Резюмета на публикациите на доц. Биляна Николова за участие в конкурс за заемане на академична длъжност „Професор“ (на английски език)

1. **Christova, N., Tuleva, B., Kril, A., Georgieva, M., Konstantinov, S., Terziyski, I., Nikolova B., Stoineva I., 2013, Chemical structure and in vitro antitumor activity of rhamnolipids from Pseudomonas aeruginosa BN10. Appl. Biochem. Biotechnol. 170, 3, 676-689. IF 1.687, Q2**

Abstract A newly isolated indigenous strain BN10 identified as Pseudomonas aeruginosa was found to produce glycolipid (i.e., rhamnolipid-type) biosurfactants. Two representative rhamnolipidic fractions, RL-1 and RL-2, were separated on silica gel columns and their chemical structure was elucidated by a combination of nuclear magnetic resonance and mass spectroscopy. Subsequently, their cytotoxic effect on cancer cell lines HL-60, BV-173, SKW-3, and JMSU-1 was investigated. RL-1 was superior in terms of potency, causing 50 % inhibition of cellular viability at lower concentrations, as compared to RL-2. Furthermore, the results from fluorescent staining analysis demonstrated that RL-1 inhibited proliferation of BV-173 pre-B human leukemia cells by induction of apoptotic cell death. These findings suggest that RL-1 could be of potential for application in biomedicine as a new and promising therapeutic agent.

2. *Nikolova, B.,* Peycheva, E., Mudrov, Ts., Dobreva, T., Matveev, M., Tsoneva, I. 2014, Current statement of electrochemotherapy in Bulgaria. Int. J. Bioautomation, 18(1), 31-44. SJR 0.134, Q3

Abstract Electrochemotherapy is one fast, easy, effective and safe method for treatment of patients with clinical and cytological diagnosis of skin tumors as Carcinoma basocellulare, Carcinoma spinocellulare, Kaposi sarkoma and Mycosis fungoides in stage I (T1N0M0). Therapy is based on the temporary formation of pores on the surface of the cell membrane, resulting from the application of electric field with appropriate intensity and duration trough which the cytostatic drug as bleomycin is introduced into the tumor cells. This work presents data on the last version of electroporator and the treated patients by the method electrochemotherapy recently.

3. Kostadinova, A., Handjiyska, P., Dimitrov, B., *Nikolova, B.,* Tsoneva, I. 2014, The effect of electroporation on cells as a new possibility of medical treatment. Science&Technology, IV, 1, 1-7.

Abstract One of the many aspects of application of the electrical pulses on the cell membrane is the ability to form temporary pores. This phenomenon is known as electroporation. The aim of this investigation is to study how electrical pulses influence on cell viability, cell-cell contact and integrate of the cells and reorganization of the actin cytoskeleton of different epithelial cell lines one of them is malignant. It is shown that the changes in adhesiveness due to the applications of the electrical pulses with different electrical intensity are dependent on the type of malignancy of epithelial cells.

4. **Atanasova, S., Lazarova, D., Nikolova, B., Zhelev, Z., Tsoneva, I., Aoki, I., Bakalova, R. 2014, In vivo visualization of electro-assisted delivery of nanoparticles using optical imaging. Anticancer Res. 34, 5761-6258. IF 1.826, Q2**

Abstract The present study was designed to investigate whether electroporation can facilitate the delivery of drugs inside tumors using quantum dot (QD)-loaded polymersomes as a model. The main goal was to increase the local concentration of anticancer drugs avoiding side-effects. The experiments were performed on colon cancer-grafted mice (*Balb/c*) using the *Maestro EX Imaging System*. Electroporation facilitated the delivery of nanoparticles inside the tumor. A significant difference in the fluorescence intensity between electroporated and non-electroporated mice was observed in the cancer area even 24 hours after treatment with nanoparticles. The data suggest that electro-assisted delivery of size-controlled long-circulating polymersomes in cancer is a promising therapeutic strategy, especially for treatment of solid tumors.

