An AMI-MS assay enables biochemical profiling of novel hydrolase inhibitors.

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Abstract

Acoustic mist ionization - mass spectrometry (AMI-MS) enables high throughput analysis of liquid samples. An AMI-MS assay was chosen as an orthogonal enzymatic assay to a primary TR-FRET probe displacement screen in an HTS campaign to identify inhibitors of a challenging hydrolase target. The assay enables readout of enzymatic activity by direct measurement of reaction products.

In parallel to the primary assay, this functional assay has been used to validate compounds from HTS and DEL hit finding campaigns. It is currently used in a screening cascade for the first time in a live project to drive the DMTA cycle.

Introduction

- A biochemical assay was developed for a challenging hydrolase target using detection by acoustic mist ionization - mass spectrometry (AMI-MS).
- Assay format enables measurement of IC50 values from 12 point concentration response curves for 30 compounds per plate.
- Here we describe the AMI-MS workflow at AZ and subsequent impact on the project.

What is AMI-MS?

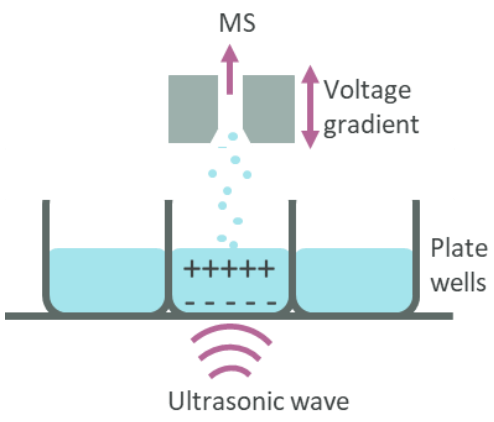


Figure 1: Acoustic mist ionisation schematic adapted from I. Sinclair *et. al. Anal. Chem.* 2019[2]. Charge separation is generated within the sample by a voltage gradient applied above the sample. Acoustic ejection generates a 'mist' of positively charged droplets in this instance.

AMI-MS enables high throughput analysis of liquid samples by mass spectrometry to directly monitor biochemical reactions[1,2]. This method is largely artefact and label free compared to other methods typically used to study biochemical reactions such an enzyme coupled systems.

The AMI-MS platform comprises of a beagle liquid handling system coupled to a Xevo G2-XS quadrupole time-of-flight mass spectrometer (Waters) via a heated transfer tube[1].

To generate a 'mist' a voltage gradient is applied above the sample well causing charge separation within the liquid and formation of a droplet. A 'mist' of charged droplets are released from the sample surface on application of acoustic energy to the base of the well[2], see figure 1. The droplets enter the capillary and are desolvated on entry to the mass spectrometer.

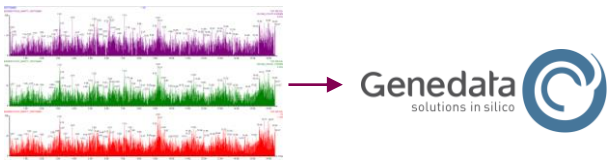
Methods

- Hydrolase + substrate reactions are incubated with compounds for 1 hour in assay ready plates (12-point concentration response, Labcyte 384 well plates).
- Internal standards are included in reaction buffers.

- Samples are analysed using the AMI-MS instrument: the Beagle liquid handling system ejects 100 nl into the mass spectrometer from each well.
- 384 wells are read in 17 minutes for this assay.

Beagle liquid handling system

- Ion counts for the mass range of the product and internal standard are extracted from raw data and converted into a format suitable for analysis in Genedata Screener.
- Ion count ratio (product/ internal standard) is plotted against compound concentration to determine IC50 in GeneData Screener.



Results

Hit Validation

Two hit campaigns were conducted for the hydrolase target:

- A high throughput screen (HTS).
- DNA encoded library (DEL) screen.

Hits from both HTS and DEL screens were entered into the project screening cascade that comprises a TR-FRET assay that utilizes a substrate competitive probe, and the AMI-MS enzymatic assay. A substrate analogue is used as a tool compound with known mechanism of action.

