Closing Progress Report

Assay Name: Identifying novel Hsp 90 inhibitors by disrupting its cochaperone interaction using an AlphaScreen based High Throughput Screening Assay

Please be sure to identify the target and include an assay description. Also, include information applicable to the last stage reached before project signoff (dose dependent curves, descriptions of hits, defining activators and inhibitors, and SAR summaries for purchased and/or synthetic analogues).

Center Probe Report

Hsp90-TPR2A(HOP) Interaction

Probe reports are generated for each compound that meets or surpasses the MLSCN requirements for a new probe. The probe report should be submitted to PubChem and copies sent to the Center Program Director, the Science Officer assigned to the assay, and the assay provider. Figures can be included within the text.

Please provide the following specific information:

Project Title: Identifying novel Hsp 90 inhibitors by disrupting its cochaperone interaction using an AlphaScreen based High Throughput Screening Assay
Grant Number: X01-MH077625-01

Screening Center Name: NCGC
Principal Investigator of Screening Center: Christopher Austin

Assay Submitter & Institution: Fang Yi, Yale University

PubChem Bioassay Identifier (AID): 595

Assay Target: Hsp90

Probe PubChem Compound Identifier (CID): ?

Mode of Action: Hsp90-TPR2A(HOP) interaction inhibitor

Please provide the following descriptions:

Significance: Heat shock protein 90 (Hsp90) is the essential molecular chaperone which regulates a variety of cellular functions including development, cell cycle, and steroid hormone signaling. The disruption of interaction between the C-terminal portion of Hsp90 and TPR2A domain of HOP may regulate the Hsp90 functions. We report here a group of small molecule inhibitory probes for the Hsp90-TPR2A interaction. These preliminary probes can be used as a research tool for further study of the function of Hsp90. (PubChem AID: 595).

Assay Implementation and Screening

PubChem Bioassay Name: qHTS Assay for Disrupters of an Hsp90 Co-Chaperone Interaction [Confirmatory Method]

PubChem Primary Assay Description/Center Summary:

Hsp90 (heat shock protein 90) is the essential molecular chaperone and it accounts for 1-2% of all cytosolic proteins and is critical for the activity of diverse cellular proteins that are involved in a variety of cellular processes, including development, cell cycle, and steroid hormone signaling. Its client proteins include signaling kinases such as IGF1R, Akt, v-Src, Raf-1; regulators of cell survival such as Bcr-Abl; and transcription factors such as mutated p53, estrogen and progesterone receptors. Hsp90 has emerged as an important target for cancer treatment due to its essential

role in maintaining transformation and regulating the function of oncogenic signaling proteins.

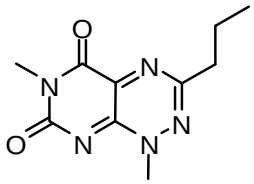
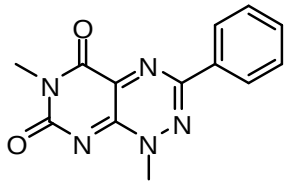
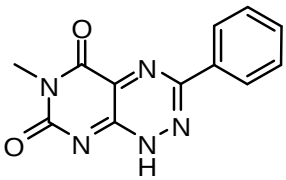
The in vivo function of Hsp90 is regulated by the binding and hydrolysis of ATP at its N-terminal. Geldanamycin binds to ATP site and inhibit Hsp90 function. Geldanamycin and its analogues are under clinical trials in cancer patients. Recent data indicate the C terminal domain of Hsp90 as an important regulator as the N terminus and may represent a second site for pharmacological intervention in chaperone function. This domain contains the binding site for tetratricopeptide repeat (TPR) domains on HOP (Hsp Organizing Protein). TPR is a structural motif used by a significant number of cochaperones by which the in vivo function of Hsp90 is regulated. TPR2A on HOP binds specifically to MEEVD motif of the C-terminal of Hsp90.

A homogeneous AlphaScreen is developed to assess the interaction between TPR2A and MEEVD motif of the C-terminal of Hsp90 by using the isolated TPR2A domain and a synthesized 20mer Hsp90 C terminal peptide. AlphaScreen is two bead-based proximity-dependent chemical energy transfer luminescent assay platform. TPR2A-His-6 and biotinylated Hsp90 C terminal peptide will bring streptavidin donor beads and nickel chelate acceptor beads to a proximity range (less than 200 nm), and subsequently lead a chemical luminescent reaction upon exciting donor beads with 680 nm laser. Inhibition of TPR2A and MEEVD motif interaction by small molecules could attenuate or abolish this chemical luminescent reaction.

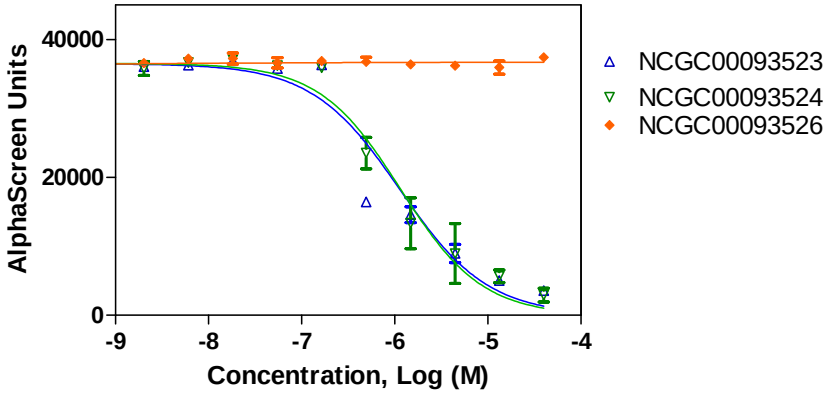
Counter screening: Biotinylated-His-6 (PerkinElmer) can be utilized as a counter screen, as it serves as a linker to bring streptavidin beads and nickel chelate beads together. The counter screen can exclude alpha screen platform false positives due to small molecules being metal chelators, biotin analogues and imidazole analogues, or due to interfering with oxygen release from donor beads. Such an assay should be utilized to qualify any activity observed in this screen.

Appendices

Comparative data on probe, similar compound structures and prior probes

PubChem ID NCGC ID	STRUCTURE	IC50 (M) Emax Hill Slope	%Puriy (PDA) %Purity (ELSD) NMR	CLogP	Supplier - ID
NCGC00093523		1.14E-06 98% -0.921	 Yes*	- 0.39	ChemDiv-D052-0127
NCGC00093524		1.16E-06 100% -0.9962	 Yes*	0.15	ChemDiv-D052-0163
NCGC00093526		Inactive 3.6 0.1803	 Yes*	0.61	ChemDiv-D052-0176

*Provided by vendor



http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=595