International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 14 Issue 3 ǁ March 2026 ǁ PP. 273-280

Design and Synthesis of Fructose-1,6-Bisphosphate Aldolase Inhibitors Against Multidrug-Resistant Pseudomonas aeruginosa

Tianyu Yang[a], Xingyong Liu[a,]*
[a]*School of Chemical Engineering, Sichuan University of Science & Engineering, Zigong 643000, China*
**Corresonding authors: (Xingyong Liu)*

Abstract

Pseudomonas aeruginosa is one of the major pathogens causing nosocomial infections in immunocompromised patients such as those with tumors. The emergence of multidrug-resistant strains poses severe challenges to clinical therapy. Fructose-1,6-bisphosphate aldolase (FBA) is a key enzyme in the glycolytic pathway. Type II FBA (FBA-II), which depends on Zn^{2+} *for catalysis, exists exclusively in microorganisms, whereas humans express type I FBA (FBA-I). This difference makes FBA-II an ideal antibacterial target. Most existing FBA inhibitors are metal chelators or substrate analogs containing polar phosphate groups, which generally suffer from poor membrane permeability and insufficient selectivity.*

In this study, 8-hydroxyquinoline-2-carboxylic acid (HCA) was used as the lead compound. Based on its co-crystal structure with FBA (PDB: 4LV4), novel inhibitors were designed using a scaffold hopping strategy. A virtual compound library containing 821 derivatives was constructed via SeeSAR software, and 500 candidate molecules were obtained after drug-likeness screening using Discovery Studio. High-precision XP molecular docking was performed with the Glide module of Schrödinger software, and 200 compounds were selected with an XP Gscore < -1 *kcal/mol. ADMET properties were predicted by QikProp. Combined with visual analysis of binding modes and synthetic feasibility, six representative compounds (FBA1–FBA6) were finally selected for synthesis and activity validation. FBA activity was determined using a Solarbio FBA activity assay kit (spectrophotometry). The results showed that FBA6 inhibited FBA by 50% at 250 μmol/L and 75.02% at 500 μmol/L, while the other five compounds almost lost activity.*

This study integrated various CADD methods including scaffold hopping, molecular docking, and virtual screening, and successfully identified FBA6, a novel FBA inhibitor with favorable binding activity. It provides a new strategy to overcome the limited membrane permeability of existing inhibitors and lays a foundation for subsequent structural optimization and antibacterial activity research. This research paradigm can also serve as a reference for drug development targeting other antibacterial targets.

Keywords: *FBA-II; 8-hydroxyquinoline-2-carboxylic acid; scaffold hopping; virtual screening; FBA enzymatic activity; antibacterial agents*

Date of Submission: 15-03-2026 Date of Acceptance: 31-03-2026

I. Introduction

Cancer patients develop impaired immune function due to the malignancy itself and treatments such as surgery, chemotherapy, radiotherapy, stem cell transplantation, and immunotherapy, making them high-risk populations for infection. Infection has become the second leading cause of death in this group. Major risk factors include neutropenia and disruption of skin and mucosal barriers[1]. Infections caused by multidrug-resistant (MDR) Gram-negative bacteria are particularly challenging in clinical management, such as carbapenemase-producing Enterobacterales (CPE) and resistant non-fermenters. The growing antibiotic resistance not only significantly reduces the efficacy of anti-infective therapy and increases mortality risk but also severely interferes with the smooth progress of anticancer treatment[2].

Among non-fermentative Gram-negative bacteria, Pseudomonas aeruginosa is the most common bacillus. Therefore, developing effective inhibitory strategies against this bacterium has important clinical value for controlling infections and improving patient prognosis.

Pseudomonas aeruginosa is an opportunistic pathogen belonging to the Pseudomonadaceae family. It is a Gram-negative, aerobic, non-spore-forming bacillus with a single polar flagellum, conferring motility. This bacterium is widely distributed in abiotic and biotic environments, detectable from soil, water bodies to plant and animal tissues[3]. It has diverse sources, isolated from various nosocomial infection foci in patients with cystic fibrosis (CF), burn wounds, urinary tract infections (UTIs), and pulmonary infections. It can also be recovered from medical devices such as inhalers, dialysis machines, ventilators, anesthesia equipment, and vaporizers, and even exists in environments such as toilets and sinks（Figure 1）[4].

