NLRP3 based platform for immune-related drug Discovery

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INTRODUCTION

Intrinsic immunity is an important line of defense against pathogenic microorganisms , which can effectively fight and remove external dangers. However, dysfunction of innate and adaptive immunity is considered to be a key step in the initiation and maintenance of autoimmune diseases. NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome is a multimeric protein complex, which can detect exogenous pathogen irritants and endogenous danger signals, which promote interleukin (IL)-1β and IL-18 secretion and pyroptosis mediated by caspase-1. Numerous studies have shown their significance in autoimmune diseases, such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), which strongly indicate that NLRP3 inflammasome complex may serve as a promising and novel therapeutic target for clinical treatment in inflammatory-related diseases.

METHODS

THP-1, PBMC and monocyte-induced macrophages are the main model cells in cellular experiments in vitro, the human whole blood was freshly collected before prior to the experiment.

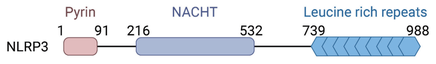
Cytokine release and cell pyroptosis in cells: PMA-induced THP-1 cells or PBMC were primed with LPS for 3 hours, the compound was incubated for 30 minutes before stimulating with nigericin for 1 hour. Supernatants were collected and cytokines quantified using ELISA. Cell pellet were used for cell Activity Testing.

The primary macrophage can be induced by PBMC-isolated monocytes, the primary Rat microglia cells were isolated from cerebral cortex, and the subsequent macrophage or microglia treatment was the same as that of PMA-induced THP-1 cells.

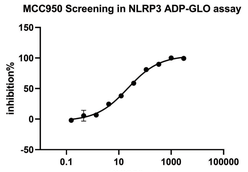
Cytokine release in the human whole blood: Freshly isolated heparinized human blood was stimulated with LPS for 3 hours, the compound was added and incubated for 30 minutes, and nigericin was then introduced and incubated for an additional 1 hour, the plasma was collected for cytokine detection.

Results 1: NLRP3 ATPase enzyme activity

A



B



Bottom	-3.115	HillSlope	0.7261
Top	104.2	IC50	22.08

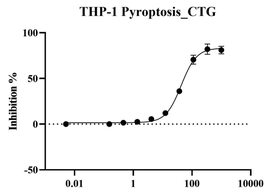
Figure 1. A, The protein structure of NLRP3. B, ADP-Glo assay was used to screen for the inhibition of NLRP3 ATPase enzyme activity by compounds.

Results 2: Cell pyropotosis

A

	LPS + nigericin									
MCC950(nM)	-	1000.00	333.33	111.11	37.04	12.35	4.12	1.37	0.46	0.15	DMSO
Cell activity	878475	702000	700950	608150	338150	145925	84525	67100	53975	47675	43425
	897300	753975	767050	669600	359950	149150	93925	67750	59225	50350	49450

B

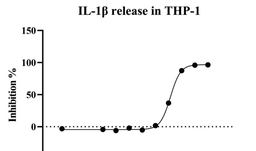


Bottom	1.605	HillSlope	1.663
Top	83.25	IC50	42.87

Figure 2. A, The raw data of cell pellet activity that were treated with LPS, compound and nigericin. B, Inhibitory activity of tool compounds MCC950 on cellular pyroptosis.

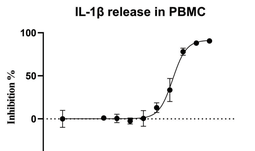
Results 3: Cytokine release in THP-1 and PBMC

A



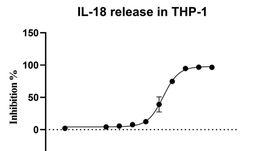
Bottom	-4.369	HillSlope	2.358
Top	96.80	IC50	43.14

B



Bottom	0.2263	HillSlope	1.768
Top	91.55	IC50	46.79

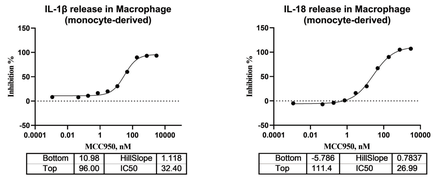
C



Bottom	4.304	HillSlope	1.565
Top	97.80	IC50	17.48

Figure 3. Data presentation of the inhibitory activity of tool compounds MCC950 to IL-1β (A) and IL-18 (C) secretion by PMA-pretreated THP-1 cells, and to IL-1β release by PBMC.

Results 4: Cytokine release in primary macrophage



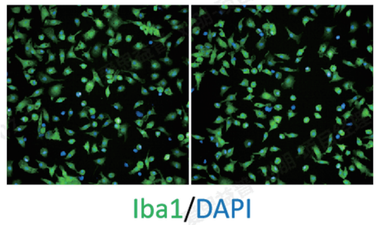
Bottom	10.98	HillSlope	1.118
Top	96.00	IC50	32.40

Bottom	-5.786	HillSlope	0.7837
Top	111.4	IC50	26.99

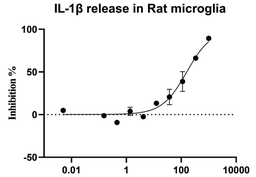
Figure 4. A, Monocyte isolated from PBMC. B, Monocyte derived macrophage. Data presentation of the inhibitory activity of tool compounds MCC950 to IL-1β (C) and IL-18 (D) secretion by monocyte-derived macrophage.

Results 5: Cytokine release in primary microglia

A



B

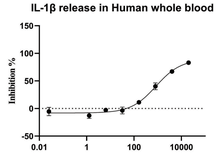


Bottom	0.2043	HillSlope	0.9682
Top	= 100.0	IC50	158.7

Figure 5. A, Isolated rat primary microglia. B, Data presentation of the inhibitory activity of tool compounds MCC950 to IL-1β secretion by rat primary microglia.

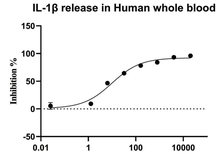
Results 6: Cytokine release in Human whole blood

A



Bottom	-8.144	HillSlope	0.8282
Top	89.15	IC50	844.5

B



Bottom	0.8490	HillSlope	0.7265
Top	92.24	IC50	9.928

Figure 6. Data on the activity of different compounds, MCC950 (A) and NT-0796 (B) in inhibiting the release of IL-1β in human whole blood.

Conclusions

Whether it is THP-1 cells, PBMC or monocyte-induced macrophages, once NLRP3 is inhibited after LPS stimulation, it can effectively inhibit the secretion of IL-1β or IL-18, as well as caspase-1 activity, and by analyzing the amount of cytokine secretion or caspase1 activity, we can assess the inhibitory activity of different types of compounds on NLRP3.

Based on the function of NLRP3, we have established different methods for evaluating the activity of various NLRP3 inhibitors in different cell systems.

References

[1] Harrison D, Billinton A, Bock MG, et al. Discovery of Clinical Candidate NT-0796, a Brain-Penetrant and Highly Potent NLRP3 Inflammasome Inhibitor for Neuroinflammatory Disorders. J Med Chem. 2023;66(21):14897-14911.

[2] Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD, Latz E. Targeting the NLRP3 inflammasome in inflammatory diseases. Nat Rev Drug Discov. 2018;17(9):688.

[3] Coll RC, Robertson AA, Chae JJ, et al. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat Med. 2015;21(3):248-255.