Inflammation and cancer are two complex pathological processes that exploit several molecular actors. The identification of new platforms able to interfere with biological targets placed at the crossroads of these two pathways is strongly needed both, for the development of promising drug candidates, and as chemical probes useful to further investigate less understood biological aspects. Three main targets, involved at different levels in inflammation and cancer, have been thoroughly investigated: the Heat Shock Factor 1 (HSF1), the Bcl-2 associated athanogene 3 protein (BAG3), and the microsomal Prostaglandin E Synthase-1 (mPGES-1). The obtained results can be summarized in the three main sections reported below according to the target of interest:

a) Discovery of new potential modulators of HSF1 by computer-aided approach.
Heat shock factor 1 (HSF1) is the master regulator of the cytoprotective Heat Shock Response (HSR) in eukaryotes. The HSR is an evolutionarily conserved mechanism triggered by proteotoxic stress and involves the rapid and transient expression of Heat Shock Protein (HSP), molecular chaperones that restore cell proteostasis. HSF1 activity is amplified in many tumor contexts in a manner that resembles a chronic state of stress, characterized by high levels of hsp gene expression as well as HSF1-mediated non-hsp gene regulation. HSF1 and its gene targets are implicated in tumorigenesis as assessed in several experimental tumor models and facilitate metastatic and resistant properties within cancer cells. HSF1 is emerged as a therapeutic target valuable in cancer related disorders although, for several reasons, it can be considered quite "undruggable". The workflow that guided us in the research of potential HSF1 binders was based on an integrated approach including computational studies, synthesis of the most promising molecules, biophysical assays, and in-cell assays. Starting from the HSF1-DBD crystallographic structure in complex with an HSE (PDB: 5D5U), two types of virtual screening were performed. The molecules with the best docking score values were purchased or synthesized and their affinity for the full-length protein was evaluated by SPR assay. These studies led us to identify several molecules potentially able to target the protein, in particular, we found that BD-1 and LAM17 are able to bind the full-length protein with a dissociation constant in the low micromolar range, and furthermore, basing on in-depth biological studies, they showed to regulate the transcriptional activity of HSF1.

b) Design, synthesis and biological evaluation of BAG3 modulators.
Bcl2-associated athanogene 3 (BAG3) protein is a member of the BAG family of co-chaperones that interacts with the ATPase domain of the Heat Shock Protein 70 (Hsp70) through the conserved BAG domain. BAG3 is the only member of the family to be induced by stressful stimuli, mainly through the activity of HSF1 on bag3 gene promoter. In addition to the BAG domain, BAG3 also contains a WW domain and a proline-rich (PXXP) repeat that mediate its binding to different partners. These multifaceted interactions underlie the BAG3 ability to modulate key biological processes like apoptosis, development, cytoskeleton organization, and autophagy, thereby mediating the cell adaptive responses to stressful stimuli. In normal cells, BAG3 is constitutively present in a very few cell types, including cardiomyocytes and skeletal muscle cells, in which the protein appears to contribute to cell resistance to mechanical stress. A growing body of evidence indicates that BAG3 is highly expressed in several tumor types where it is proven to sustain cell survival, resistance to therapy, motility, and metastatization. In some tumor types, the down-modulation of BAG3 levels was shown, as a proof-of-principle, to inhibit neoplastic cell growth in animal models. With the aim of exploring the BAG3 protein as cancer target, through a combined approach of structure-based drug design and biophysical methods, in 2017 the research group of which I am part
discovered the first selective BAG3 modulator featuring a 2,4-thiazolidinedione scaffold. Basing on these promises, I continued to explore the chemical space around the thiazolidinedione core, and, supported by computational studies, a new compounds collection has been developed. In order to assess the affinity for the target, these molecules were tested, through SPR assay, against both the full-length BAG3 protein and the BAG3-BD. LK6 proved to be the most promising molecule showing a high affinity for both the full protein (6.3 ± 0.3 μM) and the BAG domain (27.6 ± 1.9 μM). According with its favorable binding properties, LK6 showed a potent cytotoxicity and the ability to cause a cell accumulation in the G1 phase, suggesting an apoptosis/necrosis event which was confirmed by the significant dose-dependent reduction of caspase 3 and 9, the main effectors of the programming cell death, in treated cells.

c) Identification of mPGES-1 inhibitors through a multistep approach.

Microsomal prostaglandin E synthase-1 (mPGES-1) is the terminal synthase responsible for the production of prostaglandin E2 (PGE2). PGE2 is a bioactive lipid that can elicit a wide range of biological effects associated with inflammation and cancer. The pleiotropic role of PGE2 is mainly mediated by the activation of key downstream signaling cascades via transmembrane EP receptors located on the cell surface. Elevated levels of COX-2 and concomitant overproduction of PGE2 are often found in human cancers leading to the use of non-steroidal anti-inflammatory drugs (NSAIDs) as chemo preventive agents. Their long-term use, however, may be associated with gastrointestinal toxicity and increased risk of adverse effects. Targeting mPGES-1 is considered a valid alternative to the use of NSAIDs because it is an inducible enzyme able to affect only the prostanoids elicited by inflammatory stimuli without affecting those lipid mediators constitutively expressed. In the frame of my Ph.D. project, following a fragment-based approach and a structure-based drug design, a very promising mPGES-1 inhibitor was disclosed as well as a dual inhibitor of mPGES-1 and 5-lipoxygenase (5-LO). In the first case, a (4-phenyl-thiophen-2-yl)-acetic acid-based compound, SZK9, showed a high selectivity and a potent inhibitory activity against mPGES-1 (IC50 = 5.9 ± 1.0 μM). Since this molecule is endowed with a strong cytotoxic effect (IC50 = 10.1 ± 1.2 μM) comparable to the known inhibitor CAY10526 it can represent an attractive candidate for the development of new therapeutics in cancer pathology. In the second case, following a structure-based approach a 2,4-thiazolidinedione based molecule, TZ8, was identified as a potent inhibitor of both mPGES-1 and 5-LO, two key enzymes involved in inflammatory related disorders. Owing to its dual-target inhibitor profile TZ3 can be considered an interesting hit for the development of novel therapeutic interventions.