STRATAGEM
CA17104

New Diagnostic and Therapeutic Tools against Multidrug Resistant Tumours

ABSTRACT BOOK

STRATAGEM CA17104
Annual Conference
3rd MC meeting and 4th WGs meeting
Belgrade, Serbia
27th - 28th February, 2020

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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

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Welcome to Belgrade

The COST Action CA17104 STRATAGEM Annual Conference – 3rd MC meeting and 4th WGs meetings will take place in Belgrade, at the 88 Rooms Hotel in Belgrade, from 27th to 28th February, 2020. In line with the Action title "New diagnostic and therapeutic tools against multidrug resistant tumours", this meeting will provide an excellent scientific program led by international experts. Invited speakers with different expertize in cancer research, therapy, chemistry, toxicology, and bioinformatics will widen our knowledge from tumor microenvironment to tumor therapy. A talk dedicated to the memory of our honorable colleague Prof. Maurizio Botta will remind us of his work and achievements. His work inspired fruitful collaborations within our COST Action. Besides, special attention will be given to the education of young scientists through the round tables "Meet the invited speakers", "MDR research towards therapy" and "MDR research towards diagnostics". Information on how to apply for the STSM and ITCCG will also be provided during our Annual Conference. ECIs will be given a chance to present their successful STSM stories and compete for the Best Poster Award.
Belgrade – a historic capital full of beauty, history of destruction and reconstruction, famous for its traditional hospitality, food and the best time in Europe – is the perfect place to go for new ideas and collaborations.
We look forward to welcoming you at the STRATAGEM Meeting!

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STRATAGEM Action Summary

This Action will build the first multidisciplinary network, including academic laboratories, research institutes, small and medium enterprises (SMEs), with a wide range of excellent and non-overlapping expertise, aiming at improving at the same time the diagnosis and therapy of multidrug resistant (MDR) solid tumors. Until now, there are fragmented knowledge on biomarkers and therapeutic tools used against MDR tumors; there are not algorithms predictive/diagnostic of MDR tumors ex ante; all the past therapies against MDR tumors failed. The key challenge of this Action is to fill these gaps, by producing a comprehensive, open and user-friendly platform of knowledge on MDR tumors, identifying new diagnostic/predictive biomarkers, producing new and safe compounds applicable to personalized treatments of MDR tumors. Up to 70% of solid tumors are resistant at the diagnosis: this means poor life quality and poor prognosis for patients, high management costs for the European healthcare systems. This Action is working to improve diagnosis and treatment of patients with MDR tumors and reduce the costs for their management. Second, by creating fruitful collaborations between basic and industrial research, we will give impulse to the creation of new Start-up and SMEs in Europe. Finally, the Action aims at raising the level of European research on MDR, reducing the disparity in the research quality between EU countries and ITC, providing the necessary training for European early stage researchers (ESRs) to grow as future independent research leaders, regardless of location, age or gender.

Action website: https://stratagem-cost.eu/
Contact: costaction.17104@unito.it

COST is a unique means for European researchers, engineers and scholars to jointly develop their own ideas and new initiatives across all fields of science and technology through trans-European networking of nationally funded research activities.
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Acknowledgements

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Programme

Wednesday, 26th February 2020	
18:00 - 19:00	Registration (Rooftop conference room)
Thursday, 27th February 2020	
08:30	Registration (Rooftop conference room)
09:30	Welcome Message
09:40 - 11:30	**Section I: Chair Thomas Mohr**
9:40	*Invited lecture* Isaac Witz: When Circulating Tumor Cells Meet the Metastatic Microenvironment
10:10	Ana Čipak Gašparović: Oxidative stress as a factor acting on breast cancer cells of different malignancies via changes in lipid profile, aquaporin expression and Nrf2
10:30	Muriel Cuendet: 2D versus 3D co-culture multiple myeloma model: the example of with anolides
10:50	Miguel Machuqueiro: The pH-dependent mechanism underlying membrane crossing of Lewis base drugs
11:10	Coffee break
11:30 - 13:30	**Section II: Chair Catherine Passirani**
11:30	*Invited lecture* Mattia Mori: Switching from 14-3-3 to Carbonic Anhydrases through hit recycling
12:10	Philippe Bertrand: Epigenetic strategies against cancers with HDAC inhibitors alone, in combination or using drug delivery system
12:30	Andreia Valente: Ruthenium−cyclopentadienyl bipyridine−biotin based compound: P-gp inhibition and activity against resistant cancer cells
12:50	Luigi Paduano: Multimodal Iron-Oxide Nanoparticles: from Design to *in vivo* Applications
13:10	Angélica Figueroa: Novel small-molecule inhibitors against epithelial to mesenchymal transition: implication in drug resistance
13:30	Lunch and Poster Session I
15:00 - 16:30	**Parallel Sessions**
	1. Core Group Meeting (Jaspis boardroom) 2. Round Tables: Meet the Invited Speakers; Participate in the STSM; MDR research towards therapy; MDR research towards diagnosis (Rooftop conference room)
16:30	Coffee break
17:00 - 18:30	**Parallel Sessions**
	1. MC Meeting (Oniks conference room) 2. Round Tables: Meet the Invited Speakers; Participate in the STSM; Apply for the ITC grant; MDR research towards therapy; MDR research towards diagnosis
20:30	Social Event - Folklore Concert and Group Photo
Friday, 28th February 2020	
09:00	Registration (Rooftop conference room)
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09:30	*Invited lecture* Goran Mitulovic: Relevance of Proteomics Regarding Clinical Research and Clinical Application
10:00	Alfonso Taotlani Garcia Sosa: Dox and S-Dox *in silico* interaction with xenobiotic proteins Pregnane-X-receptor and Sulfotransferase
10:20	Yordan Yordanov: *In silico* and *in vitro* toxicological studies on H2S-releasing doxorubicin
10:40	José Juan García Marín: Role of transportome in liver and gastrointestinal cancer chemoresistance

11:00	Javier De Las Rivas: Pharmacogenomic methods to define druggable modules in cancer and explore drug resistance byways
11:20	Coffee break
12:00 - 13:30	**Section IV: Chair Jitka Viktorova**
12:00	*Invited lecture* Davorin Radosavljević: Targeted therapies in oncology - clinical advantage and resistance
12:30	Maria M M Santos: Development of novel p53-activating agents for cancer therapy
12:50	Wolfgang Link: Targeting FOXO proteins to extend lifespan and fight cancer and anti-cancer drug resistance
13:10	Karolina Seborova: Analysis of lncRNA expression and DNA methylation in chemotherapy response of ovarian cancer patients
13:30	Lunch and Poster Session II
15:00 - 16:10	**Section V: STSM grantees presentations**
15:00	Kornél Szőri: Synthesis of p-quinol derivates as potent MDR-selective antitumor agents
15:10	Isabella Pötsch: Inhibited IDO-enzyme in cancer cells: “Help! IDOn’t escape immune surveillance anymore!”
15:20	Epole N Ntungwe: *Plectranthus mutabilis* Codd as a source of Anti-MDR Diterpenoids
15:30	Juran Kralj: Training in mRNA profiling
15:40	Annamária Kincses: Investigation of cytotoxic and antioxidant property of salicylaldehyde thiosemicarbazones and their copper complexes
15:50	Mirna Jovanović: Investigation of inhibitory properties of Michael acceptors on thioredoxin reductase 1 inhibition *in vitro*
16:00	Anamaria Brozović: Molecular mechanisms of concomitant resistance of carboplatin resistant ovarian cancer cells to paclitaxel
16:10 - 16:40	**Section VI: Selected posters presentations**
16:10	Tanja Panić-Janković: IL-6 as a key pathway player in the secretome of human granulosa-like tumor cell line (KGN) after hormonal stimulation with different amount of FSH
16:20	Miglė Paškevičiūtėa: Application of carbonic anhydrase IX/XII inhibitors to modulate the transport of doxorubicin and its liposomal form into 2D and 3D cancer cell cultures
16:30	Nace Zidar: Synthesis, antiproliferative effect and topoisomerase II inhibitory activity of 3-methyl-2-phenyl-1H-indoles
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Synthesis of p-quinol derivates as potent MDR-selective antitumor agents

Isabella Pötsch
Inhibited IDO-enzyme in cancer cells: "Help! IDOn't escape immune surveillance anymore!"

Epole N Ntungwe
Plectranthus mutabilis Codd as a source of Anti-MDR Diterpenoids

Juran Kralj
Training in mRNA profiling

Annamária Kincses
Investigation of cytotoxic and antioxidant property of salicylaldehyde thiosemicarbazones and their copper complexes

Mirna Jovanović
Investigation of inhibitory properties of Michael acceptors on thioredoxin reductase 1 inhibition *in vitro*

Anamaria Brozović
Molecular mechanisms of concomitant resistance of carboplatin resistant ovarian cancer cells to paclitaxel

Best Poster Prize Presentations

Tanja Panić-Janković
IL-6 as a key pathway player in the secretome of human granulosa-like tumor cell line (KGN) after hormonal stimulation with different amount of FSH

Miglė Paškevičiūtė
Application of carbonic anhydrase IX/XII inhibitors to modulate the transport of doxorubicin and its liposomal form into 2D and 3D cancer cell cultures

Nace Zidar
Synthesis, antiproliferative effect and topoisomerase II inhibitory activity of 3-methyl-2-phenyl-1H-indoles

Posters

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Role of OCT1 in cholangiocarcinoma chemoresistance to sorafenib

Jelena Dinic
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Simona Dobiasová
Biological activity of silybin derivates focusing on P-glycoprotein modulation

Vladimir Dobričić
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Soner Dogan
The Effects of Different Types of Calorie Restriction Methods on Multiple Drug Resistance Associated miRNAs

Marie Ehrlichova
Comparison of *in vitro* and *in vivo* efficiency of classical and novel SB-T-taxanes with potential effect in resistant ovarian tumors

Nuray Erin
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Nuray Erin
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Abstracts

Thursday 27th February
Section 1

Oxidative stress as a factor acting on breast cancer cells of different malignancies via changes in lipid profile, aquaporin expression and Nrf2

Claudia Rodrigues[a,b] Lidija Milkovic,c Ivana Tartaro Bujak,[c] Marko Tomljanovic,[c] Graça Soveral, [a,b] **Ana Čipak Gašparović[c]**

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Oxidative stress is known for being both, cause and consequence of cancer. It leads to cancer development by causing DNA and protein damage, but also by causing lipid peroxidation. In addition, metabolic changes occur during cancer progression, which can generate ROS or change susceptibility to oxidative stress. These processes can lead to undesired adaptation of cancer cell to stress. Lipids can also, among other factors, contribute to cancer malignancy. Lipids and their peroxidation products actively contribute to modulation of cellular signaling pathways thereby affecting proliferation, differentiation and apoptosis. ROS, especially H2O2, can also activate signaling pathways and make modifications to cellular processes. Therefore, aquaporins, membrane water channels which are also hydrogen peroxide channels, can influence cellular processes that can lead or contribute to cancer development. Aquaporins are found to be upregulated in tumors and correlate with tumor grade, progression and metastatic potential. For these reasons, the role of aquaporins as potential regulators of antioxidative response was studied to elucidate their role in breast cancer cells of different malignancies. Additionally, the lipid profile and the steady state lipid hydroperoxides were studied in relation to breast cancer malignancy in order to correlate membrane saturation with sensitivity to oxidative stress. Upon oxidative challenge, increase in AQP3 and NRF2 expression was observed in hormone positive and Her2NEU positive cells, in contrast to triple negative breast cancer cell line where they were downregulated. Instead, these cells showed upregulation of AQP1 and AQP5. Finally, the lipid profile and AQP gene expression after oxidative stress in the most aggressive breast cancer cell line may represent metabolic reprogramming which could lead to stress and therapy adaptation.

References

[1]. Rodrigues C, Milkovic L, Bujak IT, Tomljanovic M, Soveral G, Cipak Gasparovic A. Oxid Med Cell Longev. 2019:2061830.

[2]. Čipak Gašparović A, Milković L, Dandachi N, Stanzer S, Pezdirc I, Vrančić J, Šitić S, Suppan C, Balic M. (2019). Antioxidants 8(12). pii: E633.

