The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

A novel and promising NLRP3 inflammasome inhibitor: Dehydroisohispanolone

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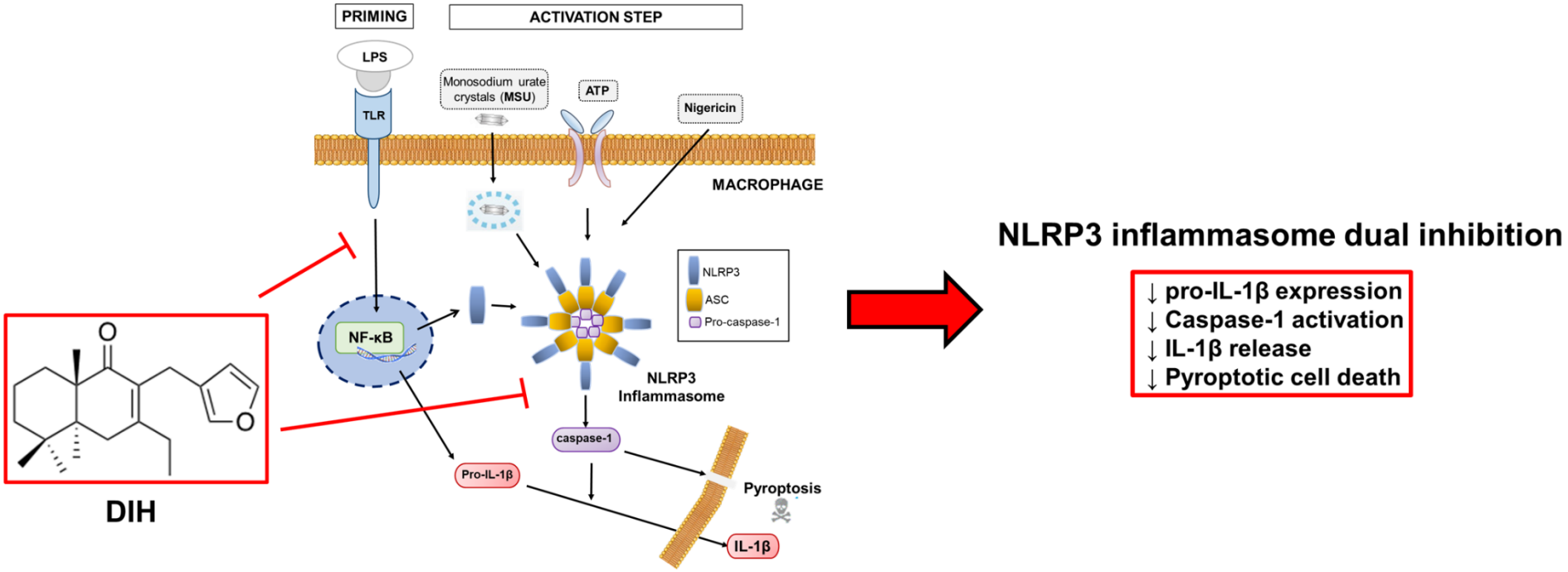
A novel and promising NLRP3 inflammasome inhibitor: Dehydroisohispanolone

Graphical Abstract



This work reproduces the published study: González-Cofrade, L., Cuadrado, I., Amesty, Á., Estévez-Braun, A., de Las Heras, B., & Hortelano, S. (2022). Dehydroisohispanolone as a Promising NLRP3 Inhibitor Agent: Bioevaluation and Molecular Docking. *Pharmaceuticals (Basel, Switzerland)*, *15*(7), 825. https://doi.org/10.3390/ph15070825

