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DEHYDROHISPANOLONE DERIVATIVE IS A POTENT INHIBITOR OF NLRP3 INFLAMMASOME ACTIVATION

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INTRODUCTION

The innate immune system is the first line of defense against pathogens and damage signals. Inflammasomes are multiprotein complexes in inmune cells that play an essential role in the modulation of the inflammatory response. Growing evidence supports **NLRP3 inflammasome** as a new therapeutic option for the treatment of inflammatory-based diseases, as neutralization of **IL-1β** has proven efficacious in the treatment of inflammation. Nevertheless, there are no drugs available clinically that specifically target NLRP3 [1].

Natural products and their derivatives have represented a successful source in drug discovery and development of new therapeutic agents, in particular, **terpenoids**. Previous studies demonstrated the therapeutical potential of diterpene hispanolone derivatives as anti-inflammmatory agents [2,3]. In this context, **dehydrohispanolone derivative (N8)** has been investigated as NLRP3 inflammasome inhibitor, and the molecular targets underlying its effects were analysed in macrophages.

[1] Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD & Latz E (2018). *Nat Rev Drug Discov* 17, 588-606.
[2] Girón N, Través PG, Rodríguez B, López-Fontal R, Boscá L, Hortelano S, de las Heras B (2008). *Toxicol Appl Pharmacol* 15, 228, 179-89
[3] González-Cofrade L, Oramas-Royo S, Cuadrado I, Amesty Á, Hortelano S, Estevez-Braun A, de Las Heras B (2020). *J. Nat. Prod* 83, 7, 2155–2164.

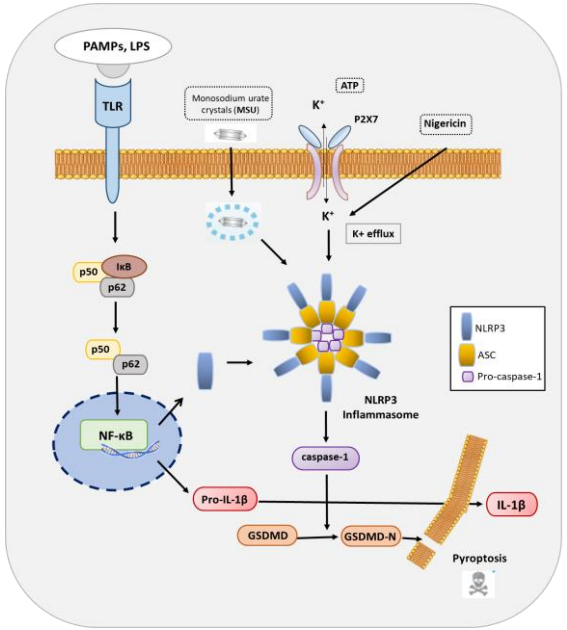

Fig. 1. Activation of NLRP3 inflammasome in macrophages.