2D versus 3D co-culture multiple myeloma model: the example of with anolides

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Multiple myeloma (MM) is a blood disease characterized by the clonal proliferation of malignant plasma cells in the bone marrow. Despite not having a strong incidence, this type of cancer is associated with a high rate of relapse and resistance to conventional therapies. The paradigm that tumors are composed of heterogeneous cell populations, namely tumoral cells and cancer stem cells (CSCs), imposes to address the effect of compounds in each cell population. Interactions with the microenvironment are also of importance as mesenchymal stem cells (MSCs) change their phenotype when in contact with MM cells, by helping the tumor cells to survive and even to become resistant to therapy. In order to evaluate the effect of potential MM treatments, a model that closely represents the disease should be used. Therefore, an in vitro model consisting of 3D co-culture spheroids was set up to better predict the *in vivo* activity. The spheroids contained malignant plasma cells and MSCs. This model presents most of the tumor complexity and includes the microenvironment. The antiproliferative activity of about 60 with anolides was evaluated in 2D and 3D models. Results obtained in 2D generally did not predict the anti-proliferative activity in the 3D model. This can be explained as compounds need to penetrate the spheroid in the 3D model and cells representing the microenvironment may influence the activity of the compound. These results show the importance of 3D co-culture models to select compounds capable of maintaining their activity in a complex tumor environment before conducting *in vivo* experiments.

The pH-dependent mechanism underlying membrane crossing of Lewis base drugs

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Targeted cancer therapeutics remains a central goal of cancer research. The tumor microenvironment (TME) is an important component of tumor development that influences several key processes such as tumor cell phenotype, proliferation, immune evasion, and drug resistance [1]. An important feature of the TME is the increased acidity of the extracellular milieu (pH 6.0-6.8), generated by enhanced anaerobic glycolysis coupled with higher levels of proton extrusion via upregulated proton pumps. This process creates a pH gradient between the extracellular and intracellular environments, potentially creating a barrier for hydrophobic Lewis base drugs to enter the cells. The high pK_a values (7.5-10) of these compounds including for example some tyrosine kinase inhibitors like sunitinib and nintedanib, require them to first undergo deprotonation before passively diffusing through the plasma membrane into the cells, which becomes more difficult in acidic microenvironments like the TME. This study aims at investigating the pH-dependent membrane insertion mechanism of several Lewis base drugs. We performed pH replica-exchange (pHRE) [2] simulations of sunitinib and nintedanib and a few other compounds, interacting with a phosphatidylcholine lipid membrane. We calculated pK_a profiles for all these systems, which capture the desolvation effect along the membrane normal [3]. Based on our data, we can also follow the average protonation and relative distribution between water and lipid phases at a given pH value. The obtained pK_a and protonation profiles along the membrane insertion pathway can help us interpret the available experimental data on how some of these compounds struggle to insert into tumor cells whereas other hydrophobic weak base drugs are highly sequestered within lysosomes [4,5].

References

[1]. Assaraf, Y. G., Brozovic, A., Gonçalves, A. G., Jurkovicova, D., Linē, A., Machuqueiro, M., Saponara, S., Sarmento-Ribeiro, A. B., Xavier, C. P. R., Vasconcelos, M. H. (2019) *Drug Resist. Updat.*, 46:100645.
[2]. Vila-Viçosa, D., Reis, P. B. P. S., Baptista, A. M., Oostenbrink, C., Machuqueiro, M., (2019) *J.Chem. Theory Comput.* , 15:3108-3116.
[3]. Teixeira, V. H., Vila-Viçosa, D., Reis, P. B. P. S., Machuqueiro, M., (2016) *J. Chem. Theory Comput.*, 12:930-934
[4] Zhitomirsky B, Assaraf YG. (2015) *Oncotarget.*, 6(2):1143-56
[5] Zhitomirsky B, Assaraf YG. (2016) *Drug Resist Updat.*, 24:23-33

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Section 2

Epigenetic strategies against cancers with HDAC inhibitors alone, in combination or using drug delivery system.

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Human histone deacetylases (HDAC) and their inhibitors (HDACi) are used to treat cancers, with four compounds approved by FDA[1,2,3,4] and one in China.[5] This presentation proposes a short overview of the epigenetics, a focus on HDACs and their inhibitors and the synthetic and in silico work achieved in our labs to obtain compounds and their polymeric nanoparticles versions. The application of this compounds/nanoparticles was against asbestos cancer and validated on mice models. Some important biological results were obtained for combinations, with cisplatin to reduce resistance, for immunotherapy strategies, or for delivery strategy to specifically target the tumor nodules.

References

1. Duvic, M.; Vu, Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. J. *Expert Opin. Investig. Drugs* 2007, *16*, 1111–1120.
2. Poole, R. Belinostat: first global approval. *Drugs* 2014, *74*, 1543–1554.
3. Fenichel, M. P. FDA Approves New Agent for Multiple Myeloma. *J. Natl. Cancer Inst.* 2015, *107*, djv165.
4. Grant, C. et al. Romidepsin: a new therapy for cutaneous T-cell lymphoma and a potential therapy for solid tumors. *Expert Rev. Anticancer Ther.* 2010, *10*, 997–1008.
5. Shi, Y. et al. Chidamide in relapsed or refractory peripheral T cell lymphoma: a multicenter real-world study in China. *J. Hematol. Oncol.J Hematol Oncol* 2017, *10*, 69.
6. Fabien Gueugnon, Pierre-François Cartron, Cedric Charrier, Philippe Bertrand, Jean-François Fonteneau, Marc Gregoire, Christophe Blanquart. New histone deacetylases inhibitors improve cisplatin antitumor properties against thoracic cancer cells. *Oncotarget* 2014, *5*, 4504-15.
7. Bensaid, D. et al. Assessment of new HDAC inhibitors for immunotherapy of malignant pleural mesothelioma. *Clinical Epigenetics* 2018, 10, 79.
8. El Bahhaj, F. et al. Histone Deacetylase Inhibitors Delivery using Nanoparticles with Intrinsic Passive Tumor Targeting Properties for Tumor Therapy. *Theranostic* 2016, *6*, 795-807.

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Ruthenium−cyclopentadienyl bipyridine−biotin based compound: P-gp inhibition and activity against resistant cancer cells

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Multidrug resistance (intrinsic or acquired) is one of the main obstacles for a successful chemotherapy, where ABC transporters play an important role. These proteins are overexpressed in cancer cells and significantly reduce the intracellular concentration of the drugs. Thus, the finding of new MDR inhibitors is a relevant clinical challenge. Over the last years, we have been engaged in the development of ruthenium-cyclopentadienyl compounds able to overcome such mechanisms of drug resistance.[1-3] In particular, a biotinylated compound (LCR134) has shown high cytotoxicity against cancer cells with different genetic profiles and a significant ability to inhibit P-gp (better than the reference inhibitor). Thus, LCR134 possesses a rare dual function as cytotoxic agent and P-gp inhibitor.[2] In addition, the preliminary toxicity assessment in a zebrafish model established that LCR134 is well tolerated at 120 hours post fertilization, showing the absence of severe toxic effects like necrosis or hemorrhage.[2] In this presentation we will disclose how small changes on the compound basic structure can drastically influence its mode of action. Moreover, preliminary data on cells lines with different types of drug resistance will highlight if LCR134 is able to overcome other MDR mechanisms.

References

[1]. Moreira T, Francisco R, Comsa E, Duban-Deweer S, Labas V, Teixeira-Gomes AP, Combes-Soia L, Marques F, Matos A, Favrelle A, Rousseau C, Zinck P, Falson P, Garcia MH, Preto A, Valente A (2019). Eur J Med Chem 168: 373-384.
[2]. Côrte-Real L, Karas B, Gírio P, Moreno A, Avecilla F, Marques F, Buckley BT, Cooper KR, Doherty C, Falson P, Garcia MH, Valente A (2019). Eur J Med Chem 163: 853-863.
[3]. Côrte-Real L, Teixeira RG, Gírio P, Comsa E, Moreno A, Nasr R, Baubichon-Cortay H, Avecilla F, Marques F, Robalo MP, Mendes P, Ramalho JPP, Garcia MH, Falson P, Valente A (2018). Inorg Chem 57: 4629−4639.

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Multimodal Iron-Oxide Nanoparticles: from Design to *in vivo* Applications

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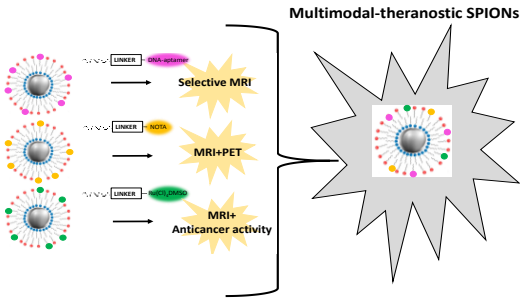
Theranostic multimodal nanoparticles (TNs) have been synthesized and characterized to obtain effective and selective vehicles that combine therapeutic and diagnostic capability. Superparamagnetic iron-oxide nanoparticles (SPIONs) have been exploited as magnetic resonance contrast agents for biomedical imaging. Besides their intrinsic physico-chemical properties, such nanoparticles (NPs) are valuable platforms able, when suitably functionalized, to deliver imaging and/or therapeutic agents in a selective way to a cellular target. Here, Fe_3O_4 NPs have been functionalized with hydrophobically-modified molecules in order to address additional tasks: probes for positron emission tomography/computed tomography (PET/CT), anticancer agents. We optimized a versatile functionalization strategy based on decorating NP surface with biocompatible lipids through hydrophobic interaction [1]. The method allows amphiphilic molecules with different functions to be easily lodged in the lipid layer. For PET/CT applications, the surface of both NPs have been functionalized with suitably designed amphiphilic NOTA derivatives as chelating agents for ^{68}Ga complexation. The anticancer activity have been achieved using the amphiphilic Ru(III) complex-based molecules we have recently synthesized and proved to have high antiproliferative activity against several tumor cells through tests in vitro [2]. Aptamers, short single-stranded oligonucleotides have been used as targeting molecules able to recognize tumor markers specifically, discriminating between health and sick cells, to overcome the severe issue of the lack of selectivity of the typical agents for cancer treatment and diagnosis. We have focused on oligonucleotide sequences bearing a hydrophobic tail in 3' or 5' position, which should be lodged in the lipid shell of NPs. An anti-VEGF DNA aptamer known to adopt a G-quadruplex structure since VEGF, being involved in the development and progression of several cancers, have been exploited both in diagnostics and as a drug target. Here, we will show a comprehensive overview from the design and physico-chemical characterization to the *in vivo* results [3].



References

[1] Luchini A., Irace C., Santamaria R., Montesarchio D., Heenan R.K., Szekely N., Flori A., Menichetti L., Paduano L. (2016) *Nanoscale*, 8: 10078-10086.

[2] Luchini A., Gerelli Y., G. Fragneto G., Nylander T., Palsson G.K., Appavoui M.S., Paduano L., (2017) *Colloids and Surfaces, B: Biointerfaces*, 151: 76-87.

[3] Misso M., Ferraro G., Riccardi, C.; Capuozzo, A.; Zarone, M.G., Mayra R.; Maione, F.; Trifuoggi, M.; Stiuso, P.; D'Errico, G.; Caraglia, M.; Paduano, L.; Montesarchio, D.; Irace, C.; Santamaria, R. (2019) *Scientific Reports*, 9: 1-15.

Novel small-molecule inhibitors against epithelial to mesenchymal transition: implication in drug resistance

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Tumor plasticity has emerged as important determinant of treatment resistance. Epithelial-to-mesenchymal transition (EMT) is associated with resistance in many different cellular and preclinical models. The alternation between epithelial and mesenchymal states may influence the search for anti-resistance strategies. Probably, the best characterized hallmark of the EMT is the loss of E-cadherin at cell-cell contacts. This protein is regulated at posttranslational level by the E3 ubiquitin-ligase Hakai, which has been shown to play an important role in EMT, tumor progression, invasiveness and metastasis *in vitro* and *in vivo*. Moreover, our recent studies show that Hakai expression is gradually increased in TNM stages of colorectal cancer, being proposed as a biomarker for colon cancer progression. These results lead to the consideration of Hakai as a potential new therapeutic target to block tumor development and metastasis in colon cancer. We have identified novel small-molecule inhibitors specifically designed against Hakai that inhibit tumor progression and metastasis *in vitro* and *in vivo*. Preclinical *in vitro* and *in vivo* studies, using tumor xenografts mouse model, indicate that our hit compound specifically inhibits Hakai activity and reduces cell proliferation, oncogenic potential, and invasion, without apparent systemic toxicity *in vivo*. More importantly, lung micrometastasis in the mice was reduced. Thus, our results constitute the preclinical proof-of-concept for the use of Hakai inhibitors targeting EMT and tumor progression, opening the possibility to use them as anti-resistance therapy strategy in combination with chemotherapeutic drugs. Given that the most efficient treatment proposed is the repeated alternation of drugs targeting epithelial or mesenchymal phenotypes compared to monotherapy or sequential therapy, these inhibitors represent an attractive strategy against drug resistant tumors.