Abstract: The NLRP3 inflammasome is a complex present in cells of the innate immune system and involved in numerous inflammatory diseases, being a potential target for their treatment. NLRP3 inflammasome regulates caspase-1 activation and subsequent interleukin (IL)-1β and IL-18 release, and a type of cell death named pyroptosis. However, no specific NLRP3 inhibitors are clinically available to date. Dehydroisohispanolone (DIH) is a natural compound derived from the diterpene hispanolone with anti-inflammatory activity via inhibition of NF-κB activation. In this study, we evaluated whether DIH modulates NLRP3 inflammasome activation in macrophages. Our findings revealed that DIH inhibited NLRP3 activation in J774A.1 macrophages triggered by diverse stimuli (LPS plus nigericin/ adenosine triphosphate/ monosodium urate crystals), as it reduced IL-1β release and caspase-1 activation. DIH treatment also diminished cleaved IL-1β and caspase-1 p10 expression, although expression of NLRP3, ASC, pro-IL-1β and pro-caspase-1 was not affected. Pyroptosis mediated by NLRP3 activation was also attenuated by DIH. In addition, we found that DIH acts as a dual NLRP3 inhibitor by inhibition of LPS-induced priming step in NLRP3 inflammasome activation. Similar results on IL-1β release were observed in nigericin-activated bone marrow-derived macrophages. Covalent molecular docking study of DIH onto the ATP-binding site revealed that DIH binds to NLRP3, forming a covalent bond with Cys415. In conclusion, our experiments show that DIH is an effective NLRP3 inflammasome inhibitor, by inhibiting both the priming and the activation steps, making DIH a promising therapeutic agent for the treatment of inflammatory-related diseases.

Keywords: caspase-1; dehydroisohispanolone; diterpene; interleukin-1β; NLRP3 inflammasome.

Introduction

NLRP3 inflammasome is a multiprotein complex that plays a key role in the pathogenesis of several inflammatory diseases (Mangan *et al.*, 2018), being a potential target for the development of new therapeutic approaches. This complex activation requires two steps: a first signal or "priming", mediated through NF-κB activation and a second signal specific to the inflammasome. This can be triggered by many diverse stimuli including nigericin (Nig), adenosin triphosphate (ATP) or monosodium urate (MSU) crystals. NLR3 inflammasome activation leads to caspase-1 activation, which in turn mediates IL-1β and IL-18 maturation and release. Caspase-1 also induces a type of cell death called pyroptosis.

Terpenoids are natural products that constitutes a successful source in drug discovery and development of new therapeutic agents. Among them, hispanolone diterpene derivatives have demonstrated anti-inflammatory potential through modulation of classical inflammatory pathways. In this context, **dehydroisohispanolone (DIH)** has been investigated as modulator of NLRP3 inflammasome activation, and the molecular targets underlying its effects were analysed in macrophages.

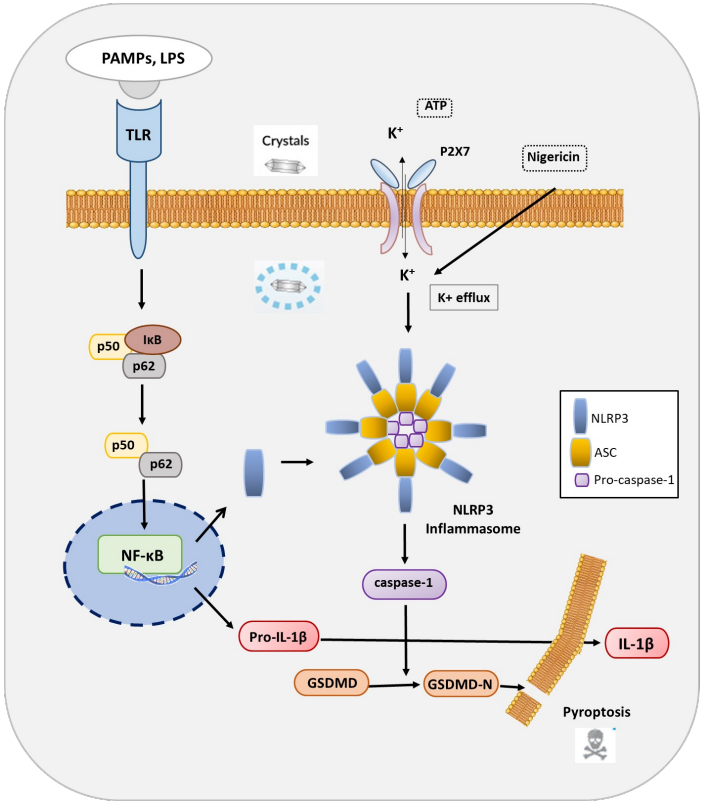


Figure 1. Activation of NLRP3 inflammasome in macrophages.