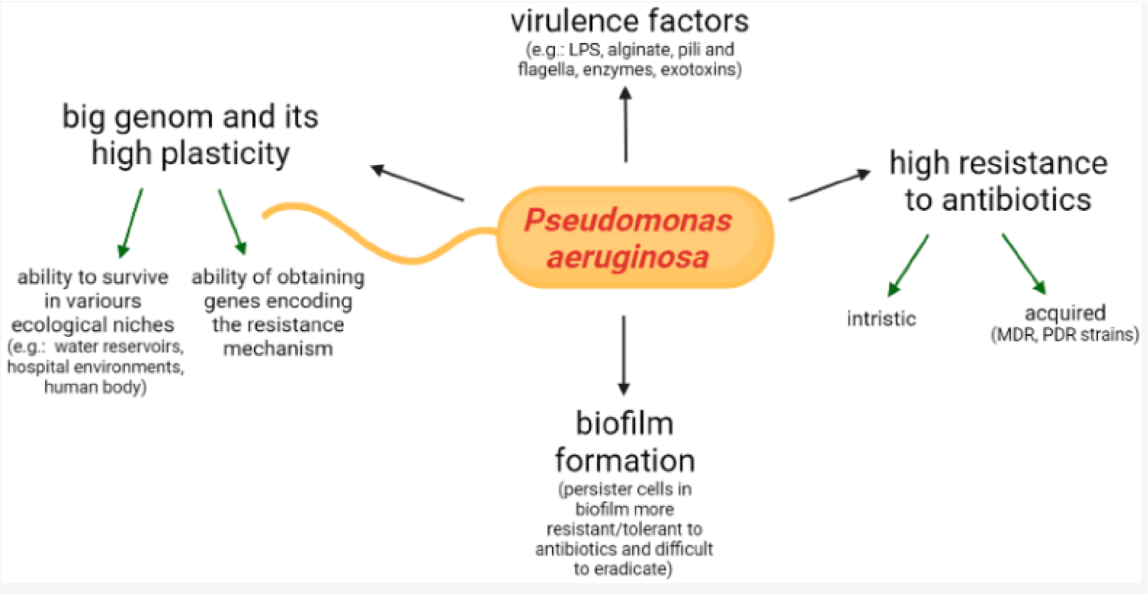


Figure 1 Factors contributing to the pathogenicity of Pseudomonas aeruginosa

MDR P. aeruginosa is frequently isolated from outbreaks in cystic fibrosis patients, cancer patients, and intensive care unit (ICU) settings. The United States reports over 51,000 healthcare-associated P. aeruginosa infections annually, with approximately 440 deaths[5]. Of these infections, more than 13% (about 6,700 cases) are MDR P. aeruginosa. The U.S. Centers for Disease Control and Prevention Antibiotic Resistance (CDC AR) Threat Report classifies MDR P. aeruginosa as a serious threat[6]. The 2017 World Health Organization list of priority pathogens for antibiotic resistance lists carbapenem-resistant P. aeruginosa among the critical threats[7]. The 2015 European Antimicrobial Resistance Surveillance Report indicated that P. aeruginosa resistance is widespread (over 10% in outbreaks) in most European countries. 13.7% of P. aeruginosa isolates were resistant to at least three antimicrobial classes, and 5.5% were resistant to all five antimicrobial classes, based on routine surveillance by the European Antimicrobial Resistance Surveillance Network (EARS-Net)[8].

Fructose-1,6-bisphosphate aldolase (FBA) is a key enzyme in glycolysis and gluconeogenesis, catalyzing the reversible cleavage of fructose-1,6-bisphosphate (FBP) into glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP) to supply ATP and metabolic intermediates for organisms. Based on catalytic mechanisms, FBA is classified into two types: FBA-I and FBA-II. Studies have revealed significant differences in structure, catalytic mechanism, and distribution between these two aldolases.

