Serine Hydrolase Profiling

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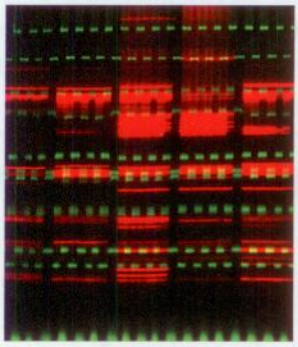
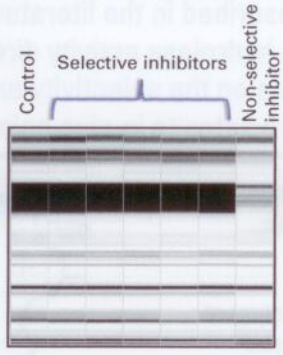
Native hydrolase profiling and inhibitor selectivity screening with activity-based probes

Inhibitor profiling in cell/tissue lysates

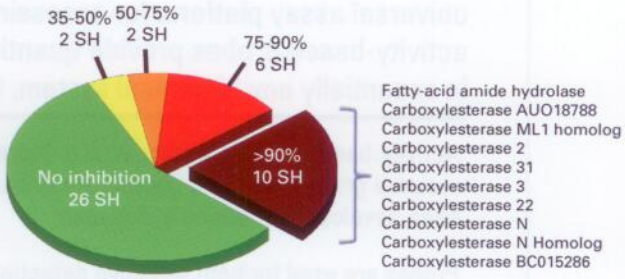
Six inhibitors were profiled in multiple proteomes using Xhibit™. One inhibitor demonstrated significant off-targert inhibitory activity and was further analyzed by XSite™ to determine the target enzymes.



Xhibit™ profiling of inhibitors in multiple proteomes



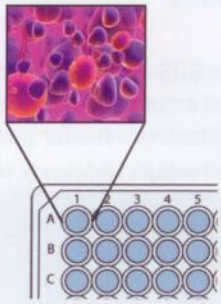
Results from individual proteome



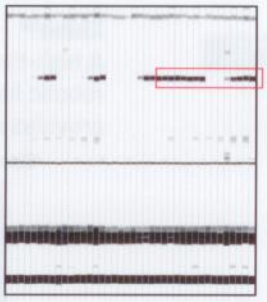
XSite™ identification of inhibitor targets for the non-selective inhibitor
Percent inhibition listed,
SH = number of serine hydrolases

Cell-based assays using Xhibit™

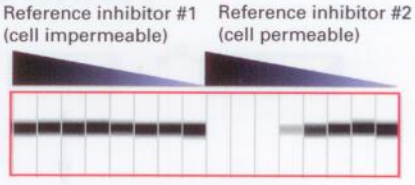
Cells were cultured in 96-well plates and treated with inhibitor prior to probe labeling. Automated cell treatment, probe labeling, lysis, SDS-PAGE, and data analysis enables rapid evaluation of cell permeability and inhibitor efficacy.



Live cell inhibitor treatment, probe labeling, etc



Xhibit™ profiling of multiple inhibitors



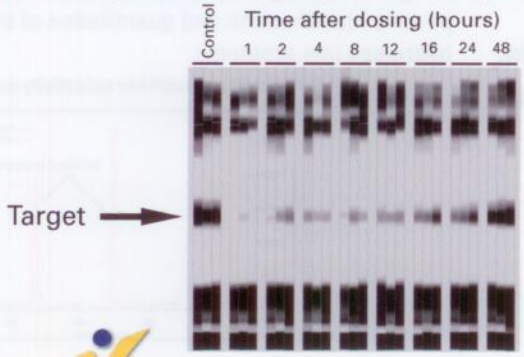
IC_{50} Values (nM)

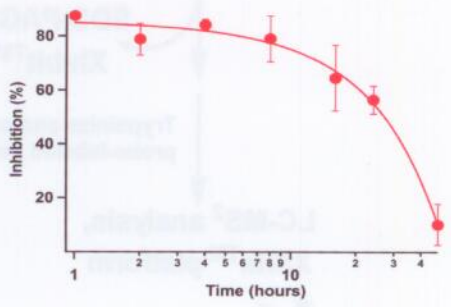
	Inh. #1	Inh. #2
Lysate	5.5	1100
Live Cell	NI	4.1

Inhibitor #1 is not cell permeable (NI = no inhibition)
Inhibitor #2 appears to concentrate within cells

Pharmacodynamic studies

Animals were treated with an inhibitor at a single dose and were sacrificed at selected time points (n = 3) followed by analysis of the target tissue using Xhibit™. Time-dependent recovery of the inhibited target is demonstrated.