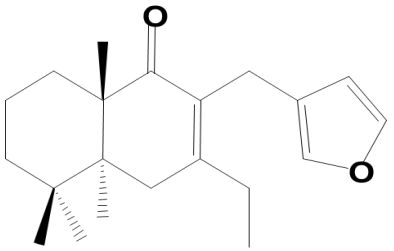


Figure 2. Dehydroisohispanolone (DIH) structure.

Results and discussion

DIH reduces IL-1β secretion following NLRP3 inflammasome activation

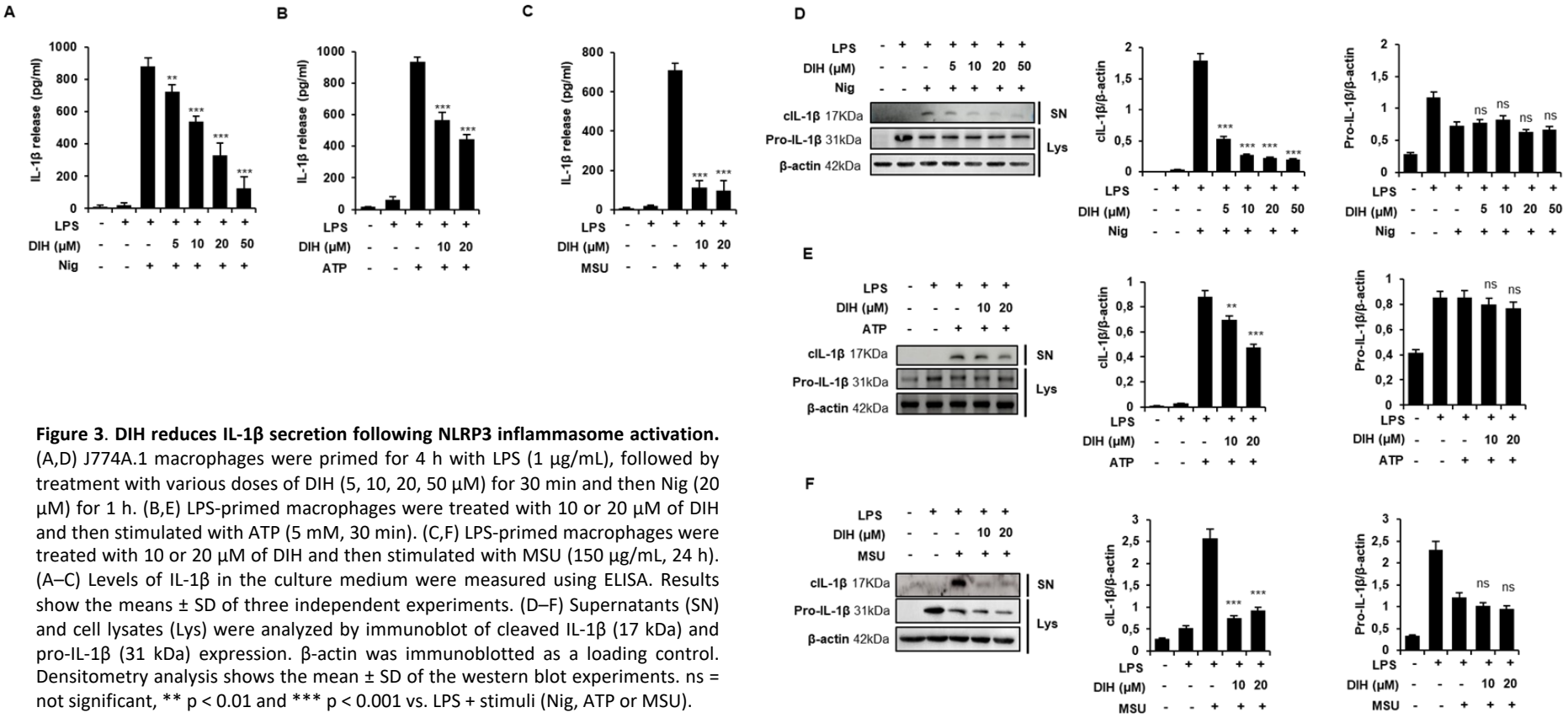


Figure 3. **DIH reduces IL-1β secretion following NLRP3 inflammasome activation.** (A,D) J774A.1 macrophages were primed for 4 h with LPS (1 μg/mL), followed by treatment with various doses of DIH (5, 10, 20, 50 μM) for 30 min and then Nig (20 μM) for 1 h. (B,E) LPS-primed macrophages were treated with 10 or 20 μM of DIH and then stimulated with ATP (5 mM, 30 min). (C,F) LPS-primed macrophages were treated with 10 or 20 μM of DIH and then stimulated with MSU (150 μg/mL, 24 h). (A–C) Levels of IL-1β in the culture medium were measured using ELISA. Results show the means ± SD of three independent experiments. (D–F) Supernatants (SN) and cell lysates (Lys) were analyzed by immunoblot of cleaved IL-1β (17 kDa) and pro-IL-1β (31 kDa) expression. β-actin was immunoblotted as a loading control. Densitometry analysis shows the mean ± SD of the western blot experiments. ns = not significant, ** $p < 0.01$ and *** $p < 0.001$ vs. LPS + stimuli (Nig, ATP or MSU).

Results and discussion

DIH treatment inhibits caspase-1 activation and attenuates inflammasome-dependent pyroptosis

