Abstract

In the last few years, computational chemistry has played an important role in disclosing novel compounds with relevant biological activity. Inflammation and cancer processes have been recently linked, and the identification of new molecular entities able to interfere with biological targets involved in these pathologies is strongly needed. The analysis of ligand-macromolecule interactions and the evaluation of possible "binding modes" are the starting points in the design and the identification of new and more powerful drugs. Also, *in silico* Virtual Screening campaigns of large libraries of compounds on a specific target allow the selection of the most promising, leading the identification of new hits. Moreover, the coupling between versatile synthetic approaches and computational protocols is a powerful tool to obtain optimal results with time optimization. Thus, in this thesis both topics are applied at the same time, as they are connected to each other.

The research work was mainly focused on mPGES-1 (microsomal Prostaglandin E_2 Synthase) as a promising target for the treatment of the pathologies above. This enzyme is a homotrimeric membrane protein involved in the arachidonic acid cascade and it acts as downstream synthase in the cyclooxygenase pathway, catalyzing the conversion of the unstable peroxidic intermediate prostaglandin H_2 (PGH_2) in prostaglandin E_2 (PGE_2). Among the three different isoforms of the enzyme, selective inhibition of the inducible mPGES-1 may be considered a valid therapeutic approach to interfere with inflammation-induced PGE_2 formation, avoiding to block the biosynthesis of constitutive prostanoids. Also, it is over-expressed in tumor pathologies. In light of this, in order to identify novel molecular platforms able to inhibit the activity of the enzyme strongly, a structure-based multi-step computational

protocol was applied starting from selected privileged scaffolds coupled to a synthetic chemical route. Specifically, new libraries of 2-aminobenzothiazole, 2-aminothiadiazole and 2-carboxamidepyrrole-based molecules were generated, and they were docked onto the 3D structure of mPGES-1 (PDB code: 4BPM) crystallized in 2014. Using the interactions with the receptor counterpart as a qualitative filter, a collection of compounds for each library was selected, synthesized and submitted to biological investigation. Finally, cell-free and cell line assays confirmed some of them (benzothiazole derivatives **1**, **3**, **6**, **9** and **13**, thiadiazole derivatives **18**-**20** and **22**, pyrrole derivatives **45** and **47**) as novel promising mPGES-1 inhibitors.

Furthermore, the same computational approach focused on mPGES-1 was applied on substituted saturated N-heterocycles, whose synthesis was performed at the Department of Chemistry and Applied Biosciences at ETH (Zurich). In detail, the libraries were docked onto the crystal structure co-crystallized with the inhibitor 6PW (PDB code: 5K0I), leading to the selection of 21 compounds (**62-82**). The synthesis was performed using the SnAP (Stannyl Amine Protocol) chemistry, which consists of a reaction between an aminotributylstannane and an aldehyde to form an imine as the first step, followed by an intramolecular cyclization. Biological investigations are in progress on the synthesized compounds.

In order to deeply combine computational tools and this versatile and suitable synthetic approach, a large database of about 1,300,000 synthetically accessible compounds was created *in silico* starting from iSnAP (Iterative SnAP) reagents, a new generation of SnAP reagents bearing a further functional group. The prepared database was then submitted to virtual screening studies on pharmacologically promising targets (mPGES-1 up to

now), in order to select the best candidates for the synthesis and the biological evaluation.

Finally, the application of multi-step computational protocols including both ligand and structure based approaches took place in the identification of two novel 3-hydroxy-3-pyrrolin-2-one-based hits (compounds **87** and **88**) as promising mPGES-1 inhibitors, starting from a large library of commercially available compounds.

A second research work regarded Hsp90, a molecular chaperone involved in the development, survival and proliferation of cancer cells, regulating the homeostasis of oncoproteins. Most of the developed Hsp90 inhibitors interact with the N-terminal domain, but this type of modulation induces toxicity issues connected to the induction of the deleterious heat shock response (HSR). Contrariwise, since C-terminus inhibitors do not produce this effect and only a few of them have been developed so far, an aim of this research work consisted of the individuation of novel C-terminus binders. Thus, a biological screening on a small library of 48 commercially available compounds was performed and two novel inhibitors were identified (compounds **100** and **103**) able to interact selectively with the C-terminal domain. Starting from these results, a computational rationalization was performed, applying Induced Fit Docking studies and Molecular Dynamic Simulations.

In conclusion, in order to perform an optimization campaign, three large libraries of derivatives of compound **103** were generated using the multi-step computational protocol above, and a total number of 21 molecules have been selected for the synthesis and the biological evaluation.