References

[1]. Shibue T and Weinberg RA (2017). Nat Rev Clin Oncol. 14 (10): 611-629.
[2]. Marcucci F, Stassi G, De Maria R (2016) Nat Rev Drug Discov 2016, 15(5):311-325.
[3]. Castosa R, Martinez-Iglesias O (2018) Sci Rep. 8(1):3466.

Acknowledgment

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Friday 28th February
Section 3

Dox and S-Dox in silico interaction with xenobiotic proteins Pregnane-X-receptor and Sulfotransferase

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Dox and S-Dox are interesting compounds that can have activity against multiresistant neoplastic cells [1]. An important consideration in drug design is the optimization of properties that can have an effect on the safety and efficacy of a compound. Some of these metabolic proteins transform compounds and their interaction with compounds can be studied in silico using their structure. Results with several programs show a different profile of interaction of Dox and S-Dox towards the pregnane-X-receptor involved in the efflux of foreign substances, as well as with sulfotransferase involved in the conjugation of sulfate groups to excrete compounds [2,3,4]. The difference in profile can be exploited for modification of compounds to improve their metabolic profile.

References

[1]. Bigagli E, Luceri C, De Angioletti M, Chegaev K, D'Ambrosio M, Riganti C, Gazzano E, Saponara S, Longini M, Luceri F, Cinci L (2018) *Invest. New Drugs* 36:985–998
[2]. Stevanovic S, Sencanski M, Danel M, Menendez C, Belguedj R, Bouraiou A, Nikolic K, Cojean S, Loiseau PM, Glisic S, Baltas M, García-Sosa AT (2019). *Molecules* 24: 1282.
[3]. García-Sosa AT (2019). *Curr. Comput.-Aided Drug Des.* 14: 131-141.
[4]. Glisic S, Sencanski M, Perovic V, Stevanovic S, García-Sosa AT (2016) *Molecules* 21: 589

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In silico and *in vitro* toxicological studies on H_2S-releasing doxorubicin

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Doxorubicin (DOX) is one of the most used drugs in anticancer therapy. Its application is limited by serious side effects and reduced efficacy due to the rapid development of drug resistance mainly through increased expression of the drug efflux P-glycoprotein (ABCB1). To overcome these limitations, new hydrogen sulfide (H2S)-releasing DOX derivatives were developed. The new compounds revealed high efficacy in DOX-resistant cancer cell lines and lower cardiotoxicity in prostate cancer xenograft mice [1, 2, 3]. This study presents ADME/Tox evaluation of the most promising compound in the series, H2SDOX [1]. The early assessment of its hepatotoxicity and cardiotoxicity represents an important issue, since these effects are among the most common toxicities driving nonclinical safety-related attrition [4]. To this aim in silico ADME-profiling of H2SDOX was initially performed and compared to that of DOX using ACD/Percepta v.2018.2.3 software. To evaluate the drug safety, Derek Nexus v.6.0.1 knowledge-based system was further applied. Few toxicity endpoints were estimated as plausible including cardiotoxicity and hepatotoxicity. Therefore, both endpoints were further evaluated. Cardiotoxicity assessed by in silico hERG inhibition prediction suggested a low probability of binding to the channel. This was supported by in vitro assay demonstrating that DOX (up to 100 μM) and H2SDOX (up to 10 μM) did not affect hERG currents recorded in hERG-HEK293 recombinant cells by using the patch-clamp technique. The in vitro hepatotoxicity was investigated on human hepatoma cells HEP G2 and on freshly isolated rat hepatocytes, by analyzing cell viability, lactate dehydrogenase leakage, the oxidative stress marker malondialdehide, and reduced glutathione. After 72 h of treatment, H2SDOX reduced cell viability with an IC50 value of 1.4 μM, one order of magnitude higher than that of DOX (0.2 μM). The early drug-like profiling of H2SDOX shows lower toxicity and better in vitro safety compared to DOX.

References

[1] Chegaev K. et al, H2S-Donating Doxorubicins May Overcome Cardiotoxicity and Multidrug Resistance. J. Med. Chem. (2016), 59, 4881−4889.
[2] Buondonno I. et al, Endoplasmic reticulum-targeting doxorubicin: a new tool effective against doxorubicin-resistant osteosarcoma. Cell. Mol. Life Sci. (2019), 76, 609–625.
[3] Bigagli E et al, New NO- and H2S-releasing doxorubicins as targeted therapy against chemoresistance in castration-resistant prostate cancer: in vitro and in vivo evaluations. Invest. New Drugs (2018), 36, 985-998.
[4] Blomme E. Toxicology Strategies for Drug Discovery: Present and Future. Chem. Res. Toxicol. (2016), 29, 473−504.

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Transportome analysis for the prediction of chemoresistance in liver cancer

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The dismal prognosis of patients with advanced liver cancers, mainly hepatocellular carcinoma and cholangiocarcinoma, is partly due to the unsatisfactory response to anticancer drugs, even the last generation of tyrosine kinase inhibitors (TKIs), such as sorafenib. Among the complex mechanisms accounting for their multidrug resistance are those involving the impairment of drug uptake, which mainly occurs through transporters of the superfamily of solute carrier (SLC) proteins, and the active export of drugs through ATP-binding cassette (ABC) proteins. Both mechanisms result in decreased amounts of active agents able to reach their intracellular targets. During the last three decades, the interest of our group of investigation on Experimental Hepatology and Drug Targeting (HEVEFARM) at the University of Salamanca (Spain) has been focused on investigating the role of the transportome in liver and gastrointestinal cancer chemoresistance and to explore the usefulness of plasma membrane transporters as targets to develop strategies aimed at sensitizing enterohepatic tumors to chemotherapy. In this line of research, we have shown that sorafenib is taken up by the organic cation transporter 1 (OCT1, gene *SLC22A1*), whose expression/function is consistently diminished in most liver tumors. Studies in experimental models, both *in vitro* and *in vivo* have demonstrated that OCT1 downregulation results in lower sorafenib uptake and impaired antitumor effect. Further investigations revealed that inactivating mutations, aberrant splicing, promoter methylation, and miRNAs activity are involved in decreasing OCT1 function in liver tumors. Moreover, induction of OCT1 expression in the mouse xenograft model resulted in enhanced drug uptake by the tumor during sorafenib treatment, together with a marked inhibition of tumor growth. Retrospective analysis of patients with advanced liver cancer receiving sorafenib revealed longer survival time if OCT1 was present at the plasma membrane of tumor cells. In conclusion, impaired expression/function of SLC proteins accounting for antitumor drug uptake could be useful for the prediction of the lack of response of liver cancer to agents that are taken up through these transporters, as is the case of the tandem sorafenib/OCT1.

Pharmacogenomic methods to define druggable modules in cancer and explore drug resistance byways

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Using pharmacogenomic tools we have performed a large-scale screening to identify biomolecular targets of cancer drugs, focused on the current collection of FDA-approved drugs for cancer, which includes about one hundred chemicals. The work integrates global gene-expression transcriptomic profiles with drug-activity profiles of a set of 60 human cell lines obtained for a collection of chemical compounds (i.e., small bioactive molecules). Using a standardized expression for each gene versus standardized activity for each drug, *Pearson* and *Spearman* correlations were calculated for all possible pairwise gene-drug combinations. These correlations provide an indirect method to establish associative links between drugs and genes, so they allow proposing putative targets for the cancer drugs (which in many cases correspond to experimentally proven protein targets and, in other cases, can reveal an indirect action over other proteins). These correlations were used to built a global bipartite network that includes 1,007 gene-drug significant associations, which allows the discovery of interesting cancer drug-target modules (that we define as "druggable modules"). All the data were integrated into an open web-tool called GEDA (Gene Expression & Drug Activity in Cancer Cells, http://cicblade.dep.usal.es/GEDA/). The results present a new relational mapping of cancer drugs and genes, disclosing new plausible cellular targets for FDA-approved drugs. The results also facilitate a better understanding of the complex links between each drug and its multiple possible targets, presenting an alternative view to tackle drug effects in cancer and to explore drug resistance byways.

Section 4

Development of novel p53-activating agents for cancer therapy

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One of the most appealing targets for developing anticancer treatments is the p53 transcription factor. This protein plays key roles in the regulation of cell cycle, cell death, cell differentiation, senescence, angiogenesis and DNA repair, being essential for the prevention of cancer. In all human cancers, p53 is inactivated, either due to mutation of TP53 or due to inhibition by negative regulators (e.g. MDM2 and MDMX). So, reactivation of the p53 tumor suppressor function is considered a promising therapeutic approach for the treatment of cancer. Medicinal chemistry approaches to reactivate p53 have been mainly focused on inhibiting the p53-MDM2 interaction. However, it is now considered that for an efficient reactivation of p53, dual inhibitors of MDM2 and MDMX are required.[1] Our research team has been actively involved on the development of novel chemotypes that activate the tumor suppressor p53 protein. Structure-activity relationship studies led to the discovery of potent inhibitors which are being optimized towards clinical candidates.[2-3] In this communication an overview of the results obtained by our team will be given.

References

[1]. Espadinha M, Conway SJ, Santos MMM (2020). *Small Molecule Drug Discovery: Methods, molecules and applications*, ed. A. Trabocchi, E. Lenci, Elsevier, in press.

[2]. Gomes S et al (2019). *Cancers* 11: 1151; b) Raimundo L et al (2018). *British J. Pharmacol.* 175: 3947.

[3] a) Amaral JD et al (2019). *Front. Chem.* 7: article 15; b) Ribeiro CJA et al (2017). *Eur. J. Med. Chem.* 140: 494.

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Targeting FOXO proteins to extend lifespan and fight cancer and anti-cancer drug resistance

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FOXO transcription factors are evolutionarily conserved proteins that orchestrate programs of gene expression known to control a variety of cellular processes such as cell cycle, apoptosis, DNA repair and protection from oxidative stress. FOXO factors are context-dependent tumour suppressor proteins commonly inactivated in human tumours and involved in anti-cancer drug resistance. Recent studies have found that FOXO3 is associated with extreme human longevity in Japanese-Americans from Hawaii, Italians, Ashkenazi Jews, Californians, New Englanders, Germans and Han Chinese. We previously developed high-throughput assay systems monitoring FOXO translocation and activity as its nuclear export to identify chemical compounds and protein targets that interfere with FOXO functions. We have identified several FOXO repressor proteins and FOXO activating compounds. Our objective is to translate our data into clinically useful tools to develop targeted strategies to improve the treatment of cancer as well as human life and healthspan.

Analysis of lncRNA expression and DNA methylation in chemotherapy response of ovarian cancer patients

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Ovarian cancer is serious heterogeneous disease with high mortality caused by late diagnosis and development of multidrug resistance. Multidrug resistance is a multifactorial process, so we decided to analyze total expression profile including long non-coding RNA (lncRNA) and DNA methylation profile in order to clarify role of lncRNA-methylation synergy in this process. We performed multiple analyses on data from TCGA database. In case of gene expression, we downloaded raw RNAseq data for whole transcriptome, which were processed by our pipeline. Methylation data were obtained as beta values. Expression and methylation data were analyzed separately and then together by emQTL (expression-methylation Quantitative Trait Loci) analysis [1]. For analysis of possible regulation by transcription factors we used UniBind [2]. We also performed whole transcriptome profiling including lncRNA profile in our pilot set of patients (n=23). We compared several approaches for processing RNA seq data, which suits the best for our dataset. Results from different analytical steps will be presented - classical alignment vs. pseudoalignment or series of differential expression tools (Sleuth, DeSeq2). We identified several candidate clusters in separate analysis of expression and methylation data and also in emQTL analysis. The next analysis using UniBind based on enrichment of specific transcription factors (TF) to each cluster revealed cluster enriched for immune related TF. These approaches could help us to identify interesting pathways and biomarkers on epigenetic and lncRNA level in association with chemotherapeutic response in solid tumors.

References

[1]. FleischerT, Tekpli X, Mathelier A, Wang S, Nebdal D, Dhakal, HP, Sahlberg KK, Schlichting E, OSBREAC, Børresen-Dale AL, Borgen E, Naume B, Eskeland R, Frigessi A, Tost J, Hurtado A, Kristensen VN (2017). *Nat Commun* 8: 1379.
[2]. Gheorghe M, Sandve GK, Khan A, Cheneby J, Ballester B, Mathelier A (2019). A map of direct TF-DNA interactions in the human genome. Nuclei Acid Res 47 (4):e21.

