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Integrated screening platform of PDE for lung fibrosis drug discovery

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PURPOSE

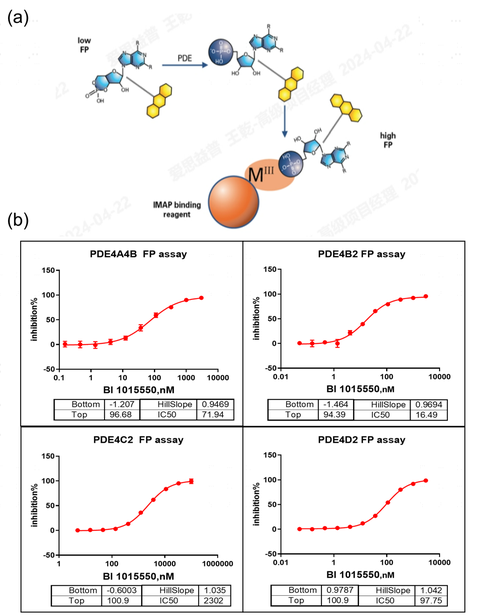
Phosphodiesterases (PDEs) are enzymes that hydrolyze cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) into AMP and GMP, respectively. Inhibitors of PDEs allow the elevation of cAMP and cGMP which lead to a variety of cellular effects including airway smooth muscle relaxation and inhibition of cellular inflammation or of immune responses. PDE4 inhibitors are potent inhibitors of inflammation, and they have been approved for the treatment of inflammatory diseases ranging from arthritis to chronic obstructive pulmonary disease (COPD). In addition, PDE4 inhibitors are approved as oral or topical treatments for psoriasis and atopic dermatitis, respectively[1]. As PDE4 is such an appealing clinical target for many additional non-dermatologic indications, additional research has continued to pursue strategies to widen the therapeutic index. Therefore, we have launched these integrated services for PDE4 screening platform from in vitro to in vivo, to get a comprehensive investigation of PDE4.

METHOD(S)

1) The IMAP fluorescence polarization PDEs platform. The principle of the IMAP assay for PDEs is shown in Figure 1. The IMAP binding reagent is a nano-particle solution containing immobilized trivalent cations that tightly bind phosphate on bio-organic molecules and peptides. Fluorescent labeled cAMP or cGMP does not bind until hydrolyzed by the PDE. Upon binding the fluorescence of the product becomes highly polarized. **2) Immune response analysis using cytokine assay and fibrosis markers detection using qPCR assay in cellular platform.** TNF-α and IL-2 release inhibition in PBMC and in human whole blood. The PBMC were pre-treated with compound for 1h before stimulating by LPS or phytohemagglutinin P. The human whole blood were pre-treated with compound for 1h and then stimulated by LPS for 7h, the TNF-α concentration in the plasma was detected. To evaluate the anti-fibrosis effect of PDE4 inhibitor, Roflumilast was used as positive drug in qPCR assay in HFL-1 cells. **3) BLM induced IPF mouse model Platform.** The aim of this study was to establish a bleomycin (BLM) -induced pulmonary fibrosis (PF) mouse model to study the alleviating effect of pirfenidone on pulmonary fibrosis. After airway exposure, 50μl bleomycin was injected into the airway to establish a pulmonary fibrosis model, and the weight and death of mice were observed. The experiment was finished after 21 days of continuous administration. The pharmacodynamic effect of the model was reflected by the detection of lung weight, lung ratio, HYP, serum factor and pathological staining.

RESULT(S)

1) The IMAP fluorescence polarization PDEs platform. The enzymatic platform was established and validated with reference compound (BI 1015550), whose IC50 values are consistent with reported data.



IC50 (nM)	PDE4A4B	PDE4B2	PDE4C2	PDE4D2
BI 1015550	71.94	16.49	2302	97.75

*BI 1015550 is a PDE4B Inhibitor and a Clinical Drug Candidate for the Oral Treatment of Idiopathic Pulmonary Fibrosis

Figure 1. The IMAP fluorescence polarization PDEs assay platform (a) Principle of the IMAP FP PDE assay system (b) Selectivity assay of BI 1015550 on diverse PDEs based on IMAP FP assay and the IC50 values are listed below.