5. **Bakalova, R., Zhelev, Z., Lazarova, D., *Nikolova, B.,* Atanasova, S., Zlateva, G., Aoki, I. 2015, Delivery of size-controled long-circulating polymerzomes in solid tumors, visualizated by quantum dots and optical imaging in vivo. Biotechnol. & Biotechnol. Eq. 29, 1, 175-180. IF 0.373, Q4**

Abstract The present study was designed to investigate whether poly-ion complex hollow vesicles (polymersomes), based onchemically modified chitosan, are appropriate for passive tumour targeting in the context of their application as drug carriers. The experiments were performed on colon cancer-grafted mice. The mice were subjected to anaesthesia and injected intravenously with water-soluble nanoparticles: (1) QD705-labelled polymersomes (average size »120 nm; size distribution »10%) or (2) native QD705. The optical imaging was carried out on Maestro EX 2.10 In Vivo Imaging System (excitation filter 435_480 nm; emission filter 700 nm, longpass). In the case of QD705, the fluorescence appeared in the tumour area within 1 min after injection and disappeared completely within 60 min. A strong fluorescent signal was detected in the liver on the 30th minute. The visualization of tumour using QD705 was based only on angiogenesis. In the case of QD705-labelled polymersomes, the fluorescence appeared in the tumour area immediately after injection with excellent visualization of blood vessels in the whole body. A strong fluorescent signal was detected in the tumour area within 16 hours. This indicated that QD705-labelled polymersomes were delivered predominantly into the tumour due to their long circulation in the bloodstream and enhanced permeability and retention effect. A very weak fluorescent signal was found in the liver area. The data suggest that size-controlled long-circulating polymersomes are very promising carriers for drug delivery in solid tumours, including delivery of small nanoparticles and contrast substances.

6. *Nikolova, B.,* Atanasova, S., Mudrov, Tz., Tsoneva, I., Zhelev, Z., Bakalova, R., Aoki, I. 2015, Image guided Electro-assisted Drug Delivery: Comparison between Two Types of Electrodes. Int. J. Bioautomation, 19(2), 259-266. SJR 0.134, Q4

Abstract: Electroporation-based cancer treatment techniques are currently after active investigations in the field of drug delivery, optimization of electrical parameters and

elucidation of the exact mechanisms at a molecular level. The present study is designed to investigate the exact in vivo redistribution and persistence of nanoparticles in the tumor tissue of colon-cancer grafted mice after electroporation with two different kinds of electrodes. The aim of the study is to avoid artifacts during electroporation due to accumulation of nanoparticles in the surrounding non-cancer tissues. The isolated electrodes are appropriate for the treatment of 3-dimensional tumors and have a large potential in this field.

7. **Kostadinova, A., *Nikolova, B.*, Handjiyska, P., Berger, MR., Tsoneva, I. 2015, Combined effect of electroporation and miltefosine on keratonocyte cell line HaCaT, Rom. Rep. Phys. 67(3), 995-1003. IF 1.367, Q2**

Abstract In this study we investigated the effect of combined treatment of HaCaT keratinocyte cells with electrical pulses (200–500V/cm) and the alkylphosholipid (ALP) miltefosine. The data show that electroporation in combination with miltefosine induces cytoskeleton disruption and increases the permeability of cell monolayers due to interruption of cell-cell junctions', as documented by fluorescent imaging of ZO-1 and actin integrity. This was accompanied with reduction of cell viability. The combination of these conditions could be considered as a method for treating several types of skin cancer or other pathological conditions affecting the skin integrity.

8. **Bakalova, R., Zhelev, Z., *Nikolova, B.*, Murayama, S., Lazarova, D., Tsoneva, I., Aoki, I. 2015, Lymph node mapping using quantum dot-labeled polymersomes. Gen. Phys. Biophys. 34, 393-398. IF 0.892, Q3**

Abstract The present study was designed to investigate whether poly-ion complex hollow vesicles (polymersomes), based on chemically-modified chitosan, are appropriate for lymph node mapping in the context of their application in the development of theranostic nanosized drug delivery systems (nano-DDS). The experiments were performed on Balb/c nude mice (colon cancer-grafted). The mice were subjected to anesthesia and quantum dot (QD705)-labeled polymersomes (d~120 nm) were injected intravenously *via* the tail vein. The optical imaging was carried out on Maestro EX Imaging System (excitation filter: 435–480 nm; emission filter: 700 nm). A strong fluorescent signal, corresponding to QD705 fluorescence, was detected in the lymph nodes, as well as in the tumor. A very weak fluorescent signal was found in the liver area. The half-life of QD705-labelled polymersomes was 6 ± 2 hours in the bloodstream and 11 ± 3 hours in the lymph nodes. The data suggest that polymersomes are very promising carriers for lymph node mapping using QD as a contrast agent. They are useful matrix for development of nano-formulations with theranostic capabilities.

9. Пехливанова, В., Кабаиванова, Л., Иванова, Ю., ***Николова, Б.*** Имунофлуоресцентно проследяване ефекта на полизахарид изолиран от щам Rhodella reticulate и/или електропорация върху реорганизацията на актиновия цитоскелет на ракови клетки. 5-6 май 2015, FOCOS 2015 Светлината в науката, МУ София, 451-455.

