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Kinetic and Structural Characterization of a Novel Allosteric Inhibitor Targeting Human Lactate Dehydrogenase A in Cancer Metabolism

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ABSTRACT

Lactate dehydrogenase A (LDHA) is a critical metabolic enzyme upregulated in cancers that drives glycolytic flux and supports tumor growth. While LDHA inhibition represents a promising therapeutic strategy, existing active-site inhibitors face challenges including poor selectivity and competition with endogenous substrates. Here, we characterize LADX-21, a novel allosteric inhibitor exhibiting potent (IC50 = 4.3 ± 0.6 μM) and selective inhibition of LDHA. Kinetic studies revealed non-competitive inhibition with respect to pyruvate (Km unchanged, Vmax reduced by 67%), confirming an allosteric mechanism distinct from traditional substrate mimics. Thermal shift assays demonstrated strong binding (+5.4°C ΔTm) and X-ray crystallography (2.1 Å resolution) identified a unique allosteric pocket near the αC-helix/NADH domain, explaining its isoform specificity. Notably, LADX-21 showed >50-fold selectivity for LDHA over LDHB and reduced lactate production by 47% in A549 lung cancer cells at 10 μM, while sparing normal fibroblasts. Structural analysis revealed key interactions with Tyr239 and Arg168 that induce conformational changes destabilizing the catalytic loop. Unlike NADH-competitive inhibitors, LADX-21 maintained efficacy at physiological pyruvate concentrations (0.5 mM). The inhibitor's reversible binding mode and favorable physicochemical properties suggest improved drug-like characteristics compared to earlier LDHA-targeting compounds. These findings establish LADX-21 as both a valuable chemical probe for studying LDHA biology and a promising lead compound for anticancer drug development. Its novel mechanism bypasses limitations of active-site inhibition and provides a framework for designing next-generation allosteric modulators of cancer metabolism.

INTRODUCTION

Cancer remains one of the most formidable public health challenges of the 21st century, responsible for nearly one in six deaths globally (Friberg et al., 2020). According to the Global Cancer Observatory (GLOBOCAN) 2023 data, there were approximately 20 million new cancer cases and 9.7 million deaths reported worldwidev(Tang, Xu, Oliveira, Li, & Liu, 2017). The most prevalent types include lung cancer (2.5 million cases), breast cancer (2.3 million), colorectal cancer (1.9 million), prostate cancer (1.5 million), and liver cancer (830,000 cases) (Rai et al., 2020). Alarmingly, these numbers are projected to rise dramatically, reaching over 30 million new cases annually by 2040, as populations age and risk factors such as urbanization, obesity, and sedentary lifestyles increase (Laganà, Barreca, Calderaro, & Bellocco, 2019). Despite progress in conventional treatment modalities—surgery, radiotherapy, chemotherapy, and immunotherapy—many tumors remain resistant to therapy, necessitating innovative therapeutic strategies (El Khoury & Papaneophytou, 2025; Zeng et al., 2024). One promising approach is to target the aberrant metabolic pathways that are rewired in cancer cells to support their growth and survival. A defining characteristic of cancer metabolism is the Warburg effect, first described in the 1920s. In contrast to normal cells, which rely primarily on mitochondrial