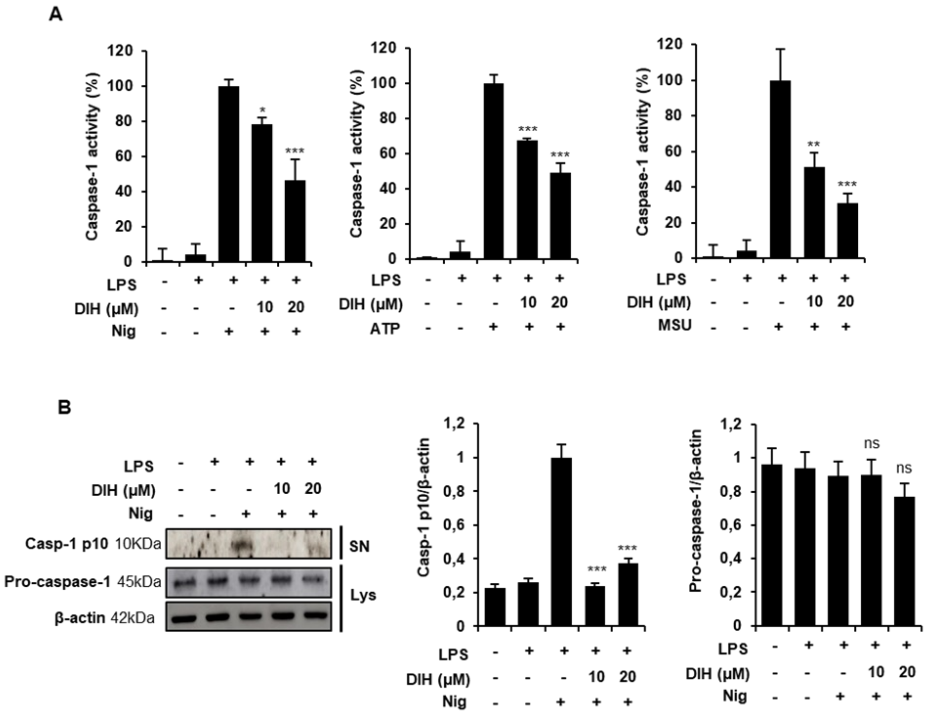


Figure 4. **DIH suppresses Caspase-1 activation.** (A) Caspase-1 activity was determined in LPS-primed J774A.1 cells stimulated with the inflammasome activators: Nig (20 μM, 1 h), ATP (5 mM, 30 min) or MSU (150 mg/mL, 24 h) in the presence of DIH (10, 20 μM). Data are expressed as means ± SD of percentage of caspase-1 activity in three independent experiments. (B) Cleaved caspase-1 (10 kDa) and pro-caspase-1 (45 kDa) expression were analysed by western blot in supernatants (SN) and cell lysates (Lys) after LPS + Nig stimulation in presence of DIH (10, 20 μM). β-actin was immunoblotted as a loading control. Densitometry analysis shows the mean ± SD of the western blot experiments. ns = not significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs. LPS + NLRP3 activator treatment.