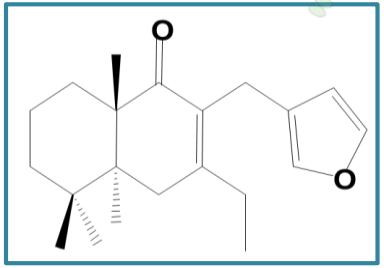

Fig. 2. Chemical structure of Dehydrohispanolone derivative **N8**

RESULTS

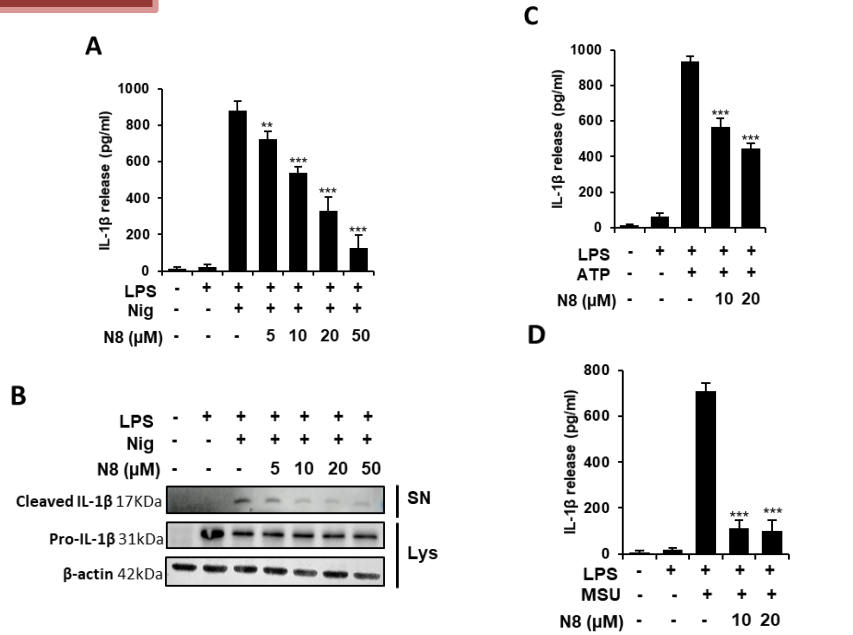

Fig. 3. N8 reduced IL-1β secretion following NLRP3 inflammasome activation by different stimuli in J774A.1 macrophages. **(A-B)** Cells were primed with LPS (1 μg/ml, 4h), followed by treatment with N8 (5, 10, 20, 50 μM, 30 min) and then Nigericin (Nig) (20 μM, 1h). **(C)** LPS-primed cells were treated with N8 for 30 min and then stimulated with ATP (5 mM, 30 min). **(D)** LPS-primed cells were treated with N8 (30 min) and then stimulated with MSU (150 μg/mL, 24 h). (**A, C, D**) Levels of IL-1β in the culture medium were measured by ELISA. Results show the means ± SD (n=3). **p<0.01, ***p<0.001 *vs* LPS + stimuli. (**B**) Inmunoblot analysis of cleaved IL-1β and pro-IL-1β and β-actin as a loading control. A representative experiment of three performed is shown.

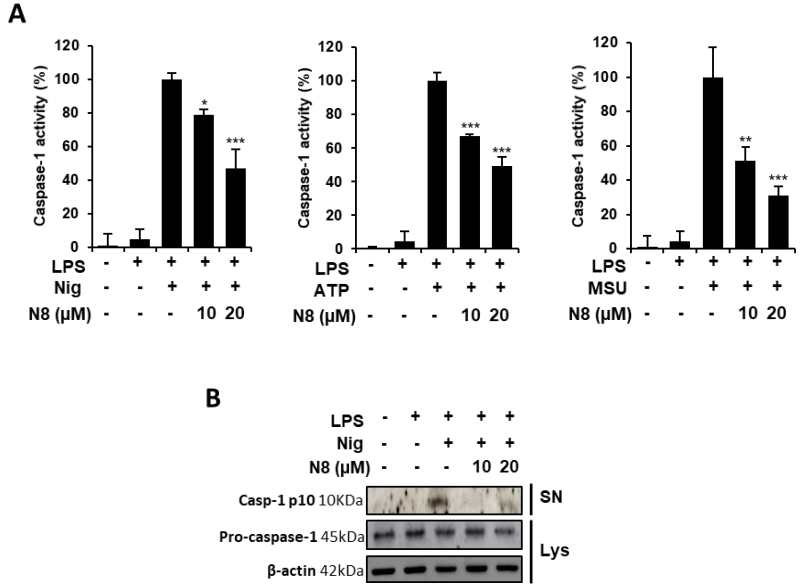

Fig. 4. N8 ameliorated caspase-1 activation macrophages. (A) Caspase-1 activity following LPS and Nig (20 μM, 1h), or ATP (5 mM, 30min) or MSU (150 mg/ml, 24h) stimulation in presence of N8. Data are expressed as means ± SD of percentage of caspase-1 activity of three independent experiments. *p<0.05, **p<0.01, ***p<0.001 *vs* LPS + Nig treatment. **(B)** Immunoblot analysis of cleaved caspase-1 and pro-caspase-1 expression. β-actin was used as a loading control. A representative experiment of three performed is shown.

