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Investigating Lung Permeability of AhR-Targeting Bacteria-derived Metabolites in Cystic Fibrosis Using an Integrated Mucosal Platform

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Microbial-derived molecules (MDM) are at the **basis of bacteria-bacteria** and **bacteria-host communication**. An exponentially **growing number of bacteria-derived molecules** are identified as **AhR activators**, highlighting the need for **systems to screen possible lead candidates**.

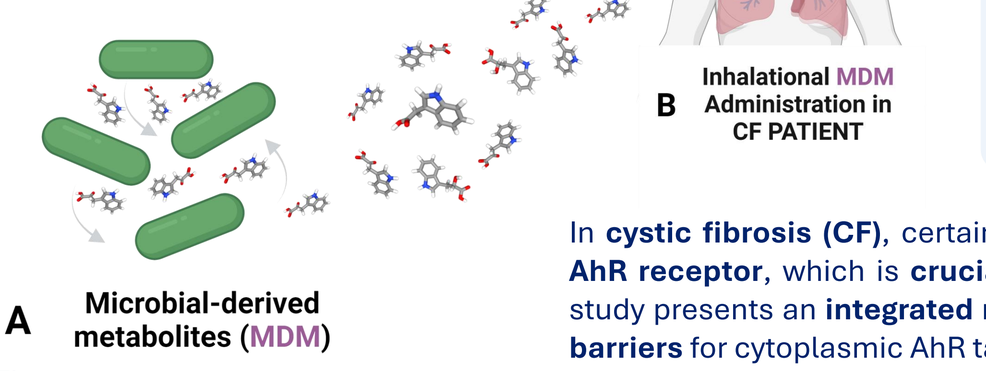
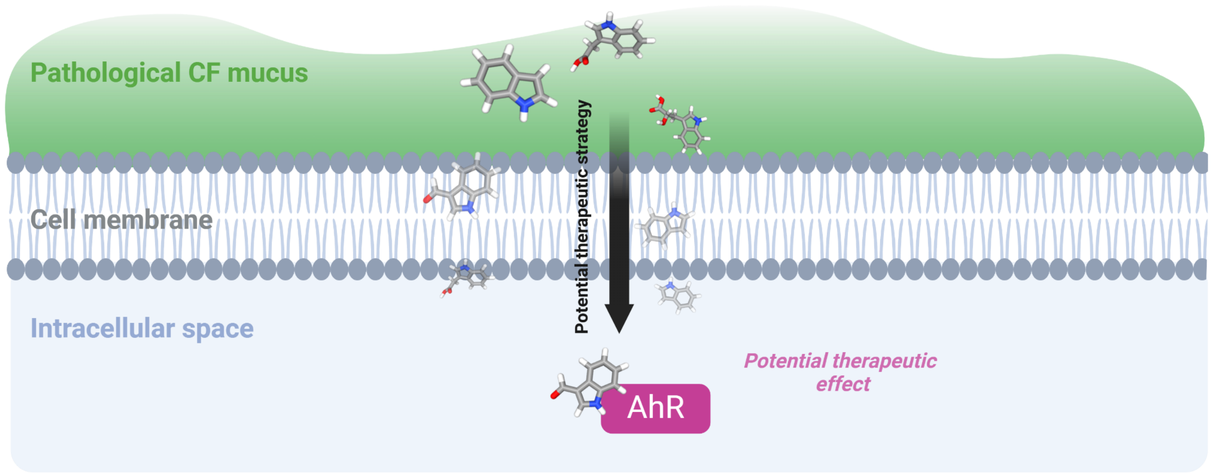


Fig. 1



In **cystic fibrosis (CF)**, certain molecules are considered **promising therapeutics** for their **ability to activate** the **AhR receptor**, which is **crucial for an effective immune response** [1] and **reducing mucus accumulation**. This study presents an **integrated mucosal platform** to **quickly identify molecules able to overcome mucus and cell barriers** for cytoplasmic AhR targeting.

