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High-Throughput Identification of Kinase Inhibitors Using Acoustic Sample Ejection and Echo® MS

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Introduction

Protein kinases are key regulators of cellular signaling, controlling processes such as growth, differentiation, and apoptosis. Their dysregulation is implicated in numerous diseases, including cancer and neurodegeneration, making them attractive drug discovery targets.

Traditional mass spectrometry–based assays often rely on chromatographic separation, which limits throughput and increases sample consumption. The SCIEX Echo® MS platform overcomes these limitations by combining acoustic droplet ejection with direct, contactless electrospray ionization, enabling rapid analysis of thousands of samples per hour.

To illustrate the applicability of this high-throughput workflow, we focused on the DYRK (dual-specificity tyrosine-regulated kinase) family, which includes DYRK1A, DYRK1B, DYRK2, and DYRK3. These kinases play important roles in neuronal development and cell-cycle regulation and have emerged as therapeutic targets in Alzheimer's disease, Down syndrome, and several cancers.

Their high sequence homology poses a challenge for identifying selective inhibitors, making them an ideal model to demonstrate both the sensitivity and selectivity of the Echo® MS assay.

Schematic overview of the Echo MS ® assay workflow

Step 0 → **Step 1** → **Step 2** → **Step 3** → **Step 4** → **Step 5**

MRM optimisation for substrate and product

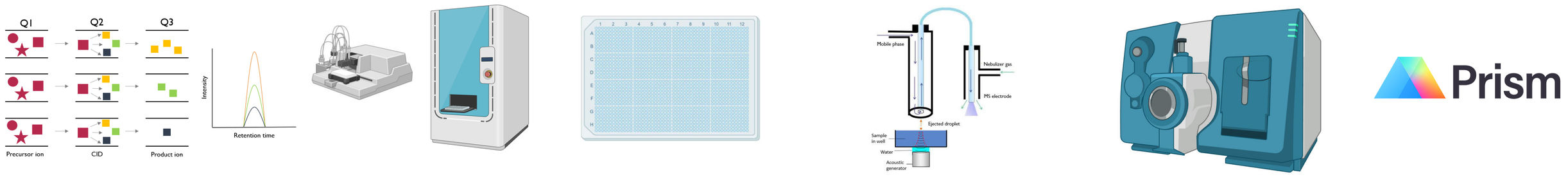
Reaction setup using liquid handlers

Reaction incubation

Acoustic sample transfer in Echo unit

MS analysis on Tripple Quad 6500+

Data processing and IC_{50} evaluation



Results

A panel of reference and exploratory compounds was evaluated for inhibition of DYRK1A, DYRK1B, DYRK2, and DYRK3 using the Echo® MS workflow. The primary screening identified several active compounds exhibiting ≥ 50 % inhibition at the tested concentration. For these hits, dose–response experiments were conducted to determine IC_{50} values and generate inhibition profiles (Fig. 1 and Fig. 2).

Among the confirmed inhibitors, Quercetin, Fisetin, and Dorsomorphin showed submicromolar activity across multiple DYRK isoforms, consistent with previously reported data, confirming assay validity. The reference compound AZ191 demonstrated strong potency and selectivity toward DYRK1B (IC_{50} = 9.6 nM), while LDN-192960 exhibited preferential inhibition of DYRK2 and DYRK3 (IC_{50} = 4.6 nM and 9.3 nM, respectively), revealing distinct selectivity patterns within the kinase family.

Compounds showing weak or negligible inhibition (IC_{50} > 1 µM) included PP242, Wortmannin, and Dauricine, supporting the specificity of the assay. Overall results are summarized in the heat map (Fig. 1), highlighting potent inhibitors (IC_{50} < 1 µM) in green.

This dataset demonstrates that the Echo® MS–based workflow enables rapid and reliable identification of nanomolar inhibitors and can distinguish subtle differences in potency and selectivity across homologous kinase targets.

Name	IC_{50} [µM]			
	DYRK1A	DYRK1B	DYRK2	DYRK3
Quercetin	0.0623	0.0634	0.1570	1.4420
LEM00000842	0.3539	0.1360	0.1964	0.3771
Dauricine	3.2760	no hit	no hit	no hit
Fisetin	0.0927	0.0367	0.0465	0.9866
PP242	3.2200	2.0600	0.1052	0.4709
Dorsomorphin	0.0419	0.0727	0.3528	1.7070
Wortmannin	1.3340	2.8640	8.9770	no hit
AZ191	0.0310	0.0096	0.3002	1.7940
LDN-192960	0.0920	0.1396	0.0046	0.0093

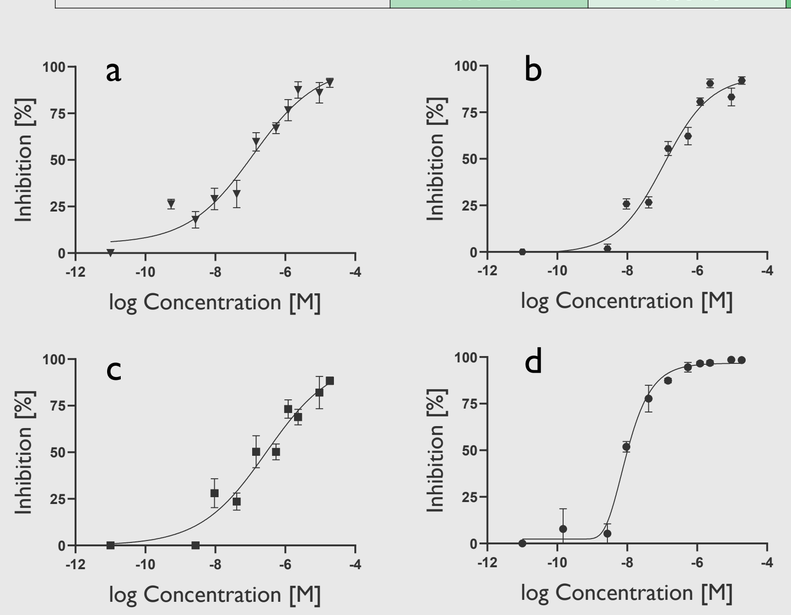


Figure 1 | Summary of IC_{50} values for all tested compounds across DYRK1A, DYRK1B, DYRK2, and DYRK3. The accompanying heat map highlights potent inhibitors (IC_{50} < 1 µM) in green.

Figure 2 | Inhibition profile of LDN-192960 showing concentration - dependent inhibition of DYRK1A (a), DYRK1B (b), DYRK2 (c) and DYRK3 (d).

Conclusion

The Echo® MS workflow enabled rapid and reliable identification of kinase inhibitors in a **chromatography-free** format, providing quantitative data within seconds per sample. Using the **DYRK kinase family** as a model system, the method successfully differentiated potent inhibitors with nanomolar IC_{50} values and revealed distinct selectivity profiles across closely related isoforms. The results confirm that Echo® MS offers sufficient **sensitivity, precision, and throughput** for both primary and secondary screening. Its ability to generate detailed inhibition profiles directly from assay mixtures establishes it as a robust analytical platform for **early-phase drug discovery and target-specific inhibitor characterization.**