This note describes the application of the technology in identifying/deconvoluting true positive targets and in understanding the action mechanism of a 'test' bait molecule SB202190.

Background and Overall Goal

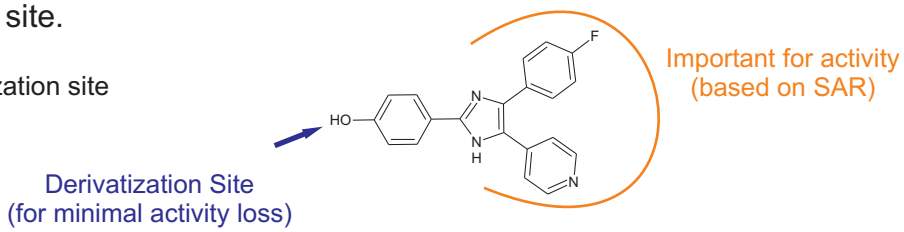
The pyridinyl imidazoles, like SB202190, are specific and potent inhibitor of p38-beta protein. It is widely shown that SB202190 induces apoptosis in the mammalian cells however non-pharmacological inhibition of p38 protein alone is insufficient to induce apoptosis in these cells. This suggests that inhibition of p38 protein alone by SB202190 may not be the sole cause of its apoptotic effect and the Sb202190 might interact with other protein target(s) in the cell to induce apoptosis.

In following experiments Shantani's technology was utilized to identify primary and secondary targets of SB202190. Later, the targets were validated through '*in-vitro*' experiments and the target information was utilized in establishing the apoptotic action mechanism of the molecule.

Development of Subcellular Location Specific Target Capturing Probes

Based on the structure-activity relationship (SAR) information the OH group on the molecule was chosen as the derivatization site.

Figure 1. SB202190 and derivatization site



Later, Shantani's subcellular location specific target capturing probes were prepared by following coupling scheme. After coupling SB202190 coupled peptide probes were purified using HPLC and characterized using Mass-spectrometry.

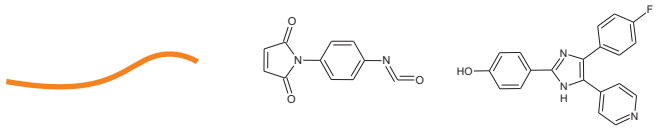
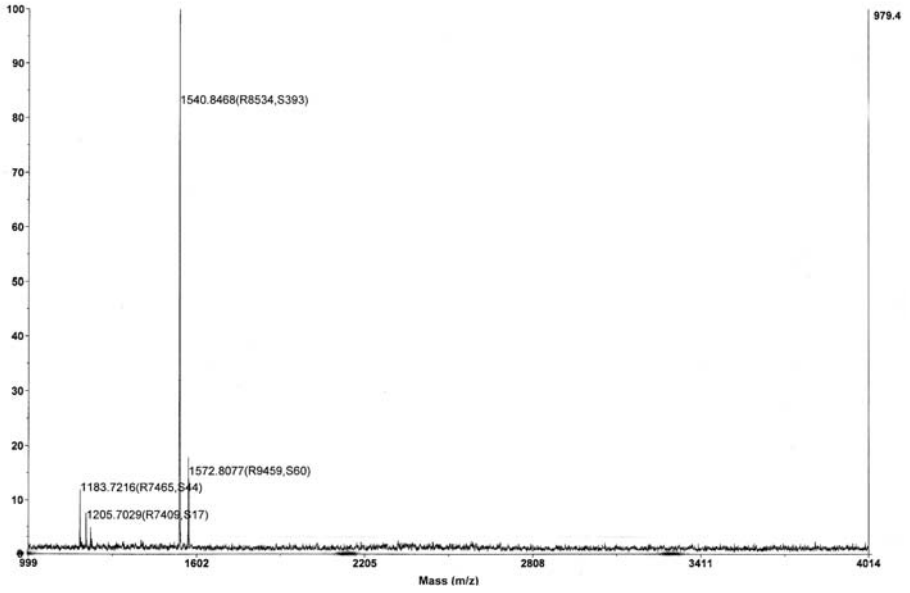


Figure 2. Coupling scheme to covalently link the molecule to peptide and typical characterization of probes



Functionality Assay and Capture, Identification and Deconvolution of Targets

Following the Shantani's technology workflow Subcellular location specific target capturing probes were tested in cell based assays to (a) measure their functional activity, (b) locate the active proteome and (c) establish a concentration range of probes that is relevant for function. Later, based on the cell based assays cytoplasmic probes were found to be most active and were further utilized for target capture experiments. Following the protocol (Technical Notes -1) proteins were identified and deconvoluted (J. Proteome Res. 2008, 7:3490-7) using the mass-spectrometry based methods. Table 1 summarizes the outcome of target deconvolution experiments.