FBA-I and FBA-II differ fundamentally in catalytic mechanism（Figure 2）: the former generates a nucleophilic enamine intermediate from DHAP using a lysine residue, while the latter relies on Zn^{2+} cations to stabilize the DHAP enolate intermediate involved in aldol condensation. These mechanistic differences, combined with distinct species distributions, provide a valuable opportunity for drug development. Notably, humans only express FBA-I, making FBA-II an ideal target for developing novel antibacterial agents[9]. Functional studies further confirm that FBA-II is essential in diverse microorganisms—gene knockout or knockdown leads to severe loss of bacterial viability or even death, fully highlighting its potential as an antibacterial target[10]. Several competitive FBA-II inhibitors have been reported to possess antifungal activity, but most are FBP substrate analogs containing polar phosphate groups. Such structures limit their cell membrane permeability and thereby compromise antifungal efficacy.

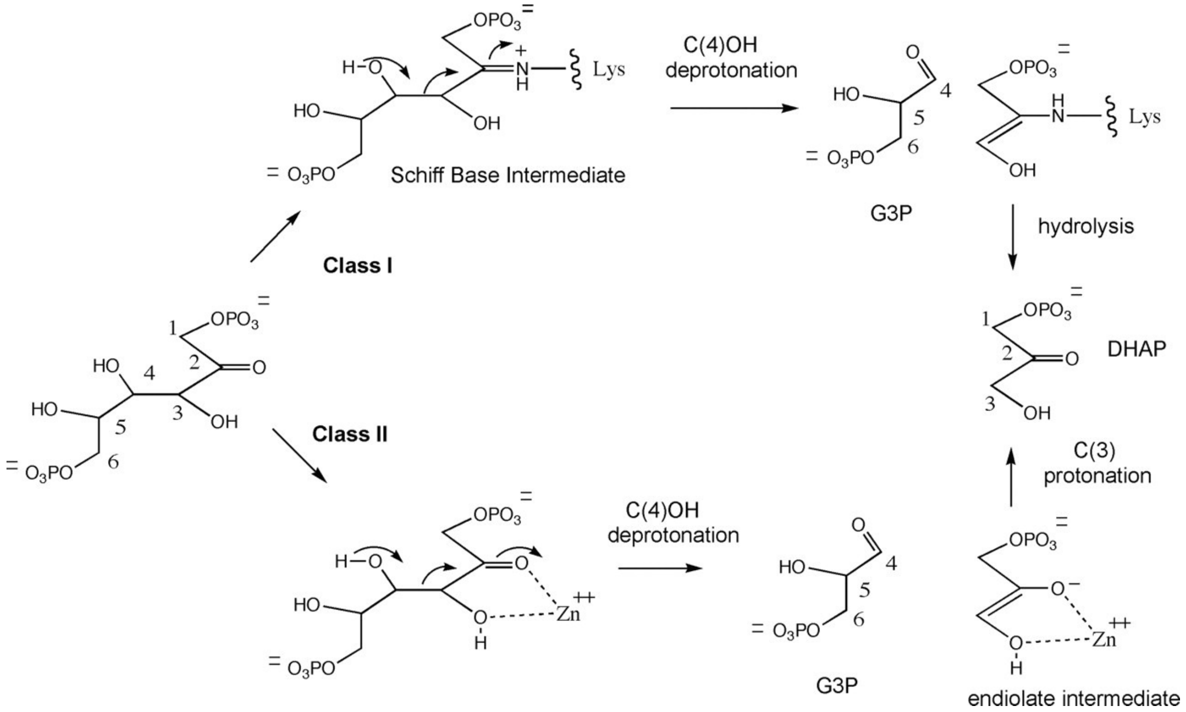


Figure 2 FBA-I and FBA-II in the catalytic mechanism

Developed Inhibitor Classes：Metal chelator inhibitors: Since the FBA-II active center depends on Zn^{2+} ions, metal chelators were the earliest explored inhibitor type. Dipicolinic acid (DPA) and its derivatives are representative compounds. Studies show that DPA exhibits moderate inhibitory activity against P. aeruginosa FBA in a time-dependent manner. Research on Mycobacterium tuberculosis FBA is more advanced: DPA has an IC_{50} of 28 μM[11], and 2,3-dimercaptopropanesulfonic acid (DMPS) has an IC_{50} of 5.2 μM[12][13][14]. These compounds competitively bind the active site with substrate FBP[15]. Notably, the inhibitory effect of EDTA on P. aeruginosa FBA can be partially reversed by adding Co^{2+} or Mn^{2+}, confirming the critical role of metal ions in FBA-II catalysis.Substrate analog inhibitors: Another major class of FBA inhibitors consists of FBP substrate analogs, usually bearing polar phosphate groups that competitively bind the enzyme active site. However, such inhibitors have significant limitations: polar phosphate groups restrict cell membrane permeability, reducing antibacterial efficacy. This suggests the need for prodrug strategies or structural optimization to improve membrane penetration[16].