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Section 5
STSM presentations

Synthesis of p-quinol derivates as potent MDR-selective antitumor agents

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Protoflavones represent a less widespread, unique class of natural flavonoids with an unusual *p*-quinol moiety on its B-ring. These compounds are potent antitumor agents both *in vitro* and *in vivo*, and they can exert selective toxicity on various multi-drog resistant cancer cell lines[1] . We have recently identified graviquinone, a cinnamic acid *p*-quinol derivative that has similar anticancer properties as protoflavones. [2] Our group is currently working on the synthesis of a chemical library of *p*-quinols, to explore structure-activity relationships and to obtain new, potent MDR-selective lead compounds. To this end, our strategy to prepare these compounds involved the oxidation of phenols with hypervalent iodine reagents. However, this type of reaction has several drawbacks. The group of Prof. Ryszard Ostaszewski, developed an alternative strategy to obtain p-quinols without the need for an oxidative step. This involves the coupling of a quinone to the other fragment of the target molecule. During the course of the joint work, a new library of potential antitumor agents was obtained. This consists of 18 compounds, among which 15 are new, not yet described in the literature. Due to their structural similarity with the pharmacophore of protoflavones all of these compounds are potentially effective antitumor agents. The structures were evaluated and confirmed by ^{1}H and ^{13}C NMR spectroscopy. A wide variety of 2,4-cyclohexadienone derivatives were prepared, bearing methyl and/or aryl substituent in positions 2 and 4. Chemical diversity of the compounds was increased by varying the nature and position of the substituents of the aryl ring; these were either electron donating or electron withdrawing groups such as methyl, methoxy, halogens, or triflouromethyl, at various positions The first bioactivity screening of the compounds carried out. With these results we able to explore structure-activity relationships in the obtained new family of p-quinols

References

[1]. Danko B. et al. Anticancer Research 2014; 34: 5955-56.
[2]. Fasi L. et al. J Med Chem 2019; 62: 1657-68.

Inhibited IDO-enzyme in cancer cells: "Help! IDOn't escape immune surveillance anymore!"

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Multidrug-resistant (MDR) cancer cells are among the major obstacles in successful anticancer chemotherapy. Noteworthy, in addition to some well-established mechanisms of MDR like enhanced efflux or reduced drug uptake, resistance mechanisms involving the immune system have recently gained attention. MDR cancer cells frequently exhibit immunosuppressive characteristics that enable them to escape immune surveillance. The heme-containing enzyme indoleamine-2,3-dioxygenase (IDO) is commonly expressed in such tumor tissue and has been shown to produce a specific immunosuppressive microenvironment of low tryptophan and high kynurenine-metabolites [1, 2]. To target MDR cancer cells and reverse immune-suppression, we developed platinum(IV) prodrugs that are based on the immunogenic anticancer compound oxaliplatin and carry an IDO-inhibitor as immune modulator. These compounds are activated on site via reduction in the malignant tissue and simultaneously release oxaliplatin and the IDO inhibitor 1-methyl-tryptophane (1MT) [3]. In the course of this short-term scientific mission (STSM) (COST action 17104), we established a method to measure the level of the downstream metabolite kynurenine *in vitro*. Following treatment with our compounds, we detected a significant reduction of kynurenine level in the supernatant of SKOV3 cells that constitutively overexpress IDO. This suggests that our compounds effectively inhibit IDO activity. Additionally, methods to investigate immunologic effects *in vitro* were performed to assess T cell function and proliferation after treatment and are currently established and translated to murine models in the labs in Austria.

References

[1]. Campia I, Buondonno I, Castella B, Rolando B, Kopecka J, Gazzano E, Ghigo D, Riganti C (2015). *PloS One* 10(5):e0126159.
[2]. Englinger B, Pirker C, Heffeter P, Terenzi A, Kowol CR, Keppler BK, Berger W (2019). *Chem Rev* 119(2):1519-1624
[3]. Jungwirth U, Kowol CR, Keppler BK, Hartinger CG, Berger W, Heffeter P (2011). *Antioxid Redox Signal.* 15(4):1085-127

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Plectranthus mutabilis Codd as a source of Anti-MDR Diterpenoids

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Cancer is one of the most important causes of mortality and morbidity worldwide. Multidrug resistance (MDR) remains a challenge in cancer therapy and is associated with the overexpression of P-glycoprotein (P-gp), resulting in increased efflux of chemotherapeutics from cancer cells. *Plectranthus* genus is comprised of medicinal plants that contain abietane diterpenoids with antitumor activities. In this work, we studied sixteen *Plectranthus* spp and searched for the compounds responsible for the biological activity of the most bioactive extracts. *P. mutabilis* had the highest ultrasound-assisted extraction yield (30.03%, dry weight % w/w). All acetonic extracts were screened for their general toxicity using the *Artemia salina* model [3]. The antitumor activity of the five most cytotoxic extracts was explored in three different cancer cell lines: HCT116, MCF-7 and NCI-H460. *P. mutabilis* showed high cytotoxic activity and was subjected to further phytochemical studies. Column chromatography on silica or polyamide, allowed to achieve the diterpenoid coleon U (**1**) and 8α,9α-epoxycoleon U quinone (**2**). The complete structure characterization was done mainly by 1D-, and 2D-NMR, and comparison with literature data. Compound **1** showed moderate cytotoxicity on sensitive and resistant (ABCB1 overexpressing) human colon adenocarcinoma and normal cell lines, showing slight selectivity towards resistant cells. Moreover the expression of ABCB1 was not able to abolish the cytotoxic effect of **1** suggesting that compound **1** is not a substrate for P-gp. Further phytochemical studies are ongoing.

References

[1]. Diogo M Nicolai M, Fernandes A, Nuno S, Lucília S, Catarina P, Rijo P, (2019). *Biomolecules*, 9, 179

[2]. Rebelo Garcia C, Eleutério C, Bastos A, Pinto F, Gaspar M, Rijo P, Reis, (2018). *Pharmaceutics* 10(4): 216

[3]. Ntungwe E, Joana M, Garcia C, Oliveira C, Cláudia O, Rijo P, *Biomed Biopharm Res*. (14) 1: 95-108

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Training in mRNA profiling

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The mRNA profiling of parental ovarian cancer cell lines and carboplatin (CBP) resistant sublines established during the treatment with different drug's concentrations (MES-OV, CBP2-CBP8) was performed by Microarray assay in Genomics & Transcriptomics Laboratory, Heinrich-Heine University of Düsseldorf, Germany and tremendous amount of data was obtained. In order to understand the background of methodology and data analysis used, the short stay in Prof. Dr. Köhrer laboratory was organized and supported by COST Action CA17104. Goals of this Short-Term Scientific Mission (STSM) were: (1) to learn complete methodology and workflow of Microarray assay; (2) to learn bioinformatics analysis of obtained gene expression data; (3) to do the analysis on already obtained and partially analysed data in order to thoroughly investigate differential gene expression related to development of acquired drug-resistance; (4) to learn other methods and software analyses (RNA sequencing, Mass Spectrometry) and (5) to enhance cooperation between the Prof. Dr. Köhrer and Dr. Brozović groups. During the STSM, I learned complete Microarray assay workflow and was trained to use three different software for raw data analysis. After the analysis training, I applied the acquired knowledge to re-analyse previously obtained Microarray (MA) data on site. I also went through the RNA sequencing protocol and got familiar with RNA sequencing data software analysis. Additionally, I learnt the basics of mass spectrometry and post-analysis by visiting Molecular Proteomics Laboratory in University of Düsseldorf. Results obtained during the STSM gave a deeper insight into gene expression patterns of established cell lines. In conclusion, the correlation of previously obtained data and data obtained during the STSM enabled us to approach to the problem of chemo resistance more specifically and aim potentially crucial target genes and pathways in further experiments.

Investigation of cytotoxic and antioxidant property of salicylaldehyde thiosemicarbazones and their copper complexes

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Thiosemicarbazones (TSC) are often show synergism with copper(II) ions. Salicylaldehyde TSC (STSC) type compounds usually do not show strong cytotoxicity, but their copper(II) complexes are much more active [1]. The activity is often related to the redox activity of these complexes. The main objective of this study was to evaluate the effect of three thiosemicarbazone ligands (STSC, KM68, KM46) and their mono copper(II) complexes, against human breast cancer cell lines (MCF-7, SUM159 and SkBr3) and human liver hepatocellular carcinoma cells (HepG2). The cytotoxicity of the tested compounds was determined using MTT method on breast cancer and liver hepatocellular cell lines. ROS (reactive oxygen species) production of compounds was measured in MCF-7 and SUM159 cell lines with DCFH-DA (2,7-dichlorodihydrofluorescein diacetate). The capacity of the compounds to influence GSH (L-glutathione) [2] and catalase production [3] was investigated by using SUM159 cell line. The tested copper complexes (STSC-Cu, KM68-Cu, and KM46-Cu) exerted remarkable cytotoxic activity on any of the tested cell lines (IC_{50}: 0.84-11.51 μM). The TSC ligands did not show significant cytotoxic effect on none of the tested cell lines (IC_{50}: >50 or >100 μM) except the KM46 on SkBr3 (IC_{50}: 19.25 μM) and MCF-7 (IC_{50}:31.41 μM) cell lines. In SUM159 cells the copper(II) complexes showed ability to produce ROS. In the case of MCF-7 cell line the complex of KM68 and KM46 were able to influence the formation of ROS. An increased catalase activity and GSH concentration was detected only for the copper(II) complex of STSC in SUM159 cells. Based on our results the copper(II) complexes were much more active in all experimental system compared to the ligands, furthermore the redox property of the complexes may play a role in the mechanism of action.

References

[1]. CR Kowol, P Heffeter, W Miklos, L Gille, R Trondl *et al.* (2012). *J Biol Inorg Chem* 17: 409–423.
[2]. F Tietze (1969). *Anal Biochem* 27: 502–522.
[3]. L Góth (1991). *Clin Chim Acta* 196: 143–151.

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Presentation of STSM: Investigation of inhibitory properties of Michael acceptors on thioredoxin reductase 1 inhibition *in vitro*

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Cancer cells have increased level of reactive oxygen species (ROS), due to metabolic changes following their growth and development; they have adapted to increase in ROS by different mechanism, in part by increasing activity of antioxidant systems. Main antioxidant systems in a living cell are thioredoxin and glutharedoxin systems. Thioredoxin system is mainly comprised of thioredoxin and thioredoxin reductase. It has been reported that cytosolic thioredoxin reductase, TrxR1, has increased activity in cancer cells. Inhibition of TrxR1 could have a detrimental effect on cancer cell survival, due to further increase of ROS. Development of new TrxR1 inhibitors gives possibilities in new therapeutic approaches in treating cancer, as an accompanying treatment to conventional treatment strategies. STSM was realized in collaboration between home institution, Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade and host institution, Latvian Institute of Organic Synthesis (Riga, Latvia). Purpose of the STSM was to evaluate inhibitory properties of 6 potential inhibitors of thioredoxin reductase 1 on neuroblastoma cell line SHSY5Y. This particular cell line was chosen as it proved to be a good model for studying Trx system. Potential inhibitors were tested for inhibitory properties of TrxR on crude protein cell lysate of SHY5Y, rat TrxR1 enzyme and on insulin assay. Main result of this STSM is selection of the best candidate for further expansion series in studying Michael acceptors as inhibitors of TrxR1 and possible applications in anti-cancer therapy. The results obtained during STSM were published in a peer-reviewed journal [1]. This STSM is a perspective start to further investigation of importance of thioredoxin reductase 1 in cancer cell survival and inhibition of TrxR1 in cancer therapy. The candidate-inhibitors will in future be tested on cancer cell lines and multidrug resistant cancer cell models, with different antioxidant capacities.

References

[1]. Jovanovic M, Zhukovsky D, Podolski-Renic A, Domraceva I, Zalubovskis R, Sencanski M, Glisic S, Sharoyko V, Tennikova T, Dar'in D, Pesic M, Krasavin M. Eur J Med Chem (2019) 181:111580.