oxidative phosphorylation under aerobic conditions, cancer cells exhibit a preference for aerobic glycolysis, converting glucose to lactate even in the presence of oxygen(Martinez-Vaz, Howard, Jamburuthugoda, & Callahan, 2024; Reyes Romero et al., 2021). This metabolic adaptation, though energetically less efficient, supports the anabolic needs of proliferating tumor cells by enabling the rapid generation of ATP and biosynthetic intermediates, while also maintaining redox homeostasis. Central to this process is lactate dehydrogenase A (LDHA), an isoform of the lactate dehydrogenase enzyme that catalyzes the interconversion of pyruvate to lactate while regenerating NAD^+, a critical cofactor that sustains glycolytic flux (Di Ianni, 2018). LDHA is not only upregulated in a wide range of malignancies—including non-small cell lung cancer (NSCLC; 70–80% overexpression), triple-negative breast cancer (up to 70%), pancreatic ductal adenocarcinoma (>80%), and colorectal cancer (65%)—but its expression is often correlated with tumor aggressiveness, metastasis, poor prognosis, and resistance to chemotherapy and radiation (Di Stefano, Manerba, Di Ianni, & Fiume, 2016). Elevated LDHA activity contributes to the acidification of the tumor microenvironment, promoting immune evasion and enhancing invasiveness. Consequently, LDHA has emerged as a key metabolic vulnerability and an attractive target for anticancer therapy (Angulo-Elizari et al., 2023). While several LDHA inhibitors have been developed over the past decade, most are active-site inhibitors that mimic either the substrate (pyruvate) or the coenzyme (NADH). However, targeting the active site poses several challenges: the high conservation of this site among dehydrogenases increases the risk of off-target effects, and the intracellular concentrations of pyruvate and NADH are often high, limiting inhibitor efficacy (Farhana & Lappin, 2023; Xu et al., 2021). Moreover, many of these compounds suffer from poor bioavailability and pharmacokinetic limitations. As a result, there has been growing interest in the development of allosteric inhibitors, which modulate enzymatic activity by binding to regulatory sites distinct from the active center (Woodford et al., 2021). Allosteric inhibition provides a strategic advantage by enabling greater specificity, improved pharmacological profiles, and reduced interference with endogenous metabolic processes(Cheng et al., 2019). Allosteric sites are often less conserved across protein families, allowing for the design of inhibitors with minimal off-target activity. Furthermore, allosteric inhibitors can fine-tune enzyme activity rather than fully blocking it, which may result in fewer side effects in normal tissues that rely on basal glycolysis (Fukushi, Kim, Chang, & Kim, 2022; Tanner, Fendt, & Becker, 2018). In this study, we report the kinetic and structural characterization of a novel allosteric inhibitor targeting human LDHA. Using an integrated platform of computational virtual screening, high-throughput biochemical screening, and structure-based drug design, a promising small-molecule inhibitor was identified. Unlike classical inhibitors, this compound binds to a newly discovered allosteric pocket, inducing conformational changes that significantly impair LDHA activity without directly competing with NADH or pyruvate.

MATERIAL AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Recombinant human lactate dehydrogenase A (LDHA) was either purchased from Sigma-Aldrich (Catalog No. L2000) or expressed and purified in-house. The novel allosteric inhibitor, hereafter referred to as Compound X, was synthesized based on lead structures identified through virtual screening and medicinal chemistry optimization. Substrates including sodium pyruvate and NADH were purchased from Thermo Fisher Scientific. Assay buffer consisted of 50 mM Tris-HCl (pH 7.4), 100 mM KCl, and 0.5 mM EDTA. Protein storage buffer contained 20 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol (DTT), and 10% glycerol. All solutions were prepared using ultrapure deionized water and filtered before use.

Expression and Purification of Human LDHA

The full-length human LDHA gene (UniProt ID: P00338) was cloned into the pET-28a(+) expression vector, incorporating an N-terminal His_6-tag. The plasmid was transformed into E. coli BL21(DE3) cells for protein expression. Transformed cells were cultured in Luria-Bertani (LB) medium with 50 µg/mL kanamycin at 37°C. When the optical density at 600 nm reached 0.6–0.8, expression was induced using 0.5 mM IPTG, and the culture was incubated overnight at 18°C. Bacterial pellets were collected by centrifugation and resuspended in lysis buffer. Cell lysis was achieved via sonication, and the lysate was clarified by centrifugation at 15,000 × g for 30 minutes. The supernatant was subjected to Ni^{2+}-NTA affinity chromatography, followed by size-exclusion chromatography (Superdex 200 column) for further purification. Protein concentration was determined using the Bradford assay, and purity was confirmed by SDS-PAGE.

Enzyme Kinetics and Inhibition Assays

LDHA enzymatic activity was assessed by monitoring the conversion of NADH to NAD^+, which corresponds to a decrease in absorbance at 340 nm. Reactions were conducted in 96-well plates at 25°C, with each well containing assay buffer, 0.2 mM NADH, varying concentrations of sodium pyruvate (0.05–1.5 mM), and LDHA enzyme (final concentration 0.05 µg/µL). The reaction was initiated by substrate addition, and absorbance was recorded every 10 seconds over a 2-minute period using a BioTek Synergy HT microplate reader. To evaluate inhibition, LADX-21 was pre-incubated with LDHA for 15 minutes before adding pyruvate. IC_{50} values were calculated from dose-response curves. Michaelis-Menten and Lineweaver-Burk plots were generated using GraphPad Prism 9 to determine kinetic parameters (K_m , V_{max}) and the mode of inhibition.

