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High Throughput Screening of a 100,000 Compound Library for Inhibitors of Influenza A Virus (H3N2)

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There are a limited number of antivirals for the control of seasonal and pandemic influenza viruses. To discover small molecules that inhibit the cytopathic effect exerted by influenza viruses, we employed our high-throughput cell-based assay (Noah et al., Antiviral Res. 2006) to screen a commercially available compound library. Using the reproducible and highly robust assay, we have screened 100,000 compounds from the ChemBridge 2 (CB2) compound library at a low and high concentration against Influenza strain A/Udorn/72 (H3N2). The "hit" rate (>50% CPE inhibition of virus) for the compounds screened at 5 μg/ml and 40 μg/ml was determined to be 0.022% and 0.37%, respectively. The hits obtained in this screen were evaluated by measuring their antiviral activity, cell toxicity and selectivity in dose response experiments. Three compounds displayed moderate activity (SI_{50} = 10–49) in reducing influenza in the presence of 5 μg/ml compound. Quite the opposite, when screening at the higher concentration, several compounds were identified that were highly active with an SI_{50} > 50. These quantitative data provide a foundation for grouping the hits into classes of compounds with similar scaffolds for structure activity relationship (SAR) analysis and make them excellent candidates for the development of new small-molecule therapeutics.

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Antiviral Activity of Reverse Transcriptase Inhibitors against Porcine Endogenous Retroviruses (PERV)

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Background: Xenotransplantation is a possible solution to the shortage of organs in transplantation. The Kagoshima strain of mini-pigs is considered to be a suitable organ donor in xenotransplantation because of its size and other favorable profiles. However, a concern about the transmission of porcine endogenous retrovirus (PERV) from donor organs to recipients has been raised. Therefore, we have evaluated various reverse transcriptase (RT) inhibitors for their inhibitory effect on PERV replication in vitro.

Materials and methods: The PERV-producing cell line PK15 and the primary kidney cells from the Kagoshima strain were used for the sources of PERV. The human cell line 293T was infected with PERV and cultured in the presence of various concentrations of test compounds. After a certain incubation period, the amount of proviral DNA in the infected cells was determined by real-time PCR. The triphosphate (TP) forms of some compounds were also examined for their inhibitory effect on PERV RT activity.

Results: Among the eight RT inhibitors examined, AZT was found to be the most active against PERV. Its EC_{50} was approximately 0.02 μM. The order of potency was AZT > PMEO-DAPy = PMPDAP > PMPA ≥ PMEA > d4T (EC_{50} = 5–10 μM). Furthermore, AZTTP proved to be a more potent inhibitor of PERV RT than d4TTP. In contrast, 3TC, 4′-ethynyl-d4T, and the nonnucleoside RT inhibitor nevirapine were inactive against PERV replication.

Conclusion: Some nucleoside HIV RT inhibitors, such as AZT, PMEO-DAPy, and PMPDAP, may be effective in the prevention of PERV transmission to humans upon xenotransplantation.

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17-AAG, an Hsp90 Inhibitor, Suppress Hepatitis C Virus (HCV) Replication

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Heat-shock protein 90 (Hsp90), which accounts for 1–2% of cytosolic protein, is one of the most abundant cellular chaperone proteins1. It functions in a multicomponent complex of chaperone proteins including Hsp70, Hop (Hsp70 and Hsp90 organizing protein), Cdc37, Hsp40 and p23. Hsp90 is involved in the folding, activation and assembly of several proteins, known as Hsp90 client proteins. As numerous oncoproteins have been shown to be Hsp90 client proteins, Hsp90 inhibitors have become a new strategy in antitumor therapy. Geldanamycin, a classical Hsp90 inhibitor, is known as a potent antitumor agent; however, it has not been used in clinical trials because of its liver toxicity. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is a new derivative of geldanamycin that shares its important biological activities but shows less toxicity.

Here, we present a new and potent strategy for the hepatitis C virus (HCV) therapy with 17-AAG, an Hsp90 inhibitor. We examine the effects of 17-AAG on full length HCV replicon cells line. The HCV RNA replication in the HCV replicon was quan-