Background: During the search for potential anticancer drugs, which are performed in our laboratory, we developed recently the new promising compounds of the structure of unsymmetrical bisacridines (UAs). These compounds were shown to exhibit high cytotoxic and antitumor activity, preferentially against experimental human solid tumors, including breast, colon and pancreatic cancers. Our group is currently involved in the studies on molecular mechanism of action of these compounds. Preliminary results indicated that our new bisacridines were metabolized rather slowly with metabolizing enzymes, DMEs, cytochromes P450 and UDP-glucuronosyltransferases, UGTs. Moreover, we demonstrated that, in contrast to the studied earlier acridine derivatives, compounds of dimer structures do not intercalate to dsDNA, although some interactions are observed.

The above results intrigued us greatly, because we did not expect that the transformation from the monomer to dimer structure not only kept high antitumor activity, but will also result in the new, not described for monomers physicochemical and biological properties. Therefore, planning the current studies we considered not only the above facts but also other results, which indicated that gene expression of drug metabolizing enzymes of P450 and UGT family undergo cellular control by transcription factors of nuclear receptors as pregnane-X-receptor, PXR, constitutive androstane receptor, CAR and several others. Moreover, there was reported that PXR and CAR activities are able to induce cell death by apoptosis. We have been also interested in the pathway of our compounds in the mice organism.

Hypothesis: Considering the above knowledge and our current results we suggest that the observed high antitumor activity of UAs would result from their interactions with, other than double helix, structures of DNA and also from their influence on the transcription of functionally important proteins. We also postulated that it is possible that nuclear receptors can modulate the levels of the selected proteins responsible for the cellular response, for example in apoptosis.

Studies proposed:

To verify the above hypothesis we propose here the studies, which will include the following tasks:

1. Determination of 3D structure(s) of UAs in aqueous media and the description of UAs complex with G-quadruplexes followed by CD spectroscopy and 2D NMR studies on the short fragments of DNA forming G4.

2. Transcriptional effects of UAs. Evaluation of functionally relevant genes and pathways (*P450*, *UGT* enzymes and nuclear receptors, NR) studied by miRNA-mRNA expression and DNA methylation.

3. Evaluation whether UAs are the substrates and/or modulators of transmembrane pumps of the ABC transporter family.

4. Metabolism of UAs in tumor cells. Their impact on the modulation of enzymatic activity in HepG2 and LS180 cells and on the expression levels of the selected P450 and UGT isoenzymes. In case of positive results from point 2, further experiments concerning the expressions and protein levels of certain nuclear receptors.

5. Cross-talk between the expression of NR and proteins involved in the cell cycle progression and apoptosis in HepG2, LS180 and DU-145 cells following bisacridines treatment.

6. The urine and blood analysis relative to metabolic kinetics of two selected new UAs in mice.

Methods: To realize the above strategy we will apply in the first step non-cellular systems for the studies on DNA-drug interactions and on membrane transport of the compound. The next studies proposed here require maintaining the cell culture, which is in our direct disposal. We will use cell lines of high and standard levels of metabolic enzymes. The cell extracts and cell medium will be analysed for metabolites by HPLC. The impact of the drugs on P450 and UGT enzymes activity and on nuclear receptor expression in tumor cells will be analysed by Western blot and RT-PCR procedures.

The project impact: The proposed studies will extend the knowledge about molecular mechanisms responsible for the extraordinary, mentioned above biological properties of new promising, highly active, antitumor unsymmetrical bisacridines, which were patented (EU and USA patents, US10,202349 B2, Feb.12, 2019). The studies will also reveal the pharmacological properties of these compounds and will extend the knowledge about their metabolism and the potent mechanism of action at the molecular as well as cellular levels. Therefore, this project will influence the development of pharmacology and biology of cancer, being strongly involved in the development of civilization.