CHANGES IN METABOLOME AND PROTEOME OF CANCER CELLS AFTER TREATMENT OF NOVEL PROMISING LIGANDS OF HUMAN STEROL HYDROXYLASES

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INTRODUCTION:

Analysis of the cell "-ome" (metabolome, proteome etc.) is of particular interest from the point of view of the influence of various bioregulators on the structural and functional properties of targets which are used in modern drug development. Cytochrome P450 (CYP) enzymes are often used as such targets. CYPs are heme-containing proteins that participate in oxidation of numerous endogenous and exogenous compounds and play an important role in the metabolism of steroids, unsaturated fatty acids, phenolic metabolites, as well as in neutralizing xenobiotics (drugs, poisons). It is established that these enzymes are involved in the metabolism of more than 60% of all drugs that get into the human body, which clearly states the conclusion that disruption of the functioning of CYPs in most cases can lead to serious consequences, including death (figure 1) .

OBJECTIVES:

In the frame of the presented research we evaluated impact of the novel perspective ligands of human sterol hydroxylases CYP7B1 and CYP17A1 on metabolome and proteome of human epithelial colorectal adenocarcinoma and lung carcinoma cell lines in order to identify mechanisms of action of these compounds on molecular and cell level.

METHOD / DESIGN:

Cytotoxicity evaluation against selected cell lines was performed for test compounds using XTT test. Next, cells were treated with test compounds (final concentration was 25 μM). Isolation of metabolome (polar and unpolar fractions) and proteome was performed simultaneously using methanol-water-chloroform extraction approach. The quadrupole time-of-flight mass-spectrometer Q-TOF 6550 ("Agilent") equipped with an electrospray ionization source (APESI+), was used for the metabolomic and proteomic analysis of samples. Chemometric analysis was performed by using MassProfiler Professional Software (metabolome analysis) and Peaks Studio 8.5 (proteome analysis).

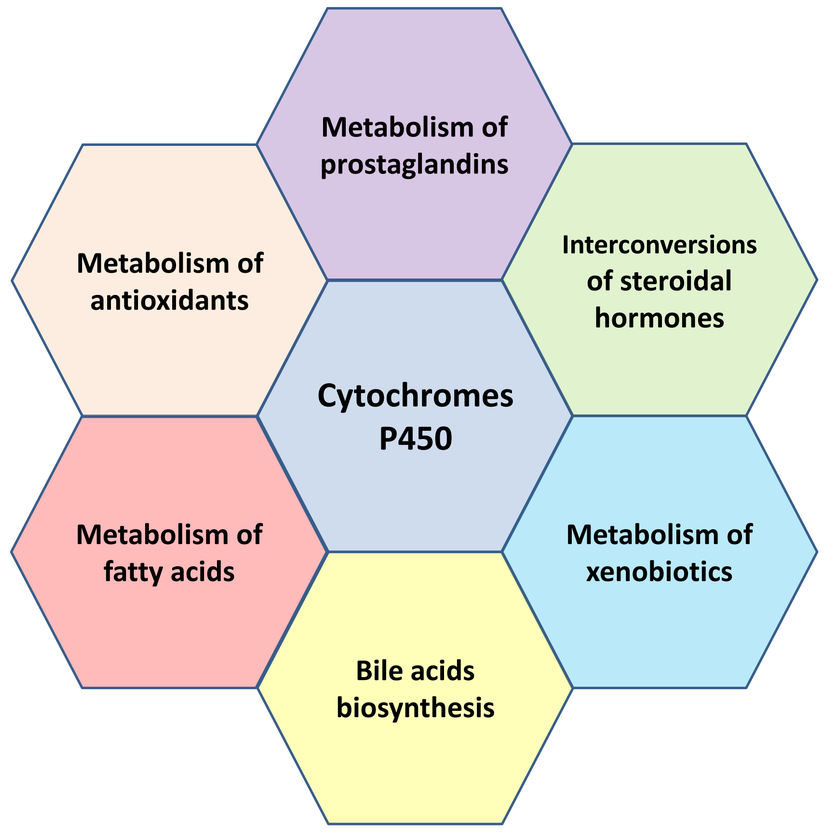
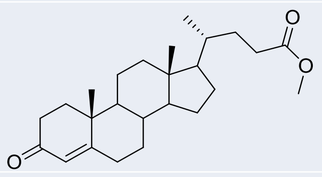
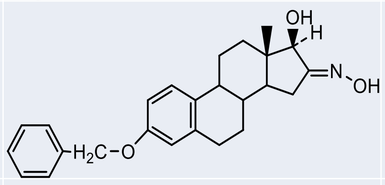
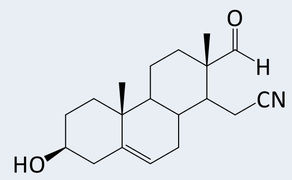


Figure 1 – Metabolic pathways including CYP enzymes

Table 1 – Novel steroidal ligands of human recombinant CYP7B1 and CYP17A1, found during *in vitro* screening, and their binding parameters.