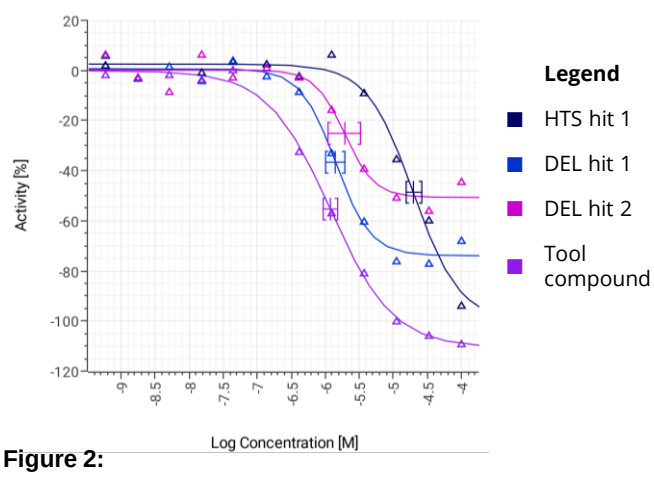


Figure 2:
IC50 curves for hydrolase tool compound and novel inhibitors.

Example curves from compounds from the hit campaigns are shown in figure 2. Validation of these compounds reveals interesting characteristics such as partial curves for some of the DEL hits versus compounds that approach full inhibition from the HTS hit series. Clearly there are several modes of inhibition at work in this compound selection.

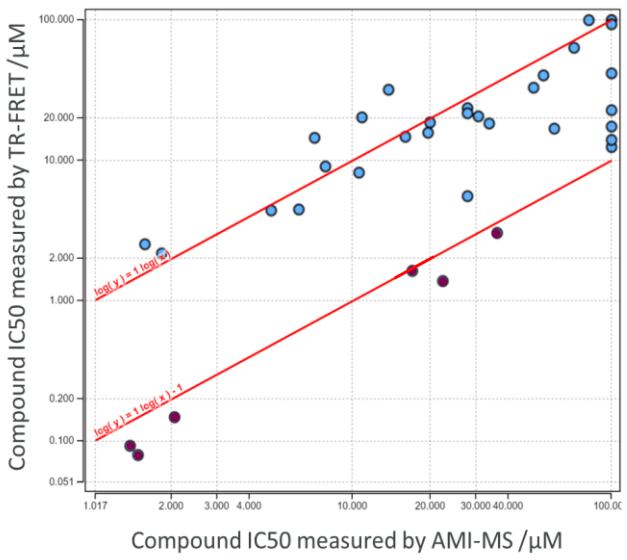


Figure 3 Correlation of initial hit validation data: TR-FRET v. AMI-MS assays:
Known substrate analogue compound IC50s (purple) measured by AMI-MS correlate to the TR-FRET with a known drop off in potency. Some novel compounds correlate with 1:1 potency between assays.

Figure 3 shows good correlation between the AMI-MS enzymatic assay versus the primary TR-FRET assay for substrate analogue compounds with known mode of action (competitive). These substrate analogues were used for assay validation and show show a known 10x drop off in potency between the two assays . However, other compounds discovered in the hit campaigns also show good correlation between the two assays but **no** drop off in potency. This interesting feature could indicate a different mode of action and will be followed up in future.

Conclusions

- ✓ We have implemented an AMI-MS assay into a robust screening cascade in a live project for the first time at Astra Zeneca.
- ✓ Compound profiling using our AMI-MS assay has validated novel hits from HTS and DEL hit finding campaigns for a challenging hydrolase target.
- ✓ AMI-MS provides functional data in the DMTA cycle for our challenging target.
- In future this assay will be used for bespoke mechanism of action studies for key compounds.

References

1. Novel Acoustic Loading of a Mass Spectrometer: Toward Next-Generation High-Throughput MS Screening. I. Sinclair *et. al. Journal of Laboratory Automation*, 2016.
2. Acoustic Mist Ionization Platform for Direct and Contactless Ultrahigh-Throughput Mass Spectrometry Analysis of Liquid Samples, I. Sinclair *et. al. Anal. Chem.* 2019.

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