Abstract Marine organisms are potential sources of highly bioactive secondary metabolites that may be useful for the development of new pharmaceuticals. In this study, the biological effect of a heteropolysaccharide isolated from the red microalgae strain Rhodella reticulata on

tumor cell A549, both alone and in combination with electroporation, was investigated. Materials and methods: The change in viability (proliferative test) and morphology (immunofluorescent staining of the cytoskeleton) of the cells after self-treatment with heteropolysaccharide and / or electrical impulses is monitored. Results: The effect of the heteropolysaccharide was shown to be dose dependent. Conclusion: These results direct interest in the polysaccharide as a substance with potential antitumor effect

Summary The methods for treatment of tumors based on electroporation are the subject of active research, and the interests are focused mainly on the following areas: local supply of antitumor agents, optimization of electrical parameters and elucidation of the exact mechanisms at the molecular level (1). The aim of the present study was to visualize the in vivo penetration, localization, and pharmacokinetics of fluorescent nanoparticles delivered passively or by electroporation in experimental mouse tumor models. The main task of the study is to evaluate the possibilities for local increase of the concentration of anticancer drugs in tumors, in combination treatment with multimodal nanoparticles and electroporation. All studies were performed on experimental models of colorectal carcinoma in mice from the Balb / c nude line. Multimodal model nanohydrogels structurally based on quantum dot (QD) are used (2). The degree and kinetics of penetration of nanoparticles into cancer cells, in the presence or absence of exposure to electrical impulses, is visualized using the Maestro EX In Vivo Imaging System (fluorescent imaging system). All data obtained in the course of the study show a promising therapeutic strategy for the treatment of solid tumors, based on the combined application of long-circulating fluorescent nanoparticles and electroporation.

10. Атанасова, С., ***Николова, Б.,*** Цонева, Я. Мураяма, Ш., Аоки, И., Желев, Ж., Бакалова, Р. Визуализиране на проникването и локализирането на флуоресцентни наночастици в тумори след електропорация: експериментални модели in vivo. 5-6 май 2015, FOCOS 2015 Светлината в науката, МУ София, 328-332.

Abstract The methods for treatment of tumors based on electroporation are the subject of active research, and the interests are focused mainly on the following areas: local supply of antitumor agents, optimization of electrical parameters and elucidation of the exact mechanisms at the molecular level (1). The aim of the present study was to visualize the in vivo penetration, localization, and pharmacokinetics of fluorescent nanoparticles delivered passively or by electroporation in experimental mouse tumor models. The main task of the study is to evaluate the possibilities for local increase of the concentration of anticancer drugs in tumors, in combination treatment with multimodal nanoparticles and electroporation. All studies were performed on experimental models of colorectal carcinoma in mice from the Balb / c nude line. Multimodal model nanohydrogels structurally based on quantum dot (QD) are used (2). The degree and kinetics of penetration of nanoparticles into cancer cells, in the presence or absence of exposure to electrical impulses, is visualized using the Maestro EX In Vivo Imaging System (fluorescent imaging system). All data obtained in the course of the study show a promising therapeutic strategy for the treatment of solid tumors, based on the combined application of long-circulating fluorescent nanoparticles and electroporation.

11. **Bakalova, R., *Nikolova, B.,* Murayama, S., Atanasova, S., Zhelev, Zh., Aoki, I., Kato, M., Tsoneva, I., Saga, T. 2016, Passive and electro-assisted delivery of**

hydrogel nanoparticles in solid tumors, visualized by optical and magnetic resonance imaging in vivo. Anal. Bioanal. Chem. 408, 905–914. IF 3.431, Q1

Abstract The present study describes a development of nanohydrogel, loaded with QD705 and manganese (QD705@Nanogel and QD705@Mn@Nanogel), and its passive and electro-assisted delivery in solid tumors, visualized by fluorescence imaging and magnetic resonance imaging (MRI) on colon cancer-grafted mice as a model. QD705@Nanogel was delivered passively predominantly into the tumor, which was visualized in vivo and ex vivo using fluorescent imaging. The fluorescence intensity increased gradually within 30 min after injection, reached a plateau between 30 min and 2 h, and decreased gradually to the baseline within 24 h. The fluorescence intensity in the tumor area was about 2.5 times higher than the background fluorescence. A very weak fluorescent signal was detected in the liver area, but not in the areas of the kidneys or bladder. This result was in contrast with our previous study, indicating that FITC@Mn@Nanogel did not enter into the tumor and was detected rapidly in the kidney and bladder after i.v. injection [J. Mater. Chem. B 2013, 1, 4932–4938]. We found that the embedding of a hard material (as QD) in nanohydrogel changes the physical properties of the soft material (decreases the size and negative charge and changes the shape) and alters its pharmacodynamics. Electroporation facilitated the delivery of the nanohydrogel in the tumor tissue, visualized by fluorescent imaging andMRI. Strong signal intensity was recorded in the tumor area shortly after the combined treatment (QD@Mn@Nanogel + electroporation), and it was observed even 48 h after the electroporation. The data demonstrate more effective penetration of the nanoparticles in the tumor due to the increased permeability of blood vessels at the electroporated area. There was no rupture of blood vessels after electroporation, and there were no artifacts in the images due to a bleeding.

