Abstract

Computational methodologies in combination with experimental techniques as Nuclear Magnetic Resonance (NMR) have become a crucial component in drug discovery process, from hit identification to lead optimization.

The study of ligand-macromolecule interactions, in fact, has a crucial role for the design and the development of new and more powerful drugs. In this project, different aspects of interaction and recognition processes between ligand and macromolecule, and streostructure assignment has been studied through this kind of combined approach with the aim to identify novel potential antitumor and/or antiinflammatory molecules.

In particular, because the strong interconnection between the tumoral and inflammatory pathology has led to the identification of new target utilizable for the therapy, in this project will be described proteins (Histone deacetilase, HDAC; Nicotinamide Phosphoribosyltransferase, NMPRTase or Nampt; microsomal prostaglandin E_2 synthase, mPGES-1; human synovial Phospholipases A_2, hsPLA_2; human Farnesoid-X-Receptor, FXR; human Pregnane-X-Receptor, PXR; Bile Acid Receptor GPBAR-1, TGR5) involved in essential cellular processes and acting at diverse levels and phases of the tumor and inflammation diseases.

The results obtained can be summarized in three main areas of activity, whose relative weight was varied according to the development of the overall project:

a) <u>Support in the design of original scaffolds for the generation of libraries potentially utilizable in therapy.</u> This work was exclusively conducted *in silico* by a molecular docking technique in order to direct the design of the new molecules basing on the analysis of ligand-target interactions and the synthetic possibilities. This kind of approach was successfully applied leading to the identification of new potential inhibitors for HDAC enzymes with ciclic (mono and bis amides, paragraph 2.2; conformationally locked calixarenes, paragraph 2.4), and linear (hydroxamic tertiary amines, paragraph 2.3) structures, and isoform selective (paragraph 2.6), and of ligands for microsomal prostaglandin E_2 synthase (mPGES)-1 (two series of triazole-based compounds; paragraphs 4.2 and 4.3).

For each of this described studied, the good qualitative accordance between the calculated and experimental data has made possible the identifications of new lead compounds, rationalizing in this way the key features to the target inhibition.

b) <u>Rationalization of the biological activity of compounds by the study of the drug-receptor interactions.</u> Molecular docking was used for the detailed study of antiinflammatory and

antitumoral compounds whose activities are known a priori. In fact, thanks to this procedure, in this thesis several rationalizations of binding modes were reported related to Ugi products derivatives of CHAP 1 (HDAC inhibitors, paragraph 2.5), new and potent inhibitor of NMPRTAse analogs of FK866 and CHS 828 (chapter 3), marine natural products as inhibitors of hsPLA_2 (BLQ and CLDA, chapter 5), 4-methylen sterols extracted from *Theonella swinhoei* as ligands of FXR and PXR (chapter 6), and known compounds as taurolitholic acid and ciprofloxacin (chapter 7), agonists of TGR5.

Through the *in silico* methodology the putative binding modes for the reported molecules was described offering a complete rationalization of docking results, evaluating the influence of the ligand target interactions (e.g. hydrophobic, hydrophilic, electrostatic contacts) on the biological activity.

c) <u>Determination of relative configuration of natural products.</u>

The complete comprehension of the three dimensional structure of synthetic or isolated molecules is fundamental to design and characterize new platform potentially utilizable in therapy. On this basis, the combined approach between the quantum mechanical (QM) calculation of NMR parameters and NMR spectroscopy was revealed a very useful mean to lead the total synthesis of natural product toward the right isomer avoiding waste of time and resources (paragraph 8.1).

Moreover, the stereostructure assignment of marine natural products conicasterol F and its analog thonellasterol I was reported in the paragraph 8.2. by a novel combined approach between the quantitative interproton distance determinations by ROE and quantum mechanical calculations of chemical shifts