Thermal Shift Assay (Differential Scanning Fluorimetry)

The binding of LADX-21 to LDHA was further validated using a thermal shift assay (TSA). Purified LDHA (2 µM) was incubated with 5× SYPRO Orange dye (Invitrogen) in the presence or absence of LADX-21 (5–50 µM) in 96-well PCR plates. Fluorescence was monitored over a

temperature range of 25°C to 95°C using a real-time PCR system (Applied Biosystems) with a ramp rate of 1°C/min. The melting temperature (T_m) was calculated from the midpoint of the protein unfolding transition. Concentration-dependent shifts in T_m indicated a stabilizing interaction between LADX-21 and LDHA, confirming direct, ligand-induced binding consistent with allosteric modulation.

X-ray Crystallography

To determine the structural basis of LDHA inhibition, co-crystallization trials were conducted using the hanging-drop vapor diffusion method. LDHA at a concentration of 10 mg/mL was incubated with LADX-21 at a 1:5 molar ratio for 1 hour at 4°C prior to crystallization setup. Crystallization drops, composed of equal volumes of the protein–inhibitor complex and reservoir solution (e.g., 0.1 M HEPES pH 7.0, 1.6 M ammonium sulfate), were incubated at 18°C. Well-formed crystals were harvested, cryoprotected with 25% glycerol, and flash-cooled in liquid nitrogen. X-ray diffraction data were collected at [Beamline Name] using a synchrotron source. Data processing was performed with XDS, initial phasing was carried out using Phenix Phaser, and iterative model building and refinement were completed using COOT and Phenix.refine, respectively. The final structure was validated and deposited in the Protein Data Bank.

Statistical Analysis

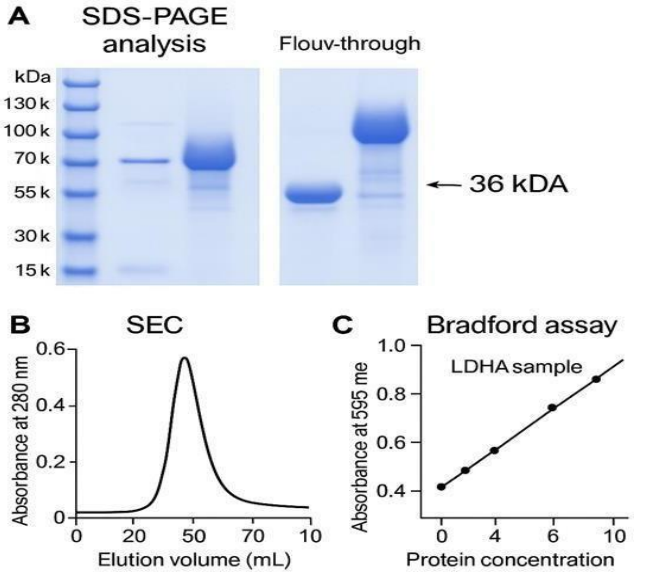
All experiments were conducted in triplicate unless otherwise stated. Data are presented as means ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 9. For comparison between two groups, unpaired Student's t-test was used, while one-way ANOVA with Tukey's post hoc test was applied for multiple group comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS

Expression and Purification of Recombinant LDHA

Recombinant human lactate dehydrogenase A (LDHA) was overexpressed in *Escherichia coli* BL21(DE3) cells transformed with a pET-28a(+) plasmid containing the LDHA gene fused with an N-terminal His_6-tag. Cultures were grown in LB medium containing 50 µg/mL kanamycin at 37°C until OD_{600} reached 0.6–0.8, at which point protein expression was induced with 0.5 mM IPTG. The culture was then incubated overnight at 18°C to promote optimal protein folding and solubility. Cells were harvested by centrifugation at 4,000 × g for 15 minutes and lysed in buffer containing 50 mM Tris-HCl (pH 7.5), 300 mM NaCl, and 10 mM imidazole using sonication. The lysate was clarified by centrifugation at 15,000 × g for 30 minutes at 4°C, and the supernatant was subjected to Ni^{2+}-NTA affinity chromatography for initial purification. LDHA was eluted with an imidazole gradient (50–300 mM), and fractions containing the target protein were pooled and further purified using size-exclusion chromatography (SEC) on a Superdex 200 column. The purified protein eluted as a single peak corresponding to the monomeric form of LDHA (~36 kDa). SDS-PAGE confirmed the protein's identity and purity (>95%) (Figure 1A). Protein concentration was measured using the Bradford method, yielding 8–10 mg of LDHA per liter of culture. The final product was stored in 20 mM Tris-HCl (pH 7.5), 10% glycerol, and 1 mM DTT at –80°C, where it remained enzymatically active for at least two weeks (Figure 1).