12. ***Nikolova, B.,*** **Borisova, E., Peycheva, E., Avramov, L., Tsoneva, I. 2016, Electrochemotherapy of skin cancer treatment results estimated by in vivo autofluorescence measurements. OAM-RC, 10, 5-6, 433-436. IF 0.470, Q3**

Abstract Electrochemotherapy is an effective method for treatment of skin tumors. To monitor the effects of application of the electrochemotherapy autofluorescence spectra are taken from the lesion and surrounding healthy skin, prior to, immediately after treatment and at the control check-ups. Patients are followed up at the first week after treatment and one month later. Here we reported the case of 82 years old woman with basal cell carcinoma, which is treated with electrochemotherapy and the effect of the treatment is verified by the optical biopsy method.

13. Kabaivanova, L., Ivanova, J., Pehlivanova, V., *Nikolova, B.* 2016, Specific antitumor effect of the combined action of algal heteropolysaccharide and electroporation. Int. J. Bioautomation, 20(3), 407-416. SJR 0.228, Q3

Abstract Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. In this study the biological effect of the freeze-dried heteropolysaccharide, isolated and purified from the red microalga Rhodella reticulata strain using electroporation wasevaluated. Two different types of cells tumor and non-tumor were treated with the heteropolysaccharide alone or together with the application of electroporation. The effect ofthe treatment was evaluated in parallel: with proliferation test for estimating cell viability and with immunofluorescent cytoskeleton staining to establish changes in morphology. Evidence for cell line specific viability reduction (70% from the control in case of cancer cell line treatment and only 30% in non-tumor cells) in a dose dependent manner was presented. These findings will arouse further interest in heteropolysaccharide as a new anticancer drug suitable for clinical trials.

14. **Atanasova, S., *Nikolova, B.*, Muraiama, Sh., Stoyanova, E., Tsoneva, I., Zhelev, Zh., Aoki, I., Bakalova, R. 2016, Electroinduced delivery of hydrogel nanoparticles in Colon 26 cells, visualized by confocal fluorescence system. Anticancer res. 36, 9, 4601-4606. IF 1.937, Q2**

Abstract. Background: Nano-scale drug delivery systems (nano-DDS) are under intense investigation. Nano-platforms are developed for specific administration of small molecules, drugs, genes, contrast agents [quantum dots (QDs)] both in vivo and in vitro. Electroporation is a biophysical phenomenon which consists of the application of external electrical pulses across the cell membrane. The aim of this study was to research electro-assisted Colon 26 cell line internalization of QDs and QD-loaded nano-hydrogels (polymersomes) visualized by confocal microscopy and their influence on cell viability. Materials and Methods: The experiments were performed on the Colon 26 cancer cell line, using a confocal fluorescent imaging system and cell viability test. Results: Electroporation facilitated the delivery of nanoparticles in vivo. We demonstrated increased voltage-dependent delivery of nanoparticles into cells after electrotreatment, without significant cell viability reduction. Conclusion: The delivery and retention of the polymersomes in vitro is a promising tool for future cancer treatment strategies and nanomedcine.

15. **Bakalova, R., Zhelev, Z., Shibata, S., *Nikolova, B.*, Aoki, I., Higashi, T. 2017, Impressive Suppression of Colon Cancer Growth by Triple Combination SN38/EF24/Melatonin: "Oncogenic" Versus "Onco-Suppressive" Reactive Oxygen Species. Anticancer res. 37 (10) 5449-5458. IF 1.865, Q2**

