17η Ημερίδα της Ελληνικής Εταιρείας Βασικής και Κλινικής Φαρμακολογίας

17 Μαΐου 2025, Τμήμα Φαρμακευτικής, ΕΚΠΑ, Πανεπιστημιούπολη Ζωγράφου

ΠΡΟΓΡΑΜΜΑ

9.00-09.30 Εγγραφές και ανάρτηση poster

9.30-11.00 ΣΥΝΕΔΡΙΑ 1η

***Στρογγυλή Τράπεζα*: Κλινικές μελέτες στην Ελλάδα – Ο ρόλος των φαρμακολόγων**

Ε. Μανωλόπουλος, Καθηγητής Φαρμακολογίας ΔΠΘ, Πρόεδρος ΕΟΦ, Συντονιστής

Λεωνίδας Κληρονόμος, Προϊστάμενος Τμήματος Κλινικών Μελετών ΕΟΦ

Το πλαίσιο των κλινικών μελετών στην Ελλάδα

Γιώργος Παπαζήσης, Καθηγητής Φαρμακολογίας ΑΠΘ

Κλινικές μελέτες πρώιμης φάσης στη Μονάδα Κλινικών Ερευνών της Ιατρικής ΑΠΘ στο Νοσοκομείο Παπαγεωργίου Θεσσαλονίκης

Κων/νος Ταμβακόπουλος, Ερευνητής Α', ΙΙΒΕΑΑ

Κλινικές δοκιμές στο ΙΙΒΕΑΑ - Ο ρόλος της Φαρμακολογίας

Διονύσης Βραχνής - Κων/νος Στρατάκης, SteroTherapeutics – IMBB-ITE

Παρειακή χορήγηση φλουαστερόνης: μια νέα θεραπεία για τις μεταβολικές επιπλοκές του συνδρόμου Cushing

11:00 – 11:45 Παρουσίαση και συζήτηση poster – Διάλειμμα

11.45-13.30 ΣΥΝΕΔΡΙΑ 2η

Προεδρείο: Ευαγγελία Παπαδημητρίου, Κωνσταντίνος Ξανθόπουλος

11.45-12.15 Γιάννης Χαραλαμπόπουλος, Καθηγητής Φαρμακολογίας, Ιατρική Σχολή, Πανεπιστήμιο Κρήτης

Novel pharmacological approaches against neurodegenerative diseases: exploiting the neurotrophins therapeutic potential.

12.15-12.45 Ταξιάρχης Κατσινέλλος, University of Cambridge, United Kingdom

Cellular systems of Tau templated-seeded aggregation in neurodegenerative diseases

12:45-13:30 Προφορικές ανακοινώσεις

ΟΑ1. Παρασκευάς Ζάμπας, ΙΙΒΕΑΑ και Τμήμα Φαρμακευτικής, ΕΚΠΑ
Protective role of MPST/H2S in the development of metabolic syndrome and vascular inflammation

ΟΑ2. Κωνσταντίνα Χανουμίδου, Ιατρική Σχολή, Πανεπιστήμιο Κρήτης
The multicellular etiology of glucose neurotoxicity in the dopaminergic system: the role of p75NTR receptor

ΟΑ3. Χαρίκλεια Ντέντη, Ιατρική Σχολή, ΑΠΘ
The impact of *glcci1* and *fkbp5* variations on ICS response in COPD patients: Clinical and histological findings from the HISTORIC study

13:30 – 13:45 Παρουσίαση και συζήτηση poster – Διάλειμμα

13:45 – 14:45 ΣΥΝΕΔΡΙΑ 3η

Προεδρείο: Αντώνης Γούλας, Κατερίνα Αντωνίου

ΟΑ4. Γιώργος Γαβριηλίδης, INEB-EKETA
Enhancing perturbation modeling in single-cell data through advanced deep learning approaches

ΟΑ5. Αναστασία Παλαιολόγου, ΙΙΒΕΑΑ
Pharmacology's silent power: how analytical chemistry provides the answers

ΟΑ6. Κωνσταντίνος Παπαδημάτος, Τμήμα Φαρμακευτικής, Πανεπιστήμιο Πατρών
Differential effect of angiogenesis inhibitors on VEGFR2 tyrosine phosphorylation and signaling

ΟΑ7. Κ. Λαφαζάνης, Τμήμα Ιατρικής, Πανεπιστήμιο Θεσσαλίας
Στόχευση των P90RSK για την ανάπτυξη νέων αντικαρκινικών θεραπειών

ΟΑ8. Νάγια Νικολάου, Τμήμα Φαρμακευτικής, ΕΚΠΑ
Mechanist insights in light chain-associated cardiovascular toxicity: implication of IRE-1/NF-kB/ICAM-1 pathway activation

14:50 – 15:15 Αφιέρωμα και βραβεία στη μνήμη της Ιωάννας Ανδρεάδου

Αντρέας Παπαπετρόπουλος, Καθηγητής Φαρμακολογίας – Μοριακής Φαρμακολογίας, Τμήμα Φαρμακευτικής, ΕΚΠΑ

15:15 – 16:30 Εκλογοαπολογιστική συνέλευση και αρχαιρεσίες για εκλογή νέου ΔΣ

ΠΡΟΦΟΡΙΚΕΣ ΑΝΑΚΟΙΝΩΣΕΙΣ

OA1

Protective role of MPST/H_2S in the development of metabolic syndrome and vascular inflammation

Paraskevas Zampas[1,4], Li Zhen[2], Antonia Katsouda[1,4], Aimilia Varela[3], Stelios Psarras[5], Constantinos H Davos[3], David J Lefer[2], Andreas Papapetropoulos[1,4]

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[5] Center of Basic Research, Biomedical Research Foundation Academy of Athens, Greece.