Compound	Structure	CYP7B1	CYP17A1
1		-*	Type I** (max 386 nm, min 419 nm) Kd = 5.6±0.4 uM ΔA = 0.062
2		-***	-
3		Type I (max 388 nm, min 420 nm), K_d= 6.9±0.8 mkM, ΔA = 0.023	Type I (max 383 nm, min 419 nm) K_d=6.4±0.5 uM ΔA = 0.040

Notes:
* - no spectral response was detected;
** - «substrate-like» binding mode;
*** - binds in the active site of human CYP7A1.

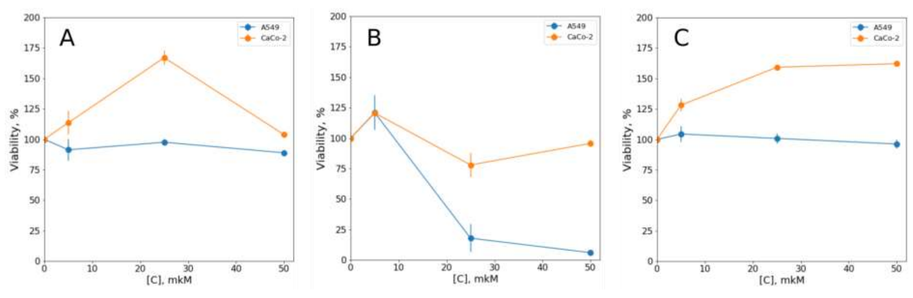
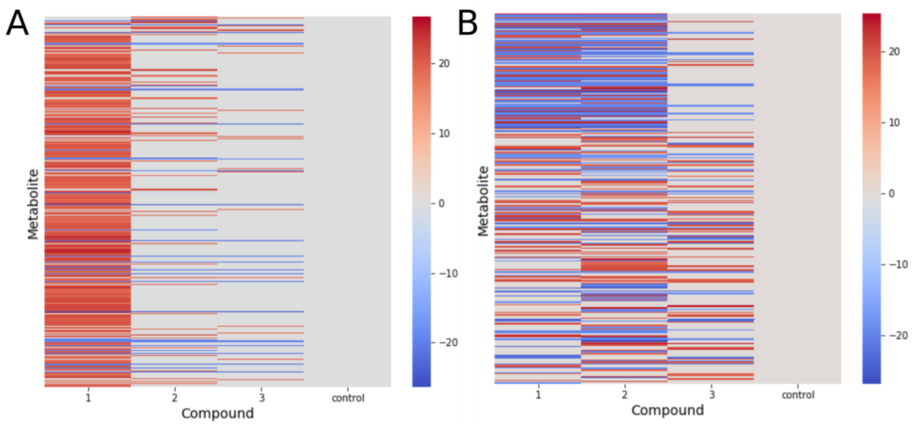


Figure 2 - Analysis of cytotoxicity of the synthetic steroid derivatives 1 (A), 2 (B), 3 (C)



The row displays metabolite and the column represents the tested compounds. Colormap represents the log base 2 of the ratio of the average metabolite abundance relative to control sample.

Figure 3 - Heatmap analysis of LC-MS metabolomics of A549 (A) and CaCo-2 (B) cells.