Indications and Advantages of FBA Inhibitors，Theoretical indications for FBA-II inhibitors include:Infections caused by MDR P. aeruginosa, especially hospital-acquired pneumonia, ventilator-associated pneumonia, burn wound infections, and pulmonary infections in cystic fibrosis patients.Combination therapy with other antibacterial agents: blocking core bacterial glucose metabolism enhances the efficacy of existing antibiotics.Compared with other antibacterial targets, FBA-II offers the following advantages:Clear mechanism: Zn^{2+}-dependent catalysis differs markedly from human FBA-I.Functional essentiality: Gene knockout causes bacterial death.Specific distribution: Exists only in microorganisms.

In this study, FBA-II was targeted. Based on the structural features of HCA (8-hydroxyquinoline-2-carboxylic acid), novel hit compounds were designed using scaffold hopping（Figure 3）. A virtual compound library was built with SeeSAR software, and multi-stage molecular docking screening was performed in the Glide module of Schrödinger 2020 to obtain candidate compounds with excellent docking scores. This systematic study of FBA-II inhibitors deepens understanding of target structure and function and provides theoretical guidance for further FBA-II-targeted drug design.

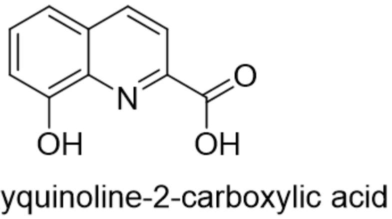


Figure 3 Structure of HCA

II. Results and Discussion

2.1 Overall Design Strategy

8-Hydroxyquinoline-2-carboxylic acid, a highly selective FBA inhibitor, was used as the lead compound. Structural modifications were performed via scaffold hopping based on its co-crystal structure with the target protein (PDB: 4LV4)[17]. First, the scaffold hopping module of SeeSAR was applied to retain the nitrogen atom from the quinoline core to form an aromatic azaheterocycle, preserving key metal ion coordination interactions. Meanwhile, the hydroxyl group on the benzene ring was replaced with other metal-coordinating groups, constructing a virtual library of 821 derivatives.

Subsequently, drug-likeness screening (based on Lipinski's Rule of Five and preliminary ADMET properties) was performed using Discovery Studio 2019, yielding 500 compounds with favorable drug-likeness. These 500 compounds were imported into the Schrödinger software suite for high-precision molecular docking using the XP mode of the Glide module. A total of 200 candidate molecules were selected with a binding free energy (XP Gscore) < -1 kcal/mol. Infinisee was then used to further predict pharmacokinetic properties, eliminating molecules with potential toxicity or pharmacokinetic liabilities, resulting in 120 qualified compounds. Finally, visual inspection was conducted, comprehensively considering novelty of binding modes, synthetic feasibility, and structural diversity. Six representative compounds (coded FBA1, FBA2, FBA3, FBA4, FBA5, FBA6) were chosen for chemical synthesis and in vitro activity validation（Figure 4）.

