to several site points within the peptide, such as inserting EK or ER motif to increase the helicity of the peptide. We here present our method used in screening of HIV-1 sequence database *in silico*. The energy of peptides those are the truncated parts (aa. 628–631) of the isolates uploaded in Los Alamos HIV-1 database were assessed. Peptides exhibiting low energies were selected as candidates firstly. Subsequently, modifications were introduced according to both the information of strong modifications previously reported and alanine scan computation. At last KYK peptides were developed. Several of these peptides were active in several different isolates, including T-20 resistant isolates. Our KYK peptides are under further studies. This concise method applied here can be used in many similar cases in which peptide inhibitors play import roles as drugs.

doi:10.1016/j.antiviral.2011.03.166

181

The 3D-Screen Technology, an Innovative Cell-based Assay to Identify Modulators that Alter Target Protein Conformation: Example with HCV

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All proteins exert their biological function through a defined tridimensional structure that determines their precise interactions with one or several specific molecular partners (proteins, DNA, etc.). Binding of natural or synthetic ligands to a given target protein induces dramatic or subtle conformational changes that influence the interaction of this protein with its cellular partners, resulting in specific modulation of the biological responses. Based on this fundamental biological principle we developed the 3D-Screen technology, an innovative human-cell based assay designed to identify small molecules that alter conformation of target proteins. Since knowledge of the biological function of the protein is not required, this highly sensitive technology can be applied to an extensive range of therapeutic targets. Moreover, screening for conformational alterations in the natural cellular environment rather than for functional alterations using purified target protein provides access to promising modulators acting through original mechanisms of action such as allosteric modulators. This technology is based on the specific recognition of the target native conformation by a short peptide sequence (3D-Sensor) that activates the expression of a reporter gene. Alteration of the target conformation prevents interaction with the 3D-Sensor, eliminating the expression of the reporter gene. In an effort to identify new classes of HCV polymerase inhibitors, high throughput 3D-Screen-based screening was performed in the hepatoma Huh-7 cell line. Optimization of the resulting hits led to compounds with potency in low nanomolar range on genotypes 1a and 1b and active against NS5B variants resistant to known inhibitors. Interestingly, activity of the series was improved by two logs on the 3D-Screen and replicon assays without modifying activity either of recombinant polymerase or of NS5B in the context of the in vitro replication complex. These results suggest a novel mechanism of action that is currently under investigation.

doi:10.1016/j.antiviral.2011.03.167

182

Synthesis and Antiviral Activity of 3-Methoxy-2-(phosphonomethoxy)propyl Nucleoside Esters Against HCV and HIV-1

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Novel therapies for hepatitis C virus (HCV) and HIV infection are greatly needed. We previously identified octadecyloxyethyl 9-(S)-[3-methoxy-2-(phosphonomethoxy)propyl]adenine (ODE-(S)-MPMPA) a potent inhibitor of HCV (EC_{50} = 1.43 μM) and HIV replication in vitro (EC_{50} = 0.03 μM). ODE-(S)-MPMPA has greatly reduced cytotoxicity compared with the analog having a 3-hydroxypropyl group, ODE-(S)-HPMPA, in Huh7 cells the CC_{50} values were 150 μM vs. 35 μM. To identify additional potent antivirals, we synthesized of MPMP-analogs of guanine, 2,6-diaminopurine, and cytosine by reaction of the corresponding nucleobase with (*R*)- or (*S*)-methyl glycidyl ether. These derivatives then reacted with alkoxyalkyl p-toluenesulfonyloxymethyl phosphonates to provide the final compounds after acidic deprotection. Antiviral evaluation of the new compounds showed that although ODE-(S)-MPMPA is the most active anti-HCV compound in genotype 1b or 2a replicons, all MPMP-purine nucleosides possess antiviral activity in a EC_{50} range between 10 and 25 μM for both (S) and (R) isomers and have the same low cytotoxicity. All MPMP-purine nucleosides are very active against HIV in MT-2 cells in vitro, with some compounds having EC_{50} values less than 10 pM. However, the ODE-(S)-MPMP pyrimidines did not show significant activity against either HCV or HIV. We also evaluated various 3-alkoxy substituents of the acyclic side chain. The ethoxy and isopropoxy analogs of (R,S)-MPMP-adenine were synthesized and evaluation of their antiviral activity shows that the methoxy group is the preferred substituent.

doi:10.1016/j.antiviral.2011.03.168

183

Herpes Simplex Virus Thymidine Kinase Inhibitor GLS122E and Its 6-Deoxy Prodrug GLS361B (Sacrovir™)—Potential for Preventing Viral Disease Recurrence *In Vivo*

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After initial infection herpes simplex viruses (HSV) establish a latent state in neural ganglia, from which future reactivation and recurrence of acute infection may occur. The current anti-HSV drugs suppress the acute infection, but do not control virus reactivation, especially subclinical reactivation and resulting transmission. HSV types 1 and 2 express virus-specific thymidine kinases (TK), which are likely required for virus proliferation in non-replicating (neural) cells and thought to be essential for reactivation from latency. Cell culture and animal model experiments have shown that HSV TK inhibitors suppress virus reactivation from established latent infections, and, therefore, could be developed as drugs to target recurrent infections and, possibly, subclinical reactivation and virus transmission. The guanine analog N^2-(3-(trifluoromethyl)phenyl)guanine (mCF_3PG, GLS122E) is a potent, specific, non-substrate HSV 1 and 2 TK inhibitor, which has been