AIM: Metabolic syndrome (MetS) is a pathophysiological condition characterized by the co-existence of obesity, insulin resistance, hypertension, and dyslipidemia. Hydrogen sulfide (H_2S) is a signaling molecule, involved in various physiological pathways and cardiometabolic diseases. Herein, we determine the role of mercaptopyruvate sulfurtransferase (MPST) -an enzyme responsible for H_2S production in mammalian tissues- in a novel model of MetS. **Methods:** C57BL6/J male mice were fed a high fat diet (HFD) for 15 weeks to induce obesity and hyperglycemia. To induce hypertension, the nitric oxide synthase inhibitor L-NAME was administered during the final 5 weeks. Body weight and glucose tolerance were monitored. Blood pressure was determined using tail cuff, while mice also underwent echocardiographic and hemodynamic assessment of left ventricular (LV) function, as well as an exercise exhaustion test. Cardiac tissues were stained with picrosirius red, and the reactivity of aortic rings was evaluated. The expression of H_2S-synthesizing and -degrading enzymes were analyzed via western blot, and H_2S levels were detected using gas spectrometry. We also examined the effects of *Mpst* gene deletion in this MetS model. Additionally, we performed an RNA-seq in aorta to identify differentially expressed genes between genotypes. Finally, mice with MetS were treated for the last 5 weeks with either SG1002, a polysulfide donor, or GYY4137, a slow releasing H_2S donor. **Results:** HFD+LNAME combination leads to MetS that caused mild LV diastolic dysfunction, cardiac fibrosis and vascular endothelial dysfunction. Free H_2S and sulfane-sulfur levels were reduced in the aorta of mice with MetS, but not the heart. MPST and thiosulfate sulfurtransferase (TST) enzymes' expression were reduced in the aorta of mice with MetS. Mice with global MPST deletion showed increased body weight and greater glucose intolerance, but no significant change in blood pressure under HFD+LNAME treatment. Also, $Mpst^{-/-}$ mice exhibited exacerbated cardiac fibrosis. Interestingly, RNA-seq data analysis in aortas showed upregulation of proteins involved in immune response, especially correlated with T-cell activation in $Mpst^{-/-}$ mice with MetS. Finally, the administration of SG1002 ameliorated obesity and improved glucose metabolism, while GYY4137 showed additional antihypertensive effects, reversed cardiac fibrosis and improved the endothelium-dependent relaxation of aortic rings. **Conclusion:** MetS leads to dysregulation of key enzymes involved in H_2S metabolism in the aorta, resulting in reduced endogenous H_2S levels. MPST deficiency worsens metabolic and cardiovascular parameters and contributes to vascular inflammation and cardiac fibrosis. Pharmacological restoration of H_2S levels may offer a promising therapeutic strategy for MetS.

Acknowledgments: The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 2nd PhD Fellowship grant (Fellowship Number: 1087)

OA2

The multicellular etiology of glucose neurotoxicity in the dopaminergic system: the role of p75NTR receptor

Konstantina Chanoumidou[1,2], Maria Anna Papadopoulou[1,2], Chrystalla Konstantinou[1,2], Alexandros Tsimpolis[1,2], Anne Grünewald[3], Achille Gravanis[1,2], Ioannis Charalampopoulos[1,2]

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Background: Hyperglycemia is a hallmark of Diabetes Mellitus, a complex metabolic disorder associated with significant neurological complications. Epidemiological data and findings from animal models indicate that diabetes can alter the dopaminergic system, thereby increasing the risk for Parkinson's Disease; however, the underlying molecular mechanisms remain poorly understood. To this end, we examined both direct and glial-mediated effects of hyperglycemia on dopaminergic neurons using a human-induced pluripotent stem cell (iPSC)-derived model. Given the established pro-apoptotic function of the pan-neurotrophin receptor p75NTR in neurons, and its previously reported involvement in diabetic peripheral neuropathy and retinopathy, we investigated its potential contribution to glucose-induced neurotoxicity within the central nervous system. **Methods:** Neurons, astrocytes, and microglia were differentiated from human iPSCs and exposed to high glucose for 48h to simulate hyperglycemia. Cytotoxicity assays, molecular and transcriptomic analyses were used to decipher the mechanisms of hyperglycemia-induced neurotoxicity and the role of p75NTR. ELISA was used to quantify pro-NGF in high glucose-treated neurons. Neurons were treated with astrocyte- and microglia-conditioned media to investigate changes in neuronal-glia communication in hyperglycemia. **Results:** Hyperglycemia led to DNA damage, activation of the JNK pathway and cell death of dopaminergic neurons in a dose-dependent manner. Furthermore, glucose overload increased neuronal susceptibility to the cytotoxic effects of 6-OHDA and Amyloid-β. We found up-regulated levels of p75NTR and its pro-apoptotic ligand pro-NGF in high glucose-treated neurons. Notably, modulation of p75NTR activity prevented neuronal cell death, revealing p75NTR receptor as a novel mediator of glucose neurotoxicity. We tested BNN27, a synthetic NGF mimetic which activates p75NTR and TrkA receptors, against HG-induced toxicity and we highlight its neuroprotective action in hyperglycemia. Finally, we demonstrated the contribution of glial cells to neurodegeneration. Exposure of iPSC-derived astrocytes and microglia to high glucose induced the secretion of neurotoxic factors with a secondary pro-apoptotic effect on dopaminergic neurons, depicting the complexity of cellular communication under neurodegenerative conditions. **Conclusions:** Our study provides insights into the mechanism of glucose neurotoxicity in human dopaminergic neurons and suggests the therapeutic potential of targeting the pro-apoptotic pro-NGF/p75NTR axis. Additionally, we demonstrate the involvement of glial cells in dopaminergic neurodegeneration in hyperglycemia. Our findings underline the importance of glycemic control in patients with PD predisposition to delay the deterioration of the dopaminergic system.