Selected molecules

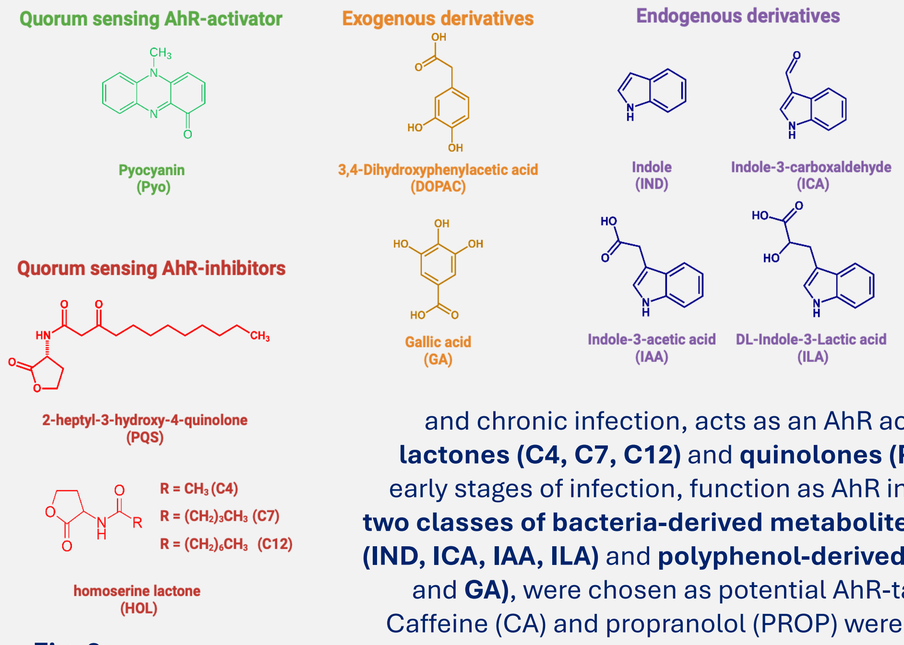


Fig. 2

Three classes of molecules were selected as **representatives of *P. aeruginosa* quorum sensing compounds affecting AhR** activity. **Pyocyanin (PYO)**, secreted during biofilm formation and chronic infection, acts as an AhR activator. **Homoserine-lactones (C4, C7, C12)** and **quinolones (PQS)**, produced in the early stages of infection, function as AhR inhibitors. Additionally, **two classes of bacteria-derived metabolites**, **indole derivatives (IND, ICA, IAA, ILA)** and **polyphenol-derived postbiotics (DOPAC and GA)**, were chosen as potential AhR-targeting therapeutics. Caffeine (CA) and propranolol (PROP) were included as standard molecules with high permeability.

Experimental workflow

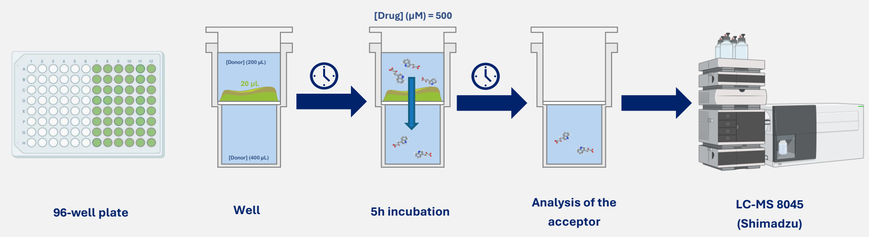


Fig. 3

In this study, artificial phospholipid membranes (**PermeaPad®** or **PAMPA**) were used to **mimic the cellular barrier**, while an **in vitro cystic fibrosis (CF) mucus model** was developed by incorporating pathological concentrations of mucin into alginate-based gels, replicating the rheological properties of CF mucus [2]. The system was designed to evaluate **how the mucus layer impacts the permeability of quorum sensing (QS) molecules and AhR-targeting bacteria-derived metabolites (BDMs)**, with mass transport measured after 5 hours and analyzed through LC-MS and HPLC techniques to determine molecule concentrations in the acceptor compartment.