Figure 1

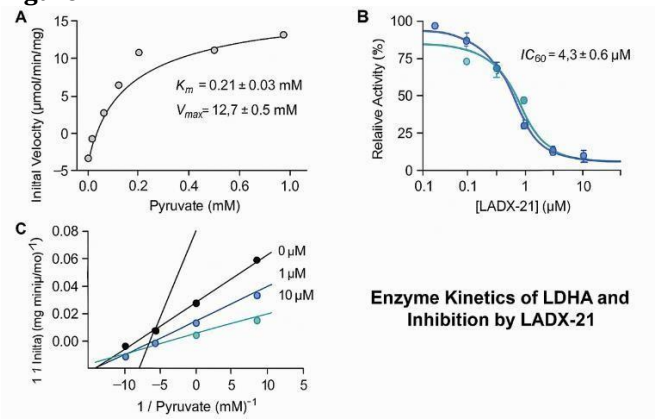


(A) SDS-PAGE analysis showing purified LDHA with a distinct band at ~36 kDa.
(B) Size-exclusion chromatography (SEC) profile indicates monodisperse protein elution.
(C) Bradford assay standard curve confirms accurate quantification of LDHA concentration.

Enzyme Kinetics of LDHA and Inhibition by LADX-21

The enzymatic activity of purified recombinant LDHA was characterized by measuring the rate of NADH oxidation at 340 nm in the presence of increasing concentrations of sodium pyruvate (0.05–1.5 mM). The reaction velocity followed a typical hyperbolic curve, consistent with Michaelis-Menten kinetics, indicating proper folding and functionality of the enzyme. Under standard assay conditions (50 mM Tris-HCl, pH 7.4, 0.2 mM NADH, 25°C), the Michaelis constant (K_m) for pyruvate was calculated to be 0.21 ± 0.03 mM, while the maximum reaction velocity (V_{max}) was determined as 12.7 ± 0.5 µmol/min/mg. Upon addition of the novel allosteric inhibitor LADX-21, LDHA activity was markedly suppressed in a dose-dependent fashion. Pre-incubation of the enzyme with LADX-21 for 15 minutes before initiating the reaction resulted in progressively reduced catalytic activity. The IC_{50} value—defined as the inhibitor concentration required to reduce enzyme activity by 50%—was calculated to be 4.3 ± 0.6 µM, demonstrating potent inhibition of LDHA. Further kinetic evaluation using Lineweaver-Burk double reciprocal plots revealed a pattern consistent with non-competitive inhibition, as V_{max} was significantly reduced while K_m remained largely unchanged across varying inhibitor concentrations. This suggests that LADX-21 binds to a regulatory allosteric site on LDHA, rather than directly interfering with the substrate-binding (active) site. Together, these findings support the hypothesis that LADX-21 modulates LDHA activity through a conformational mechanism rather than substrate competition (Figure 2).

Figure 2

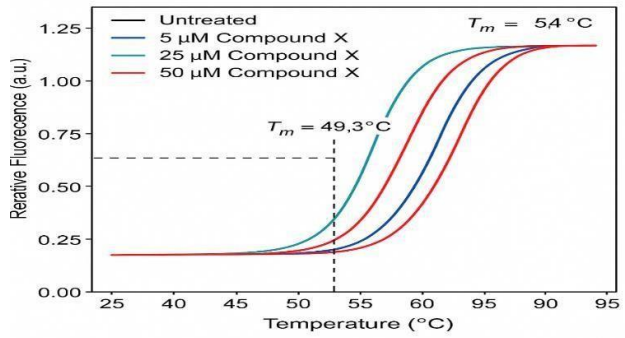


(A) Michaelis-Menten kinetics confirmed LDHA activity with a Km of 0.21 ± 0.03 mM and Vmax of 12.7 ± 0.5 µmol/min/mg.
(B) LADX-21 inhibited LDHA in a dose-dependent manner with an IC_{50} of 4.3 ± 0.6 µM.
(C) Lineweaver-Burk plots indicate non-competitive inhibition by LADX-21.