OA3

The Impact of *GLCCI1* and *FKBP5* Variations on ICS Response in COPD Patients: Clinical and Histological Findings from the HISTORIC Study

Charikleia Ntenti[1,2,3,5], Eleni Papakonstantinou[1,2], Leticia Grize[1], Antonis Goulas[3,4], Daiana Stolz[1,2]

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Introduction: Variations in the *FKBP5* and *GLCCI1* genes -both implicated in the GC-GR cascade- have been associated with response to corticosteroid treatment in various diseases. **Methodology:** A total of 165 COPD patients from the HISTORIC study, a double-blind, randomized, placebo-controlled trial, were genotyped for the functional SNVs rs4713916 (*FKBP5*) and rs37973 (*GLCCI1*). Baseline clinical and histological data from endobronchial biopsies, along with patients' responses to inhaled corticosteroids (ICS)—assessed by FEV1 changes and health-related QoL tests—were examined in relation to the two SNVs. **Results:** AG carriers of rs4713916 exhibited significant FEV1 change in both intergroup and intragroup analyses ($p=0.022$ and $p<0.0001$, respectively) (Fig. 1). A significant, novel association of rs37973 with the expression of the active GR receptor subunit (GRα), was also observed ($p \leq 0.045$) (Fig. 2). **Conclusion:** Our findings underscore the importance of genetic variations on response to ICS, revealing key associations and revitalizing interest in pharmacogenetics in COPD, which has waned due to inconsistencies in recent research.

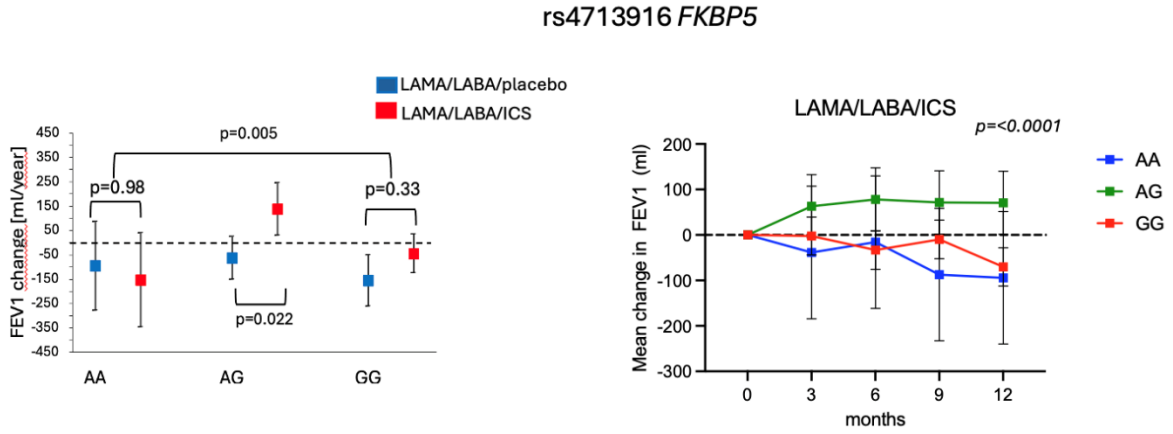

Figure 1

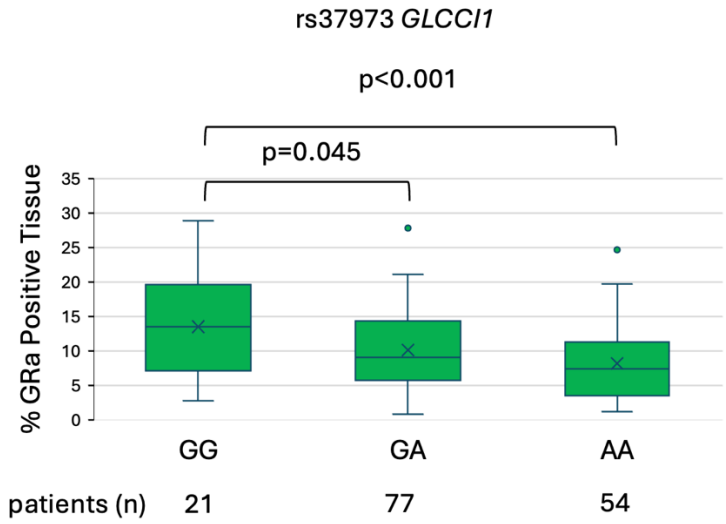
rs37973 GLCCI1
p<0.001
p=0.045
% GRa Positive Tissue
35
30
25
20
15
10
5
0
GG
GA
AA
patients (n)
21
77
54

