

*IX Giornate Italo-Francesi di Chimica
IX Journées Franco-Italiennes de Chimie
Genova
April 16-18, 2018*

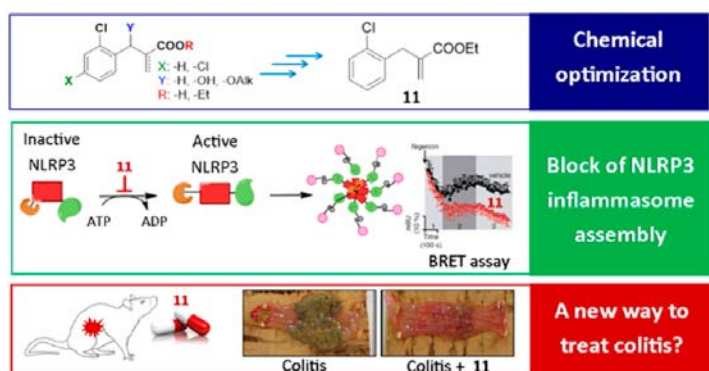
New tools for the development of direct NLRP3 inflammasome inhibitors

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In the last decades, several studies have highlighted the pivotal role of inflammasomes in the molecular control of inflammatory processes and the pathological role of NLRP3 inflammasome has been well established in different pathological settings. The discovery of agents able to prevent inflammasome activation is a promising therapeutic strategy to decrease chronic inflammation and associated damage. To date, different approaches have been pursued, among which reversible or irreversible modification of reactive cysteine residues of relevant proteins seems to be the prevalent one. In view of the above, we designed a library of electrophilic warheads bearing α,β -unsaturated nitrile and carbonyl-substituents.¹ These compounds were able to significantly prevent NLRP3-dependent pyroptosis of THP-1 cells. We demonstrated via a thiol trapping kinetic assay and ¹H NMR experiments that the biological activity of this series of compounds is dependent on their reactivity as Michael acceptors. The most promising warheads identified also proved able to directly inhibit the NLRP3 ATPase activity of isolated enzyme; unfortunately, these compounds exerted a certain degree of cytotoxicity on THP-1 cells, which could be related to their high reactivity. Thus, we developed new series of safer compounds by tuning down the reactivity of the electrophilic warhead through the chemical modulation of the acrylate scaffold. Thanks to this approach, we were able to obtain INF39 (**11**), a noncytotoxic molecule able to counteract NLRP3 activation through direct irreversible interaction with NLRP3 protein.² We better investigated his mechanism of action, and **11** has revealed able to block the conformational change required for inflammasome activation (Bioluminescence Resonance Energy Transfer experiment on recombinant NLRP3). Our preliminary in vitro ADME studies showed that INF39 was stable in simulated gastric and intestinal fluids, and it was absorbed into the

intestinal epithelium, where it can act locally and generate a nontoxic active metabolite. On the basis of the preliminary ADME profile, INF39 was selected for in vivo studies in a model of DNBS-induced colitis in rats. After oral administration, INF39 significantly reduced systemic and colonic inflammation.



[1] Cocco, M. et al. Electrophilic Warhead-Based Design of Compounds Preventing NLRP3 Inflammasome-Dependent Pyroptosis. *J. Med. Chem.* 57, 10366-10382 (2014).

[2] Cocco, M. et al. Development of an Acrylate Derivative Targeting the NLRP3 Inflammasome for the Treatment of Inflammatory Bowel Disease. *J Med Chem* 60, 3656-3671 (2017).

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