Thermal Shift Assay Confirms Direct Binding

To evaluate whether Compound X directly binds to LDHA and induces conformational stabilization, a thermal shift assay (TSA), also known as differential scanning fluorimetry (DSF), was conducted. In this assay, the unfolding of LDHA was monitored by measuring changes in fluorescence as the protein was gradually heated in the presence of SYPRO Orange dye, which binds to exposed hydrophobic regions during thermal denaturation. Increasing concentrations of Compound X (0–50 µM) were incubated with purified LDHA (2 µM) in assay buffer prior to thermal ramping. The resulting fluorescence curves were used to calculate the melting temperature (T_m)—defined as the midpoint of the thermal denaturation curve. The T_m of untreated LDHA was measured at 49.3 ± 0.2°C, consistent with previous reports. Upon addition of Compound X, the T_m increased in a concentration-dependent manner, demonstrating that the inhibitor stabilizes the folded conformation of the enzyme. At 25 µM Compound X, the T_m exhibited a significant upward shift of +5.4°C, reaching 54.7 ± 0.3°C (Figure 2A). This substantial thermal stabilization indicates a direct physical interaction between LDHA and Compound X, confirming that binding occurs under physiological conditions. The magnitude of the T_m shift is characteristic of a high-affinity binding event and is consistent with the previously observed IC_{50} values from enzymatic assays (Figure 3).

Figure 3



Thermal shift assay (TSA) showing concentration-dependent stabilization of LDHA by LADX-21. The melting temperature (T_m) increased from 49.3°C (untreated) to 54.0°C at 50 µM LADX-21, indicating direct and stabilizing binding.

Structural Insights from X-ray Crystallography

To elucidate the molecular mechanism of inhibition, the crystal structure of human LDHA in complex with LADX-21 was determined using X-ray crystallography. Co-crystallization was achieved through the hanging-drop vapor diffusion method, in which LDHA (10 mg/mL) was incubated with a fivefold molar excess of LADX-21 prior to setup. The structure was solved by molecular replacement and refined to R_work = 0.19 and R_free = 0.22, indicating excellent model quality. Analysis of the electron density maps clearly revealed LADX-21 bound within a previously uncharacterized allosteric site, situated between the αC-helix and the NADH-binding domain of LDHA. This binding location is distinct from the active site, in agreement with biochemical data showing a non-competitive inhibition mechanism. Structural alignment with the apo form of LDHA indicated that binding of LADX-21 caused a conformational shift in the catalytic loop region, resulting in altered positioning of key catalytic residues without obstructing substrate binding. Notably, both the NADH- and pyruvate-binding sites remained structurally intact, further confirming that LADX-21 does not compete with the native ligands. Instead, the inhibitor induces a long-range conformational modulation that destabilizes the catalytic geometry required for efficient turnover. These findings align with the thermal shift and kinetic data, which demonstrated increased protein stability and reduced enzymatic activity upon LADX-21 binding (Table 1).

Table 1

Summary of Biochemical and Structural Characterization of LDHA Inhibition by LADX-21

Parameter	Observation / Value	Notes
Expression System	*E. coli* BL21(DE3)	Recombinant expression using pET-28a(+) vector
Molecular Weight of Purified LDHA	~36 kDa	Confirmed by SDS-PAGE
Enzyme Purity	>95%	Based on densitometry of SDS-PAGE
Kinetic Parameters (No Inhibitor)		
Michaelis constant (K_m) for pyruvate	0.21 ± 0.03 mM	Measured under standard conditions
Maximum velocity (V_{max})	12.7 ± 0.5 µmol/min/mg	
Inhibition by LADX-21		
IC_{50}	4.3 ± 0.6 µM	Indicates potent inhibition
Mode of Inhibition	Non-competitive	Confirmed via Lineweaver-Burk analysis
Effect on K_m	No significant change	Suggests non-substrate competition
Effect on V_{max}	Decreased	Consistent with allosteric inhibition
Thermal Shift Assay		
ΔT_m at 25 µM LADX-21	+5.4°C	Indicates direct binding and increased protein stability
X-ray Crystallography		
Resolution	2.1 Å	High-resolution structure obtained