Abstract The study aimed to investigate the effect of multi-targeted combinations (SN38/EF24; SN38/EF24/melatonin) on the growth of colon cancer in experimental animals and their impact on the ratio "oncogenic"/"onco-suppressive" reactive oxygen species (ROS) – a crucial factor for triggering of carcinogenesis, as well as for development of effective therapeutic strategies. The experiments were conducted on colon-cancer grafted mice – nontreated, SN38/EF24-treated and SN38/EF24/melatonin-treated within 22 days. The balance between different types of ROS was measured in vivo by nitroxide-enhanced magnetic resonance imaging (MRI), as well as on isolated tissue specimens by conventional analytical tests. Both combinations significantly suppressed the tumor growth. Impressive anticancer effect was observed in SN38/EF24/melatonin-treated mice – almost complete destruction of the tumor. Both types of ROS (superoxide and hydroperoxides) were elevated in cancer, but the MRI data suggest that the ratio between them tends towards superoxide. SN38/EF24 2 decreased the level of superoxide, but did not affect the level of hydroperoxides in the cancerous tissue, while SN38/EF24/melatonin decreased the level of superoxide below the control and increased significantly the level of hydroperoxides. The most important observations are that: (i) colon cancer was characterized by a vicious cycle, which ensures a permanent domination of "oncogenic" ROS (as superoxide) over "onco-suppressive" ROS (as hydrogen peroxide); (ii) the anticancer effect of the triple combination EF24/SN38/melatonin was accompanied by decreasing "oncogenic" and increasing "onco-suppressive" ROS; (iii) the ratio between both types of ROS could be a new onco-target for combined therapy; and (iv) nitroxide-enhanced MRI is a valuable tool for analyzing of this ratio.

16. **Semkova, S., *Nikolova, B.*, Zhelev, Zh., Tsoneva, I., Aoki, I., Bakalova. R. 2018, Loading efficiency of polymersomes with contrast agents and their intracellular delivery: Quantum dots versus organic dyes. Anticancer res. 38(2): 825-831. IF:1.935, Q2**

Abstract Background/Aim: Contrast nanocarriers as drugdelivery systems, capable of selective delivery to cancer cells and solid tumors, are essential for the development of new diagnostic and therapeutic (theranostic) strategies. The present study aimed to investigate the loading efficiency of chitosan-based polymersomes with fluorescent contrast substances [quantum dots (QDs) and conventional organic dyes] and the possibility to control their release from the polymer matrix into the cells by chemical modifications and electroporation. Materials and Methods: All investigated fluorophores were retained within the polymer globule via electrostatic and hydrophilic–hydrophobic interactions, without conjugation with the polymer. The fluorophoreloaded polymersomes were characterized by dynamic light scattering, zeta-potential titration, and fluorescence spectroscopy. The release of fluorophore from the polymersomes, passively or after electroporation, was detected by 5-step spin-ultrafiltration, combined with fluorescence spectroscopy of the upper phase (supernatant) of the filter unit. Passive intracellular delivery of the nanoparticles to HeLa cells was detected by fluorescence confocal microscopy. Results: The QDs were retained tightly and continuously in the polymer matrix, while the organic fluorophores [fluorescein isothiocyanate (FITC), FITCdextran10,000 and FITC-dextran70,000] were released rapidly from the polymersomes. The detergent Brij significantly increased the retention of FITC-dextran10,000 in the polymer globule. Electroporation up to 1000 V/cm did not induce release of QDs from the polymersomes, but accelerated the release of FITC-dextran10,000 Brij from the polymer matrix. High-voltage pulses (over 750 V/cm) induced also fragmentation or aggregation of the nanoparticles. QD_labeled polymersomes penetrated passively in cancer cells after 24-hour incubation. Conclusion: The results suggest that QD-labeled polymersomes are appropriate fluorescent probes and a nano-drug delivery system with high tracing opportunities for in vitro and in vivo applications. Furthermore, loading polymersomes with organic dyes with different molecular weights (such as FITCdextrans) is a simple model for visualizing and predicting the rate of release of small organic molecules (e.g. conventional drugs, other contrasts, stabilizers, and supplements) from the polymer matrix.

17. **Ivanova, D., Zhelev, Z., Getsov, P., *Nikolova, B.* Aoki, I., Higashi, T., Bakalova, R. 2018, Vitamin K: Redox-modulation, prevention of mitochondrial dysfunction and anticancer effect. Redox Biology, 16, 352-358. IF:7.793, Q1**

Abstract This review is directed to the redox-modulating properties and anticancer effect of vitamin K. The concept is focused on two aspects: (i) redox-cycle of vitamin K and its effect on the calcium homeostasis, "oncogenic" and "onco-suppressive" reactive oxygen species and the specific induction of oxidative stress in cancer; (ii) vitamin K plus C as a powerful redox-system, which forms a bypass between mitochondrial complexes II and III and thus prevents mitochondrial dysfunction, restores oxidative phosphorylation and aerobic glycolysis, modulates the redox-state of endogenous redox-pairs, eliminates the hypoxic environment of cancer cells and induces cell death. The analyzed data suggest that vitamin C&K can sensitize cancer cells to conventional chemotherapy, which allows achievement of a lower effective dose of the drug and minimizing the harmful side-effects. The review is intended for a wide audience of readers - from students to specialists in the field.