Molecular mechanisms of concomitant resistance of carboplatin resistant ovarian cancer cells to paclitaxel

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Most epithelial ovarian cancer patients are diagnosed with advanced-stage disease due to the late appearance of symptoms and lack of early diagnostic methods/markers. The major problem for a successful therapy is development of intrinsic tumour drug resistance during carcinogenesis (20-30%). Moreover, about 70-80% of patients, who respond to the initial treatment, experience a recurrence of cancer within months to years [1]. Platinum complexes and taxanes are the first-line therapy. Epithelial-mesenchymal transition (EMT) is involved in cancer progression and metastasis [2]. Epigenetic changes in tumours are associated not only with cancer development and progression, but also with resistance to chemotherapy. Aberrant DNA methylation at CpG islands, histone deacetylation and associated epigenetic silencing are observed during the acquisition of drug resistance [3, 4]. It is known that many EMT regulators, such as members of miRNA-200 family and their downstream targets are epigenetically regulated [5]. We established several ovarian cancer cell lines resistant to carboplatin (CBP) (OVCAR-3 CBP, MES-OV CBP and SK-OV-3 CBP) by treatment of their parental OVCAR-3, MES-OV and SK-BR-3 cell lines with increasing doses of CBP. All CBP resistant cell lines show a mesenchymal like phenotype and two of them (OVCAR-3 CBP and MES-OV CBP) are cross-resistant to paclitaxel. Our preliminary data indicate that miR-200c/miR-141-mediated increased expression of tubulin beta III (TUBBIII) could be one of the major molecular mechanisms of concomitant resistance of CBP-cells to paclitaxel. Since it is known from the literature that the miR-200c/141 promotor is epigenetically regulated [5] our goal is to investigate a possible permanent CBP-induced aberrant regulation of either DNA methyltransferase or histone deacetylase, which could further epigenetically regulate silencing of miR-200c and 141 promotor and downstream TUBB expression. The ultimate goal is to investigate CBP-induced event(s) which triggered cross-resistance to paclitaxel in CBP resistant ovarian cancer.

References

[1]. Coleman RL, Monk BJ, Sood AK, Herzog TJ (2013) *Clin Oncol* 10:211-224.
[2]. Huber MA, Kraut N, Beug H (2005) *Curr Opin Cell Biol* 17:548-558.
[3]. Steele N, Finn P, Brown R, Plumb JA (2009) *Br J Cancer* 100:758-763.
[4]. Zeller C, Brown R (2010) *Ther Adv Med Oncol* 2:319-329.
[5]. Lim YY, Wright JA, Attema JL, Gregory PA, Bert AG, Smith E, Thomas D, Lopez AF, Drew PA, Khew-Goodall Y, Goodall GJ (2013) *J Cell Sci* 126:2256-2266.

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Section 6
Best Poster Prize Presentations

IL-6 as a key pathway player in the secretome of human granulosa-like tumor cell line (KGN) after hormonal stimulation with different amount of FSH

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Introduction: The major functions of granulosa cells (GCs) include the production of steroids, as well as a myriad of growth factors to interact with the oocyte during its development within the ovarian follicle. Insufficiencies in granulosa cell function leads to many pathologies including infertility, defects in oocyte development and abortion. Many of these pathologies are caused by hormonal imbalances acting directly on granulosa cells. Progress in the emerging field of proteomics allows analyzing proteins derived from small samples. Here we report about our efforts to analyze the secretome of human granulosa cells after stimulation with various gonadotropins used in infertility treatment.
Material and Method: The secretome of human granulosa cells and the granulosa carcinoma cell line KGN under gonadotrophic hormonal stimulation were compared versus controls. Protein precipitation of samples was performed by Methanol/Chloroform following trypsin digestion. Separation of digested proteins was performed by liquid chromatography and mass spectrometry. The database search is performed using ProteomeDiscoverer 2.4 and MSGF+.
Results: A total of 2480 different cell secreted proteins were identified. After hormonal stimulation the secreted protein pattern changes. The changes in relative intensities of Interleukin 6 were clearly observable between the control and treated samples. The higher concentration of FSH resulted in lower expression of IL6. These changes were also confirmed by measuring the IL6 concentration using the routine analysis procedure in a clinical laboratory. Although the mechanism of IL6 involvement in KGN cells development is still poorly characterized, there are hints that it regulates protein phosphorylation, calcium homeostasis, cell growth and differentiation and, especially, the inflammatory response.
Conclusion: The proteome based analyses of secreted proteins provide a useful tool to understand the processes during hormonal stimulation in human granulosa cells. It may help to define granulosa cell derived distinct protein profiles causative for human pathologies with regard to infertility and reproduction. The role of IL6 has still to be clarified and we will focus on its role in fertility.

Application of carbonic anhydrase IX/XII inhibitors to modulate the transport of doxorubicin and its liposomal form into 2D and 3D cancer cell cultures

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The extracellular tissue of tumors is usually acidic [1]. In the acidic environment, weakly basic drugs become protonated, thus their ability to penetrate cell membrane decreases [2]. Carbonic anhydrase (CA) is a transmembrane protein that catalyzes the reversible hydration of carbon dioxide to bicarbonate, hence increasing extracellular acidity [3]. We hypothesize that the inhibition of CA IX and XII, reduces extracellular acidity, thus enhancing their delivery into a tumor. The aim of our study was to evaluate the influence of two CA inhibitors methazolamide (MTZ) and U-104 on doxorubicin (DOX) and its pegylated liposomal form (PLD) delivery into monolayer-cultured 4T1 murine breast cancer cells and tumor spheroids at pH 6.0 and 7.4. The effect of CA inhibitors on cell viability was evaluated by MTT assay. DOX penetration into cancer cells and spheroids was assessed using fluorescence microscopy. Student's t-test was used, and p-values were calculated. A value of $p<0.05$ was considered as the level of significance. At physiological pH, none of the tested CA inhibitors (100 μM) increased DOX (5 μM) and PLD (concentration corresponding to 5 μM DOX) delivery into 2D cell cultures. In acidic conditions both MTZ and U-104 increased the delivery of DOX and PLD into cell nucleus up to 1.8-fold compared to the control group. MTZ did not enhance the transport of DOX and PLD into spheroids. U-104 increased the amount of DOX and PLD spheroids up to 2.4-fold compared to control at pH 6.0. MTZ and U-104 enhance DOX and PLD delivery into 2D cancer cell cultures in an acidic environment. Only U-104 enhances DOX and PLD penetration into tumor spheroids at acidic pH. U-104 is worth further studies as a transport modulator of weakly basic drugs.

References

[1]. Zhang X, Li, Y, Gillies, RJ (2010). J. Nucl. Med 51 (8), 1167–1170.
[2]. McCarty MF, Whitaker J (2010). Altern. Med. Rev. 15 (3), 264–272.
[3]. Stadie WC, O'Brien H (1933). J Biol Chem 103(2):521-529.

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Synthesis, antiproliferative effect and topoisomerase II inhibitory activity of 3-methyl-2-phenyl-1*H*-indoles

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Cancer is one of the leading causes of mortality worldwide and its incidence is rapidly growing. The use of conventional chemotherapeutic agents, such as alkylating cytostatics, nucleoside analogues, anthracyclines and compounds that stabilise microtubules is often limited because of their severe side effects. One of the important targets for anticancer drug discovery is human DNA topoisomerase II.[1-2] A series of 3-methyl-2-phenyl-1*H*-indoles was prepared and investigated for antiproliferative activity on three human tumour cell lines, HeLa, A2780 and MSTO-211H, and some structure-activity relationships were drawn up. Compounds were designed based on the 3-methyl-2-phenyl-1*H*-indole hit, which was identified through screening of in-house library of compounds and showed good antiproliferative activity, with growth inhibition values (GI_{50}) in the micromolar concentration range. Based on this hit compound, a series of analogues was designed and synthesized. The GI_{50} values of the most potent compounds were lower than 5 μM in all tested cell lines. For the most biologically active derivatives, the effect on human DNA topoisomerase II relaxation activity was investigated, which highlighted good correlation between the antiproliferative effect and topoisomerase II inhibition. The most potent compound was shown to induce the apoptosis pathway. The obtained results highlight 3-methyl-2-phenyl-1*H*-indole as a promising scaffold for further optimisation of compounds with potent antiproliferative and anti-topoisomerase II activities.[3]

References

[1]. Dang CV, Reddy EP, Shokat KM, Soucek L. (2017) *Nat Rev Cancer* 17: 502-508.
[2]. Skok Ž, Zidar N, Kikelj D, Ilaš J. (2019) *J Med Chem*: https://doi.org/10.1021/acs.jmedchem.9b00726.
[3]. Zidar N, Secci D, Tomašič T, Peterlin Mašič L, Kikelj D, Passarella D, Garcia Argaez AN, Hyeraci M, Dalla Via L (2020). *ACS Med Chem Lett*: https://doi.org/10.1021/acsmedchemlett.9b00557.

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Posters

Role of OCT1 in cholangiocarcinoma chemoresistance to sorafenib

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Although the multi-tyrosine kinase inhibitor sorafenib is moderately useful in the treatment of hepatocellular carcinoma, most cholangiocarcinoma (CCA) patients are refractory to this drug. Among other mechanisms of chemoresistance, impaired uptake via OCT1 (gene *SLC22A1*) has been suggested. Here we have investigated the events accounting for this phenotypic characteristic and have evaluated the interest of selective gene therapy strategies to overcome this limitation. Gene expression and DNA methylation of *SLC22A1* were analyzed using biopsies from intrahepatic (iCCA) and extrahepatic (eCCA) cholangiocarcinoma. Decreased *OCT1* mRNA correlated with the hypermethylation status of the *SLC22A1* promoter. Treatment of CCA cells with decitabine (demethylating agent) or butyrate (histone deacetylase inhibitor) restored OCT1 expression and increased sorafenib uptake. MicroRNAs able to induce *hOCT1* mRNA decay were analyzed in paired samples of tumor and adjacent non-tumor tissue. Consistent upregulation in tumor tissue was found for several miRs with predicted interaction with *SLC22A1* pre-mRNA. When they were expressed in CCA cells, downregulation of OCT1 was induced. A high proportion of aberrant *OCT1* mRNA splicing in CCA was also seen. Lentiviral-mediated transduction of eCCA (EGI-1 and TFK-1) and iCCA (HuCCT1) cells with OCT1 enhanced sorafenib uptake and cytotoxic effects. In chemically induced CCA in rats reduced rOct1 expression was accompanied by impaired sorafenib uptake. In xenograft models of eCCA cells implanted in mouse liver, poor response to sorafenib was observed. However, tumor growth was markedly reduced by co-treatment with sorafenib and adenoviral vectors encoding OCT1 under the control of the *BIRC5* promoter, a gene highly upregulated in CCA. ***Conclusions****:* The reason for impaired OCT1-mediated sorafenib uptake by CCA is multifactorial. Gene therapy capable of selectively inducing OCT1 in tumor cells can be considered a potentially useful chemosensitization strategy to improve the response of CCA to sorafenib.

Pyrazolo[3,4-d]pyrimidine derivatives, Si306 and pro-Si306, inhibit the growth of sensitive and multidrug resistant glioblastoma

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Glioblastoma (GBM) is the most frequent and aggressive brain tumor in adults. Main characteristics of GBM include high proliferation rate, infiltrating nature, and resistance to chemotherapy and radiation. GBM have high expression of c-Src tyrosine kinase which has a key role in regulating survival, proliferation, angiogenesis and invasiveness of tumor cells. Thus, c-Src emerged as a potential target for GBM therapy. Anticancer properties of c-Src tyrosine kinase inhibitors Si306 and its prodrug pro-Si306, pyrazolo[3,4-d]pyrimidines were assessed in human GBM cell line U87, its multidrug resistant (MDR) counterpart U87-TxR, and human primary GBM culture. Si306 and pro-Si306 triggered ROS generation and DNA damage in sensitive and MDR GBM cell lines, as well as primary GBM cells. Both compounds induced a prominent cell death in primary GBM culture, while the effect on GBM cell lines was predominantly antiproliferative, characterized by decrease in Ki-67 expression and cell cycle disturbance. Moreover, the investigated compounds made primary GBM culture more prone to anoikis. In addition, Si306 and pro-Si306 showed strong antiproliferative effect in U87 xenografts in zebrafish embryo model. The antiglioblastoma effects of investigated c-Src inhibitors were more prominent when compared to dasatinib, a well-known tyrosine kinase inhibitor. The presence of the MDR phenotype did not diminish the activity of the compounds. The investigated pyrazolo[3,4-d]pyrimidines displayed significant anticancer potential in GBM which makes them good candidates for further development regarding treatment of this cancer type.