2) Immune response analysis using cytokine assay and fibrosis markers detection using qPCR assay in cellular platform. The compound can inhibit the TNF-α release in PBMC and human whole blood or IL-2 release from PBMC, and the IC50 of which is consistent with the previous reported paper. In the presence of PGE2, Roflumilast can inhibit the expression of fibrotic biomarkers as α-SMA and CoL1A1, this assay can be applied to anti-fibrosis screening of PDE4 inhibitors.

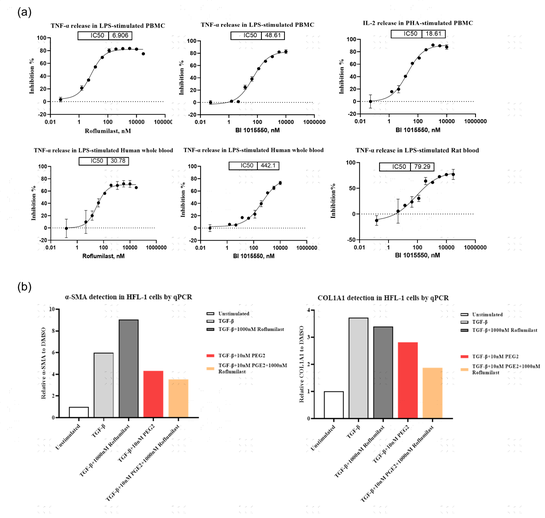
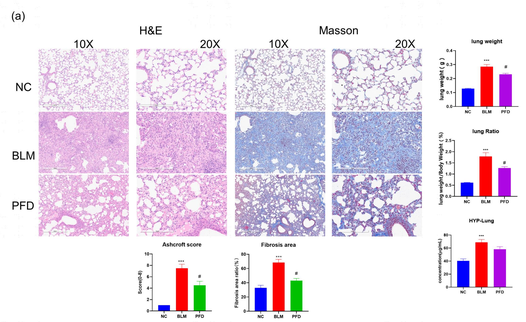


Figure 2. (a) TNF-α and IL-2 release inhibition in PBMC and in human whole blood. (b) Fibrosis markers (α-SMA and CoL1A1) detection in HFL-1 cells using qPCR assay.

3) BLM induced IPF mouse model Platform. Through continuous observation, pirfenidone can alleviate the weight loss after bleomycin injection, and delay the death time and mortality. Through the analysis of the experimental end point of lung weight and calculated lung coefficient, it was found that the lung weight and lung coefficient could be reduced, reflecting the degree of pulmonary fibrosis. Pirfenidone can reduce the pathological score induced by bleomycin, relieve the thickening of alveolar septum, the infiltration of inflammatory cells and reduce the collagen deposition of lung tissue. The decrease of HYP content can indirectly reflect the significant decrease of collagen content in lung tissue.



RESULT(S)

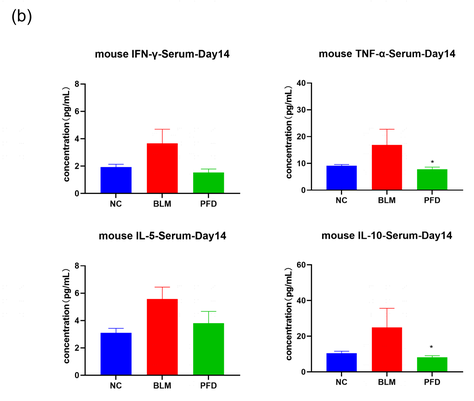


Figure 3. (a) A bleomycin (BLM) -induced pulmonary fibrosis (PF) mouse model was established to study the alleviating effect of pirfenidone on pulmonary fibrosis. (b) The expression levels of different inflammatory factors such as IL-5, IL-1β TNF-α, and TNF-γ in serum of mice at different periods were detected by MSD.

CONCLUSION(S)

In general, we constructed an experimental cascade from in vitro to in vivo, including of biochemical assays, cellular assays, and animal modeling. That can satisfy the mechanism study of PDEs as well as efficient and comprehensive screen of PDEs inhibitor, thus accelerate the novel drug discovery.

REFERENCES

[1] Hsien Lai S, Zervoudakis G, Chou J, Gurney ME, Quesnelle KM. PDE4 subtypes in cancer. Oncogene. 2020 May;39(19):3791-3802. doi: 10.1038/s41388-020-1258-8. Epub 2020 Mar 20. PMID: 32203163; PMCID: PMC7444459.

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