New inhibitors of NLRP3 inflammasome activation: pharmacological characterization in differentiated THP-1.

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**Background**

The NLR family protein NLRP3 is an intracellular signalling molecule activated by many pathogen-, environmental- and host-derived factors. NLRP3 activates caspase-1, a proteolytic enzyme that induces the cleavage and the release of proinflammatory cytokines, interleukin 1β (IL-1 β) and IL18, and causes a type of cell death known as pyropotosis. The fine regulation of inflammasome makes it a central player in the pathophysiology of numerous autoimmune and inflammatory diseases such as type 2 diabetes, gout, obesity, atherosclerosis, cryopyrinopathies, chronic inflammatory bowel diseases but also Alzheimer's and Parkinson's disease [1].

**Methods**

Several NLRP3 inhibitory compounds have been synthetized by SynBioMed group of the Department of Drug Science and Technology of Turin through the chemical modulation of a benzo[d]imidazol-1-one sub-moiety, which was identified as a weak inhibitor of ATPase activity. The inhibition of NLRP3 activation by these compounds was evaluated in THP-1 cell lines, differentiated with PMA and primed with LPS. NLRP3 was activated using different stimuli, such as ATP and MSU. Their protective effect on pyroptosis was evaluated by measuring LDH levels using LDH cytotox 96 non radio cytotoxicity assay (Promega). Moreover, the release of proinflammatory cytokines in supernatant and the expression of proteins involved in the signalling pathway activated by NLRP3 were evaluated. Finally, the cytotoxicity of these inhibitors was evaluated after 72h of treatment through the MTT assay.

**Results**

The most promising compound inhibited pyroptosis and IL-1b release in a dose-dependent manner, with inhibition of 58.7 ± 7.6% and 35 ± 1.2% respectively at the maximum concentration tested.
All the compounds were not cytotoxic at the concentration used to prevent NLRP3 activation.

**Conclusions**

Future studies are required in order to perform a more accurate characterization of NLRP3 inhibitory activity and to understand the possible role of these new inhibitors in the treatment of autoimmune and inflammatory diseases.

**References**

1) Awad et al. Pharmacol Ther 2018, 187:133-149