Abstract

Inflammation and cancer are two complex pathological processes, involving a variety of molecular actors. The deeply connection and crosstalk between cancer and inflammation is well-known and the modulation of these processes is one of the main goals of modern medicinal chemistry. The identification of new molecular entities able to interfere with biological targets placed at the crossroads of these two pathways is strongly needed, both for the development of new 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: bromodomain (BRD) containing proteins, microsomal Prostaglandin E_2 Synthase-1 (mPGES-1) and Heat-shock protein 90 (Hsp90). The results obtained can be summarized in the three main sections, reported below according to the target of interest:

a) Discovery of new modulators of human bromodomains by structure-based and computer-aided combined approaches. BRDs are evolutionary conserved modules which act as readers of the histone code, by recognizing acetyl-lysine (Kac) residues on histone tails. The contribution of BRD containing proteins has recently emerged in a number of diseases, especially in cancer processes. With the aim of identifying a new Kac mimetic chemotype, a structure-guided approach was undertaken starting from small fragment-like 9*H*-purine scaffolds. One of the initial identified fragments (**2a**), that was shown to be a BRD binder, was systematically modified employing organic synthesis approaches in order to gather a structure activity relationships profile to be exploited in the next structural optimization process. These studies allowed to disclose potent nanomolar ligands for BRD9 (compounds **7d** and **11)**, showing only residual micromolar affinity towards BRD4. Binding of **7d** and **11** to BRD9 was investigated by crystallography and flexible docking experiments and resulted in an unprecedented rearrangement of residues forming the Kac cavity, affecting plasticity of the protein in an induced-fit pocket. Finally, the compounds did not exhibit any cytotoxic effect in HEK293T cells and displaced the BRD9 bromodomain from chromatin in bioluminescence proximity assays without remarkably affecting the BRD4/histone complex.

b) Identification and structural optimization of DHPM-based mPGES-1 inhibitors. mPGES-1 is a homotrimeric membrane protein involved in the arachidonic acid cascade, which acts as downstream synthase in the cyclooxygenase (COX) pathway by catalyzing the

biosynthesis of Prostaglandin (PG) E_2 from the PGH_2 precursor. Inhibition of mPGES-1 can represent a valid therapeutic approach to interfere with inflammation-induced PGE_2 formation without affecting the constitutively formed prostanoids. In order to find a new molecular platform for mPGES-1 modulation, a structure-based design approach was carried out on a focused collection of 3,4-dyhidropyrimidin-2(1H)-one (DHPM)-based molecules, docked in the first high resolution X-ray crystal structure of the enzyme in its active form (PDB code: 4AL0). The key interactions with the receptor counterpart were introduced as a qualitative filter for the selection of the most promising compounds to be synthesized. Biological results were consistent with the computational suggestions and disclosed two molecules (**48** and **49**) showing a promising *in vitro* mPGES-1 inhibitory activity. The most recently crystallized structure of mPGES-1 with the inhibitor LVJ (PDB code: 4BPM) was used to optimise compound **48** (IC_{50} = 4.16 ± 0.47 μM) to give compound **53**, a 10-fold more potent mPGES-1 inhibitor (IC_{50} = 0.41 ± 0.02 μM).

In order to deeply investigate this complex enzyme, a heterologous expression of human His_6-tagged mPGES-1 and two-dimensional crystallographic studies were also carried out.

c) The DHPM core as new chemotype for Hsp90 C-terminal modulation. Hsp90 is a molecular chaperone highly involved in the development, survival and proliferation of cancer cells. Traditional inhibitors of Hsp90 target its N-terminal domain. Nevertheless, this type of modulation produces scheduling and toxicity issues connected to the induction of the deleterious heat shock response. Although less explored, C-terminal inhibition of Hsp90 represents a very promising approach for developing new potential anti-cancer drugs as it is devoid of the negative effects triggered by the heat shock response. In an attempt to identify non-natural inspired modulators of Hsp90 C-terminus, a collection of DHPM derivatives was synthesized. The rationale for targeting Hsp90 C-terminal domain by DHPMs derives from the structural analogy between the DHPM core and uridine triphosphate (UTP), a nucleotide shown to selectively interact with the chaperone C-terminal site, but not with its N-terminus. Biological evaluation revealed that the privileged DHPM core can be considered as a new template for the modulation of Hsp90 chaperoning function, through the binding to its C-terminal region. In particular, compound **54** was identified as a novel promising antiproliferative agent against Hsp90 C-terminus.