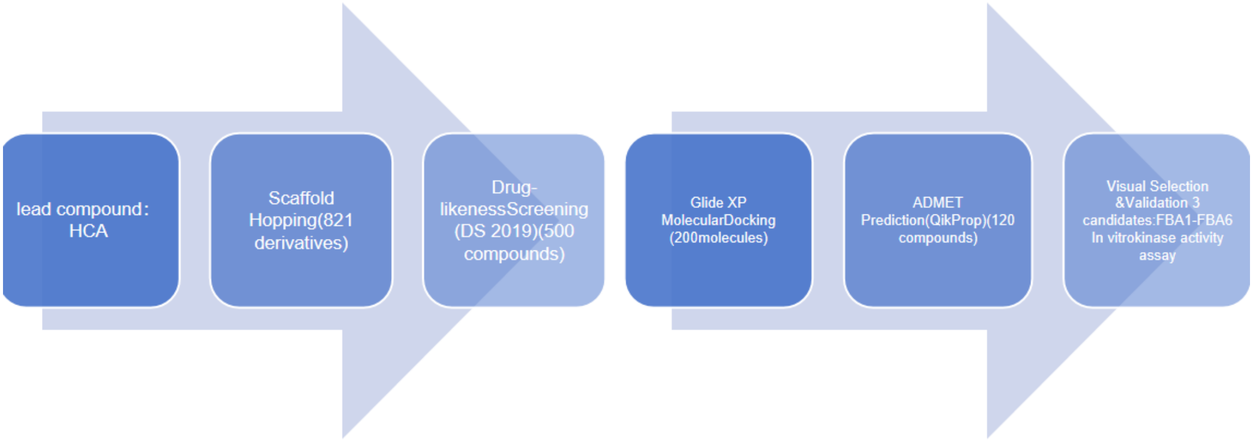


Figure 4 Screening process of small molecule inhibitors of FBA

2.2 Molecular Docking Screening and Binding Mode Analysis

To evaluate the binding capacity of hit compounds to FBA, high-precision molecular docking was carried out for the 500 compounds retained after drug-likeness screening using the Extra Precision (XP) mode of the Glide module in the Schrödinger software suite. The XP mode achieves more accurate discrimination between active and inactive compounds through more comprehensive energy scoring and conformational search.

In this study, an XP Gscore < -1 kcal/mol was set as the preliminary cutoff, determined empirically and based on known active compounds (e.g., XP Gscore of HCA $= -2.804$ kcal/mol). Finally, 200 candidate molecules meeting the energy criterion were screened from 500 compounds.

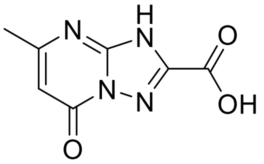
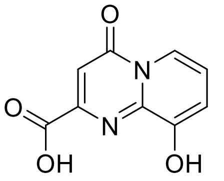
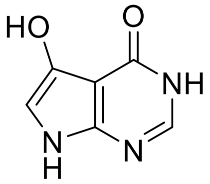
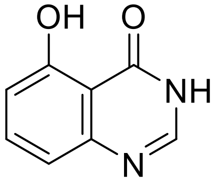
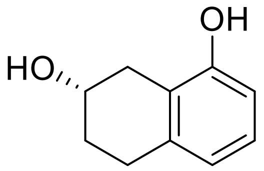
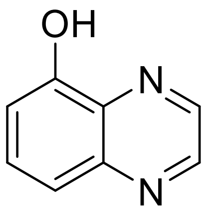
The ADMET properties of these 200 compounds were then predicted using the QikProp module, focusing on key pharmacokinetic parameters including oral absorption, CYP450 inhibition risk, blood–brain barrier penetration, and compliance with Lipinski's Rule of Five. Molecules with obvious toxicity risks or pharmacokinetic disadvantages were excluded, leaving 120 compounds with overall favorable properties.

Each molecule's binding mode at the FBA active site was inspected individually in Schrödinger's Maestro visualization interface. Key focus areas included formation of a critical hydrogen bond with the backbone carbonyl of Glu142, formation of a metal coordination bond with Zn^{2+} 500, and successful insertion into the hydrophilic pocket formed by Leu146, Gly147, Ala178, Ala179, Tyr256, and other residues. Through the above multi-dimensional screening, six representative compounds with novel structures, clear interaction patterns, and moderate synthetic difficulty—FBA1, FBA2, FBA3, FBA4, FBA5, and FBA6—were finally selected.