18. *Николова, Б.,* Цонева, Я., Христова-Панушева, К., Кръстева, Н. 2018, Актуални изследвания в областта на електроиндуцираните явления и адхезивното поведение на клетките. Списание на БАН, 3, 29-35.

Abstract Exposure to an external electric field can cause significant biochemical or physiological changes in individual cells, tissues or organs. In recent years, it has found increasing biomedical applications in tissue engineering, regenerative and reproductive medicine, for electrotransfection and electrotransformation of cells, electrofusion,

sterilization, the production of DNA vaccines, as well as in the treatment of some genetic diseases and tumors. Tissue engineering and regenerative medicine, in turn, are highly dependent on the interaction of cells with biomaterials. From the adhesive behavior of the cells, important conclusions can be drawn about the biocompatibility and future application of the test material. Therefore, for the needs of tissue engineering and regenerative medicine, in-depth studies are needed both on the behavior of cells in contact with biomaterials and under the influence of an external electric field.

19. Kadinov, B., *Nikolova, B.*, Semkova, S., Kabaivanova, Tsoneva, I., Dimitrova, D., 2021, Trehalose lipid biosurfactant reduced cancer viability, but not affected the isometric concentration of rat mesenteric arteries in vitro. Int. J. Bioautom. 24, 1, 79-86. SJR (Scopus):0.242, Q3.

Abstract: Trehalose lipid biosurfactant from Nocardia farcinica strain is a naturally derived substance with potent anticancer activity. The increasing interest in naturally derived substances-based modality of cancer treatment requires investigations of the possible adverse effects of these substances, including the effects on vasculature. Therefore the present study was designed to investigate the effect of Trehalose lipid on isometric contraction of isolated rat mesenteric arteries. The contractile responses of arteries under Trehalose lipid was studied using wire myography for small blood vessels. The isometric contractions of rat mesenteric artery rings with intact endothelium were examined. The effect of this biosurfactant was assessed in arteries precontracted with 42 mM KCl as a vascular smooth muscle depolarizing stimulus. The results showed that Trehalose lipid (75 μM) failed to change high K+-induced contractions. The observed lack of effect of Trehalose lipid biosurfactant on the contractility of rat mesenteric arteries in vitro together with finding of reduced cancer cells viability makes it to be a suitable for potential medical application.

20. ***Nikolova B.,*** **Semkova, S., Tsoneva, I., Antov, G., Ivanova, J., Vasileva, I., Kardaleva, P., Stoineva, I., Christova, N., Nacheva, L., Kabaivanova, L. 2019, Characterization and potential antitumor effect of a heteropolysaccharide produced by the red alga Porphyridium sordidum. ENG LIFE SCI. Special Issue: Plant Cells and Algae in Bioreactors, 2019, 978-985, IF 1.934, Q2.**

Abstract Taking into account the rising trend of the incidence of cancers of various organs, effective therapies are urgently needed to control human malignancies. However, almost all chemotherapy drugs currently on the market cause serious side effects. Fortunately, several studies have shown that some non-toxic biological macromolecules, including algal polysaccharides, possess anti-cancer activities or can increase the efficacy of conventional chemotherapy drugs. Polysaccharides are characteristic secondary metabolites of many algae. The efficacy of polysaccharides on the normal and cancer cells is not well investigated, but our investigations proved a cell specific effect of a newly isolated extracellular polysaccharide from the red microalga Porphyridium sordidum. The investigated substance was composed of xylose:glucose and galactose: manose:rhamnose in a molar ratio of 1:0.52:0.44:0.31. Reversible electroporation has been exploited to increase the transport through the plasma membrane into the tested breast cancer tumor cells MCF-7 and MDA-MB231. Application of 75 μg/mL polysaccharide in combination with 200 V/cm electroporation induced 40% decrease in viability of MDA-MB231 cells and changes in cell morphology while control cells (MCF10A) remained with normal morphology and kept vitality.

21. ***Nikolova, B.,*** **Antov, G., Semkova, S., Tsoneva, I., Christova, N., Nacheva, L., Kardaleva, P., Angelova, S., Stoineva, I., Ivanova, J., Vasileva, I., Kabaivanova, L. 2020, Bacterial Natural Disaccharide (Trehalose Tetraester): Molecular**

Modeling and in Vitro Study of Anticancer Activity on Breast Cancer Cells. Polymers, 12(2), 499. IF 4.329, Q1.

