ENAMINE, Confidential

Quotation Date: May 31, 2023

Attn.: [redacted]
[redacted] group
Institute of Organic Chemistry and Biochemistry of the CAS
Flemingovo nám. 2
166 10 Praha 6
Czech Republic
T [redacted]
[redacted]

Quotation #BEIO20230531YH-1

This Quote is pursuant to the Framework Service Agreement ("Agreement") between Enamine Ltd ("Enamine") and Institute of Organic Chemistry and Biochemistry of the CAS dated July 10, 2019. The parties agree that Enamine shall perform the work in accordance with this Quote and subject to all the terms and conditions of the Agreement.

#	Study title	Report delivery time*	Rate, EUR	Qty	Price, EUR
1.	Shake-Flask Aqueous Solubility Assay (kinetic solubility)	2 weeks	80.00	27	2160.00
2.	Hepatic Microsomal Stability (human)	2-3 weeks	170.00	7	1190.00
3.	Hepatic Microsomal Stability (rat)	2-3 weeks	160.00	7	1120.00

TOTAL amount, EUR:	**4,470.00**

***After receipt of the test articles at Enamine facility

Notes: A minimal accurately weighable quantity of each dry compound (~2 mg) or 100 uL of 20 mM stock solution in DMSO will be sufficient to run all listed assays**.** We do not need to know structures of the molecules for testing. However, we ask our customers to provide brutto formulas, for all studies involving MS detection.
We keep all DMSO stocks for test articles at the analytical lab for up to 3 months, in case any additional in vitro ADME tests are required.

Shake-Flask Solubility Assay

Background: Determining compound solubility is an essential tool for early stages of the drug discovery process, as well as for lead optimization. Low solubility can lead to unpredictable and unreliable results during *in vitro* testing, thereby increasing the development costs. Solubility issues at the later stages of the drug discovery may lead to poor bioavailability, underestimated toxicity and other obstacles, lowering the chances of a given drug candidate for success. Solubility can be measured either as a kinetic or thermodynamic value. Typically, for early-stage drug discovery the kinetic solubility method is used, as it is fast and well suited for HTS format. In this case, solid compounds are first

dissolved in DMSO and then linear serial dilutions of each compound are added to an aqueous buffer and observed for precipitate formation when the compound is not completely soluble. Precipitate appearance can be evaluated by light scattering (laser nephelometry method). For better precision, the solution can be subjected to high-speed centrifugation or filtration using special solubility filter plates and then the compound concentration is measured in the saturated solution directly by UV or LC-MS using separately built calibration curves. Thermodynamic solubility is important for lead optimization and drug formulation stages. It is usually determined for pure compounds: crystalline powders, amorphous substances and liquids. In this modification of solubility assay long (24 hours or more) incubations are required. Measurements are usually performed by the shake flask method with UV-Vis or LC-MS detection.

Service Details: This commonly used shake flask protocol is based on the use of Millipore Multiscreen solubility filter plates or centrifugation followed by UV-Vis quantitation of dissolved compounds. Microplate reader SpectraMax Plus (Molecular Devices) is used in our lab for UV-Vis measurements. LC-MS/MS quantitation (API3000 mass detector, AB Sciex) can also be done for poorly UV-absorbing compounds, mixtures, and compounds prone to degradation. Kinetic solubility measurements are performed starting from DMSO stock solutions of the test articles; powders are used for thermodynamic solubility measurements.

Typical assay conditions are as follows:

- Kinetic solubility measurements: 2 h shaking time at 25ºC in an aqueous buffer;
- Thermodynamic solubility measurements: 4 h and 24 h shaking time at 25ºC.

The assay is run in duplicates. One or two reference compounds are included in each test batch. Assay/protocol customization is available upon request.

Deliverable: Full study report is provided, including solubilities and calibration curves for test and reference compounds.

Sample Submission: Sample requirement is at least 60 µL of 20 mM stock compound solutions in DMSO for kinetic solubility measurements, and 5 x 3 µmol of dry compound for thermodynamic solubility measurements. We do not need to know structures of the molecules for ADME testing. However, brutto formulas have to be provided for all studies involving MS detection.

Hepatic Microsomal Stability (human, rat, or mouse)

Background: Liver is a primary site of drug metabolism, and drug metabolic transformations may have significant impact on its efficacy and safety. For this reason, drug candidates are screened early in the discovery process for metabolic stability. Microsomes from human or animal liver are useful models to quickly and inexpensively predict hepatic clearance *in vitro* for the corresponding species. Stability experiments can be done either with hepatic microsomal fraction to investigate only the cytochromes P450-mediated (Phase I) metabolism or with the S9 fraction, which consists of both hepatic microsomes and cytosol. The advantage of using S9 fraction is that it contains both Phase and Phase II enzymes and can be used to investigate Phase II metabolic pathways

in vitro, when supplemented with the corresponding cofactors such as UDPGA (for glucuronidation) and PAPS (for sulphation)**.**

Service Details: Metabolic stability assays are typically performed using mouse, rat, or human microsomes or S9 fraction (microsomes from other species are available upon request). Test compounds are incubated with microsomes supplemented with cofactors at 37°C. Typical conditions are the compound concentration of 2 µM and 5 sampling time points over 40 min, in two independent replicates. At each time point, the reactions are terminated with acetonitrile. The samples are centrifuged and the relative parent compound concentrations are evaluated by LC-MS/MS. The incubation of two control drugs with microsomes and blank control reaction without co-factors are used as controls.

Deliverable: Data include parent compound percent remaining, half-life ($t_{1/2}$), and intrinsic clearance (Cl_{int}) values. Full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 µmol) or 50 µL of 10-20 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 do not need to know structures of the molecules for ADME testing. However, brutto formulas have to be provided for all studies involving MS detection.