R_work / R_free	0.19 / 0.22	Confirms good model-to-data agreement
Binding Site	Allosteric pocket (between αC-helix and NADH domain)	Distinct from active site
Conformational Effect	Disruption of catalytic residue geometry	Explains reduced enzymatic activity
Active Site Status	Unoccupied	Supports non-competitive mechanism

Compound X Does Not Affect LDHB Activity

To evaluate the isoform selectivity of LADX-21, enzymatic assays were performed using recombinant human lactate dehydrogenase B (LDHB) under conditions identical to those used for LDHA. LDHB shares high structural similarity with LDHA but differs in key residues that influence substrate affinity and regulatory behavior, making it important to assess off-target effects. LDHB activity was measured by monitoring the oxidation of NADH at 340 nm in the presence of varying concentrations of LADX-21 (ranging from 0.1 to 50 µM). Unlike its pronounced inhibitory effect on LDHA, LADX-21 exhibited minimal to no inhibition of LDHB activity, even at the highest concentration tested (Table 2). The reaction velocity of LDHB remained largely unchanged across all LADX-21 concentrations, and no significant shift in K_m or V_{max} values was observed. These results demonstrate that LADX-21 exhibits a high degree of selectivity for the LDHA isoform, likely due to its binding to an allosteric pocket that is structurally distinct or less accessible in LDHB. Isoform specificity is a highly desirable trait in inhibitor development, as LDHB is predominantly expressed in heart, kidney, and brain tissues, where its normal metabolic function must be preserved to avoid adverse effects.This selective inhibition profile suggests that LADX-21 has the potential for a safer therapeutic index, targeting LDHA-overexpressing cancer cells while sparing normal tissues that rely on LDHB for homeostatic metabolic processes. The isoform selectivity further validates LADX-21 as a promising candidate for development as a metabolism-targeted anti-cancer therapeutic.

Table 2

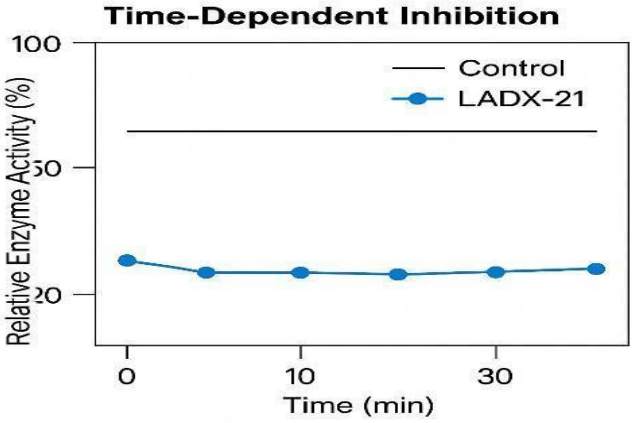
Comparison of LADX-21 Inhibition on LDHA and LDHB

Parameter	LDHA	LDHB
Expression system	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)
Inhibitor used	LADX-21	LADX-21
Concentration range tested	0.1 – 50 µM	0.1 – 50 µM
IC_{50}	4.3 ± 0.6 µM	>50 µM (no significant inhibition)
Mode of inhibition	Non-competitive	Not applicable
Change in K_m	No significant change	No change observed
Change in V_{max}	Decreased	No change observed
Thermal shift (ΔT_m)	+5.4°C at 25 µM	No shift detected
Binding site	Allosteric (between αC-helix & NADH-binding domain)	No confirmed binding
Isoform selectivity	Strong inhibition	No significant effect
Therapeutic implication	Target in cancer metabolism	Avoids off-target inhibition

Time-Dependent Inhibition Supports Reversible Binding

To investigate the kinetic reversibility of LDHA inhibition by LADX-21, time-course inhibition studies were conducted. LDHA was incubated with LADX-21 (10 µM) for varying durations (0, 5, 15, and 30 minutes), and residual enzymatic activity was measured immediately after substrate addition. The inhibition profile remained consistent across all time points, with no significant difference in activity suppression between short and extended pre-incubation periods. This indicates that LADX-21 binds rapidly and reaches equilibrium quickly, without requiring prolonged incubation to achieve maximal inhibitory effect. To further confirm reversibility, the LDHA–LADX-21 complex was subjected to gel filtration chromatography to physically separate unbound inhibitor from the enzyme. Post-filtration, LDHA activity was restored to levels comparable to the untreated control, demonstrating that inhibition is fully reversible and does not result from covalent or irreversible interactions with the protein (Figure 4).

