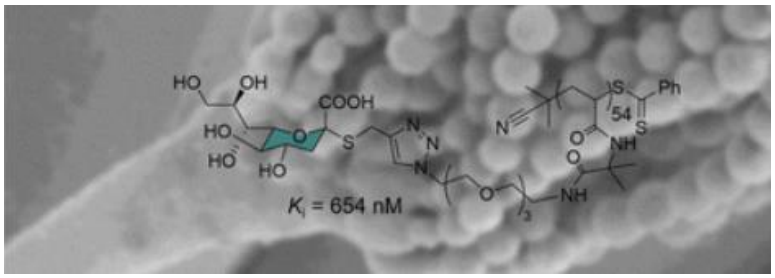
DEVELOPMENT OF POTENT *AF*KDNASE INHIBITORS AS POTENTIAL ANTIVIRULENCE AGENTS FOR *ASPERGILLUS FUMIGATUS*

Mathieu Scalabrini[a], Denis Loquet[a], Camille Rochard[b], Mélyne Baudin Marie[a], Coralie Assailly[a], Yoan Brissonnet[a], Franck Daligault[c], Amélie Saumonneau[c], Annie Lambert[c], Cyrille Grandjean[c], David Deniaud[a], Paul Lottin[d], Sagrario Pascual[d], Laurent Fontaine[d], Viviane Balloy[b], Sébastien Gouin[a]

[a] Nantes Université, CNRS, CEISAM, UMR 6230, F-44000 Nantes, France
mathieu.scalabrini@univ-nantes.fr
[b] Sorbonne Université, Inserm, Centre de Recherche Saint-Antoine, CRSA, Paris, France
[c] Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France
[d] Institut des Molécules et Matériaux du Mans (IMMM), UMR 6283 CNRS, Le Mans Université, Av. O. Messiaen, 72085 Le Mans cedex 9, France

Aspergillus fumigatus is a deadly opportunistic pathogen responsible for severe infections in immunocompromised individuals. Recently, *Af*KDNAse, an exoglycosidase that hydrolyzes the rare sugar 3-deoxy-D-galacto-D-glycero-nonulosonic acid (KDN), was identified as a key player in fungal cell wall morphology and virulence [1]. However, the precise function of *Af*KDNAse remains unclear, necessitating the development of potent inhibitors to better understand its biological role and its potential as a target for antivirulence strategies.
In this work, we report the design and synthesis of a novel set of *Af*KDNAse inhibitors based on thio-KDN motifs, which are enzymatically stable and capable of fitting into the unique KDN-binding pockets of the enzyme. Two classes of inhibitors, C2- and C9-linked heterodi-KDN, were developed, alongside a polymeric compound containing an average of 54 KDN motifs, synthesized using click chemistry. Enzymatic assays demonstrated that these compounds inhibited AfKDNAse with moderate to strong potency, with the poly-KDN showing a remarkable more than 900-fold improvement in inhibitory activity (IC_{50}= 1.52 ± 0.37 µM, 17-fold on a KDN molar basis) over a monovalent KDN reference.
Our findings suggest that multivalency is a key strategy for enhancing KDNase inhibition. Importantly, poly-KDN demonstrated a strong reduction in *A. fumigatus* filamentation when co-cultured at micromolar concentrations, offering promising prospects for the development of novel antivirulence agents targeting fungal pathogenesis [2].



References:
1. J. C. Telford, J. H. F. Yeung, G. Xu, M. J. Kiefel, A. G. Watts,S. Hader, J. Chan, A. J. Bennet, M. M. Moore and G. L. Taylor, *J. Biol. Chem.*, **2011**, 286, 10783–10792
2. M.Scalabrini, D. Loquet, C. Rochard, M. Baudin Marie, C. Assailly, Y. Brissonnet, F. daligault, A. Sumonneau, A. Lambert, C. Grandjean, D. Deniaud, P. Lottin, S. Pascual, L. Fontaine, V. Balloy, S. Gouina, *Org. Biomol. Chem.*, **2024**, 22, 5783-5789