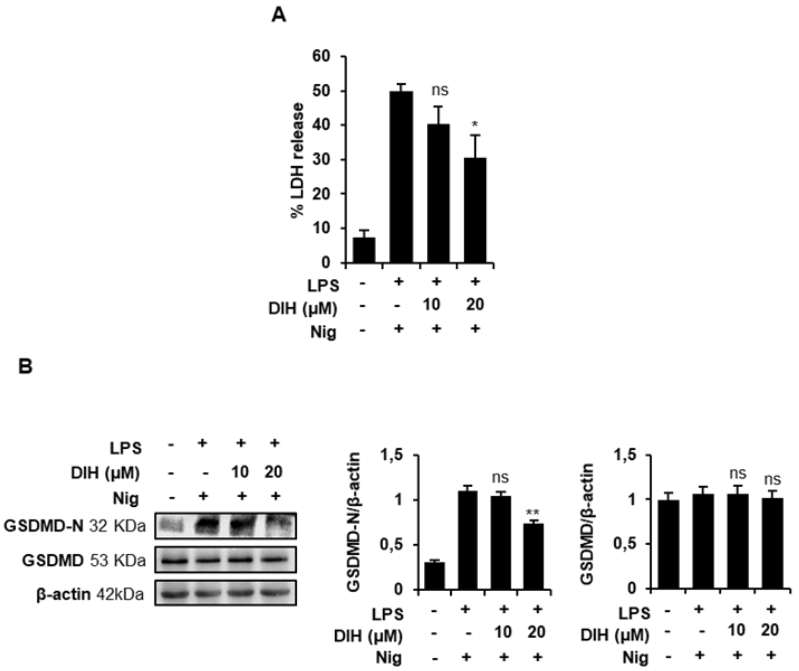


Figure 5. DIH attenuates NLRP3-dependent pyroptotic cell death. (A) LDH release was measured using a CytoTox® kit in the supernatants of LPS-primed J774A.1 macrophages treated with DIH (10, 20 μM) and stimulated with Nig (20 μM, 1 h). Results are expressed as means ± SD (n = 3). (B) Immunoblot analysis of GSDMD-N and GSDMD expression in cell lysates. β-actin was immunoblotted as a loading control. Densitometry analysis shows the mean ± SD of the western blot experiments. ns = not significant, * $p < 0.05$ and ** $p < 0.01$ vs. LPS + Nig treatment.

Results and discussion

DIH has no effects on NLRP3 inflammasome components when preincubating after LPS stimulation