Biological activity of silybin derivates focusing on P-glycoprotein modulation

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Silymarin is known for its biological activity for a long time. Despite the fact, not everyone knows that the major component with potentially the most significant activity is silybin. The minor part of this special complex is also dehydrosilybin [1]. Our investigation focused on complex monitoring of biological activities of silybin and 2,3-dehydrosilybin isomers. In the study, the antioxidant capacity of compounds was determine as an ability to scavenge oxygen radicals to protect fluorescein oxidation (ORAC) and also to scavenge radicals generated in the living cells. Isomer A and B of both stereomeric mixtures were equally involved in the antioxidant activity. All these substances effectively reduced the production of inflammatory first-response marker (NO) and release of IL-6 production in dose-dependent manner. The potential to modulate the multidrug resistance (MDR) was evaluated in two different ways – inhibition of P-gp ATPase activity and regulation of mRNA expression level of ABC proteins [2]. In dose dependent manner, all the compounds showed strong ability to inhibit the P-gp pump, especially stereomeric mixture silybin AB and dehydrosilybin AB. Moreover, dehydro-compounds provided the most effective sensitization of P-gp positive ovarian carcinoma at the 10 μM concentration. Despite this significant effect, the silybin B was the only compound affecting directly P-gp and also downregulating the expression of MDR genes. This compound altered the expression of P-gp (*ABCB1*), MRP1 (*ABCC1*) and BCRP (*ABCG2*). On the other side, dehydro-compounds showed the most effective inhibition of acetylcholinesterase activity.

References

[1]. Trappoliere M., Caligiuri A., Schmid M., Bertolani C., Failli P., Vizzutti F., Novo E., di Manzano C., Marra F., Loguercio C., Pinzani M. (2009): Silybin, a component of sylimarin, exerts anti-inflammatory and anti-fibrogenic effects on human hepatic stellate cells. Journal of Hepatology, 50, 1102–1111.
[2]. Nanayakkara A. K., Follit C. A., Chen G., Williams N. S., Vogel P. D., Wise J. G. (2018): Targeted inhibitors of P-glycoprotein increase chemotherapeutic-induced mortality of multidrug resistant tumor cells. Scientific reports, 8, 1–18.

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Design of novel thiourea derivatives of naproxen with potential antitumor activity

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In the search for potent biologically active molecules, thiourea and other structure-related derivatives such as thiosemicarbazones have attracted great attention. In the past two decades, thiourea derivatives have been recognized as promising class of anticancer drugs due to their inhibitory activity against various targets, such as protein kinases and topoisomerases [1,2]. In this work, molecular docking analyses were performed on 20 thiourea derivatives of naproxen, previously designed by our group, in order to find their potential mechanisms of action. Designed derivatives contain amino acids and aromatic amines in the side chains. Following 3D structures of selected protein kinases involved in multidrug resistance were taken from PDB: 1M17 (EGFR), 3E87 (AKT2), 3HNG (VEGFR1) and 4JSV (mTOR). The receptor sites were prepared using MAKE Receptor 3.2.0.2 software [3]. Ligands were prepared in OMEGA 2.5.1.4 [4,5] and multiconformational binary files were generated. The FRED 3.2.0.2 software [6-8] was used for the analysis of binding poses into the receptor sites. The key binding interactions were observed for derivatives **1** (with AKT2 and mTor) and **20** (with EGFR and VEGFR1). Therefore, these derivatives possess the best multitarget potential and represent potential candidates for targeting multidrug resistant tumors (**Figure 1**).

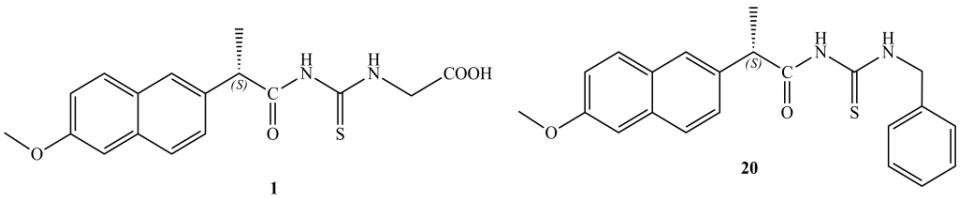

Figure 1. Chemical structures of **1** and **20.**

References

[1]. Li HQ, Yan T, Yang Y, Shi L, Zhou CF, Zhu HL (2010). *Bioorg Med Chem* 18: 305-313.
[2]. Zhao Y, Wang C, Wu Z, Fang J, Zhu L (2012). *Invest New Drugs* 30: 17-24.
[3]. OpenEye Scientific Software, Inc., Santa Fe, NM, USA; https://www.eyesopen.com/.
[4]. OMEGA 2.5.1.4: OpenEye Scientific Software, Santa Fe, NM, USA; http://www.eyesopen.com.
[5]. Hawkins PCD, Skillman AG, Warren GL, Ellingson BA, Stahl MT (2010). *J Chem Inf Model* 50: 572-584.
[6]. FRED 3.2.0.2: OpenEye Scientific Software, Santa Fe, NM, USA; http://www.eyesopen.com.
[7]. McGann M (2011). *J Chem Inf Model* 51: 578-596.
[8]. McGann M (2012). *J Comput Aid Mol Des* 26: 897-906.

The Effects of Different Types of Calorie Restriction Methods on Multiple Drug Resistance Associated miRNAs

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Roles of calorie restriction (CR) have been reported in various physiological and pathophysiological functions such as asthma, neurogenesis, cardio vascular diseases (CVD) and development of various cancer types. Usually, two types of CR are being applied in studies; chronic or intermittent calorie restriction. Although, in most studies chronic calorie restriction (CCR) have been applied both in animal and human studies, researchers have started studying the effects of application of intermittent calorie restriction (ICR) in recent studies. In this context, it has been reported that ICR is more effective than CCR for numerous pathological conditions. On the other hand, involvement of micro RNAs (miRNAs) in apoptosis, tumorogenesis, cell invasion, migration, angiogenesis, CVD and neurogenesis has been reported in numerous studies. Roles of miRNAs in the preventive effects of CR have also been reported. However, there are limited research about the effects of CR on miRNAs which are related to Multiple Drug Resistance (MDR) genes. Therefore, the aim of the current study was to determine the effects of two different types of calorie restriction on miRNAs which are related to MDR genes in mouse model. In this study, 10 weeks old female C57BL/6 mice were fed ad libitum, CCR (15% CR compared to AL group) or ICR (three weeks of AL application followed by one week 60% CR compared to AL group in a cyclic manner). Serum samples were collected at 10, 49/50 and 81/82 weeks of mouse age. Then, blood RNA samples were isolated and Affymetrix GeneChip™ miRNA 4.1 array strip microarray was performed to evaluate changes in miRNA expression levels in blood in response to the different types of CR application from week 10 up to week 50. Total of nine different miRNAs which were related to MDR genes were differentially expressed among the dietary groups. The expression levels of two miRNAs, miR-7071-5p, and miR-6395 were significantly different between CCR and ICR-R groups. These differentially expressed miRNAs among the different dietary groups predicted to target five different MDR related genes (ABCA2, ABCA5, ABCB9, ABCC1, ABCG4). These results implies that the way calorie is consumed may play important role in regulation of MDR genes. Using GO (Gene Ontology) and KEGG analysis, the physiological roles and signaling pathways in which these miRNAs are involved will also be presented at the meeting.

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Comparison of *in vitro* and *in vivo* efficiency of classical and novel SB-T-taxanes with potential effect in resistant ovarian tumors

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Taxanes are successfully used in therapy of different carcinomas, especially breast and ovarian carcinomas. However, inherited or acquired drug resistance of tumor cells to classical taxanes (paclitaxel, docetaxel) presents one of the major obstacles in successful therapy. Drug resistance is a multifactorial process that may be related to drug transport, metabolism or alterations in apoptosis induction by taxanes. Certain novel taxanes (e.g. SB-T-taxanes of second- and third-generation) possess extremely high potency against drug-resistant cancer cells expressing the multidrug resistance (MDR) phenotype [1, 2]. The aim of our study was to explore and compare the effect of classical and novel taxanes on cell death, molecular mechanism of their action and transport in our established experimental model of ovarian carcinoma. Our experimental model (*in vitro*) composed of ovarian tumor cell lines; paclitaxel sensitive OVCAR-3 and paclitaxel-resistant NCI/ADR-RES. The effect of second-generation taxanes (e.g. SB-T-1216, SB-T-1214) and third-generation taxanes (SB-T-12602, SB-T-121605, SB-T-121606) on the growth and survival of sensitive OVCAR-3 and resistant NCI/ADR-RES cells was higher as compared to that of paclitaxel. Novel taxanes demonstrated higher sensitivity (50-1000-fold) to NCI/ADR-RES cells that are highly expressing P-glycoprotein. Transport of paclitaxel in cell lines was significantly different. Resistant cells accumulated paclitaxel 20-fold less than sensitive cells. On the contrary, transport of second-generation taxanes was similar in both cell lines. SB-T-121605 was selected for further *in vivo* experiments. We applied $2x10^6$ NCI/ADR-RES cells to mice and monitored the tumor growth. Taxane treatment was initiated after tumors reached a size of cca 100 mm^3. Paclitaxel alone was not effective in this our established resistant *in vivo* model.

References

[1] Ojima Y et al. (2008). ; *J Med Chem*.51(11): 3203-3221.
[2] Ehrlichova M et al. (2012). *Naunyn Schmiedebergs Arch Pharmacol.* 385(10):1035-1048.

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Activation of protein kinase C epsilon (PKCε) and delta (PKCδ) inhibits chemokine secretion enhancing anti-tumoral effects of CXCR2 antagonist

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Activation of CXCR2, receptor for CXCL1 and CXCL2 chemokines, increases aggressiveness of breast cancer and induces chemoresistance. We previously observed that CXCR2 also has an auto receptor function and inhibition of CXCR2 induces reactive increases in CXCR2 ligands which may inhibit anti-tumoral effects of CXCR2 antagonist. Further studies demonstrated that activators of protein kinase C epsilon (PKCε) and delta (PKCδ), members of the novel family of PKC isozymes, may prevent reactive increases in chemokine secretion. We have used SB 225002, an CXCR2 antagonist; FR 236924, selective PKCε activator and t7α-acetoxy-6β-benzoyloxy-12-O-benzoylroyleanone (Roy-Bz), a selective small-molecule activator of PKCδ. The effects of inhibitors and activators were determined using 4TBM and 4THM cells. The 4TBM and 4THM cells were previously established using brain and heart metastatic lesions originated from 4T1 primary tumors. Changes in cell proliferation and release of human homologues of CXCL1 (KC) and human homologues of CXCL2 (MIP-2) was determined. FR 236924 (10μM) markedly inhibited cell growth due to autocrine factors without inducing major changes in MIP-2 and KC secretion. Similarly, FR 236924 (10μM) further enhanced the anti-proliferative effects of CXCR2 inhibitor. FR 236924 also prevented SB 225002-induced increases in MIP-2 and KC secretion. Similar to FR 236924, Roy-Bz (10μM) markedly inhibited cell growth and further enhanced the anti-proliferative effects of CXCR2 inhibitor. Roy-Bz also prevented SB 225002-induced increases in KC secretion. Hence activators of PKCε and PKCδ may prevent emerging of resistance to CXCR2 inhibitors which are in clinical trials.

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Inhibition of bone morphogenetic protein 1 activity decreases proliferation of metastatic breast carcinoma cells; an effect independent of transforming growth factor-β

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Triple negative breast cancer (TNBC) is the most malignant subtype of breast cancer in which drug resistance is observed commonly. Transforming growth factor-β (TGF-β) by inducing cancer stem cell population leads to multidrug resistance. BMP-1 (bone morphogenetic protein 1) is a metaloprotease involved in release of TGF-ß from extracellular macromolecular complexes. We previously observed that level of BMP-1 in exosomes of metastatic murine breast cancer cells is higher compared to non-metastatic cells. The aim of the present study was to evaluate the effects of BMP-1 inhibitors on anti-tumoral effects of doxorubicin. In addition, we also evaluated the effects of TGF-ß antagonist SB 431542, in order to determine whether BMP-1 inhibitor mimics the effects of TGF-ß inhibition or not. We have used UK 383367 which is a selective inhibitor of BMP-1. SB 431542 is a potent and selective inhibitor of the (TGF-β) type I receptor/ALK5. The 4TBM and 4TLM cells were treated with various concentrations of the inhibitors alone or in combination with Pegilated form of liposomal Doxorubicin in the presence of 5% or 0.2% FBS. Changes in cell proliferation was determined using WST-1 after 72 or 144 hours. The 4TBM and 4TLM cells were derived from brain and livers metastatic lesions originated from 4T1 primary tumor with which is a murine model for TNBC. Inhibition of BMP-1 activity with UK 383367 dose dependently decreased proliferation of 4TLM and 4TBM cells 72 hours after treatment. This affect was observed under both high and low serum conditions. When the treatment duration increased to 144 hours, UK 383367 was more effective in suppressing the cell growth. UK 383367, however did not enhance the growth inhibitory effects of doxorubicin. This effects of UK 383367 seemed to be independent of release extracellular TGF-β because SB 431542 did not alter the cell growth 72 hours after treatment.