Figure 2

OA4

Enhancing Perturbation Modeling in Single-Cell Data through Advanced Deep Learning Approaches

George Gavriilidis[1], Aspasia Orfanou[1], Vasileios Vasileiou[1,2], Theodoros Kantzalis[3], Myrthe van Baardwijk[4], Jiedan Xao[5], Naveed Ishaque[6], Andrew Stubbs[4], Pericles A. Mitkas[3], Fotis Psomopoulos[1]

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Perturbation modelling in single-cell data is crucial for studying molecular changes elicited due to molecular knockouts, chemical compounds, and biological stimulants across health and disease phenotypes. Perturbation modelling is confounded by scarce biological explainability, statistical uncertainty in Deep Learning (DL) predictions in extreme perturbation scenarios, hyperparameter optimization, and limited scalability to multi-omic single-cell data. We present the Mongoose project (Multi-Objective Network Generator Of Optimized Single-cell Experiments) to explore enhancing perturbation modelling in complex single-cell datasets through Multi-Task Learning (MTL). Mongoose combines (i) UnitedNet, a DL framework that employs MTL to simultaneously perform joint group identification and cross-modal prediction with Sharpley values (SHAP) as an explainability component and (ii) perturbation modelling tools like SCING and GenKI, which reconstruct and perturb cell type-specific gene-regulatory networks (GRNs). UnitedNet contains an encoder-decoder-discriminator structure which approximates the statistical characteristics of each modality without prior assumptions. Hence, we claim that UnitedNet can facilitate biologically informed decisions on conducting ensuing digital KOs across reverse-engineered GRNs by providing (i) better cell-type clusters and (ii) SHAP values of significant cross-modal/cell-type associations from complex single-cell and spatial omic datasets. We will showcase the Mongoose approach on complex multi-omic mRNA/protein datasets like the Perturb-CITE-seq (CRISPR knockouts) and the spatial DBiT-seq mouse embryo dataset, where mRNA-protein associations and spatial niche identification are expected to play pivotal roles in perturbations like in-silico GRN digital KOs. We anticipate that Mongoose will provide perturbational insights closer to ground truths, ultimately highlighting critical transcription factors and signalling pathways with potential translational value.

OA5

Pharmacology's silent power: how analytical chemistry provides the answers

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Introduction: Analytical chemistry plays a pivotal role in pharmacological research, offering precise and reliable tools to tackle complex drug related questions. Its methods are essential for quantifying therapeutic agents, verifying their presence in biological matrices, and supporting drug development through robust analytical evidence. By elucidating both the identity and concentration of compounds within complex systems, analytical chemistry directly contributes to the advancement of pharmacological hypotheses and therapeutic strategies. Here we present two case studies: in the first we quantified a novel antisense oligonucleotide (ASO) drug targeting Parkinson's disease in brain tissue[1]; in the second, we measured a newly developed senolytic agent targeting senescent cells in cell cultures[2]. **Methods:** Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed among other analytical techniques due to its high specificity, sensitivity and adaptability for complex biological matrices. For each study, a custom LC-MS/MS method was developed and optimized, including the use of an appropriate internal standard. The ASO method was validated in accordance with EMA bioanalytical guidelines, including parameters such as linearity, accuracy, precision, matrix effects and selectivity. Sample preparation for the ASO involved tissue homogenization, protein digestion with Proteinase K and solid-phase extraction. For the senolytic compound, preparation involved cell lysis followed by protein digestion with cold acetonitrile. Chromatographic separation was tailored for each analyte using the appropriate reversed-phase column and mobile phase composition and adjusting the optimum gradient elution. Detection was performed in multiple reaction monitoring (MRM) mode to ensure reliable identification and quantification. **Results:** In the first study, the novel

antisense oligonucleotide was successfully quantified in rat brain homogenates, achieving an LLOQ of 1 ng/mg tissue. The compound remained detectable for up to 45 days post-administration, indicating sustained CNS exposure which is essential for maintaining therapeutic efficacy. In the second study, the senolytic compound was quantified in cell lysates. The LLOQ was established at 0.05 ng and analysis revealed significantly elevated concentrations in senescent cells compared to non-senescent controls. This selective accumulation supported the hypothesis of targeted action, underscoring the compound's therapeutic potential. **Conclusions:** In both applications, analytical chemistry effectively addressed key pharmacological questions by confirming the presence of therapeutic agents in relevant biological systems. The high selectivity of the LC-MS/MS methods enabled specific and robust detection, making them highly suitable for such investigations. Moreover, the prolonged detection of the ASO in brain tissue and the selective uptake of the senolytic compound in senescent cells underscore the value of these methods in advancing pharmacological research and therapeutic development.

(1) Palaiologou, A.; Naki, M.; Pantazopoulou, M.; Kattan, F.-G.; Stefanis, L.; Doxakis, E.; Tamvakopoulos, C. A Bioanalytical Liquid Chromatography Tandem Mass Spectrometry Approach for the Quantification of a Novel Antisense Oligonucleotide Designed for Parkinson's Disease: A Rat Brain Biodistribution Study. *ACS Pharmacol. Transl. Sci.* **2025**. https://doi.org/10.1021/acsptsci.4c00698.

