of the assay was verified by a screen of a small peptidomimetic compound library, with the discovery of three novel HIV-1 gp41 inhibitors. The best hit has a molecular weight below 500, binds with a K_i of 1.2 μM, and inhibits syncytium formation at micromolar concentration in vitro.

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Development of ATP/Luminescence Assays For Profiling Compounds Against A Panel of Positive-Strand RNA Viruses

Edwin Gong*, Tania Ivens, Christel Van den Eynde, Sabine Hallenberger, Kurt Hertogs

Tibotec BVBA, Generaal De Wittelaan L11B3, 2800 Mechelen, Belgium

We designed an ATP-based assay platform to profile antiviral compounds against a number of positive strand RNA viruses including yellow fever virus (flavivirus), West Nile virus (flavivirus), Coxsakie B virus (picornavirus) and sindbis virus (alphavirus). This assay platform is based upon the bioluminescent measurement of ATP in metabolically active cells. Antiviral efficacy was determined by measuring the ATP level in cells that were protected from the viral cytopathic effect (CPE) by the presence of antiviral reference or test compounds.

The bioluminescent ATP-detection method utilizes luciferase which catalyses the formation of light from ATP and luciferin. The emitted light intensity is linearly related to the ATP concentration in live cells and was measured using a ViewLux apparatus (Wallace). In these assays, Vero cells were seeded in 96-well plates in the presence of various concentrations of test compounds and infected with different viruses. The assay is homogeneous (mix and measure) and amenable for high-throughput screening.

The ATP/luminescence assay parameters were optimized and the assays were validated using different reference compounds to determine intra- and inter-assay reproducibilities. The signal to noise ratios for yellow fever virus and West Nile virus were 7.5 and 36, respectively. This compares favorably to the signal to noise ratio of only 1.5 in the neutral red dye uptake assay for yellow fever virus, an alternative readout for CPE inhibition. For Coxsakie B and sindbis virus, the signal to noise ratios were 40 and 50, respectively.

In conclusion, we have validated the ATP/luminescence assay for profiling antiviral compounds against yellow fever virus, West Nile virus, sindbis virus and Coxsackie B virus, representing three virus families. These assays are robust, high-throughput, reproducible and give much better signal to noise ratios than that of dye uptake assays.

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Discovery of Two Novel Classes of Inhibitors of Hepatitis C Virus (HCV) Replication utilizing a Dicistronic Reporter HCV Replicon High Throughput Assay

Weidong Hao*, Peter Weady, Fausto Maldonado, Amy Patick, Rohit Duggal

Pfizer Global Research and Development, San Diego, CA 92121, USA

Background: We report the discovery of two novel classes of HCV replication inhibitors using the HCV subgenomic replicon system in a high throughput screen (HTS).

Methods: The HCV genotype 1b subgenomic replicon was modified to include a humanized Renilla luciferase (hRLuc) followed by the antibiotic resistance gene neo^R. Antiviral activity was monitored using the hRLuc gene as an endpoint. A stable cell line was obtained by the introduction of the modified subgenomic replicon into Huh-7 cells and subsequent clonal selection. The firefly luciferase (FLuc) gene was subsequently introduced into the chromosome of the replicon-containing cell line to monitor compound cytotoxicity. Robustness of both signals from this dual reporter cell line has provided the flexibility of screening in the HTS or low-throughput, multidose assay formats as well the simplicity of obtaining antiviral activity and cytotoxicity data in a single well.

Results: Greater than 1.5 million compounds were screened using this dual reporter HCV replicon cell with a calculated Z' value of 0.8 indicating low variability of both reporter signals. Approximately, 18,000 confirmed hits were generated with a confirmation rate of 40%. Two novel classes of compounds were identified from this screening effort. One series of compounds contain hydroxamic acid residues and have 50% effective concentrations (EC_{50}) values ranging from 50 to 1500 nM and therapeutic indices (TIs) ranging from 19 to 1980. The second series of compounds contain a urea-thiazole motif and have EC_{50} values ranging from 11 to 16,000 nM and TIs ranging from 1 to >1230. Efforts are currently underway to investigate the potential molecular target(s) for these two series of compounds.

Conclusions: We describe the construction of a modified HCV subgenomic replicon as well as the generation of a replicon cell line capable of detecting antiviral activity and cytotoxicity simultaneously in a single well. We will also report the identification, in vitro antiviral activity and initial structure activity relationship of two classes of small molecule inhibitors of HCV replication from an HTS screen.

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