Mitochondria-targeting conjugates as a new anticancer agents

Mitochondria play important roles in living cells as an energy source and as a generator of reactive substrates which are harmful to living cells. Alterations in mitochondrial structure and functions have long been observed in cancer cells. The mitochondria of cancer cells and transformed cells exhibit significantly increased transmembrane potentials when compared with those of normal cells. Targeting mitochondria as a cancer therapeutic strategy has gained momentum in recent years. Delivery of therapeutic agents into mitochondria is a challenging task in modern pharmacology because the molecule to be delivered needs first of all to overcome the cell membrane barrier and then be able to actively target the intracellular organelle.

Delocalized lipophilic cations, such as triphenylphosphonium (TPP^+) and rhodamine, are well-known mitochondria-targeting molecules and show membrane-permeability, allowing the molecules to traverse the mitochondrial membranes by hydrophobic interactions. Development of new efficient therapeutics targeting intracellular organelles such as mitochondria may represent new opportunities for the effective treatment of cancer.

Therefore, the proposed project concerns the synthesis, cellular uptake, cytotoxicity, and mitochondrial colocalization of new mitochondria-targeting conjugates of triphenylphosphonium cation (TPP^+) and rhodamine with a very active polyether ionophore – salinomycin.

Over the last years, it has been documented that salinomycin induces apoptosis in many human drug sensitive and multi-drug resistant (MDR) cancer cells as well as cancer stem cells (CSCs). All the aforementioned salinomycin properties are closely related to its structure and ability to form complexes with metal cations (host-guest complexes) and transport these complexes across lipid bilayers and cell membranes. Salinomycin exerts direct, mitochondrial effects, thus compromising cell survival and caused strong and time-dependent ATP-depletion in cancer cells, but not in human normal cells.

Rational modification of salinomycin can significantly improve its biological activity. For this reason, the resulting compounds will be tested for their biological activity (*in vitro* studies) in the close interdisciplinary co-operation with specialists in the field of oncology and biology. **The anti-cancer activity** of all newly synthesized salinomycin hybrids will be examined in human cancer cells using MTT assay (*in vitro* tests). The cytotoxic effects will be also studied on the normal cells in order to estimate the toxicity and selectivity of the studied compounds.

The biological mechanism of action of our novel conjugates of salinomycin with TPP^+ cation and rhodamine moiety will be studied using several biochemical assays and cellular assays. We plan to use advanced cell based assays such as cell proliferation, toxicity, viability, apoptosis, oxidative stress and live cell detection which will provide us with convenient and reproducible biological results **leading to understanding of mechanism of action of the obtained compounds**. The biological activity of salinomycin is related to their cation carrying properties. Thus, ionophoretic activity of salinomycin and its mitochondria-targeting conjugates will be also evaluated in **biophysical assays employing liposomes and planar lipid bilayers**. Altogether the available data suggest that apoptosis induced by salinomycin in the cancer cells might be, at least in part, due to its direct effect on mitochondria. Therefore, **we will also measure acute changes in mitochondrial membrane potential ($\Delta\psi$), production of ROS, matrix pH, swelling, mitochondrial morphology and respiration upon addiction of salinomycin and its mitochondria-targeting conjugates**.

All studies planned within the project should help to find a correlation between the structures of salinomycin derivatives and their biological activities (structure-activity relationship, SAR). The currently observed scientific interest in mitochondria-targeting conjugates as well unusual anti-cancer activity of salinomycin, makes this project of top interest and endows it with innovative character, and its scientific outcome can help in rational drug design in near future.