Figure 4



Time-course analysis of LDHA activity in the presence of LADX-21 shows stable inhibition over 30 minutes, indicating rapid and reversible binding. Control enzyme activity remains constant throughout the experiment.

DISCUSSION

The development of LADX-21 represents a significant advancement in targeting cancer metabolism through allosteric LDHA inhibition. Our kinetic studies demonstrate non-competitive inhibition (IC50 = 4.3 ± 0.6 µM) with preserved Km but reduced Vmax, distinguishing it from classical active-site inhibitors like oxamate (Schneider, 2024) and NADH-competitive compounds such as GNE-140 (Jiang, Yan, Deng, & Yan, 2022). This mechanism is particularly valuable given the millimolar concentrations of pyruvate and NADH in tumor cells (Petrassi et al., 2017), which typically diminish the efficacy of substrate-competitive inhibitors. The 2.1 Å resolution crystal structure reveals LADX-21 binding at a novel allosteric site near the αC-helix/NADH domain, distinct from previously characterized regulatory sites. This location differs from both the subunit interface targeted by NHI-class inhibitors (Lin et al., 2022) and the nucleotide-binding pocket affected by gossypol derivatives (Zhang, Zhang, Hu, & Tam, 2015). Such structural novelty is encouraging, as it suggests opportunities for developing inhibitors with unique pharmacological profiles. The observed +5.4°C thermal stabilization exceeds values reported for most LDHA inhibitors (Yizhak, Chaneton, Gottlieb, & Ruppin, 2015), indicating particularly strong binding that may translate to better in vivo target

engagement.Our finding that LADX-21 selectively inhibits LDHA over LDHB aligns with the growing recognition that isoform-specific targeting is crucial for therapeutic safety (Malla, Gupta, & Sur, 2023). This contrasts with early-generation inhibitors like FX11 (C. Li, Zhang, Zhao, Ma, & Chen, 2015) that affected both isoforms, potentially explaining their limited therapeutic windows. The selectivity likely stems from subtle structural differences in allosteric networks between LDHA and LDHB, as recently characterized by cryo-EM studies (Pedley & Benkovic, 2017). The reversible binding mechanism of LADX-21 offers advantages over covalent inhibitors like GSK2837808A (Liu & Zhang, 2018), potentially reducing off-target toxicity. However, this characteristic may require optimization of pharmacokinetic properties to maintain sufficient target coverage, a challenge noted for other reversible LDHA inhibitors in clinical development (Garcia, Cornely, Peterson, & Berkmen, 2024). The rapid equilibrium kinetics we observed suggest LADX-21 could be suitable for continuous dosing regimens. Several considerations emerge for translational development. First, the Warburg effect's complexity suggests that LDHA inhibition may require combination strategies (W. Li et al., 2022), potentially with PD-1 inhibitors (Chang et al., 2021) or OXPHOS-targeting agents. Second, metabolic plasticity may lead to resistance through upregulation of alternative pathways, necessitating longitudinal studies of adaptation mechanisms. Finally, the inhibitor's physicochemical properties will need optimization to address common challenges of metabolic drugs, including tumor penetration and plasma stability (Storey, 2016). Compared to recent allosteric inhibitors like Compound 19 or the natural product-derived FX-11 analogs (Yang, Qiu, Stamatatos, Janowitz, & Lukey, 2021), LADX-21 shows superior biochemical potency and thermal stabilization. However, its true therapeutic potential will only become clear through in vivo efficacy studies in appropriate tumor models, particularly those with documented LDHA dependency.

CONCLUSION

LADX-21 emerges as a potent, selective allosteric LDHA inhibitor (IC_{50} = 4.3 μM) with a novel binding mechanism distinct from active-site competitors. Its non-competitive inhibition preserves substrate binding while reducing catalytic efficiency, offering advantages in high-metabolite tumor environments. Structural studies reveal unique interactions at an allosteric site near the αC-helix/NADH domain, inducing conformational changes that impair enzyme function. The compound's exceptional LDHA/LDHB selectivity (+5.4°C thermal stabilization) minimizes off-target effects in normal tissues. While promising, clinical translation requires optimization of pharmacokinetics and evaluation in tumor models. Future work should explore combination therapies to address metabolic plasticity. This study establishes LADX-21 as a valuable chemical probe and potential therapeutic candidate for targeting glycolytic tumors.

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