2.3 FBA Enzymatic Activity Validation

After chemical synthesis of FBA1, FBA2, FBA3, FBA4, FBA5, and FBA6, their inhibitory activities were assessed using the Solarbio Fructose-1,6-Bisphosphate Aldolase (FBA) Activity Assay Kit (Spectrophotometry). The results showed that FBA6 exhibited good inhibition of FBA. At a test concentration of 250 μmol/L, FBA6 inhibited FBA activity by 50%, and at 500 μmol/L by 75.02%. In contrast, compounds FBA1–FBA5 almost completely lost effective inhibitory activity against FBA. (Table 1).

Table 1 Inhibition rate of compounds against FBA

Compound	Structure	FBA inhibition(%)	
		250μm	500μm
FBA1		N/A	10.02
FBA2		N/A	15.05
FBA3		N/A	4.97
FBA4		N/A	10.2
FBA5		N/A	7.89
FBA6		50.04	75.02

In contrast, compounds FBA1, FBA2, FBA3, FBA4, and FBA5 almost completely lost effective inhibitory activity against FBA enzyme.

III. Materials and Methods

3.1 Computer Hardware, Software and Databases

The Schrödinger Suite 2022-4 (Schrödinger, LLC, USA) was used for protein structure preparation, molecular docking, and ADME property prediction, including the Protein Preparation Wizard, LigPrep, Glide, and QikProp modules. SeeSAR software (BioSolveIT, Germany) was used for scaffold hopping and virtual library construction. Discovery Studio 2019 (Dassault Systèmes, France) was used for preliminary drug-likeness screening. Molecular visualization and binding mode analysis were performed in Maestro 12.8.

The 3D crystal structure of the FBA-II domain was obtained from the RCSB Protein Data Bank (PDB: 4LV4), with a resolution of 2.08 Å, corresponding to the co-crystal complex of 8-hydroxyquinoline-2-carboxylic acid bound to FBA-II.

3.2 Protein Structure Preparation

The 4LV4 crystal structure was preprocessed using the Protein Preparation Wizard: all crystal water molecules were removed (except those potentially forming hydrogen bonds with the active site and participating in ligand interactions), missing hydrogen and side-chain atoms were added, the hydrogen bond network was optimized, and energy minimization was performed. Energy minimization was conducted with the OPLS4 force field, with a convergence criterion of RMSD = 0.30 Å for non-hydrogen atoms.

3.3 Compound Library Construction

Using 8-hydroxyquinoline-2-carboxylic acid as the lead, structural modifications were performed via the scaffold hopping module of SeeSAR. Modification strategies: retain the nitrogen atom from the quinoline core to form an aromatic azaheterocycle, preserving key metal ion coordination interactions; replace the hydroxyl group on the benzene ring with other metal-coordinating groups. An initial virtual library of 821 compounds was constructed. These 821 designed compounds were imported into the LigPrep module for ionization and energy optimization.

3.4 Drug-Likeness Screening

The 821 compounds processed by LigPrep were imported into Discovery Studio 2019. Drug-likeness was evaluated based on Lipinski's Rule of Five and preliminary ADMET parameters. A total of 500 compounds meeting drug-likeness criteria were selected for molecular docking.

3.5 Molecular Docking

A docking grid (10 Å × 10 Å × 10 Å) was generated centered on the co-crystallized ligand 8-hydroxyquinoline-2-carboxylic acid at the active site, covering 10 key amino acid residues and Zn^{2+}. High-precision molecular docking was performed using the XP mode of Glide. The 500 compounds were imported into Glide XP for docking. A cutoff of XP Gscore < -1 kcal/mol selected 200 candidate compounds. Docking validation confirmed that parameters reliably reproduced the experimental binding mode.

3.6 ADMET Property Prediction

The 200 compounds from XP ranking were imported into QikProp for pharmacokinetic prediction. Parameters assessed included molecular weight, logP, H-bond donors/acceptors, predicted oral absorption, CYP2D6/CYP3A4 inhibition risk, and blood–brain barrier penetration. Molecules with liabilities were removed, leaving 120 compounds with favorable overall properties.

