the dye/poly r(A-U) combinations was not due to increased interferon induction, direct viral inactivation or cytotoxicity. Bright field and fluorescence micrographs of human foreskin fibroblast monolayers co-incubated with active members of each of the 3 families of dyes, poly r(A-U) alone or with the dye/poly r(A-U) combinations exhibited three basic patterns: 1. the dye entered the cell as well as the nucleus and the accumulation of the dye in the nucleus was greatly potentiated by the poly r(A-U), (AQ); 2. the dye entered the cell and was sequestered in the cytoplasm, but readily accumulated in the nucleus in the presence of poly r(A-U), (EPC); 3. the dye entered the cell and was sequestered in cytoplasmic organelles (mitochondria) and only the poly r(A-U) and minute quantities of the dye accumulated in the nucleus when the dye was combined with poly r(A-U) (XAN). These results suggested that the potentiated antiviral activity of the AQ (or EPC)/poly r(A-U) combinations was due to the altered kinetics of uptake and subcellular distribution and the subsequent modulation of one or more nuclear (nucleolar) processes. Xanthene dyes have been shown to exert a cytostatic effect on cultured cells due to their disruption of mitochondrial energy production and the subsequent inability of cells to attain a critical content of essential components, such as rRNA, necessary for cell entrance into the pre-replicative compartment of G_1 phase. Thus it appears that xanthene dyes potentiated the antiviral activity of poly r(A-U) by interfering with cellular processes.