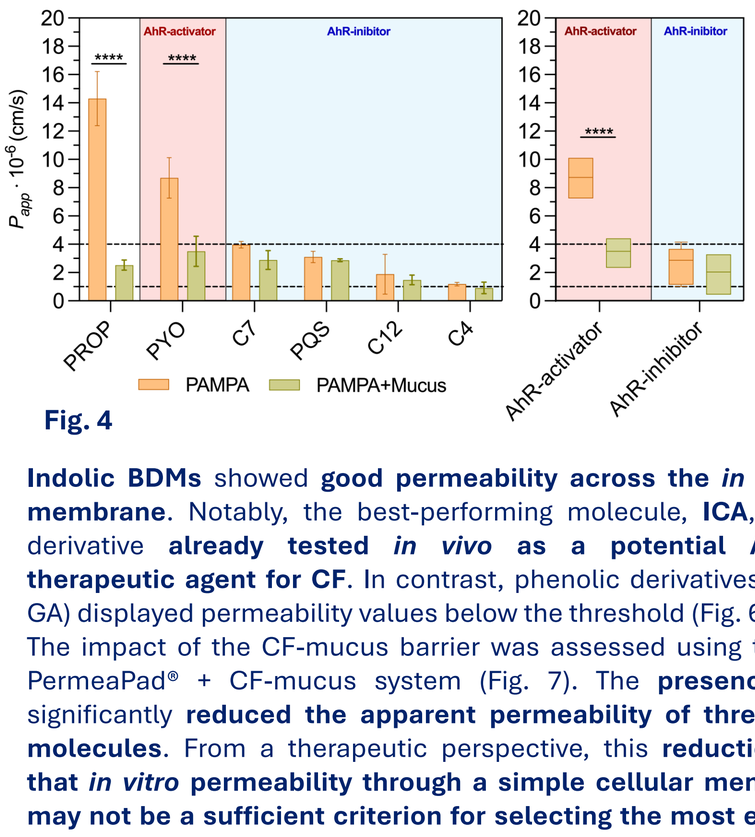


Fig. 4

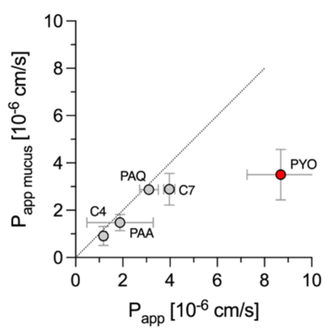


Fig. 5

The role of mucus in *P. aeruginosa* infections was assessed using the integrated PAMPA + CF-mucus system. **Pyocyanin (PYO)** showed a **60% reduction in Papp**, while quinolones (PQS) and lactones (C4, C7, and C12) were not significantly affected (Fig. 4). These *in vitro* results (Fig. 5) highlight the **critical role of mucus** in impairing the immune system's ability to counter *P. aeruginosa* infections in cystic fibrosis. In particular, the **reduced permeability of PYO** suggests that *P. aeruginosa* may **exploit quorum sensing and biofilm production to evade AhR-dependent immune responses**, promoting chronic bacterial persistence.

Indolic BDMs showed **good permeability across the *in vitro* cellular membrane**. Notably, the best-performing molecule, **ICA**, is an indole derivative **already tested *in vivo* as a potential AhR-targeting therapeutic agent for CF**. In contrast, phenolic derivatives (DOPAC and GA) displayed permeability values below the threshold (Fig. 6).

The impact of the CF-mucus barrier was assessed using the integrated PermeaPad® + CF-mucus system (Fig. 7). The **presence of mucus** significantly **reduced the apparent permeability of three out of five molecules**. From a therapeutic perspective, this **reduction highlights that *in vitro* permeability through a simple cellular membrane alone may not be a sufficient criterion for selecting the most effective AhR-targeting drug candidates**.