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Natural product-derived compounds for targeting MDR

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Multidrug resistance (MDR) in cancer is one of the foremost impediments for a successful chemotherapy. One of the most significant mechanism of MDR is related to the overexpression of ABC transporter proteins, which act as efflux pumps for chemotherapeutic agents. The three most involved in MDR are P-glycoprotein (P-gp), multidrug resistance protein (MRP1) and breast cancer resistance protein (BCRP). Aiming at finding effective natural product-derived compounds for reversing MDR in cancer, we have been assessing the ability of a great number of compounds, with different scaffolds, as ABC transporter modulators. Among the most relevant results are those obtained for some terpenoids, namely macrocyclic diterpenes and triterpenoids, monoterpene indole alkaloids and flavonoid derivatives. Continuing our search for plant-derived compounds that can circumvent MDR in cancer treatment, in this work three triterpenoids, featuring a unique 5/6/3/6/5-fused pentacyclic carbon skeleton, were isolated from the African medicinal plant *Momordica balsamina*. They were assessed for their ability as P-gp modulators, using a mouse T-lymphoma MDR1-transfected cell model, by the rhodamine-123 accumulation assay. The three compounds showed very strong P-gp modulation activity, pointing out their potential as MDR reversers [1-4].

References

[1]. Mónico A, Ramalhete C, André V, Spengler G, Mulhovo S, Duarte MT, Ferreira MJU (2019). *J Nat Prod* 82:2138-2143.
[2]. Reis MA, Ahmed OB, Spengler G, Molnár J, Lage H, Ferreira MJU (2017). *J Nat Prod* 80: 1411-1420.
[3]. Paterna A, Kincses A, Spengler G, Mulhovo S, Molnár J, Ferreira MJU (2017). *Eur J Med Chem* 128: 247-257.
[4]. Ferreira RJ, Baptista R, Moreno A, Madeira PG, Khonkarn R, Baubichon-Cortay H, Dos Santos DJ, Falson P, Ferreira MJU. (2018) *Future Med Chem* 10:725-741.

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Activity of novel Cd(II) complex against pancreatic cancer stem cells (CSCs)

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Novel binuclear Cd complex with pyridine-based hydrazone ligand (**1**) reveals a strong proapoptotic activity against pancreatic AsPC-1 CSCs. While apoptosis undergoes mostly caspase-independent, **1** stimulates the activation of intrinsic pathway with noteworthy down regulation of caspase-8 activity in respect to non-treated controls. Distribution of cells over mitotic division indicates that 1 caused DNA damage, which is confirmed in DNA interaction studies. Compared to 1, cisplatin does not achieve cell death in 2D cultured AsPC-1 cells, implying that these two compounds do not share similar mechanism of action. Additionally, 1 acts as a powerful inducer of mitochondrial superoxide production with dissipated trans-membrane potential in the majority of the treated cells already after 6 h of incubation. On 3D model, 1 displays a superior activity and at 100 μM induces disintegration of spheroids within 2 days of incubation. Fluorescence spectroscopy, along with molecular docking show that compound 1 binds to the minor groove of DNA.

References

[1]. Bjelogrlić, S. K, Todorović, T. R, Cvijetić, I. N, Rodić, M., Vujčić, M, Marković, S, Araškov, J, Janović, B, Emhemmed, F, Muller, C. D, Filipović N. R (2019). *J Inorg Biochem* 190: 45-66.

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International PhD Program "Translational Oncology" (IPPTO)

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IPPTO is a new PhD excellence program supported by the Austrian Science Fund's doc.funds initiative. Starting in March 2020, IPPTO will operate within the framework of the Medical University of Vienna's graduate training program. In total, 14 students will join the IPPTO faculty and work closely together to study therapy resistance, which has been one of the hardest problems facing cancer research. The projects will focus on three research areas: 1) Cellular mechanisms of resistance: The most straightforward cause of therapy resistance are cellular alterations that prevent the drug to act on its target. In some cases, mutations alter the target or activate compensatory pathways (e.g. target or bypass mutations of driver genes). Alternatively, resistance mechanisms change the "cellular pharmacology" of the cancer cell, influencing the uptake, metabolism or the efflux of the drugs. 2) Cellular plasticity: Resistance to anticancer drugs can also arise through non-genetic modifications of metabolism and differentiation. Understanding the underlying mechanisms of this phenotypic adaptation is crucial for development of novel therapeutic strategies. Here we aim to better understand the non-genetic plasticity of cancer cells. One important process hijacked by cancer cells is the epithelial to mesenchymal transition (EMT), the initial step of the metastatic cascade, associated with the acquisition of cancer stem cell properties and drug resistance. 3) The tumor microenvironment: In addition to tumor cell-autonomous mechanisms of drug resistance, the tumor microenvironment also elicits innate resistance to many therapies through stromal and immune cells. Cancer immunotherapy has emerged as a promising therapeutic intervention. However, complete and durable responses are only seen in some cancer patients. A lack of appropriate models recapitulating the complexity of the tumor microenvironment hampers the investigations of drug resistance in this field.

References

Homepage: https://phd-ippto.meduniwien.ac.at/

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Synthesis of artemisinin-thymol hybrid molecules

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Nowdays, design and synthesis of novel organic molecules or isolation of natural products from plants have been gained big importance for the treatment of cancer and other diseases. Artemisinin and its semi-synthetic derivatives are a group of drugs that possess the most rapid action of all current drugs against malaria. Last decades, there have been many research including biological properties of artemisinin and their derivatives [1]. However, there are a few studies for the design and synthesis of novel artemisinin derivatives. Artemisinin structure has been displayed anti-cancer activity for different kind of cancer cells [2]. Therefore, scientists have been tried to find best hiybrit molecules consisting artemisinin and biologically known organic molecules. Thymol are also well known biologically important natural organic molecules. They have been used as anti-bacterial, anti-parasitic, anti-cancer, anti-inflammatory and anti-tumor agents. In the present study, we design and synthesis novel artemisinin-thymol hybrit molecules.

References

[1]. Liu C-X. (2017). *Chinese Herbal Medicines*, 9(2): 101-114.
[2]. Ma, J., Katz, E., Kyle, D.E., Ziffer, H., (2000). *J. Med. Chem*, 43: 4228-4232.

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Synthesis and evaluation of Isoquinolinequinone *N*-oxides as multidrug resistant anticancer agents

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The isoquinolinequinone (IQQ) pharmacophore is a privileged framework in known cytotoxic natural product metabolites, caulibugulones and mansouramycins both isolated from marine sponges [1]. Both series exhibit cytotoxicity in the sub-micromolar range across multiple cancer cell lines including renal, breast and ovarian. A multitargeted approach is often adopted to explain the IQQ's potent cytotoxicity. This includes mitochondrial effects through redox cycling and enzyme inhibition through electrophilic addition to critical amino acids *in vivo* for example in Cdc25 isoforms, whose function is crucial in normal cell cycle regulation [2,3]. We report on the discovery of a potent novel anticancer *N*-oxide derived framework (Figure 1). A library of novel IQQ's were synthesised exhibiting nanomolar cytotoxic activity against breast, melanoma and ovarian cancer cell lines. A lead compound has been identified to conduct further mechanistic studies in view of progression towards clinical development [4].

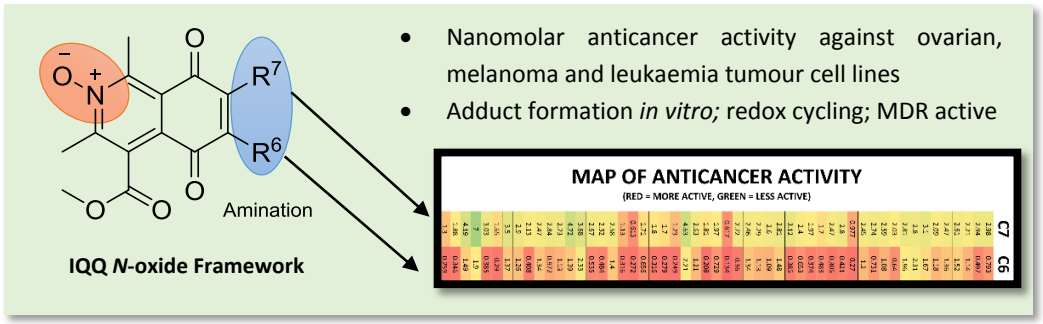

Figure 1: Novel IQQ framework derived from marine metabolites families, Mansouramycin and Caulbugulone

References

[1]. Milanowski, J.D. *et al*., (2004) *Journal of Natural Products*, 67, 70-73. Hawas W.U. *et al*., (2009) *Journal of Natural Products*, 72, 2120-2124.
[2]. Kuang S. *et al*., (2017) *Oncotarget*, 61, 104057-104071.
[3]. Brisson M. *et al*., (2007) *Molecular Pharmacology*, 71, 184-192.
[4]. Kruschel R. *et al*., (2020) *Organic and Biomolecular Chemistry*, 18, 557-568

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Combination of Cisplatin with small-molecule inhibitors to reduce chemoresistance in lung cancer cells

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Lung cancer is first in incident and the leading cause of cancer-related death worldwide. Almost 85% of all lung cancer cases are diagnosed as non-small-cell lung cancers (NSCLC). Platinum-based chemotherapy, such as Cisplatin, has been used as standard first-line systemic treatment for advanced NSCLC for decades. However, this approach has shown modest increase in survival rates and is often ineffective due to the development of resistance [1]. This indicates that additional adjuvants are necessary to achieve effective results. Evading apoptosis is an important mechanism for developing resistance to chemotherapeutic drugs. Survivin, a member of the inhibitor of apoptosis proteins family, is highly expressed in most cancers but undetectable in differentiated normal tissues. Survivin is known to inhibit cancer cell apoptosis and promote cell proliferation [2]. In addition, the PI3K/AKT survival pathway which is often overactivated in cancer, partially controls Survivin expression [3] and serves as a convergence point for activation of many of the oncogenes involved in NSCLC. In this study we hypothesized that the combination of Cisplatin and small molecule inhibitors will show increased effectiveness in NSCLC cells. To test this, we incubated lung cancer cells with Cisplatin alone and in combination with p-AKT inhibitor MK2206 and Survivin inhibitors, YM155 and TPGS. We discovered that Cisplatin synergistically reduced viability of H460 in combination with MK2206. Cisplatin and YM155 reduced H1975 cancer cell viability in a synergistic manner, as revealed by the MTT proliferation assay. In addition, the phosphorylation of p-AKT was affected in the presence of both Cisplatin and MK2206 in H460 cells. These results indicate that the addition of AKT and/or Survivin inhibitors in a chemotherapeutic regimen may show promise for the sensitization of lung cancer cells to platinum-based drugs.

References

1. Ghosh, S., *Cisplatin: The first metal based anticancer drug.* Bioorg Chem, 2019. 88: p. 102925.
2. Li, F., et al., *Control of apoptosis and mitotic spindle checkpoint by survivin.* Nature, 1998. 396(6711): p. 580-4.
3. Neophytou, C.M., et al., *D-alpha-tocopheryl polyethylene glycol succinate (TPGS) induces cell cycle arrest and apoptosis selectively in Survivin-overexpressing breast cancer cells.* Biochem Pharmacol, 2014. 89(1): p. 31-42.

The synthesis of Ciprofloxacin derivatives *via* Passerini reaction for MDR tumor treatment.

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One of the most widely prescribed of the fluoroquinolone class of antibiotics is ciprofloxacin (CIP) which is on the World Health Organization (WHO) model list of essential medicines containing the most effective and safe medicines needed in health system.[1] Due to its notable antimicrobial activity and excellent pharmacokinetic properties is common used antibiotic to treat bacterial infections caused by Gram-negative bacteria. It functions by inhibiting DNA gyrase and a type II topoisomerase, topoisomerase IV, necessary to separate bacterial DNA, thereby, inhibiting cell divisions. However, emergency and widely spread drug resistance pathogens making lower activity of ciprofloxacin. Thus, necessary is discover new antibacterials compounds with enhanced activity. A number of derivatives of ciprofloxacin have been reported that have shown improved activity and potency.[2] The development an efficient method for the synthesis of derivatives of ciprofloxacin has of great interest of chemists.[3] Recent evidences pay attention on the possible antitumor effects of ciprofloxacin and other derivative of fluoroquinolones.[4]
The multicomponent reaction, such as Passerini reaction, has been of special interest of chemists and biologist because of the large number of applications in medicinal chemistry and drug discovery, polymer, combinatorial chemistry, argochemistry and natural product chemistry.[5]
In the present studies, we will report a new synthetic strategy based on Passerini reaction leading to formation of ciprofloxacin peptidomimetics for Multi Drug Resistant tumor treatment.

