Abstract

Background: L-type amino acid transporter 1 (LAT1) is upregulated in many cancers and supports tumor growth through uptake of essential amino acids, making it an important therapeutic target. However, functional LAT1 assays often rely on radiolabeled substrates or costly proprietary kits that limit accessibility for routine screening.

Methods: We developed a simplified fluorescence-based assay to measure LAT1-mediated amino acid uptake in live A549 cells using boronophenylalanine (BPA) as a substrate and a detection reagent to generate a fluorescent signal measurable on a standard microplate reader. The assay was validated through substrate concentration and time-course experiments as well as pharmacologic inhibition with the known LAT1 inhibitor JPH203. Fluorescence output was also compared with that of a leading commercial assay.

Results: The assay produced measurable fluorescence consistent with intracellular BPA accumulation. Signal decreased in a dose-dependent manner with JPH203, confirming detection of LAT1 inhibition. Fluorescence increased with BPA concentration and plateaued near 1000 µM, suggesting saturable uptake. Trends were comparable to those observed with a commercial assay. Cost analysis demonstrated a greater than 3,400-fold reduction in per-test reagent expenses.

Conclusions: This low-cost fluorescence assay provides an accessible alternative for functional LAT1 analysis in live cells and may enable broader high-throughput screening of LAT1-targeted compounds, particularly in resource-limited research settings.