(2) Magkouta, S.; Veroutis, D.; Papaspyropoulos, A.; Georgiou, M.; Lougiakis, N.; Pippa, N.; Havaki, S.; Palaiologou, A.; Thanos, D.-F.; Kambas, K.; Lagopati, N.; Boukos, N.; Pouli, N.; Marakos, P.; Kotsinas, A.; Thanos, D.; Evangelou, K.; Sampaziotis, F.; Tamvakopoulos, C.; Pispas, S.; Petty, R.; Kotopoulos, N.; Gorgoulis, V. G. Generation of a Selective Senolytic Platform Using a Micelle-Encapsulated Sudan Black B Conjugated Analog. *Nat Aging* **2025**, *5* (1), 162–175. https://doi.org/10.1038/s43587-024-00747-4.

OA6

Differential effect of angiogenesis inhibitors on VEGFR2 tyrosine phosphorylation and signaling

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Forming new blood vessels is a significant process in physiological situations, such as development, and pathologies, such as cancer. Several studies on the mechanisms involved have highlighted vascular endothelial growth factor A (VEGFA) and its tyrosine kinase receptor VEGFR2 as significant mediators in the regulation of angiogenesis, leading to the development of numerous drugs that inhibit binding of VEGFA to VEGFR2 or activation of VEGFR2 following VEGFA binding. Other membrane molecules significantly regulating angiogenesis involve $\alpha_v\beta_3$ integrin, cell surface nucleolin (NCL), and receptor protein tyrosine phosphatase zeta 1 (PTPRZ1). Inhibitors of these molecules also inhibit VEGFA-induced endothelial cell activation and angiogenesis, though it remains unclear whether and how they affect VEGFA signaling downstream of VEGFR2. In the present work, we used pharmacological inhibitors of $\alpha_v\beta_3$ integrin, cell surface NCL, and PTPRZ1 to study their effect on VEGFR2 tyrosine phosphorylation and downstream signaling. All the inhibitors used were found to abolish $VEGFA_{165}$-induced endothelial cell migration. Inhibition of cell surface NCL by the pseudopeptide N6L was found to significantly inhibit the $VEGFA_{165}$-induced tyrosine phosphorylation of VEGFR2 at tyrosines 951, 996, 1059, 1175, and 1212, as well as the downstream activation of phospholipase C gamma (PLCγ) and ERK1/2. The $\alpha_v\beta_3$ integrin inhibitors, LM609 (neutralizing antibody) and $PTN_{112\text{-}136}$, have a minor effect on VEGFR2 tyrosine 1175 phosphorylation and partially inhibit PLCγ and ERK1/2 activation induced by $VEGFA_{165}$. A peptide or an antibody that inhibits VEGFA binding to PTPRZ1 partially inhibits VEGFR2 tyrosine 996 and 1175 phosphorylation (only the peptide), does not affect ERK1/2 activation, but abolishes $VEGFA_{165}$-induced Akt activation. These data support the notion that $\alpha_v\beta_3$ integrin, cell surface NCL, and PTPRZ1 functionally interact and affect VEGFR2 signaling to regulate endothelial cell migration and angiogenesis.

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ΟΑ7

Στόχευση των P90RSK για την ανάπτυξη νέων αντικαρκινικών θεραπειών

Κ. Λαφαζάνης[1], Α. Σακελλαροπούλου[2], Φ. Κουτσουγιάννη[1], Δ. Αλεξοπούλου[1], Κ. Δεμέτζος[2], Γ. Λεονταρίτης[3], Ν. Σακελλαρίδης[1], Κ. Δήμας[1]