Protein Annotation	Unique Peptide	Sequest Xc for highest matched peptides	Tandem e value for highest matched peptides	Specificity Ratio
MAPK11 (p38 β)	9	5.51	0.000	1.00
Isoform 1 of GSK3 (GSk3-β)	5	5.49	0.000	1.00
Lactoglutathione lyase (GLO1)	5	5.31	0.003	1.00
Isoform 1 of Casein Kinase (CSNKI-δ)	3	4.35	0.000	1.00
STE20/SPE1-realted (STK39)	2	6.13	0.000	1.00
Isoform 2 of MAPK9 (JNK2)	5	6.54	0.005	0.83

Table 1. Identified and Deconvoluted Specific Binding Partners, the target(s) of SB202190

The primary target, p38-beta, of SB202190 was effectively captured using the described workflow. At the same time secondary targets of the molecule were also identified. Interaction of SB202190 with identified targets was validated using 'in-vitro' kinase inhibition assays (Table 2).

Protein Annotation	Interaction Efficiency
MAPK11 (p38 β)	Inhibition 95.95% (kinase Panel), IC50 = 38 nm
Isoform 1 of GSK3 (GSk3-β)	Inhibition 68.65% (kinase Panel)
Lactoglutathione lyase (GLO1)	ND
Isoform 1 of Casein Kinase (CSNKI-δ)	Inhibition 88.72% (kinase Panel)
STE20/SPE1-realted (STK39)	Inhibition 63.25% (kinase Panel)
Isoform 2 of MAPK9 (JNK2)	Inhibition 72.25% (kinase Panel)

Table 2. '*In-vitro*' validation of Identified and Deconvoluted Specific Binding Partners, the target(s) of SB202190

Because only a few but true positive targets of the SB202190 were identified the information can be effectively utilized in understanding the action mechanism of the molecule using *'in silico'* tools.

(1) Proteins were rank ordered based on their abundance and interaction ability
(2) Using the borrowing neighborhood analysis, identified proteins were mapped on canonical pathways
(3) *'in-silico'* inhibition of pathway predicted a high score probability of molecule being apoptotic

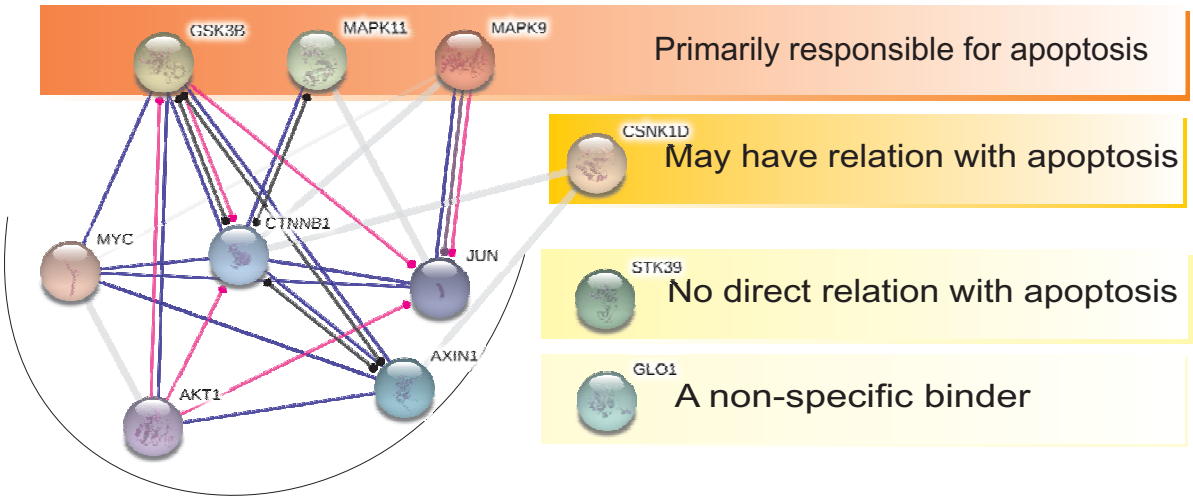


Figure 3. Identified and Deconvoluted Specific Binding Partners, the targets, of SB202190 (highlighted in colors) and their implication in apoptosis related pathways. (Note: String 9.05 was used for 'in-silico' analysis)

It was concluded that along with inhibition of p38 protein, inhibition of JNK2 and GSK3b by SB202190 can explain its apoptotic behavior.

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