Capture Compound® Mass Spectrometry: Elucidating Off-Target Binding to Deconvolute Drug Toxicity

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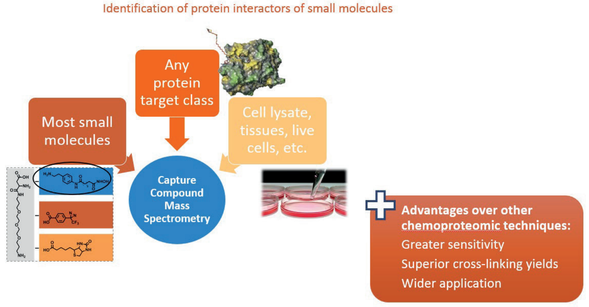
1 CCMS INTRODUCTION

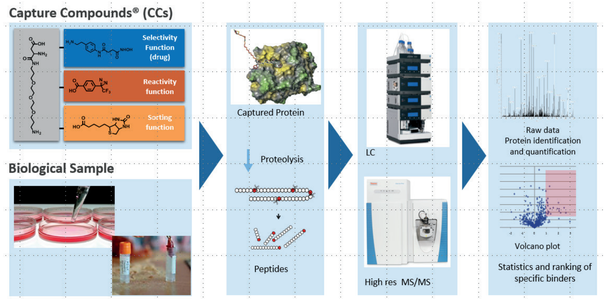
Capture Compound Mass Spectrometry (CCMS) is an unbiased, proteome-wide approach for the identification of specific on- and off-target binding protein targets for small molecules of interest.

Capture Compounds® (CCs) are unique tri-functional probes designed to interrogate proteins in their native environments. The distinct molecular architecture of the Capture Compounds® enables a three-stage process of binding, capture and isolation



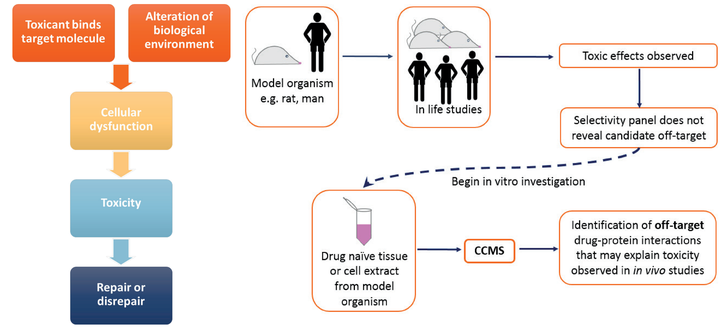
2 THE CCMS EXPERIMENT

A panel of CCs are synthesized with the selectivity function in a position compliant with on-target SAR and in alternative orientations to allow generation of a full interaction profile. CCs are incubated with a biological sample whereby binding of the selectivity function to interacting proteins occurs before covalent capture via photo-irradiation.
Competition experiments include incubation of the sample with both the CC and an excess of the free ligand to allow specifically interacting proteins to be determined. Sample analysis by high-resolution LC-MS/MS reveals specific binding partners.



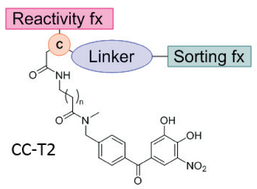
3 CCMS IN DRUG SAFETY

During Drug Discovery and Development, the timely assessment of the off-target liabilities of a lead molecule or the cause of an unexpected toxic event in vitro or in vivo can de-risk a molecule. With CCMS, both toxic pharmacological interactions and drug-protein adducts can be detected.

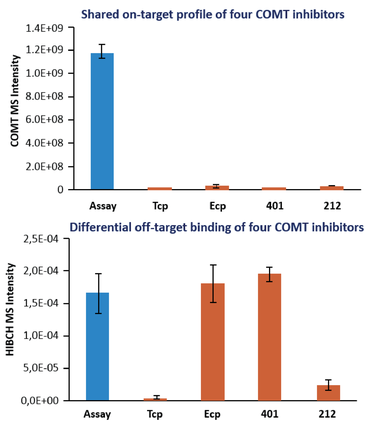


4 CASE STUDY: CCMS IDENTIFIES OFF-TARGET MECHANISM OF TOLCAPONE TOXICITY

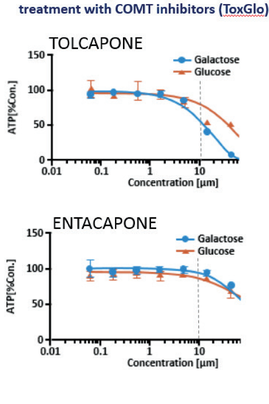
CCMS was used to determine on- and off-target interactions of the catechol-O-methyl transferase (COMT) inhibitor tolcapone in a human liver cancer cell line (HepG2).



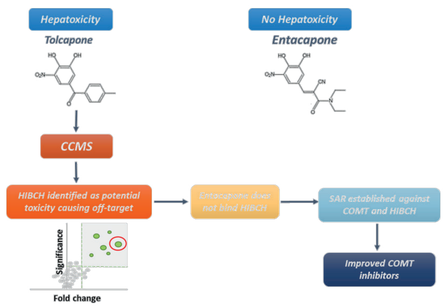
Inhibitors and CCs	IC_{50} [nM], rec. human COMT
Tolcapone	127
Tolcapone CC - T1	>6,000
Tolcapone CC - T2	448
Entacapone	386
Cmpd 212	179
Cmpd 401	54



Differential binding profiles were seen against on- and off target proteins. These binding profiles correlated with cellular hepatotoxicity.



Compound	COMT Affinity	HIBCH Affinity	Cellular Hepatotoxicity
Tolcapone	✓	✓	✓
Entacapone	✓	×	×
Cpd 212	✓	✓	✓
Cpd 401	✓	×	×



CCMS is a powerful chemoproteomic tool for profiling protein interactions of small molecules

- Unbiased screening of the entire proteome versus traditional selectivity screening
- Can be used to probe the potential mechanisms causing toxicity by identifying specific binding off-targets
- CCMS can support projects at a variety of stages throughout drug discovery and development.