An Ultra High Throughput Screen of the Venenum Discovery Library for PARP-1 Inhibitors

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ABSTRACT

Poly(ADP-ribose)polymerase-1 (PARP-1) plays an important role in the regulation of inflammation in the context of ischemia-reperfusion injury. Numerous PARP-1 inhibitors are being investigated in a variety of in vivo models and in clinical trials for solid tumors, leukemia, lymphoma, and ischemia-reperfusion injury. Our proprietary collection of 5.5 million compounds derived from ECLiPS technology (Encoded Combinatorial Libraries on Polymeric Support) was designed for diversity and druggability (Lipinski’s rules). We ran an HTS using the ECLiPS collection on purified recombinant PARP-1 in a 1536-well, 6 mL assay format. The HTS ran with an overall Z’ factor = 0.68, Z-factor = 0.65, and identified hits in 19 of the 129 libraries within ECLiPS. “Synthon Frequency Plots” gave initial indications of SAR within the collection. Promising hits in 19 of the 129 libraries within ECLiPS. “Synthon Frequency Plots” gave initial indications of SAR within the collection. Promising hits in 19 of the 129 libraries within ECLiPS.

PARP-1 IN ISCHEMIC BRAIN INJURY

• Rationale: Current therapies for ischemic stroke require administration within 3 hours of onset of ischemia, which is a limitation for many stroke patients. Therapies capable of supporting a wider treatment window are needed. Targeting the post-ischemic inflammation, mediated by activated microglia, offers an opportunity to widen the treatment window since microglia require several hours to become fully activated. NF-κB requires PARP-1 as a co-activator in inflammatory activation and toxicity. Hence, PARP inhibitors have the potential to reduce the post ischemic inflammation that leads to more widespread neuronal death.

• Target Validation: In 2011, Matsuura and coworkers demonstrated efficacy of a PARP-1 inhibitor, MP-124, in ameliorating ischemic brain injury in a non-human primate model with treatment after 3 and 6 hours after middle cerebral artery occlusion.

VENENUM DISCOVERY COLLECTION - ECLIPS

The library is derived from combinatorial chemistry and consists of ~8 million compounds clustered into 135 Libraries and 4000 sub-libraries, each containing ~1500 compounds. Compounds are screened in solution after elution from the beads used in synthesis. The beads are retained in separate plates, and compound and corresponding bead well locations are tracked. Beads corresponding to hits can then be retrieved for “decoding” the hit structure.

PARP-1 UHTS ASSAY

The PARP-1 UHTS assay involved three additions: PARP1 (1.5 µL), nicked DNA (1.5 µL), and coupling system (3 µL). The assay was validated in two ways:

1. by comparing hits obtained from 3 independent screens of the Prestwick FDA Approved Drug collection (1120 compounds)
2. by determining IC50s of PARP inhibitors and comparing them to literature values

PARP-1 catalyses the polymerization of NAD+ into poly(ADP-ribose) on a variety of acceptor protein substrates, including histones, which in this assay are present in the form of a “nicked” DNA. The coupling enzyme system detects the amount of unutilized NAD+. Thus, complete PARP-1 inhibition produces a full assay signal, providing a positive readout that minimizes the false positive hit rate.

PARP-1 UHTS ASSAY VALIDATION

• R1
• R2
• R3

RESULTS

PARP1
ECLiPS UHTS Multiple Compound/Well

- 320 1536 well plates
- 14 screening days
- HTS Statistics
  - Z' = 0.68
  - Z = 0.65
- 19 Libraries with active compounds
- 10 Libraries selected for Single bead per well screen

PARP1
ECLiPS UHTS Single Compound/Well

- 127 1536 well plates
- 4 screening days
- All 10 libraries produced active hits
- Good distribution of inhibition
- Identified the top 5 libraries for re-synthesis and structure confirmation

SYNTHON FREQUENCY PLOTS FOR OTHER TOP LIBRARIES

• The PARP-1 UHTS of the Venenum Discovery Library performed very well, characterized by good statistical measures and identification of “Hits” in 19 different libraries.

• The single compound per well screen confirmed that Hits in the 10 best libraries produced inhibition data similar to the multi-compound per well screen (MCW), indicating the activity seen in the MCW was not due to additive effects.

• Analysis of the “Decoded” Hit structures indicated that SAR exists within the best five hit series, as reflected in Synthon Frequency Plots, and provides guidance for Hit optimization efforts.

• The next steps are to re-synthesize compounds and quantify their potency (i.e. determine IC50s).

CONCLUSION


• Baldwin, J. J.; Horlbeck, E. G. Direct dividing method for synthesis of combinatorial libraries (Pharmacopeia, Inc.) WO9535503, 1995


• Luo, X. and Kraus, W. L. On PARP with PARP- cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes & Development. 2012, 26, 417-432

• Hamby, A.M.; Suh, S.W.; Kauppinen, T.M.; Swanson, R.A. Use of a poly(ADP-ribose) Polyamine Inhibitor to Suppress Inflammation and Neuronal Death After Cerebral Ischemia-Reperfusion Stroke, 2007, 38, 632-636


REFERENCES

VENENUM DISCOVERY WORKFLOW

Multi-compound/well screen: 10 million compounds @ 240 µL

Single-compound/well screen: 60 compounds @ 1.5 µL

UHTS Normalized Data visualized in 96-well charts

PARP-1 catalyses the polymerization of NAD+ into poly(ADP-ribose) on a variety of acceptor protein substrates, including histones, which in this assay are present in the form of a “nicked” DNA. The coupling enzyme system detects the amount of unutilized NAD+. Thus, complete PARP-1 inhibition produces a full assay signal, providing a positive readout that minimizes the false positive hit rate.

EXEMPLARY SCREENING AND DATA ANALYSIS

UHTS SAR – SYNTHON FREQUENCY PLOT LIB-5, SUB-11

• 63 options
• 36 options
• 40 options

Within Sub-11, best potency seen in R2 = synthon-19 R3 = any number of synthons = good for optimizing other pharmacological features