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Antiviral Activity of the Proteasome Inhibitor VL-01 against Human and Avian Influenza A Viruses

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In silico Screening of Compounds Targeting Human Cyclin T1 and *In vitro* Evaluation of their Anti-HIV-1 Activity

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The gene expression and transcription step from the integrated proviral DNA is essential for HIV-1 replication. However, drugs blocking this step have not been clinically approved yet. The cellular protein complex p-TEFb (cyclin T1/CDK9) interacts with HIV-1 Tat and the TAR RNA, which plays a crucial role in HIV-1 transcription. Therefore, the interaction among p-TEFb, Tat, and the TAR RNA is considered to be a potential target for inhibition of HIV-1 replication. To identify a lead compound having selective anti-HIV-1 activity, *in silico* screening of compounds targeting cyclin T1 was performed using the molecular docking simulation software MOE (Chemical Computing Group Inc., Quebec, Canada). Since the complex structure of human p-TEFb/Tat/TAR is not available to date, a model structure of human cyclin T1 was constructed by homology modeling based on the complex structure of equine cyclin T1, equine infectious anemia virus (EIAV) Tat, and EIAV TAR RNA (Protein Data Bank ID: 2w2h) and used for the docking study. A putative binding pocket, where small molecule compounds could be bound, was identified within human cyclin T1 model structure. Approximately 3 million compounds were screened according to the primary condition (molecular weight: 350–600 Da, the number of hydrogen bond donor/acceptor < 13, rotatable bonds < 7, and log *P*: 0–6). Then, the selected compounds were further examined for their *in silico* binding to the pocket within cyclin T1 by MOE. Based on the docking score obtained by the screening, 254 molecules were synthesized and examined for their anti-HIV-1 activity in cell cultures. Consequently, 2 compounds with similar chemical structures showed selective inhibition of HIV-1 replication in chronically infected cells (OM-10.1 and U1) stimulated with TNF-α. When their derivatives were synthesized and evaluated for the anti-HIV-1 activity, one compound was found to have higher activity. Studies including their molecular mechanism of action are in progress.

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Novel 2-Styryl-8-hydroxyquinolines (8SQs) Derivatives with Anti-HIV-1 Activity Targeting Viral Integrase and Protease

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Earlier it has been reported about anti-HIV-1 integrase activity of styryl-quinolines (Mekouar K. et al., 1998). In Bulgaria six novel 2-styryl-8-hydroxyquinolines (8SQs) derivatives were synthesized and evaluated for anti-HIV-activity in cell culture. The aim of this work was to target anti-HIV-1 activity of novel substances. Supernatants of chronically infected H9/HTLVIIIB were used as a source of HIV-1. Cytotoxicity tests and microtiter infection assays for HIV cytopathic effect in MT4 cells were performed (MTT uptake assay). Reverse transcriptase (RT) activity in supernatants and directly on recombinant RT (Cavidi, Sweden) were checked. Protease as a target was studied by modified screening method using direct spectrophotometric reading of specific substrate utilization by native HIV-1 protease in the absence and presence of the substances studied. To check the integrase as a target, mutants were obtained by serial passaging of virus with continuous exposure to increasing concentrations of active compounds followed by sequencing of *in* region. Mitochondrial toxicity was evaluated by RealTime-PCR as mtDNA:nDNA ratio. Two novel 8SQs: 105B and 241, differing in substitutes at C_2 in the phenol ring, demonstrated inhibition of HIV replication (105B > 95% and 241–70%). 105B showed mitochondrial toxicity accompanied by reducing of both mtDNA and nDNA (mtDNA:nDNA = 0.82 compared to 1.01 and 0.97 in MT4 uninfected and HIV-1 infected cells without inhibitor, resp.). 241 showed no mitochondrial toxicity. Both derivatives exposed no RT inhibition but anti-protease activity (105B–21% and 241–25% resp.). As far as the latter did not explain anti-HIV effect in microtiter assay, we looked for mutations in IN gene in passages 30–32 for 105B and 241. The molecular sequencing found mutations in 105B (N17S, D231I) and in 241 (E10D, D231I), all in *in* region. Evidence demonstrated that 105B and 241 target viral protease and integrase.

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A Novel Method—Amenable for High-throughput Screening Purposes—to Quantify Antiviral Activity Against Viruses that Induce Limited CPE

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For antiviral screening purposes, infection of cell cultures with the virus under study, should ideally result in the induction, within just a few days, of (nearly) complete CPE and allow the calculation of acceptable Z′ factors (>0.5). The human Corona virus (NL63) causes only limited CPE on different cell lines (Schildgen et al., J Virol Methods, 2006). Following infection of Vero118 cells, virus-induced CPE was too low to allow readout based on classical