Identifying the cellular location of target protein that is responsible for drug action is an important task in drug discovery. Rightly identified location can assist in rational designing of the drug molecules so that the molecule can specifically reach to the cellular location of the target. This note demonstrates the capability of subcellular location specific probes in identifying the location of the activity of the target protein in particular cellular compartment

Background and Overall Goal

Bisindolylmaleimide-III (Bis-III) is a inhibitor of GSK3-β protein and it induces apoptosis in the cancerous cell-lines. However GSK3-β protein is present both in cytoplasm as well as in nucleus. In absence of the information that interaction of Bis-III at which location of GSK3-β is responsible for apoptosis, drug moelcule can not be further optimized from the perspective of its probable cellular and may be nuclear entry.

In following experiments Shantani's technology was utilized in identifying the location of the target that is responsible for apoptosis after interacting with Bis-III compound.

Development of Subcellular Location Specific Target Capturing Probes

Based on the SAR (Structure-Activity Relationship Data) the primary amine at the molecule was chosen as the derivatization site.

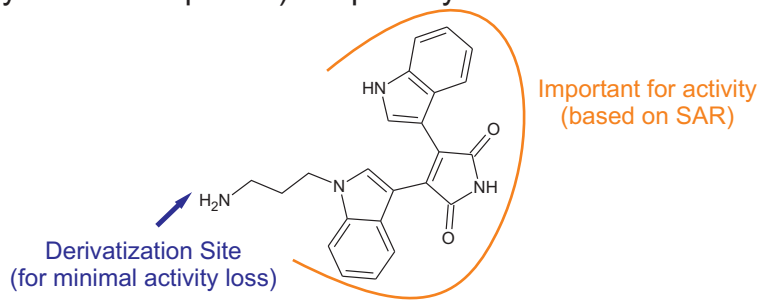
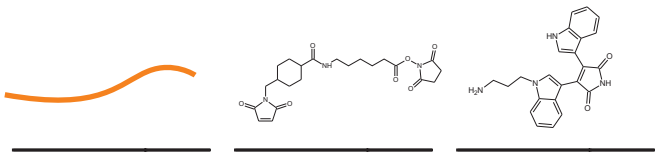


Figure 1. Bis-III derivatization site

Later, Shantani's subcellular location specific target capturing probes were prepared by following coupling scheme. After coupling, Bis-III coupled peptide probes were purified using HPLC and characterized using Mass-spectrometry.



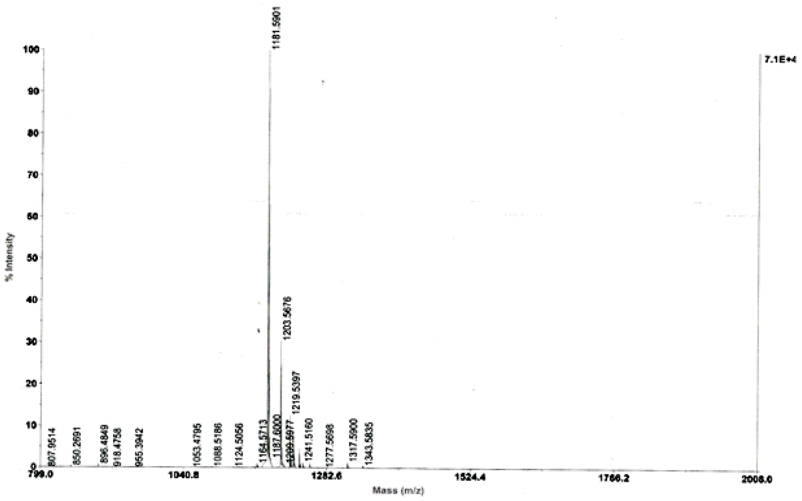


Figure 2. Coupling scheme to covalently link the molecule to peptide and typical characterization of probes

Functionality Assay

Following the Shantani's technology workflow Subcellular location specific target capturing probes of Bis-III along with the respective controls were incubated with He-La cells for 48 hours. Cell-viability was measured at the end of 48 hours using resazurine based assay (Figure 3).

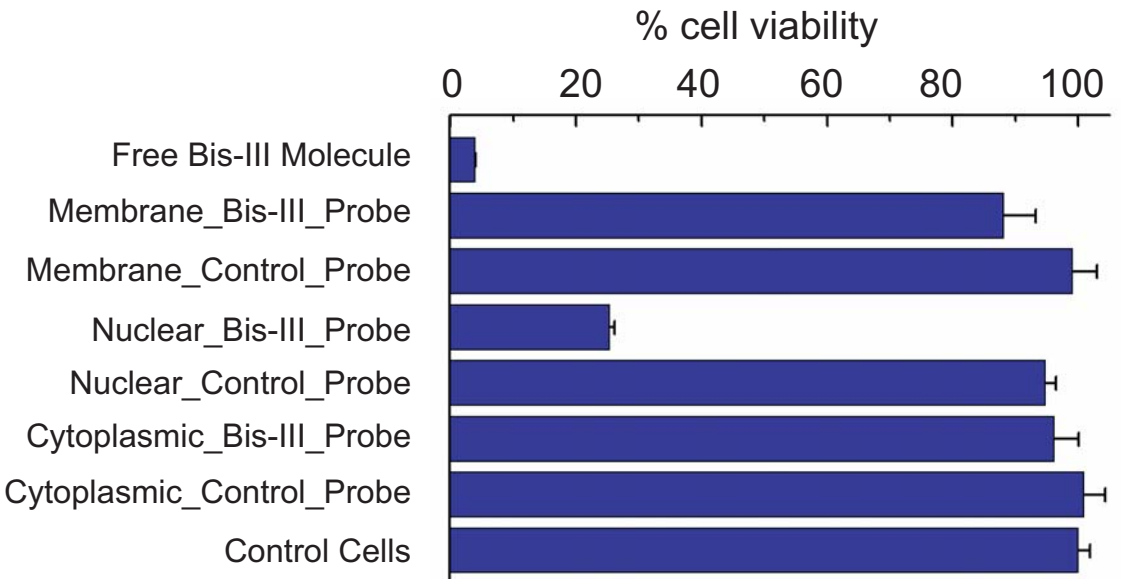


Figure 3. Functional Assay - Cell-Viability after 48 hours after incubating the cells with only Bis-III molecule or different subcellular location specific Bis-III probes and control probes.

Conclusion on Location of Target Activity

Date represented above shows that nuclear location specific Bis-III probes induced the most cell death. This simple experiment clearly demonstrate the capability of subcellular location specific probes in identifying the location of the activity of the target protein in particular cellular compartment. The information that Bis-III interaction with GSK3-β **only in nucleus** is responsible for the cell death is very important. This suggests that new potential molecule that are aimed at GSK3-β and are designed for the purpose of cyto-toxicity should reach to the nucleus to exert its effect.

Contact Us

Shantani Proteome Analytics Pvt. Ltd.
100 National Chemical Laboratory Innovation Park
Dr. Homi Bhabha Road, Pune, MH, India 411 008
Phone: +91-20-64103918, +91-20-64009991, +91-997 544 7489
Email: bd@shantani.com
Online: www.shantani.com