statins inhibit HCV RNA replication [Ikeda et al., 2006. Hepatology, 44]. We confirmed the *in vitro* anti-HCV activity of statins and we identified mevastatin as the most potent inhibitor in this series. We studied various combinations of statins with selective HCV inhibitors by performing clearance-rebound assays and antiviral combination assays. We also studied whether mevastatin can delay or prevent the development of resistance against inhibitors of HCV replication. For the clearance-rebound assays, Huh-9-13 replicon containing cells were cultured for six consecutive passages in the presence of a selective HCV inhibitor, alone or in combination with mevastatin and in absence of neomycin selection. During the rebound phase, the inhibitor was removed and cells were cultured for three passages in the presence of neomycin. If antiviral therapy is able to clear the replicon from the culture, the cells will not survive when cultured in the presence of neomycin in the rebound condition. Several antiviral combination assays were performed as described before using Huh-5-2 replicon containing cells [Paeshuyse et al., 2006. Hepatology, 43]. For combination resistance selection, Huh-9-13 replicon containing cells were cultured in the presence of neomycin selection and in the presence of mevastatin or HCV-796, or a combination of mevastatin and HCV-796 at various ratios. Replicon cells that formed visible colonies were selected for further characterization. Neither mevastatin nor the selective HCV inhibitors were able to cure the cells from replicon after six passages of antiviral pressure. However, the combination of mevastatin with polymerase or protease inhibitors resulted in an efficient clearance of the cells from replicon. Combination of mevastatin with IFN-a, with selective HCV polymerase or protease inhibitors elicited an additive anti-HCV effect. Mevastatin was able to decrease (at low concentrations) or prevent (at high concentrations) the emergence of antiviral drug resistance against HCV-796.

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Comparative Virtual and Experimental Medium Throughput Screening for Hepatitis C Virus Polymerase Inhibitors

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Although many search aim at the development of actives antiviral drugs, for most patient infected by one genotype, persistent HCV infection cannot be controlled by antiviral therapy. This has encouraged the search for more potent new antivirals to HCV. Previous work has demonstrated that the NS5B polymerase is a target of choice for multi-therapy programs. Although very promising in vitro, none of the non-nucleoside inhibitors (NNI) discovered so far gives concluding results in the clinic, either because of adverse side effects or little efficacy in patients, due to the rapid selection of mutant virus leading to resistance. Screening campaigns, involving million of compounds issued from several original libraries, are still ongoing with the hope to discover new active compounds. In order to decrease the elevated cost of such research, it is tempting to first virtually screen libraries to select and eventually predict, a list of the best binder molecules that would potentially be inhibitors. The known 3D structure of the polymerase combined to the availability of powerful in silico docking programs allowed such a strategy of antiviral development. In this work, we screened in parallel the same in-house library by an in vitro experimental and an in silico virtual screening. We then compared the results and determined: (i) the best virtual screening protocol and (ii) its predictive power. Our results show that most of our hits are active inhibitors on mutant polymerase harboring the well-known M423T mutation selected by inhibitors targeting the B NNI site. Furthermore, our virtual screening brings an added value to the understanding of the binding mechanism of those hits and some important insight on the potential resistance profile.

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MIV-170, A Novel NNRTI Exhibiting Tight Binding to HIV-1 Reverse Transcriptase (RT)

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The NNRTI MIV-170 has been found to be a very efficient inhibitor of wtHIV and HIV mutant strains resistant to the NNRTIs used in the clinic. To better understand the interaction between MIV-170 and HIV-1 the details of this have been studied by different methods. The kinetics of the interaction between MIV-170 and HIV-1 RT was analysed using a biosensor assay. The association and dissociation rates were determined using immobilized wtRT or RT mutants and MIV-170 as analyte. The results demonstrated that MIV-170 had both a faster association and a slower dissociation rate than efavirenz, nevirapine and delavirdine, thus exhibiting a higher affinity than these compounds. The strength of the interaction between the NNRTIs and RT and RT mutants in the biosensor assay was compared to the reversibility of inhibition in cell culture experiments. In these experiments virus and infected cells were incubated with MIV-170 and other NNRTIs for various times and after removal of the compounds the remaining infectivity was assayed. X-ray analysis of the binding of MIV-170 to HIV-1 RT displayed extensive interactions, not only between the compound and the lining amino acids but also between these residues, turning the binding cavity into a rigid entity and explaining the tight binding in the biosensor assay and the inactivation of HIV.

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