Abstract

The identification of small molecules targeting specific protein-protein interactions (PPIs) involved in the regulation of the cell cycle is a recent and promising approach for the development of new anticancer agents. Targeting PPIs has long been viewed as a very challenging task because protein-protein interfaces tend to be *large* and *flat*. However, the discovery of so-called "hot spots" supported the hypothesis that many PPIs are "druggable" by small molecules.

This PhD thesis describes the development of new modulators of the p53/MDM2/MDM4 interaction, and new microtubules targeting agents.

The p53 protein is a transcription factor with a critical role in safeguarding genome integrity by activation of cell cycle checkpoints. Many different types of cancer show a high incidence of TP53 mutations, leading to the expression of mutant p53 proteins, which can promote cancer progression both by loss of their tumor-suppressor function, or acquisition of pro-oncogenic properties. However, human cancers retaining wild-type p53 frequently show overexpression of MDM2 and/or MDM4 proteins. MDM2 and MDM4 are negative regulators of the p53 protein, and their overexpression compromise p53 transcriptional activity. Thus, in these cases, inhibition of the p53/MDM2 (and/or p53/MDM4) interaction by small molecules is a promising anticancer strategy.

Recently, biological and computational studies supported the hypothesis that a tetrasubstituted pyrrole derivative (4-benzoyl-5-methyl-1-(4-methylbenzylbenzyl)-1H-pyrrole-2 carboxylic acid 3-chlorobenzylamide, **1**) is a modulator of p53/MDM2 interaction. In this thesis, the design and synthesis of new pyrrole derivatives analogues of **1** were described. The antiproliferative activity of some of them was investigated *in vitro* on three cell lines: the A375 human melanoma cell line, the HCT-116 colon cancer cell line as well as on the HaCaT *human keratinocyte* cell line. Among all tested compounds, 1-(4-amino-benzyl)-4-benzoyl-5-methyl-1H-pyrrole-2-carboxylic acid 3-chloro-benzylamide was found to be the most active pyrrole derivative, with IC_{50} values in a micromolar range.

Microtubules constitute a well-validated cancer drug target because of their central role in cell division. Many microtubule targeting agents (MTAs) currently in clinical use (e.g. taxol, vinca alkaloids) have shown several limitations (i.e. resistance, severe toxic side effects and low oral bioavailability), therefore innovative MTAs are needed.

Pyrrolo-1,5-benzoxazepine (PBOX) compounds have been reported as tubulin-targeting agents. In particular, PBOX-6 (namely 7-[(dimethylcarbamoyl) oxy]-6-(2-naphthyl)pyrrolo-[2,1-*d*][1,5]benzoxazepine potently induces apoptotic cell death in a variety of human cancer cell lines,

indicating its potential in the treatment of both solid tumors and tumors derived from the hematopoietic system. However, recent studies revealed PBOX resistance, mainly related to autophagy induction. In this project, PBOX-6 was chosen as a starting point for chemical modifications in order to improve its pharmacological profile and extend SAR analysis of this class of compounds. A small set of PBOX-6 analogues was generated by introducing substituents with different lipophilic, steric and electronic properties on the benzo-fused ring of our lead. Furthermore, their ability to affect the cell cycle and induce apoptosis on the HL60 (human promyelocytic leukemia) cell line was determined by flow cytometric analysis.