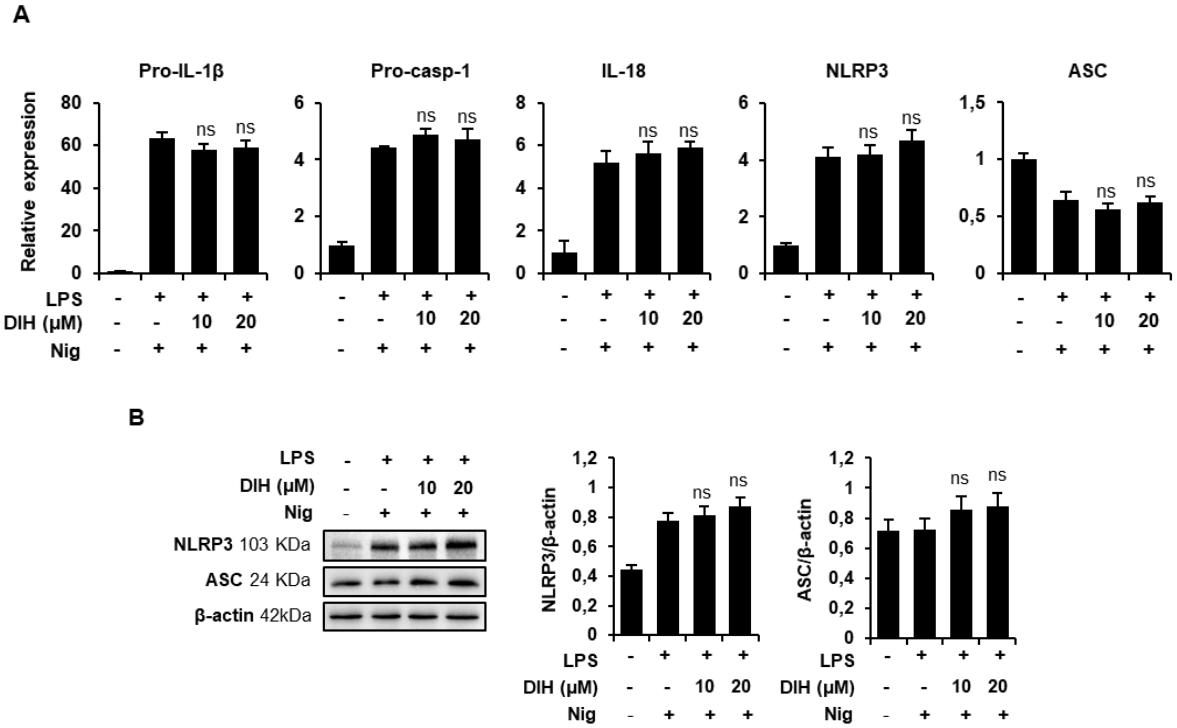


Figure 6. **DIH has no effects on NLRP3 inflammasome components**. J774A.1 macrophages were primed with LPS (1 μg/mL), then treated with DIH (10, 20 μM) and then stimulated with Nig (20 μM, 1 h). (**A**) Fold change of mRNA levels of inflammasome complex components. Results are expressed as means ± SD (n = 3). (**B**) Cell lysates after treatments were determined by immunoblot analysis of NLRP3 (103 kDa) and ASC (24 kDa) expression. β-actin was immunoblotted as a loading control. Densitometry analysis shows the mean ± SD of the western blot experiments. ns = not significant *vs*. LPS + Nig treatment.

