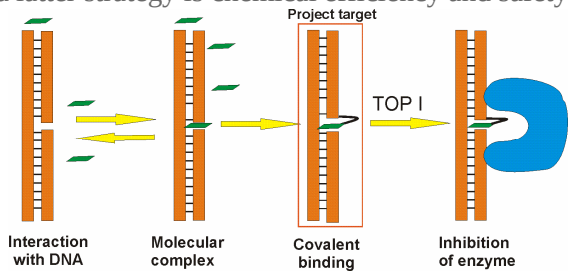
Research project objectives/Hypothesis

The project concerns disclosure and description of physicochemical features which specify the mechanism of phenomenon of targeted chemotherapy significant by selective and strong binding of a potential drug from camptothecin family in a strictly defined site of biomolecule having biological importance.

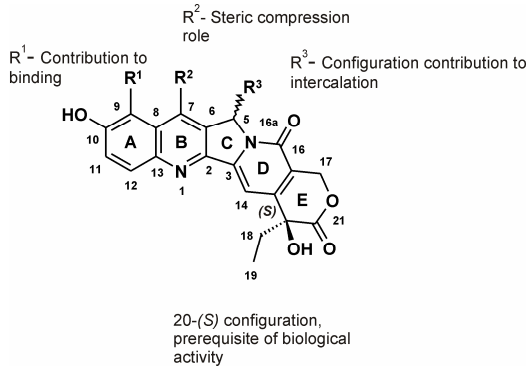
The advantage of a tumor targeted chemotherapy (on the right part) in case of inhibition of Top I *vs.* normal drug ction (on the left part) is shown in a scheme below. It can be compared to a strategy of combating the enemy by massive attack *vs* single precise shot of a sniper. Immediately obvious advantages of a latter strategy is chemical efficiency and safety of a therapy.



In case of camptothecin family derivatives acting as the Top I inhibitors this site is defined as a nick in one strand of a duplex DNA in DNA/Inhibitor complex. In a recent research the authors initiated basic research on both subjects (grant no. 2012/07/B/ST4/00566). This research yielded so far the international publication of a patent application in . regional european phase in 2017 – no. EP 2 912 039 B1 validated in PL, D,F,GB and CH and in a national phase USPTO no. US 9,682,992 B2.

In this project the authors undertake in-depth inerdisciplinary **mechanistic study**, with the aim of finding rationale determining the dependence of biological activity upon substituents R^1, R^2, R^3 in new **5-substituted and 5,9-disubstituted** derivatives of 10-hydroxycamptothecin and factors which in a concerted manner govern the **site specific intercalation** of a ligand in a nick, **the mechanism of interaction** (covalent binding *vs* intercalation) and **regioselectivity of reaction with nucleophilic centers** of aromatic bases in DNA

Basic research planed to solve the hypotheses concern, inter alia, the following problems elaborated using



potential of NMR, LC-MS, ESI-MS techniques and bioassays *in vitro*; **1** - Inventing the reaction conditions leading to formation of 5-substituted and 5,9-disubstituted 10-hydroxycamptothecins. **2** – Finding out the absolute configuration of R^3 substituent in 5-substituted 10-hydroxycamptothecins. **3** - Designation the half lifetime $t_{1/2}$ in biological conditions, water, pH 7, for compounds bearing the alkylamino substituents. **4** - Recognition of specific preassociation and binding of the new derivatives to G-C base pair in natural duplex of oligonucleotide and to G-C/ A-T base pairs flanking a nick in nicked DNA duplex. **5** - Finding out the role of a binding, *i.e.* alkylation *vs* intercalation of substituents in a new 5,9-disubstituted derivatives with respect to their strength of biological activity. Mapping the surface interface in a biological complex Top I/DNA/Inhibitor. **6** - Discussion on the mechanism of action of new derivatives on a cellular level (interpretation of *in vitro* bioassays). Mechanisms of concern are; alkylation, strength of intercalation, direct nucleophilic substitution on C9-**Cα** carbon atom (in R^1) and *retro*-Mannich reaction on C-9 carbon.

Motivation to undertake the research is due to the fact, that targeted chemotherapy is a key element of a personalized medicine , which is currently on the frontier line of a research on anticancer drugs.

Origins	Cell lines	IC_{50}, µmole/L					
		SN38	BN-MOA	BN-49B1	BN-49B2	BN-37B	Irinotecan
Blood	HL-60	0,004	0,022	0,007	0,011	0,009	4,240
Normal cell	CRL1790	4,98	1077,0			39,09	90,25

- BN-MOA – 7-ethyl-9-(N-morpholinyl)methyl-10-hydroxycamptothecin, Irinotecan, SN38 – camptothecin derivatives.

With respect to this our preliminary bioassays show that studied new derivatives (coded in green in a table) are few orders of magnitude more cytotoxic against blood cancer than used in clinic Irinotecan and are equally efficient against breast, lung, colon and leukemia cancer cells. Simultaneously they are order of magnitude less toxic against normal cells than SN38. Introduction to treatment of such medicines would secure a breakthrough in the effectiveness of the drug, elimination of metastases to mentioned organs and safety of the patient.