Computer-aided drug design of novel Elastase B inhibitors

Mohammad Walid Shahrour [1,2,3], Angeliki Vgenopoulou [1,2,3], Antoine Lacour [1,2,3,4], Dominik Kolling [1,2,5], Raphael Klein [6], Jörg Haupenthal [1,2,3], Jesko Köhnke [5], Andrea Volkamer [1,3,4], Anna K.H. Hirsch [1,2,3]

[1]*Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) – Helmholtz Centre for Infection Research (HZI), 66123, Saarbrücken, Germany.*
[2]*Department of Pharmacy, Saarland University, 66123, Saarbrücken, Germany.*
[3]*PharmaScienceHub, Saarland University, 66123, Saarbrücken, Germany.*
[4]*Data Driven Drug Design, Center for Bioinformatics, Saarland University, 66041, Saarbrücken, Germany.*
[5]*Institute for Food Chemistry, Leibniz University Hannover, 30167, Hannover, Germany.*
[6]*BioSolveIT GmbH, 53757, St. Augustin, Germany.*

Antimicrobial resistance poses a significant threat to global public health, urging the scientific community to put greater effort into searching for novel and effective antimicrobial compounds. Pathoblockers, which target bacterial virulence factors, disarm pathogens rather than killing them directly, representing a promising approach [1]. *Pseudomonas aeruginosa,* a Gram-negative bacterium associated with serious respiratory and eye infections, secretes several virulence factors including elastase B (LasB), a zinc-dependent metalloprotease which destroys tissue components and interferes with the host defense mechanisms, presenting itself as a valuable drug target [2,3].

While our group has previously reported the discovery and optimization of different LasB inhibitors with zinc-chelating groups [4,5], we observed a new allosteric site through an X-ray-based fragment screening campaign. This observation paves the way for inhibitors with a new binding mode bypassing zinc chelation which reduces the risk of unwanted binding to off-targets such as human matrix metalloproteases (MMPs) [4,5].

In this work, we employed Chemical Space Docking® [6] to explore Enamine REAL Space with over 76 billion make-on-demand molecules. Synthons were template-based docked onto crystallographic fragments. Those that shared a high maximum common substructure (MCS) and had a promising predicted binding mode were extended based on the chemistry rules encoded in REAL Space. Out of 2.5 million virtual candidates, we selected and experimentally tested 33 hits following a rational selection workflow encompassing visual inspection and consideration of drug-like properties and chemical diversity.

Two hits exhibited moderate LasB inhibition at 100µM (25.9% and 35.7%). In the presence of an orthosteric inhibitor, inhibition increased markedly to 67.5% and 58.9%, compared with 51.9% for the orthosteric inhibitor alone at 50 µM. Molecular dynamics simulations of the hits are in progress to further explore the stability of the protein-ligand complexes before performing X-ray crystallography to confirm their binding modes.

These findings highlight the potential for new allosteric non-zinc binding LasB inhibitors that could contribute to the fight against resistant *Pseudomonas aeruginosa.*

Bibliography :
[1] I. Gajic; N. Tomic; *et al.* Antibiotics 14 (2025) 221.
[2] M.J. Everett; D.T. Davies; *et al.* Drug Discov. Today 26 (2021) 2108–2123.
[3] V. Camberlein; G. Jézéquel; *et al.* Antibiotics 11 (2022) 1060.
[4] J. Konstantinović; A.M. Kany; *et al.* ACS Cent. Sci. 9 (2023) 2205–2215.
[5] A.F. Kiefer; C. Schütz; *et al.* Adv. Sci. 12 (2025) 2411807.
[6] J. Müller; R. Klein; *et al.* J. Med. Chem. 65 (2022) 15663–15678.