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Εισαγωγή: Ο καρκίνος του παγκρέατος αποτελεί την τέταρτη αιτία θανάτου από καρκίνο παγκοσμίως. Οι 90 kDa ribosomal S6 kinases (RSKs) δρουν ως καθοδικοί τελεστές στο σηματοδοτικό μονοπάτι MAPK (RAS/RAF/MEK/ERK). Οι ενεργοποιημένες RSK κινάσες εμπλέκονται σε διάφορες κυτταρικές διεργασίες, όπως αυτών της μεταγραφής, της μετάφρασης, της ρύθμισης του κυτταρικού κύκλου και της κυτταρικής επιβίωσης. Στη διεθνή βιβλιογραφία φλαβονοειδή της σειράς της κεμπφερόλης (π.χ. SL0101) αναφέρονται να εμφανίζουν σημαντική αντικαρκινική δράση in vitro η οποία προκύπτει ως αποτέλεσμα της δυνατότητας τους να αναστέλλουν τη δράση της στην RSK-2. Προηγούμενα πειράματα στο εργαστήριο φαρμακολογίας έδειξαν ότι και το ακετυλιωμένο παράγωγο του Τιλιροσίδη (Tac), έχει αξιοσημείωτη αντικαρκινική δράση, τόσο in vitro όσο και in vivo (xnografts) εναντίον της κυτταρικής σειράς HC116 (human colorectal cancer), ασκώντας, τουλάχιστον εν μέρει, τη δράση του μέσω αναστολής της δράσης της RSK-2 και κατά συνέπεια της αναστολής της λειτουργίας του MAPK μονοπατιού σηματοδότησης. **Σκοπός:** σκοπός της παρούσας εργασίας είναι η στοχευμένη αναστολή των RSK και κατ' επέκταση του MAPK σηματοδοτικού μονοπατιού με τη χρήση φαρμακολογικών αναστολέων με απώτερο στόχο την ανάπτυξη νέων αντικαρκινικών θεραπειών πιο αποτελεσματικών και περισσότερο ασφαλών για το πορογενές παγκρεατικό αδενοκαρκίνωμα (pancreatic ductal adenocarcinoma, PDAC). **Υλικά και Μέθοδοι:** η κυτταροτοξική δράση επιλεγμένων αναστολέων (Tac, SL0101, FMK, PF4708671, BI-D1870 & BX-912) μελετήθηκε στις ανθρώπινες παγκρεατικές σειρές AsPC-1, Bx-PC3, MIA Paca-2 και PANC-1 (εγκαθιδρυμένες) και στην EANM–att021013 (πρωτογενής κυτταρική σειρά) με την τεχνική SRB (sulforhodamine B). Η ανάλυση του κυτταρικού κύκλου έγινε με τη χρήση κυτταρομετρίας ροής (FACS) μετά από χρώση με Propidium Iodide. Για τη μελέτη του τύπου του κυτταρικού θανάτου που επάγεται μετά τη θεραπεία των κυττάρων με Tac αξιολογήθηκαν τα επίπεδα της προ-κασπάσης 8, της προ-κασπάσης 9, της προ-κασπάσης 3 και της PARP1, με τη χρήση Western Blot (WB). Τέλος, με τη χρήση WB διερευνήθηκε η επίδραση του Tac στην οδό σηματοδότησης MAPK με τη μελέτη της έκφρασης της ολικής RSK2 και των επιπέδων φωσφορυλίωσης της Ser-227 της κινάσης. **Αποτελέσματα:** Ο ελέγχος κυτταροτοξικότητας κατέδειξε ότι από τους αναστολείς που δοκιμάσθηκαν ο Tac είχε την ισχυρότερη *in vitro* αντικαρκινική δράση. Τα αποτελέσματα από την κυτταρομετρία ροής έδειξαν ότι το φλαβονοειδές αυτό επάγει ισχυρή κυτταρόσταση στη G1 φάση. Με βάση τα επίπεδα των κασπασών και της PARP1 φαίνεται ότι ο κύριος μηχανισμός κυτταρικού θανάτου είναι η επαγωγή απόπτωσης μέσω του ενδογενούς μονοπατιού με δόσο και χρόνο εξαρτώμενο τρόπο. Τέλος, η επώαση των κυττάρων με Tac μείωσε σημαντικά τα επίπεδα φωσφορυλίωσης της Ser-227 με δόσο και χρόνο εξαρτώμενο τρόπο καταδεικνύοντας ότι η υπό μελέτη ένωση ασκεί τουλάχιστον εν μέρει τη δράση της στοχεύοντας την RSK2. **Συμπεράσματα:** ο Tac παρουσίασε ισχυρή *in vitro* κυτταροτοξική δράση σε ανθρώπινες κυτταρικές σειρές παγκρεατικού καρκίνου, η οποία φαίνεται να μεσολαβείται τουλάχιστον εν μέρει από την αναστολή της δράσης της RSK2 με δοσο- και χρονο-εξαρτώμενο τρόπο. Περεταίρω μελέτες για πιο λεπτομερή διερεύνηση του μηχανισμού δράσης της ένωσης αυτής και της in vivo αντικαρκινικής δράσης του σε ζωικά πρότυπα παγκρεατικού καρκίνου σε πρωτογενή

ξενομοσχεύματα από όγκους ασθενών (Patient Derived Xenografts, PDX) είναι ήδη σε εξέλιξη στο εργαστήριο μας.

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OA8

Mechanist insights in light chain-associated cardiovascular toxicity: implication of IRE-1/NF-kB/ICAM-1 pathway activation.

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+ We wish to dedicate this work to the memory of Professor Ioanna Andreadou, who died prematurely and unexpectedly while supervising this work, and to whom we are all profoundly indebted, for her substantial impact on recent advances in the field. Above all, we cherish her guidance, friendship, and the inspiration she provided to us all.

Background: Light chain amyloidosis (AL) is a plasma cell dyscrasia where cardiac involvement is a major determinant of prognosis. Immunoglobulin light chains (LCs) are known to contribute to cardiotoxicity, but the precise mechanisms remain unclear. This study aimed to investigate LC-induced cardiotoxicity in vitro and in vivo and explore potential protective therapies. **Methods:** Amyloidogenic LCs from seven AL patients with cardiac involvement and isotype-matched LCs from two healthy donors were produced and characterized for their amyloidogenicity. Cardiotoxicity was initially assessed in primary adult murine ventricular cardiomyocytes (pAVMCs). In vivo, male C57BL6 mice received intramyocardial injections (IMC) of LCs (1 mg/mouse) or vehicle, while male and female NSG