Abstract: Isolation and characterization of new biologically active substances a_ecting cancer cells is an important issue of fundamental research in biomedicine. Trehalose lipid was isolated from Rhodococcus wratislaviensis strain and purified by liquid chromatography. The e_ect of trehalose lipid on cell viability and migration, together with colony forming assays, were performed on two breast cancer (MCF7—low metastatic; MDA-MB231—high metastatic) and one "normal" (MCF10A) cell lines. Molecular modeling that details the structure of the neutral and anionic form (more stable at physiological pH) of the tetraester was carried out. The tentative sizes of the hydrophilic (7.5 Å) and hydrophobic (12.5 Å) portions of the molecule were also determined. Thus, the used trehalose lipid is supposed to interact as a single molecule. The changes in morphology, adhesion, viability, migration, and the possibility of forming colonies in cancer cell lines induced after treatment with trehalose lipid were found to be dose and time dependent. Based on the theoretical calculations, a possible mechanism of action and membrane asymmetry between outer and inner monolayers of the bilayer resulting in endosome formation were suggested. Initial data suggest a mechanism of antitumor activity of the purified trehalose lipid and its potential for biomedical application.

22. ***Nikolova, B.,*** **Semkova, S., Tsoneva, I., Stoyanova, E., Lefterov, P., Lazarova, D., Zhelev, Zh., Aoki, I., Higashi, T., Bakalova, R. 2020, Redox-related molecular mechanism of sensitizing colon cancer cells to camptothecin analog SN38. Anticancer res. 40 (9) 5159-5170. IF 2.480, Q2**

Abstract Background/Aim: The aim of this study was to elucidate the possibility of sensitizing colon cancer cells to the chemotherapeutic drug SN38 and investigate its mechanism of action after combined treatment with electroporation (EP). Materials and Methods: Cells were treated with SN38, EP and their combination for 24/48 h. The cell viability, actin cytoskeleton integrity, mitochondrial superoxide, hydroperoxides, total glutathione, phosphatidyl serine expression, DNA damages and expression of membrane ABC transporters were analyzed using conventional analytical tests. Results: The combination of EP and SN38 affected cell viability and cytoskeleton integrity. This effect was accompanied by: (i) high production of intracellular superoxide and hydroperoxides and depletion of glutathione; (ii) increased DNA damage and apoptotic/ferroptotic cell death; (iii) changes in the expression of membrane ABC transporters – up-regulation of SLCO1B1 and retention of SN38 in the cells. Conclusion: The anticancer effect of the combined treatment of SN38 and EP is related to changes in the redox-homeostasis of cancer cells, leading to cell death via apoptosis and/or ferroptosis. Thus, electroporation has a potential to increase the sensitivity of cancer cells to conventional anticancer therapy with SN38.

23. **Dimov, S., Mavrova, A., Yancheva, D., *Nikolova, B.,* Tsoneva, I. 2021, Thieno [2,3-d] pyrimidin-4(3H)-one Derivatives of Benzimidazole as Potential Anti-Breast Cancer (MDA-MB-231, MCF-7) Agents. Anti-cancer Agents Med Chem. 21(11), 1441–1450, IF 2.505, Q3.**

Abstract Aims: The purpose of this study was the synthesis of some new thienopyrimidine derivatives of 1,3-disubstituted benzimidazoles and the evaluation of their cytotoxicity against MDA-MB-231, MCF-7, and 3T3 cells lines. Background: An overexpression or mutational activation of TK receptors EGFR and HER2/neu is characteristic of tumors. It has been found that some thieno[2,3-d]pyrimidines exhibited better inhibitory activity against epidermal growth factor receptor (EGFR/ErbB-2) tyrosine kinase in comparison to aminoquinazolines. Breast cancer activity towards MDA-MB-231 and MCF-7 cell lines by inhibiting EGFR was