Results and discussion

DIH also affects the priming step of inflammasome activation

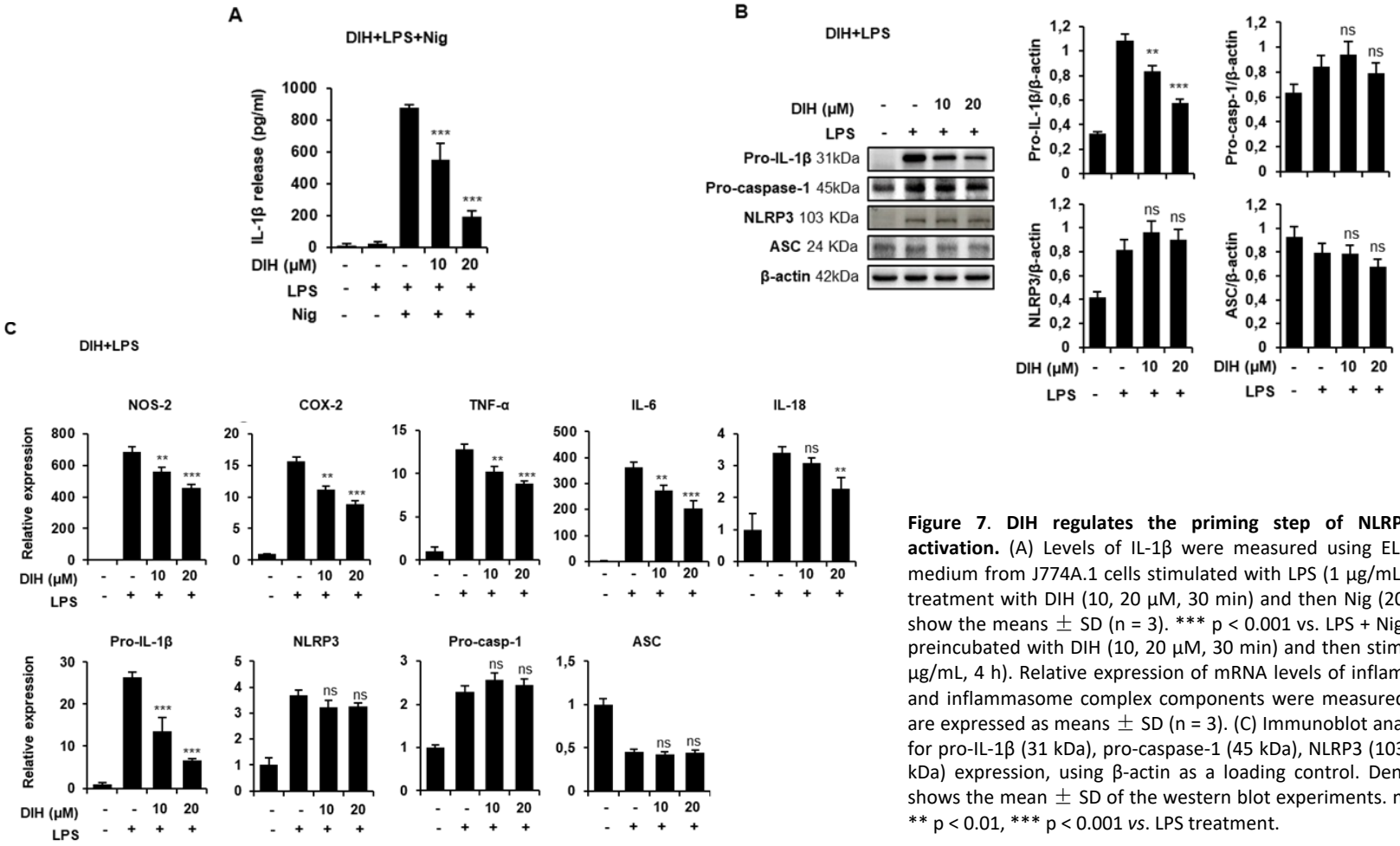


Figure 7. DIH regulates the priming step of NLRP3 inflammasome activation. (A) Levels of IL-1β were measured using ELISA in the culture medium from J774A.1 cells stimulated with LPS (1 μg/mL, 4 h), followed by treatment with DIH (10, 20 μM, 30 min) and then Nig (20 μM, 1 h). Results show the means ± SD (n = 3). *** $p < 0.001$ vs. LPS + Nig. (B) J774A.1 were preincubated with DIH (10, 20 μM, 30 min) and then stimulated with LPS (1 μg/mL, 4 h). Relative expression of mRNA levels of inflammatory mediators and inflammasome complex components were measured by qPCR. Results are expressed as means ± SD (n = 3). (C) Immunoblot analysis of cell lysates for pro-IL-1β (31 kDa), pro-caspase-1 (45 kDa), NLRP3 (103 kDa) and ASC (24 kDa) expression, using β-actin as a loading control. Densitometry analysis shows the mean ± SD of the western blot experiments. ns = not significant, ** $p < 0.01$, *** $p < 0.001$ *vs*. LPS treatment.