mice received an AL LC (200 µg daily, intraperitoneally) for one or two months. Cardiac function was evaluated by echocardiography, followed by histological, biochemical, and molecular analyses. Based on identified toxicity mechanisms, pharmacological interventions were tested in vitro. **Results:** Five AL-derived LCs significantly reduced pAVMC viability and exhibited amyloid formation in vitro. In vivo, IMC administration led to cardiac damage, with lambda-type LCs significantly decreasing ejection fraction, whereas kappa-type LCs did not impair systolic function. LC-induced toxicity was associated with endoplasmic reticulum stress (ERS) via the IRE-1/NF-kB axis. In NSG mice, LC treatment prolonged isovolumic contraction time and significantly elevated NT-proBNP, urea, and creatinine. Histology revealed microvascular damage and perivascular fibrosis, while proteomics on the myocardium of NSG mice indicated VCAM-1 upregulation and NF-kB/p38/ICAM-1 activation. Amyloid deposits were absent. Treatment with the IRE-1α inhibitor STF-083010 and the ERS/NF-kB modulator TUDCA mitigated LC-induced cardiotoxicity in vitro. **Conclusions:** AL-derived LCs induce cardiovascular damage through ERS and NF-kB/p38/ICAM-1 pathways, independent of amyloid deposition. These findings provide insights into AL-associated cardiac dysfunction and highlight potential therapeutic targets.

ΑΝΑΡΤΗΜΕΝΕΣ ΑΝΑΚΟΙΝΩΣΕΙΣ (POSTER)

Poster 1

Inflammatory macrophage infiltration reduces subcutaneous adipocyte MPST levels through IL-1β secretion

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Introduction: MPST sulfurtransferase is a sulfide-species generating enzyme downregulated in adipose tissue depots of obese mice and humans[1,2]. *Mpst* genetic ablation in mice fed a high fat diet leads to increased body weight, excessive inguinal white adipose tissue (iWAT) fat accumulation and upregulation of inflammatory pathways[3]. Adipose tissue macrophages (ATMs) constitute the most abundant infiltrating immune cells cross-talking with adipocytes. During obesity, ATMs differentiate into inflammatory M1-like phenotype and sustain low-grade inflammation by secreting TNFα, IL-1β and IL-6 cytokines[3]. **Aim**: Our study aimed to characterize the infiltration of adipose tissue macrophages under *Mpst* deletion and examine their potential contribution into the observed MPST reduction. **Methods:** WT and *Mpst*$^{-/-}$ C57BL/6 male mice fed normal (10%kcal) or high fat (45%kcal) diet for 16 weeks. iWAT was collected and ATMs phenotypes were characterized using FACS analysis for CD11b, F4/80, CD11c markers and TNFα, IL-1β, IL-6 cytokines. Bone marrow monocytes and iWAT pre-adipocytes were isolated from WT mice and differentiated into macrophages and mature adipocytes. Bone marrow-derived macrophages (BMDMs) were polarized to M1-like phenotype by LPS (100ng/ml) and IFNγ (20ng/ml) for 24hrs. iWAT-derived adipocytes were treated for 48hrs with BMDMs conditioned media or TNFα, IL-1β, IL-6 cytokines (100ng/ml). Gene expression of *Mpst*, *TNFα, IL-1β, IL-6* was detected by RT-qPCR. MPST protein levels were analyzed by Western blot. **Results:** Total numbers of $CD11b^+$ $F4/80^+$ ATMs infiltrating iWAT increase during obesity. The population of inflammatory $CD11c^+$ M1-like ATMs is upregulated in *Mpst*$^{-/-}$ compared to WT obese mice (9.9±1.1% vs 16.0±2.1%, *$p<0.05$), as well as ATMs IL-1β secretion (3.8±0.3% vs 6.4±1.0%, *$p<0.05$). Adipocyte MPST protein levels are decreased by TNFα and IL-1β by 35.1±7.6% and 25.4±7.4%, respectively. In particular, M1-like BMDMs conditioned media downregulates MPST protein levels in adipocytes by 51.0±7.0% because of increased IL-1β content.**Conclusions:** We demonstrate that *Mpst* deletion potentiates the infiltration of IL-1β-producing inflammatory macrophages in iWAT during obesity. Furthermore, IL-1β release by macrophages leads to reduced MPST protein in adipocytes. Our findings propose a cross-correlation between MPST and IL-1β secreting macrophages in adipose tissue and contribute to a better understanding of the complex immune-related mechanisms driving obesity pathogenesis and progression.

