



UNIVERSITÀ DEGLI STUDI DI SALERNO



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***DESIGN AND SYNTHESIS OF PEPTIDES THAT  
MODULATE APOPTOTIC PROCESS***

settore scientifico disciplinare di afferenza: CHIM/08

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Oncogenic activation of tyrosine kinases is a common feature in cancer, and its regulation represents an excellent antitumoral target. Tyrosine phosphorylation is also controlled by protein-tyrosine phosphatases (PTPs). Recent evidence has shown that PTPs can function as tumour suppressors. An improved understanding of how these enzymes function and how they are regulated might aid the development of new anticancer agents.

It has been shown that cross-regulation of kinases/phosphatases and caspases allows for fine-tuning of the apoptotic threshold, as well as the opportunity to amplify apoptotic signals. The signaling pathways involved in the control of cell proliferation, adhesion and migration are governed by the balanced action of protein tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs).

For this reason my PhD thesis focused the attention on three different targets PTPRJ a receptor protein tyrosine phosphatase, GRK2 G protein-coupled receptor kinase 2, CaMKII, Ca<sup>2+</sup>/Calmodulin-dependent protein kinase II.

PTPRJ is down regulated in tumor cells and its over-expression suppresses cell growth, both in vivo and in vitro, concomitant with the reduction of the activity of MAP-kinase (ERK1/2) and phosphorylation of PLC- $\gamma$ .

Therefore, the identification of agonist peptides would be a valid approach in antitumoral therapy. By means of a phage display library screening, we recently identified two peptides able to bind and activate PTPRJ, [CHHNLTHAC]-OH and [CLHHYHGSC]-OH. Focused the attention on [CHHNLTHAC]-OH my research project was based on the design of different peptide libraries using several approaches like Alanine scanning approach and changes in disulfur bridge.

GRK2, G protein-coupled receptor kinase 2 is a relevant signaling node of the cellular transduction network, playing major roles in the physiology of various organs/tissues including the heart and blood vessels. Emerging evidence suggests that GRK2 is up regulated in pathological situations such as heart failure, hypertrophy, hypertension and is involved in the progression of cellular cycle. Therefore, its inhibition offers a potential therapeutic solution to these diseases.

During my PhD thesis a SAR study and a NMR conformational analysis of peptides derived from HJ loop of GRK2 and able to selectively inhibit GRK2 were performed. Moreover, we explored the GRK2 inhibitory activity of a library of cyclic peptides derived from the HJ loop of GRK2. The design of these cyclic compounds was based on the conformation of the HJ loop within the X-ray structure of GRK2. One of these compounds, potently and selectively inhibited the GRK2 activity, being more active than its linear precursor.

CaMKII, Ca<sup>2+</sup>/Calmodulin-dependent protein kinase II constitutes a family of closely related multifunctional serine/threonine kinases that transduces elevated Ca<sup>2+</sup> signals in cells to a number of target proteins ranging from ion channels to transcriptional activators. Among processes regulated by CaMKII are neuronal growth and functions related to brain development, synaptic plasticity as well as the formation and maintenance of memory, cell proliferation and apoptosis, proper function of the immune system, and the central control of energy balance. Current knowledge about CaMKII control on physiological or pathological functions is largely based on experiments with pharmacological inhibitors. As part of our current interest in the study of CaMKII-dependent cell signaling, we directed our efforts toward the identification of novel CaMKII peptide inhibitors.

Starting from a potent CaMKinase II inhibitor, CaM-KNtide, we designed different CaM-KNtide analogues and evaluated the inhibitory activity and specificity.