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RESULTS

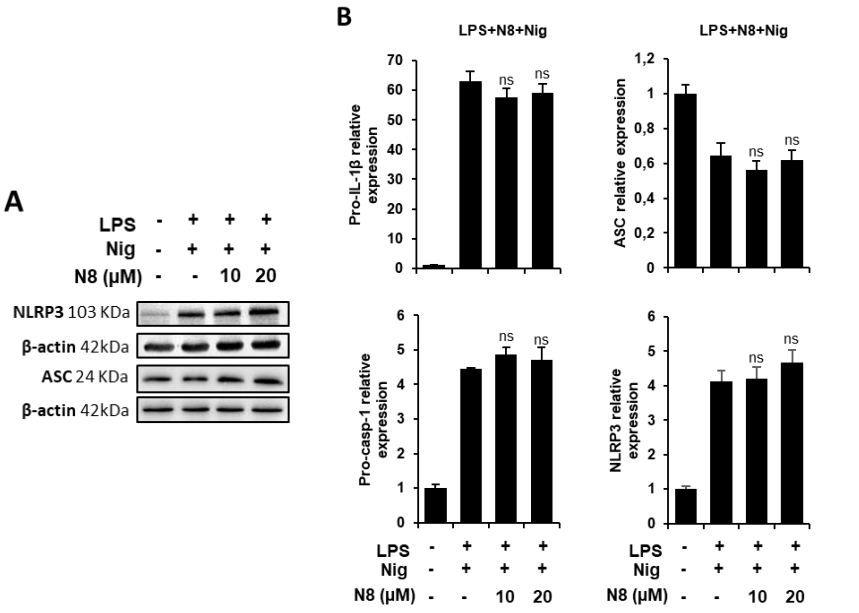

Fig. 5. NLRP3 inflammasome components expression was not impaired by derivative N8. Macrophages were primed with LPS, then treated with N8 (30 min) and then stimulated with Nig (20 μM, 1h). **(A)** Immunoblot analysis of NLRP3 and ASC expression. β-actin was used as a loading control. A representative experiment of three performed is shown. **(B)** Fold change of mRNA levels of inflamasomme complex components. Results are expressed as means ± SD of three independent experiments. Not significant (ns) *vs* LPS + Nig treatment.

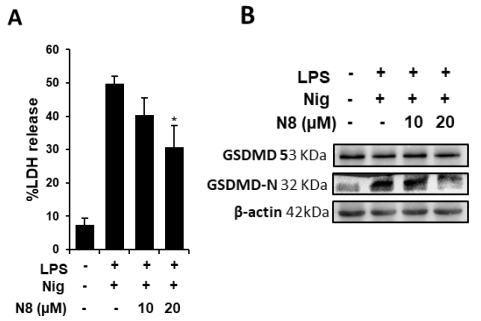

Fig. 6. N8 attenuates NLRP3-dependent pyroptotic cell death. LPS-primed cells were treated with N8 (30min) and then stimulated with Nig (20 μM, 1h) **(A)** LDH release in the culture supernatants was measured by CytoTox assay. Results are expressed as means ± SD of percentage of total LDH release (n=3). *p<0.05 *vs* LPS + Nig treatment. **(B)** Inmunoblot analysis of GSDMD and GSDMD-N expression. β-actin was used as a loading control. A representative experiment of three performed is shown.

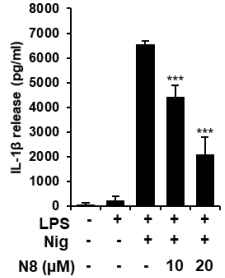

Fig. 7. N8 reduces IL-1β release after NLRP3 inflammasome activation in bone marrow-derived macrophages (BMDMs). Cells were primed for 3 h with LPS (1 μg/mL), followed by treatment with N8 for 30 min and then Nig (20 μM) for 1h. Levels of IL-1β in the culture medium were measured by ELISA. Results show the means ± SD of six independent experiments run in duplicate. ***p<0.001 *vs.* LPS + Nig treatment.

CONCLUSIONS

1. **N8** specifically blocked NLRP3 inflammasome activation both in J774A.1 and in bone marrow-derived macrophages, as it significantly reduced IL-1β release.
2. **N8** attenuated caspase-1 activity and pyroptosis without affecting NLRP3 complex components expression in J774A.1 macrophages.
3. **Dehydrohispanolone derivative N8** is a promising NLRP3 inflammasome specific inhibitor.