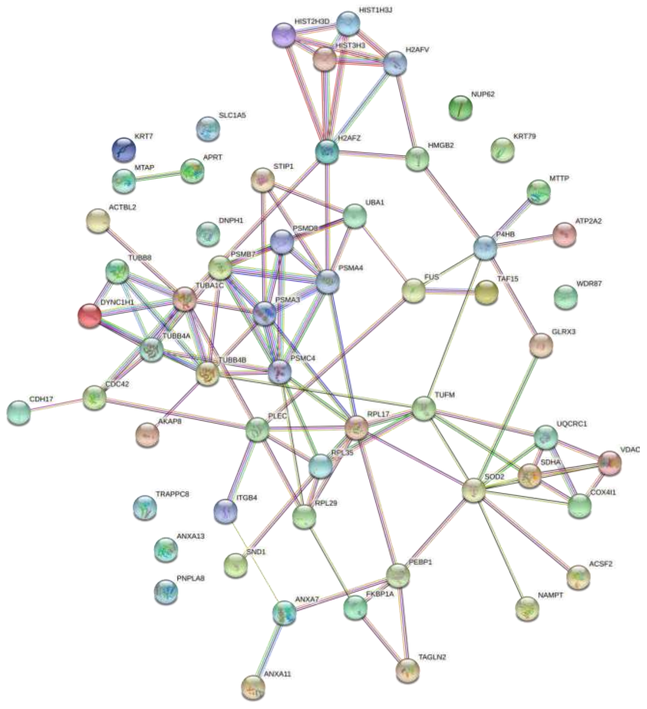
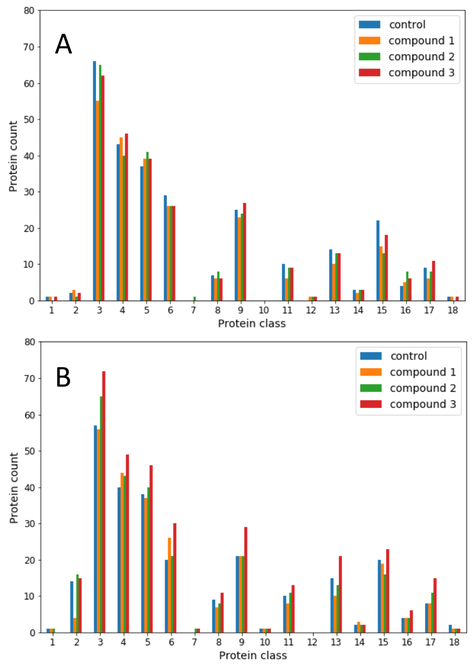


Figure 4. Results of analysis of unique proteins for CaCo-2/compound **3** sample using STRING database.



1, intercellular signal molecule
2, membrane traffic protein
3, metabolite interconversion enzyme
4, chromatin/chromatin-binding, or -regulatory protein
5, translational protein
6, chaperone
7, cell adhesion molecule
8, scaffold/adaptor protein
9, cytoskeletal protein
10, extracellular matrix protein
11, protein modifying enzyme
12, structural protein
13, transporter
14, transfer/carrier protein
15, nucleic acid metabolism protein
16, calcium-binding protein
17, protein-binding activity modulator
18, gene-specific transcriptional regulator

Figure 5. Common classes of proteins have been detected during proteome analysis

Key results.

- Analysis of cytotoxicity (figure 2) of the tested compounds showed that estrane derivative (compound **2**) expresses significant cytotoxic effect against A549 cells, while bile acid derivative (compound **1**) and D-secoandrostane (compound **3**) do not show significant effect;
- Treatment of A549 cells with bile acid derivative **1** results in an increasing of level of different metabolites (figure 3);
- In case of CaCo-2 cells the most significant changes were detected for the samples after treatment by compounds **1** and **2** (figure 3);
- After incubation of CaCo-2 cells with compound **2**, increased levels of ceramides and phospholipids were detected. The same situation is for A549 cells/compound **2** sample, but in a lesser degree;
- The higher differences between protein levels were detected for CaCo-2 cells, treated with compound **3** (Figure 4): 54 unique proteins were detected among which 5 are core histones, 7 proteins specific for colonic cancer cells, 7 proteins involved in process of proteasome degradation, 4 participate in cellular response to interleukine-7 and 4 belong to interleukin-12-mediated signaling pathway. 26 proteins are localized in extracellular space, extracellular organelle or in extracellular vesicle;
- Main differences in proteome of A549 and CaCo-2 cells are connected with levels of membrane-traffic proteins, extracellular matrix proteins and structural proteins (figure 5);
- A549 cells after treatment with modified steroids showed increased level of annexin A4 – a protein potentially involved in chemo-resistance in several types of cancer;
- In A549/compound **2** sample increased level of protocadherin Fat 1 was detected. It is well-known that overexpression of the protein decreases the expression of tumor-initiating markers;
- In general, proteome analysis of samples showed increased level on oncogenic proteins and well-known oncomarkers (ALDH X, HSP75, protocadherin Fat 1, 60S ribosomal protein L7a, RNA cytosine C(5)-methyltransferase, insulin-like growth factor 2 mRNA-binding protein 3, S100-A6 protein and others).

Acknowledgements. This study was supported by Belarus-Serbia bilateral project (No. X20SRBG-004 / 337-00-00612/2019-09/04), which is being realized between IBOCh of NAS of Belarus and University of Novi Sad Faculty of Sciences, as well the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2021-14/ 200125).