3.7 Candidate Compound Selection

Detailed visual inspection was performed on the 120 compounds retained after ADMET prediction. Selection criteria: formation of a key hydrogen bond with the backbone carbonyl of Glu142; metal coordination with Zn^{2+} 500; insertion into the hydrophilic pocket; synthetic feasibility and potential for further modification. Based on these criteria, six representative compounds (FBA1, FBA2, FBA3, FBA4, FBA5, FBA6) were selected.

3.8 Chemical Synthesis

Compounds FBA1–FBA6 were prepared by standard organic synthesis. Synthetic routes used substituted aromatic or heterocyclic amines as starting materials, constructing the target scaffold via amide condensation, cyclization, etc. All target compounds were purified by silica gel column chromatography. Structures were confirmed by ^1H NMR, ^{13}C NMR, and HRMS; purity was ≥ 95% by HPLC..

3.9 FBA Enzymatic Activity Assay

The Solarbio FBA Activity Assay Kit (Spectrophotometry) was used to evaluate inhibitory activity. The principle is based on FBA-catalyzed cleavage of FBP, followed by NADH consumption via a coupled enzyme system, leading to decreased absorbance at 340 nm (ΔA_{340} as the activity index).

Assays were performed in 96-well plates (100 µL per well). Purified FBA was diluted in PBS (pH 8.0) to working concentration. Test compounds were serially diluted in DMSO. Absorbance at 340 nm was monitored continuously for 5–10 min using a microplate reader. ΔA_{340} was calculated, blank-corrected, and relative inhibition was calculated with DMSO control as 100% activity. IC_{50} values were obtained by four-parameter logistic regression.

IV. Conclusions

This study addresses the clinical challenge of MDR P. aeruginosa infections by designing and screening novel inhibitors targeting fructose-1,6-bisphosphate aldolase (FBA). FBA is a key glycolytic enzyme classified into FBA-I and FBA-II. Humans express only FBA-I, while pathogens rely on Zn^{2+}-dependent FBA-II, making FBA-II an ideal antibacterial target. Reported FBA inhibitors are mainly metal chelators and FBP substrate analogs, which suffer from poor selectivity and membrane permeability.

Using 8-hydroxyquinoline-2-carboxylic acid (HCA) as the lead and the co-crystal structure (PDB: 4LV4), scaffold hopping was applied for structural modification. A virtual library of 821 derivatives was built; 500 candidates were obtained by drug-likeness screening. High-precision XP docking selected 200 compounds. ADMET prediction retained 120 molecules. Visual analysis and synthetic feasibility identified six compounds (FBA1–FBA6) for synthesis and testing.

Enzymatic activity results showed that FBA6 inhibited FBA by 50% at 250 µmol/L and 75.02% at 500 µmol/L; the other five compounds were inactive.

This study established a complete CADD workflow for FBA inhibitor discovery and identified FBA6, a novel scaffold inhibitor with favorable activity. It provides a new strategy to overcome poor membrane permeability of existing FBA inhibitors and supports further structural optimization and antibacterial research. This paradigm can guide drug development for other antibacterial targets.

References

[1] Nanayakkara, A.K.; Boucher, H.W.; Fowler, V.G., Jr.; Jezek, A.; Outterson, K.; Greenberg, D.E. Antibiotic resistance in the patient with cancer: Escalating challenges and paths forward. *CA Cancer J. Clin.* 2021, 71, 488 – 504.

[2] Perez, F.; Adachi, J.; Bonomo, R.A. Antibiotic-resistant gram-negative bacterial infections in patients with cancer. *Clin. Infect.* Dis. 2014, 5 (Suppl. 5), S335 – S339.

[3] Crone, S.; Vives-Flórez, M.; Kvich, L.; Saunders, A.M.; Malone, M.; Nicolaisen, M.H.; Martínez-García, E.; Rojas-Acosta, C.; Catalina Gomez-Puerto, M.; Calum, H.; et al. *The environmental occurrence of Pseudomonas aeruginosa.* Apmis 2020, 128, 220 – 231.