ACTIVX®

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Serine Hydrolase Profiling

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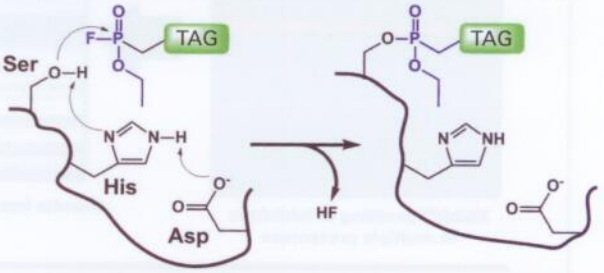
Native hydrolase profiling and inhibitor selectivity screening with activity-based probes

Serine hydrolases comprise one of the largest and most widely distributed enzyme families. Members of this family are therapeutic targets for a wide range of indications including nervous system disorders (pain, anxiety, and insomnia), diabetes, and cancer. A large fraction of serine hydrolases have no known substrates and no substrate-based assays described in the literature. Activity-based probes provide a universal assay platform for assessing serine hydrolase activity directly in complex proteomes. Moreover, activity-based probes provide quantitative data on the selectivity and potency of serine hydrolase inhibitors in essentially any biological system, from cell lysates to in-vivo animal models and beyond.

Activity-based probes composed of a fluorophosphonate reactive group linked to a fluorescent or biotin tag have been developed for serine hydrolases

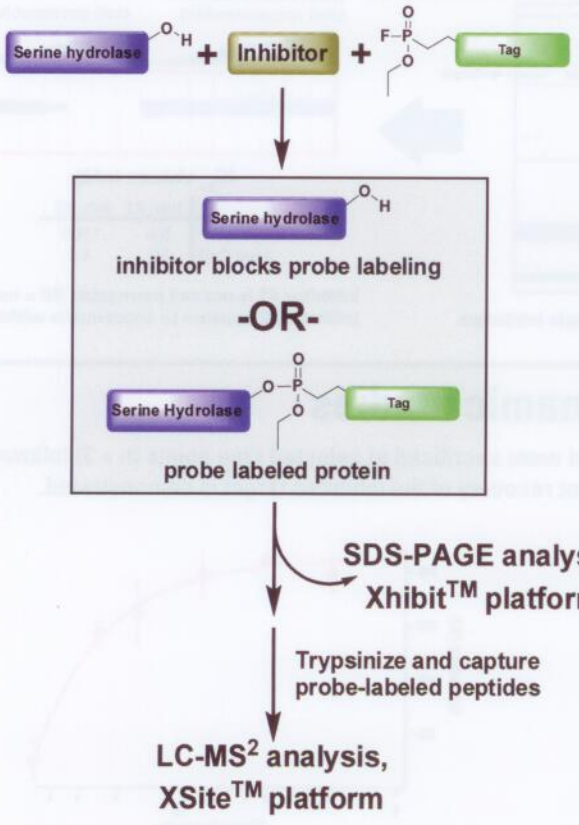
Probes are used for both selective detection and enrichment of serine hydrolases from proteomes. The activity-based nature of labeling enables simultaneous evaluation of inhibitor selectivity and potency

Cell permeable probes permit live cell assays to monitor the efficacy and permeability of inhibitors



Covalent modification of the active-site serine nucleophile by a fluorophosphonate

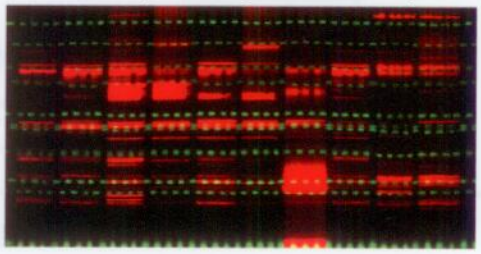
ActivX activity-based probe technology platforms



Xhibit™

A high-throughput 96-lane SDS-PAGE platform incorporating robotic liquid handling and automated data analysis. Xhibit™ provides rapid and cost-effective inhibitor profiling.

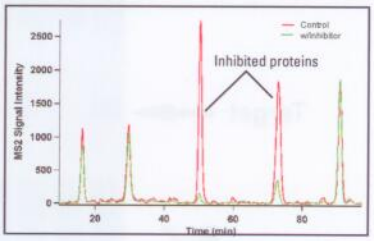
Simultaneous analysis of multiple inhibitors in 10 proteomes



XSite™

A high information content mass spectrometry platform that permits identification and quantitation of every active serine hydrolase in a proteome.

Identification of inhibitor selectivity using XSite™



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