Results and discussion

DIH fits well and forms a covalent bond with Cys415 on the ATP binding-site of NLRP3

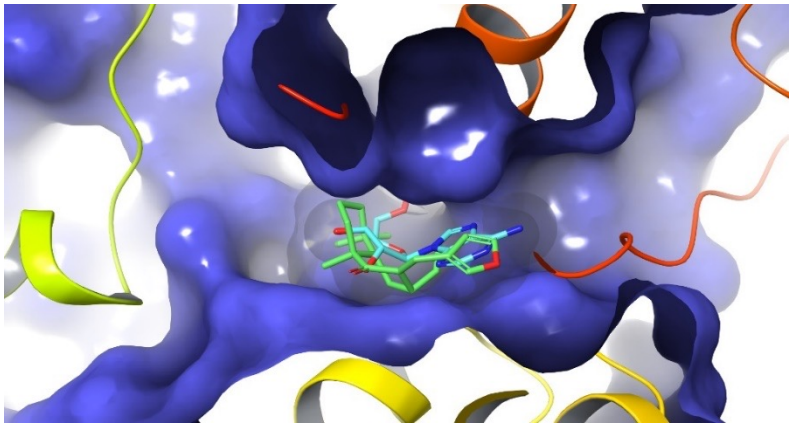
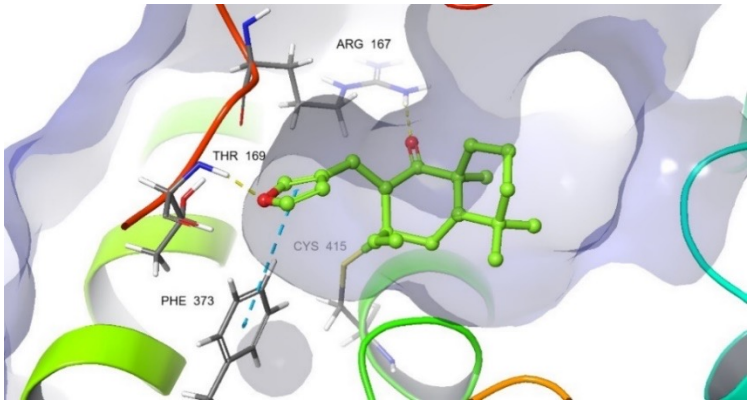


Figure 8. Covalent Docking of DIH occupying a large part of the ATP binding site (PDB 7ALV).



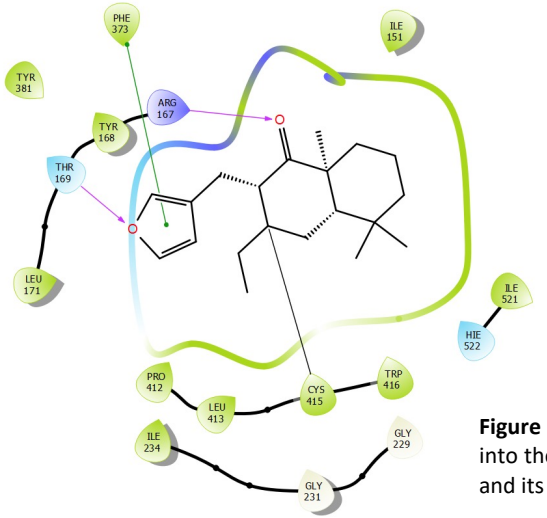


Figure 9. Covalent docking of DIH into the ADP binding site (PDB 7ALV) and its key interactions.

Conclusions

- **DIH** inhibits NLRP3 inflammasome activation with reduction of caspase-1 activation and IL-1β secretion and amelioration of pyroptosis.

- **DIH** links covalently to Cys415 in the ATP binding pocket of NLRP3, thus suggesting that it directly target NLRP3 by inhibiting its ATPase activity.

- **DIH** can target NLRP3 inflammasome in a dual manner to exert its anti-inflammatory activity, by both inhibiting NF-κB and NLRP3.

Acknowledgments

Research Module 5. Pharmacology, Pharmacognosy and Botanics Department. Faculty of Pharmacy (UCM).

Dr. Beatriz de las Heras
Dr. Irene Cuadrado

Pharmacological Therapies Unit. IIER, ISCIII.

Dr. Sonsoles Hortelano

IUBO-AG. Universidad de La Laguna (Tenerife).

Dr. Ana Estevez-Braun
Dr. Angel Amesty

Funding:

FPU17/03519

RTI2018-094356-B-C21

PI17CIII/00012
PI20CIII/00018

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