In vitro Metabolite Profiling and Identification

Background: The enzymatic biotransformation of drugs in living systems strongly affects their biological activity, sometimes resulting in metabolites with decreased bioavailability and enhanced toxicity. The knowledge of specific sites of metabolic transformation is useful for guiding synthetic optimization of the lead compounds or drug candidates to overcome the stability and toxicity issues.

Service Details: To elucidate the main routes of hepatic metabolism, incubations of new chemical entities are carried out with liver microsomes (mouse, rat or human), that provide a major range of the metabolizing enzymes (phase I metabolism). Additionally, S9 fractions from mouse, rat and/or human liver are used for a broader coverage of possible biotransformations (both phases I and II of metabolism). Some compounds are unstable in blood plasma; therefore plasma stability test is usually included in the service. A typical protocol includes incubation of the test compound (at concentration of 2 μM) with one chosen metabolically active test-system (liver microsomes, liver S9 or plasma) in the presence (or absence, in the case of plasma) of cofactors at 37°C, sampling at 2 time points (0 and 60 minutes), reaction quenching with acetonitrile and centrifugation. The analysis of the incubation sample and comparison to the control (quenched at time 0 min) is performed using liquid chromatography/tandem mass spectrometry (LC-MS/MS, API3000, AB Sciex). Our approach to drug metabolite identification integrates both software- and knowledge-based predictions of metabolic pathways allowing for list-dependent search of metabolites (1), as well as standard tandem mass spectrometry protocols (2) including MS/MS spectra of parent compound and metabolites, precursor ion and neutral loss scans to ensure the detection of unexpected metabolites formed by less common metabolic reactions.

Deliverable: Data include ion chromatogram of parent compound and metabolites, table containing metabolites references and molecular formulas where possible, names of biotransformations, masses, m/z, mass differences from the parent, and retention times. The structure identification data comprise MS/MS spectra and details of the product ion fragments for parent compound and metabolites, as well as structural assignment based on the key fragments observed. Finally, we provide a detailed report containing proposed metabolites structures and expected metabolic pathways.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 μmol) or 50 μL of 20 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient, depending on the particular project. We need to know structure of the molecules for metabolite profiling studies.