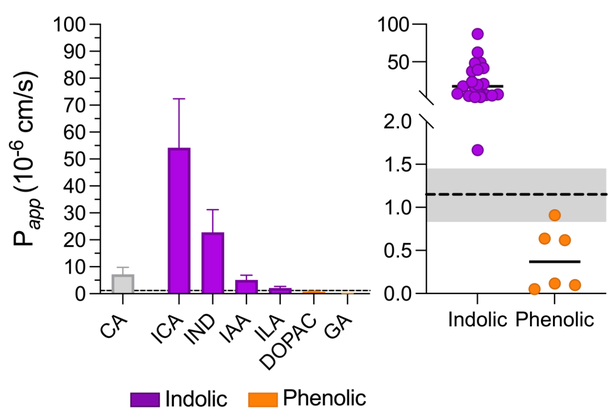


Fig. 6

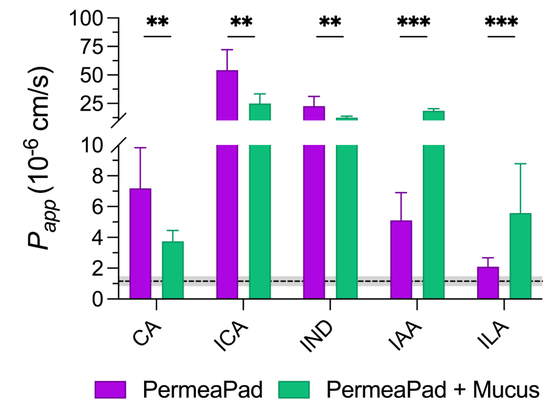


Fig. 7

To gain deeper insight into these interactions, we calculated the **molecular charge at physiological pH**, as well as the **solubility** and **Van der Waals (VDW) volume** of the selected molecules. These parameters were analyzed **to explore potential correlations with their permeability behavior across the** integrated CF-mucus and membrane **system**. As shown in Fig. 8, interestingly, our analysis suggests that the **diffusion percentage** appears to **correlate with solubility**, which is consistent with expectations.

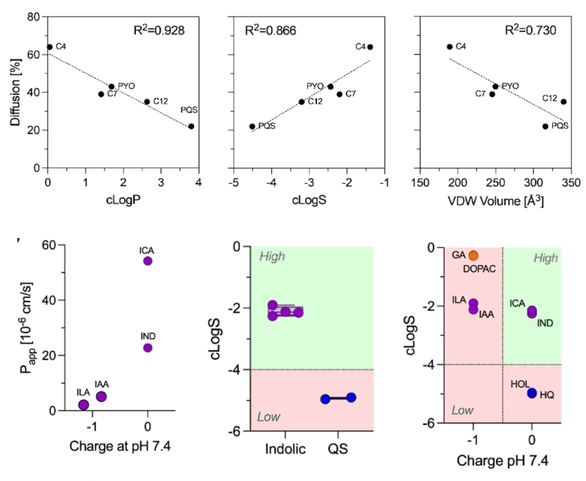


Fig. 8

Take home messages

This **innovative engineered approach allows** for the **investigation of the pathological role of QS in *P. aeruginosa* chronic infections** and **provides a precise evaluation of the ability of potential BDM therapeutics to reach AhR under disease-relevant conditions**. Given its significant impact on permeability, **incorporating the CF mucus barrier into *in vitro* models is crucial in the early stages of the drug discovery pipeline**, including pharmacokinetic assessments, rational drug design, and drug candidate screening.

[1] M. Puccetti, G. Paolicelli, V. Oikonomou, A. De Luca, G. Renga, M. Borghi, M. Pariano, C. Stincardini, L. Scaringi, S. Giovagnoli, M. Ricci, L. Romani, T. Zelante, *Mediators of Inflammation 2018.*

[2] C. Butnarasu, G. Caron, D. Pacheco, P. Petrini, S. Visentin, *Mol. Pharmaceutics 2022, 19, 520-531.*