[4] de Sousa, T.; Hébraud, M.; Dapkevicius, M.; Maltez, L.; Pereira, J.E.; Capita, R.; Alonso-Calleja, C.; Igrejas, G.; Poeta, P. Genomic and Metabolic Characteristics of the Pathogenicity in Pseudomonas aeruginosa. Int. J. Mol. Sci. 2021, 22, 12892.

[5] Petenkova, A., Auger, S.A., Lamb, J. et al. Prenylcysteine oxidase 1 like protein is required for neutrophil bactericidal activities. Nat Commun 14, 2761 (2023).

[6] Boulant T, Boudehen Y-M, Filloux A, Plesiat P, Naas T and Dortet L (2018) Higher Prevalence of PldA, a Pseudomonas aeruginosa Trans-Kingdom H2-Type VI Secretion System Effector, in Clinical Isolates Responsible for Acute Infections and in Multidrug Resistant Strains. Front. Microbiol. 9:2578.

[7] P. aeruginosa is listed as a pathogen of a serious threat (Level 2) by the US Center for Disease Control and Prevention as it causes 51,000 infections per year; of which 6,700 are multi-drug resistance with 440 deaths (CDC, 2013).

[8] Tomic T, Henman M, Tadic I, Antic Stankovic J, Santric Milicevic M, Maksimovic N, Odalovic M. Antimicrobial utilization and resistance in Pseudomonas aeruginosa using segmented regression analysis: a comparative study between Serbia and eight European Countries. Int J Clin Pharm. 2023 Aug;45(4):989-998.

[9] [9]Racha Daher and Michel Therisod.Highly Selective Inhibitors of Class II Microbial Fructose Bis-phosphate Aldolases.*ACS Medicinal Chemistry Letters* 2010 1 (3), 101-104.

[10] Characterization, Kinetics, and Crystal Structures of Fructose-1,6-bisphosphate Aldolase from the Human Parasite, Giardia lamblia.*Journal of Biological Chemistry*, 2006; 282, 4859-4867.

[11] Lewis, D. J., & Lowe, G. (1973). Phosphoglycolohydroxamic acid: an inhibitor of class I and II aldolases and triosephosphate isomerase. J. Chem. Soc. Chem. Commun., 713-715.

[12] Ramsaywak, P. C., Labbe, G., Siemann, S., Dmitrienko, G. I., & Guillemette, J. G. (2004). Molecular cloning, expression, purification, and characterization of fructose 1,6-bisphosphate aldolase from Mycobacterium tuberculosis - A novel Class II tetramer. Protein Expr. Purif., 37, 220-228.

[13] Fonvielle, M., Weber, P., Dabkowska, K., & Therisod, M. (2004). New highly selective inhibitors of Class II fructose-1,6-bisphosphate aldolases. Bioorg. Med. Chem. Lett., 14, 2923-2926.

[14] Hall, D. R., Leonard, G. A., Reed, C. D., Watt, C. I., Berry, A., & Hunter, W. N. (1999). The crystal structure of Escherichia coli Class II fructose-1,6-bisphosphate aldolase in complex with phosphoglycolohydroxamate reveals details of mechanism and specificity. J. Mol. Biol., 287, 383-394.

[15] Cooper, S. J., Leonard, G. A., McSweeney, S. M., Thompson, A. W., Naismith, J. H., Qamar, S., Plater, A., Berry, A., & Hunter, W. N. (1996). The crystal structure of a Class II fructose-1,6-bisphosphate aldolase shows a novel binuclear metal-minding active site embedded in a familiar fold. Structure, 4, 1303-1315.

[16] Wang, L., & Dmitrienko, G. (2009). Approaches to the Inhibition of Class II Fructose-1,6-Bisphosphate Aldolase. Master's thesis, University of Waterloo.

[17] Capodagli, G.C., Sedhom, W.G., Jackson, M., Ahrendt, K.A., Pegan, S.D.A Noncompetitive Inhibitor for Mycobacterium tuberculosis's Class IIa Fructose 1,6-Bisphosphate Aldolase.Biochemistry 53: 202-213