References
[1]. Brunner M, (2004) Antimicrob Agents Chemother 48:3850–3857.
[2]. Azéma J, et al (2009) Bioorganic & Medicinal Chemistry 17: 5396–5407.
[3]. Akhtaret R, et al (2016) Synth. Commun 46: 1849–1879.
[4] Koltai T., DOI: 10.13140/RG.2.1.3255.1920
[5]. Domling A, Ugi I., (2000)Angew. Chem. Int. Ed. 39: 3168-3210.

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In silico study to elucidate possible interactions of Hsp90 inhibitors with P-gp

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Heat Shock Protein 90 (Hsp90) is an ATP-dependent molecular chaperone which interacts with a broad range of client proteins involved in cancerogenesis and cancer progression. However, Hsp90 inhibitors were unsuccessful as anticancer agents because of their limitations in physicochemical properties, safety profiles and efflux by membrane transporters responsible for multidrug resistance (MDR) such as P-glycoprotein (P-gp). In the efforts to develop dual targeting molecules with potential to act against both, deregulated cancer metabolism by Hsp90 inhibition and MDR mechanism by P-gp inhibition, eleven Hsp90 inhibitors containing an isoxazolonaphtoquinone core were synthesized and evaluated in sensitive and corresponding resistant cancer cells with P-gp overexpression [1]. Three compounds were identified as dual Hsp90 and P-gp inhibitors. This presentation describes *in silico* studies that were undertaken to elucidate possible interactions of the dual inhibitors with P-gp. In particular, docking simulations were performed using the recently resolved structures of the human P-gp extracted from Protein Data Bank (www.rcsb.org). These structures provide an excellent opportunity for comparison of substrate- and inhibitor-bound structures in the drug-binding cavity of P-gp [2]. Different docking protocols were compared and the one with the best performance on re-docking of the X-ray taxol and zosuquidar structures was selected in terms of: (i) similarity between the generated poses and the corresponding structures in the crystal complex, and (ii) calculated scores, that approximate the binding affinity. The P-gp-ligand interactions were analyzed to outline key residues potentially involved in binding. Based on the results, it was suggested that the binding sites of the studied compounds may partially overlap with a binding site of the P-gp substrate Rhodamine 123, implying that these compounds may act as its competitive inhibitors. The *in silico* results are in accordance with the experimental findings and contribute to the elucidation of the mechanism action of the dual inhibitors.

References

[1]. Dinić J, Podolski-Renić A, Jovanović M, Musso L, Tsakovska I, Pajeva I, Dallavalle S, Pešić M (2019). *Int J Mol Sci* 20, 4575.
[2]. Alam A, Kowal J, Broude E, Roninson I, Locher KP (2019) *Science* 363: 753–756.

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New pyrazolo[3,4-d]pyrimidine derivatives reverse multidrug resistance in cancer cells by inhibiting P-glycoprotein activity

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Multidrug resistance (MDR) represents the leading cause of cancer treatment failure. One of the main causes of MDR is overexpression of P-glycoprotein (P-gp). As a member of the ATP-binding cassette (ABC) transporter family, P-gp is responsible for reduced intracellular accumulation of both targeted therapies and classic chemotherapeutics. Tyrosine kinase inhibitors (TKIs) have been reported to interact with ABC transporters either as their substrates or inhibitors depending on the concentration range applied. We have investigated the anticancer potential of novel TKIs pyrazolo[3,4-d]pyrimidines and their prodrugs against two pairs of sensitive and MDR cancer cell lines with P-gp overexpression: non-small cell lung carcinoma (NCI-H460 and NCI-H460/R) and colorectal carcinoma (DLD1 and DLD1-TxR). The tested compounds displayed significant cell growth inhibition that was not compromised by the MDR phenotype. Treatment with the compounds inhibited P-gp activity in concentration- and time-dependent manneras revealed by the increase in accumulation of the P-gp substrate rhodamine 123. TKIs directly interacted with P-gp and inhibited its ATPase activity. The investigated pyrazolo[3,4-d]pyrimidines enhanced the efficacy of doxorubicin and paclitaxel in MDR cancer cells. The potential for reversing P-gp-mediated MDR makes investigated TKIs prospective candidates for further development regarding the treatment of resistant cancers.

Using the cinnamic pharmacophore for the design of multitarget compounds

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It is well known the implication of inflammation in numerous diseases such as cancer and senile dementia Alzheimer's type. Lipoxygenase (LOX) and Cycloxygenase (COX) pathways play an important role in inflammatory sites in correlation with the reactive oxygen species (ROS) produced during the inflammation by phagocytic leukocytes. ROS are involved in the LOX and COX mediated conversion of arachidonic acid into pro-inflammatory intermediates. Acetylcholinesterase (AchE) was used as another target implicated in the dementia and Alzheimer's disease. The aim of our study is to synthesize compounds bearing the cinnamic and curcumin pharmacophore, the coumarin and thiazole nucleus, the azomethine linkage as well as hybrids of cinnamic acids and drug like moieties. Computer-aided drug design was used for the candidates' synthesis selection, which was partly based on published procedures. The curcumin, cinnamic, coumarin, thiazole and hydrazone derivatives play a vital role in the formation of commercially important intermediate molecules which are necessary for the production of different bioactive compounds and drugs. Cinnamic acid derivatives present a wide range of biological activities: antituberculosis, antidiabetic, antioxidant, antimicrobial, hepatoprotective, central nervous system stimulant (CNS), antidepressant, anticholesterolemic, antimalarial, antiviral, anxiolytic, cytotoxic, and anti-inflammatory. Furthermore, the combination of appropriate pharmacophore groups led to conjugates with multi-target activities. In recent years, intensive research on hybrids has been conducted in order to create new multifunctional drugs. The results of our synthetic efforts are several groups of newly synthesized compounds that were subjected to further optimization. The new derivatives were characterized based on the structural characteristics and physicochemical properties of the molecules. Preliminary antioxidant and AChE inhibitory activity *in vitro* tests have been performed followed by inhibition of soybean LOX and COX. The physicochemical properties of the compounds were analyzed in terms of Lipinski's rule. Further investigation is in progress concerning their multi-target profile.

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Cytotoxic royleanones from *Plectranthus madagascariensis* as agents to overcome multidrug resistant in cancer

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The development of multidrug resistance (MDR) is the main cause of failure in cancer chemotherapy. *Plectranthus* spp. were recognized as potential sources of antitumor lead compounds, some of them with ability to inhibit the activity of MDR efflux pump P-glycoprotein (Pg-p) [1]. In this work, the polyphenol rosmarinic acid (**1**) and the abietane diterpenes 7α,6β-dihydroxyroyleanone (**2**), 7α-formyloxy-6β-hydroxyroyleanone (**3**), 7α-acetoxy-6β-hydroxyroyleanone (**4**), and coleon U (**5**) were isolated from *P. madagascariensis*, using different solvents. Additionally, 6,7-dehydroroyleanone (**6**) was obtained as the main constituent of the essential oil of *P. madagascariensis*. The cytotoxic activity of compounds **1** to **5** was evaluated in several human cancer cell lines. The cytotoxicity of **6** was previously established, specifically its ability to evade the P-gp activity [2]. Royleanones **2** and **4** exhibited similar growth inhibition of NCI-H460 and the MDR NCI-H460/R cancer cell lines, suggesting that **2** and **4** are not substrates for P-gp. Royleanones **4** and **6** were used to develop analogues with enhanced anti-P-gp activity. Eight new derivatives were obtained (**7** to **15**) and their P-gp inhibition potential was also evaluated. Derivatives 7α-acetoxy-6β-(4-chloro)benzoyloxy-12-*O*-(4-chloro)benzyl-royleanone (**7**) and 7α-acetoxy-6β-benzoyloxy-12-*O*-royleanone (**8**) showed promising potential anti-P-gp activity, with similar efficacy as Dex-VER (well stablished P-gp inhibitor). Moreover, molecular docking study suggested that the presence of benzoyl substituent in position C-6 seems particularly relevant for improving the anti-P-gp activity [3]. Thus, a suitable approach for further generation of novel derivatives may involve a selective modification of position C-12 with alternative substituents while keeping the benzoyl substituent at position C-6.

References

[1]. Matias D, Nicolai M, Saraiva L, et al. (2019) *ACS Omega* 4 (5): 8094–8103.
[2]. Garcia C, Silva CO, Monteiro CM, et al. (2018) *Future Med Chem* 1 (10): 1177–1189.
[3]. Garcia C, Isca VMS, Monteiro CM, et al. *Frontiers*: in submition.

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Aquaporins as emergent drug targets for cancer therapeutics

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Aquaporins (AQPs) are transmembrane protein channels that facilitate the diffusion of water and glycerol across cell membranes, crucial for water and energy homeostasis. A few AQP isoforms are overexpressed in different cancer cells and tissues, being involved in cell proliferation and migration, tumor formation, and angiogenesis, suggesting their great potential as novel drug targets for cancer treatment. In particular, AQP3 and AQP5 are strongly expressed in cancer tissues and revealed promising targets in several cancer types. Detecting AQPs expression and function in cells and tissues and screening modulators for the development of efficient medicines is of utmost importance. We have disclosed inhibition of AQP3 by copper- and gold-based compounds [1] and highlighted the relation between AQP3 expression, glycerol permeation across cell membranes and cancer cell proliferation [2]. The mechanism of inhibition has been recently described using molecular dynamics, combined with density functional theory and electrochemical studies [3]. Subsequently, we developed nanoformulations of a potent copper-based aquaporin inhibitor with cytotoxic effect against skin cancer cells [4] and reported their promising therapeutic potential *in vivo* in murine melanoma models [5]. AQPs are also involved in tumor invasion, metastasis and growth. Interestingly, we have detected aberrant expression of AQP5 in pancreatic tumors of high malignancy, suggesting it may also be used as a biomarker for early diagnosis of pancreatic adenocarcinoma [6]. Our recent studies showed that AQP5 transports H_2O_2 in addition to water and modulates cell resistance to oxidative stress. Moreover, by facilitating hydrogen peroxide permeation across membranes, AQP3 and AQP5 are regulators of cell migration and are involved in cell oxidative stress response in human pancreatic cancer cells [7]. Altogether, our studies unveil a major role of AQPs in cancer, highlighting their potential as drug targets for cancer therapeutics.

References

[1] Martins, A. P., Marrone, A., Ciancetta, A., Galan Cobo, A., Echevarria, M., Moura, T. F., Re, N., Casini, A., Soveral, G. (2012). *PloS one 7*: e37435.

[2] Serna, A., Galan-Cobo, A., Rodrigues, C., Sanchez-Gomar, I., Toledo-Aral, J. J., Moura, T. F., Casini, A., Soveral, G., Echevarria, M. (2014). *J Cell Physiol 229*: 1787-1801.

[3] de Almeida, A., Mosca, A. F., Wragg, D., Wenzel, M., Kavanagh, P., Barone, G., Leoni, S., Soveral, G., Casini, A. (2017). *Chem Commun (Camb) 53*: 3830-3833.

[4] Nave, M., Castro, R. E., Rodrigues, C. M., Casini, A., Soveral, G., Gaspar, M. M. (2016). *Nanomedicine (Lond) 11*: 1817-1830.

[5] Pinho, J. O., Amaral, J. D., Castro, R. E., Rodrigues, C. M., Casini, A., Soveral, G., Gaspar, M. M. (2019). *Nanomedicine (Lond) 14*: 835-850.

[6] Direito, I., Paulino, J., Vigia, E., Brito, M. A., and Soveral, G. (2017). *J Surg Oncol 115*: 980-996.

[7] Rodrigues, C., Pimpao, C., Mosca, A. F., Coxixo, A. S., Lopes, D., da Silva, I. V., Pedersen, P. A., Antunes, F., Soveral, G. (2019). *Cancers (Basel) 11*.

MENSADB: A major Structural Analysis of Membrane Protein Dimers

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In this work, we used *in silico* tools to perform a comprehensive and thorough structural and physico-chemical analysis of a curated collection of membrane protein dimer structures. Due to the significant lack of in-depth analysis of fundamental features of membrane proteins surface and their interfacial regions, this work brings essential insights about conservation of residues, Accessible Solvent Area (ASA) descriptors, average B-factors, intermolecular contacts at 2.5 Å and 4.0 Å distance cutoffs, salt-bridges, hydrogen-bonds, hydrophobic, π-π interactions, t-stacking and cation-π interactions. The data, easily assessed and downloaded by all the users is freely available through a real-time, user-friendly and interactive web-application, the MEmbrane protein dimer Novel Structure Analyser database (MENSAdb) - www.moreiralab.com/resources/mensadb.

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