revealed by a novel 2-arylbenzimidazole. This motivated the synthesis of new thienopyrimidines possessing benzimidazole fragments in order to evaluate their cytotoxicity to the above-mentioned cell lines. Objective: The objectives of the study were to design and synthesize a novel series of thieno[2,3-d]pyrimidines bearing biologically active moieties, such as 1,3-disubstituted-benzimidazole heterocycle, structurally similar to diaryl ureas in order to evaluate their cytotoxicity against MDA-MB-231, and MCF-7 breast cancer cell lines. Methods: N,N-disubstituted benzimidazole-2-one carbonitriles were synthesized by Aza-Michael addition and used as precursors to generate some of the new thieno[2,3-d]pyrimidines in acidic medium The interaction of chloroethyl-2-thienopyrimidines, 2-amino-benzimidazole and benzimidazol-2-one nitriles under solid-liquid transfer catalysis conditions led to new thienopyrimidines. MTT assay for cell survival was performed in order to evaluate the cytotoxicity of the tested compounds. A fluorescence study was conducted to elucidate some aspects of the mechanism of action. Results: The effects of nine synthesized compounds were investigated towards MDA-MB-231, MCF-7 and 3T3 cell lines. Thieno[2,3-d]pyirimidine-4-one 16 (IC50 – 0.058 μM) and 21 (IC50 – 0.029 μM) possess high cytotoxicity against MDA-MB-231 cells after 24h. The most cytotoxic compounds against breast cancer MCF-7 cells was compound 21 (IC50 – 0.074 μM), revealing lower cytotoxicity against mouse fibroblast 3T3 cells with IC50 – 0.20 μM. SAR analysis was performed. Fluorescence study of the treatment of MDA-MB cells with compound 21 was carried out in order to clarify some aspects of the mechanism of action. Conclusion: The relationship between cytotoxicity of compounds 14 and 20 against MCF-7 and 3T3 cells can suggest a similar mechanism of action. The antitumor potential of the tested compounds proves the necessity for further investigation to estimate the exact inhibition pathway in the cellular processes. The fluorescence study of the treatment of MDA-MB cells with compound 21 showed a rapid process of apoptosis.

24. **Semkova, S., Antov, G., Iliev, I., Lefterov, P., Christova, N., Nacheva, L., Stoineva, I., Kabaivanova, L., Tsoneva, I., Staneva, G., *Nikolova, B.* 2021 Rhamnolipid biosurfactants - possible natural anticancer agents and autophagy inhibitors. Separations, Separations 2021, 8, 92. https://doi.org/10.3390/separations8070092, IF 2.777, Q2.**

Abstract: Background/Aim: A number of biologically active substanceswere proved as an alternative to conventional anticancer medicines. The aim of the study is in vitro investigation of the anticancer activity of mono- and di-Rhamnolipids (RL-1 and RL-2) against human breast cancer. Additionally, the combination with Cisplatin was analyzed. Materials and Methods: Breast cell lines (MCF-10A, MCF-7 and MDA-MB-231) were treated with RLs and in combination with Cisplatin. The viability was analyzed using MTT assay, and investigation of autophagy was performed via acridine orange staining. Results: In contrast to the healthy cells, both tested cancer lines exhibited sensitivity to RLs treatment. This effect was accompanied by an influence on the autophagy-related acidic formation process. Only for the triple-negative breast cancer cell line (MDA-MB-231) the synergistic effect of the combined treatment (10 μM Cisplatin and 1 μg/mL RL-2) was observed. Conclusion: Based on studies on the reorganization of membrane models in the presence of RL and the data about a higher amount of lipid rafts in cancer cell membranes than in non-tumorigenic, we suggest a possible mechanism of membrane remodelling by formation of endosomes. Shortly, in order to havea synergistic effect, it is necessary to have Cisplatin andRL-2 as RL2 is a molecule inducingpositive membrane curvature.

25. Dimitrova, D.Z., *Nikolova, B.*, Bogoeva, V., Tsoneva, I., Dimitrov, S., Kadinov, B. 2021, Do Mistletoe (Viscum album L.) Lectins Influence Isometric Contraction of Non-diseased Human Mesenteric Arteries ex vivo? Int. J. Bioautom. 25(1), 41–52. Q4

Abstract: Mistletoe (Viscum album L., VA) lectins (MLs) are plant lectins with potent anticancer activity. Although wide use of VA extracts in curing cancer, the effects of purified MLs on human vasculature in term of possible side effect of the lectin has not yet been reported. The present study was aimed to investigate isometric contractions of isolated human mesenteric arteries during MLs application. The contractile response of arteries was studied using Mulvany-Halpern myograph and the isometric contractions under MLs' treatment were examined in artery segments with either intact endothelium or after endothelium removal. Furthermore, the effect of the lectin was assessed in arterial preparations in basal tension, in arteries precontracted with 42 mM KCl as a depolarizing stimulus or endothelin-1 (ET-1) as a potent receptor-operated agonist of vascular smooth muscle contraction. The results showed that MLs (1 to 100 nM) failed to affect the high K+-induced contractions of both endothelium-intact and endothelium-denuded arteries. The contractions of tissue preparations without endothelium in basal tone or after ET-1 (1 nM) treatment were also not affected by the application of MLs. The observed mild effect of MLs on the contractility of human vasculature may potentially be beneficial with MLs-based anticancer therapy without vascular side effects.