Identification of Small Synthetic Anti-Pyroptotic Compounds

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Pyroptosis is a pro-inflammatory form of programmed cell death and the common endpoint for many stimuli able to activate different inflammasomes in macrophages and other cell types. An abnormal activation of inflammasomes has been shown in several disorders of the innate immune system, characterized by chronic inflammation. Pharmacological agents counteracting pyroptosis could be a novel class of highly effective anti-inflammatory therapeutics for these disorders. To date only few substances, including parthenolide (isolated from *Tanacetum parthenium*; Figure 1A) and bromoxone (from a species of acorn worm belonging to the genus *Ptychodera*, found in deep underwater caves on the island of Maui; Figure 1B), have demonstrated to exert anti-pyroptotic effects in cellular models. The aim of this study was to identify new small synthetic anti-pyroptotic compounds. Some of the reported anti-pyroptotic agents share the ability to behave as Michael acceptors. Therefore, we have designed and synthesized a library of 23 small electrophilic alkenes (Figure 1C) *via* the Morita-Baylis-Hillman reaction. These compounds were screened to assess their Michael acceptor activity and cytotoxicity. In addition, their anti-pyroptotic effects were evaluated in the model of lipopolysaccharide/ATP-triggered death of phorbol myristate acetate-differentiated THP-1 cells. In general, these compounds formed Michael adducts with the sulfhydryl group of cysteine in GSH. Significant quantitative differences were determined when their effects on cell viability of human immortalized tubular epithelial cells and on pyroptosis were measured. The properties of the EWG and R_1 emerged as major determinants of the biological activity, while the analysis of the structure-activity relationship revealed a complex role for the R_2 substituent. Our results indicate that the anti-pyroptotic effects of these synthetic Michael acceptors may be related to the inhibition of the NALP3 inflammasome activity. This study not only highlights an easily accessible structural moiety suitable for further developments, but also indicates the presence of druggable targets in the signaling network underling pyroptosis.

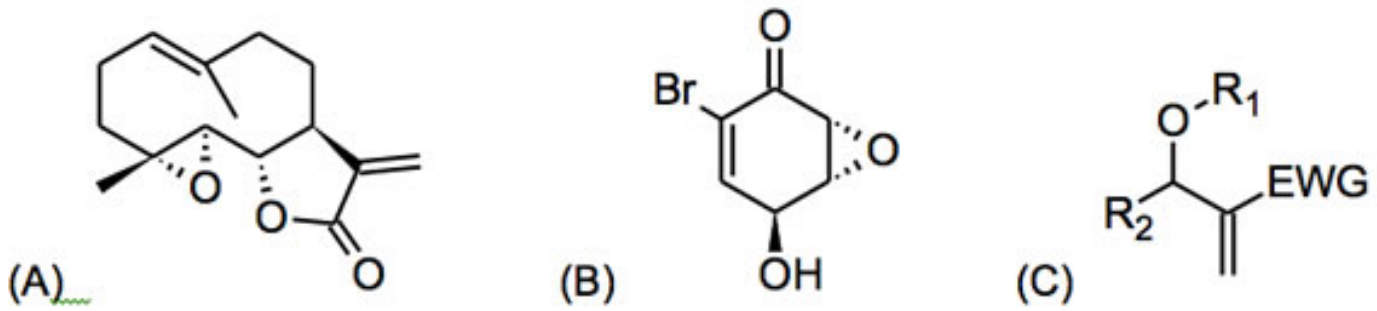

Figure 1. (A) Parthenolide, (B) bromoxone and (C) model of the synthesized compounds (EWG: electron withdrawing group).