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Poster 2

Η γ-λυάση της κυσταθειονίνης επάγει τη φαιοποίηση του λευκού λιπώδους ιστού

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Τα μπεζ λιποκύτταρα αναπτύσσονται στον λευκό λιπώδη ιστό (white adipose tissue, WAT) σε απάντηση σε εξωτερικά ερεθίσματα και προάγουν τη δαπάνη ενέργειας μέσω της θερμογένεσης, μιας διαδικασίας που καταλύεται από την πρωτεΐνη αποσύζευξης-1 (uncoupling protein-1, UCP1). Δεδομένου του υψηλού μεταβολικού ρυθμού που παρουσιάζουν, η επαγωγή του σχηματισμού τους στον WAT (φαιοποίηση) δύναται να μετατρέψει τον λευκό λιπώδη ιστό από ένα όργανο αποθήκευσης ενέργειας σε ένα όργανο δαπάνης αυτής και επομένως να αποτελέσει στρατηγική βελτίωσης της μεταβολικής υγείας. Η γ-λυάση της κυσταθειονίνης (cystathionine γ-lyase, CTH), ένα ένζυμο που συμμετέχει στο μονοπάτι της δια-θείωσης (transsulfuration pathway), παρουσιάζει ισχυρή αρνητική συσχέτιση με την έκφραση του UCP1 στον ανθρώπινο WAT. Απαλοιφή του γονιδίου *Cth* σε μύες ($Cth^{-/-}$) συνεπάγεται αύξηση των επιπέδων έκφρασης του *Ucp1* και δημιουργία συστάδων μπεζ λιποκυττάρων στον WAT. Τα $Cth^{-/-}$ πειραματόζωα παρουσιάζουν μειωμένη μάζα λευκού λιπώδους ιστούς και αυξημένη ευαισθησία στη γλυκόζη. Η ρυθμιστική δράση του CTH στην έκφραση του *Ucp1* επιβεβαιώθηκε επιπροσθέτως με τη χρήση του φαρμακολογικού αναστολέα αυτού, προπαργυλογλυκίνη. Η φαιοποίηση του WAT που παρατηρήθηκε σε μύες με ειδική απαλοιφή του *Cth* στα λιποκύτταρα ($Cth^{Adipoq\text{-}KO}$), επιβεβαίωσε την εξειδικευμένη δράση του ενζύμου στον συγκεκριμένο πληθυσμό κυττάρων; οι $Cth^{Adipoq\text{-}KO}$ μύες παρουσίασαν αυξημένη έκφραση UCP1, μειωμένη μάζα λευκού λίπους και βελτιωμένη ευαισθησία στη γλυκόζη. *In vitro* προσεγγίσεις με τη χρήση πρωτογενών κυτταροκαλιεργειών, υπέδειξαν τη διαδικασία της δια-*διαφοροποίησης (trans-differentiation)* των λευκών λιποκυττάρων ως την υπεύθυνη για τη δημιουργία του παρατηρούμενου φαινοτύπου. Προκειμένου να διερευνηθούν οι μοριακοί μηχανισμοί που συνδέουν την αναστολή του CTH με την ανοδική ρύθμιση του *Ucp1* στον λευκό λιπώδη ιστό, χρησιμοποιήθηκε προσέγγιση πολλαπλών-ωμικών αναλύσεων (multi-omics) ακολουθούμενη από βιοπληροφορική ανάλυση. Τα αποτελέσματα που προέκυψαν από την RNA αλληλούχιση (RNA sequencing) αποκάλυψαν την ύπαρξη εκτεταμένου μεταγραφικού επαναπρογραμματισμού στον WAT των $Cth^{-/-}$ μυών, ενώ η πρωτεωμική ανάλυση και οι αναλύσεις του προφίλ των σουλφυδριλιωμένων (persfulfidomics) και φωσφορυλιωμένων πρωτεϊνών (phosphoproteomics) αποκάλυψαν την ύπαρξη σημαντικών αλλαγών σε μεταφραστικό και μετα-μεταφραστικό επίπεδο. Η απαλοιφή του *Cth* οδήγησε σε μείωση των επιπέδων πληθώρας σουλφυδριλιωμένων πεπτιδίων μεταξύ των οποίων συγκαταλέγεται μεγάλος αριθμός πρωτεϊνών με δράση κινάσης ή φωσφατάσης. Η λειτουργική σημασία της παραπάνω παρατήρησης επικυρώθηκε με την ανάλυση φωσφοπρωτεομικής, όπου παρατηρήθηκε αύξηση στα επίπεδα φωσφορυλίωσης σημαντικού αριθμού πρωτεϊνών, συμπεριλαμβανομένων αρκετών μεταγραφικών παραγόντων. Ο GABPA **(**GA-binding protein

alpha chain) ταυτοποιήθηκε ως ρυθμιστής της επαγόμενης από το CTH-θερμογένεσης. Η πρόσδεση του GABPA στον υποκινητή του Ucp1 επιβεβαιώθηκε με τη χρήση της ανοσοκατακρήμνισης χρωματίνης (Chromatin Immunoprecipitation, ChIP), ακολουθούμενης από αλληλούχιση (ChIP-Seq). Τα αποτελέσματα της συγκεκριμένη μελέτης αποδεικνύουν το ρυθμιστικό ρόλο του ενζύμου CTH στην φαιοποίηση του λευκού λιπώδους ιστού και υποδεικνύουν νέους στόχους για την ανάπτυξη θεραπευτικών προσεγγίσεων με στόχο τη βελτίωση της μεταβολικής υγείας.

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Poster 3

Cth/Mpst double ablation results in early onset fatty liver disease in lean mice

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Aim: Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by increased liver mass as a result of liver steatosis and is the hepatic manifestation of the metabolic syndrome. Hydrogen sulfide (H_2S), a gasotransmitter, is endogenously generated by cystathionine-γ lyase (CTH), cystathionine-β synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (MPST) enzymes. The liver tissue (and hepatocytes in culture) expresses all three major H_2S-producing enzymes, CTH, CBS and MPST and has one of the highest capacities for H_2S production among mammalian tissues. *Cth* global or hepatocyte-specific deletion in mice leads to increased hepatic lipid deposition under high fat diet (HFD) conditions. HFD feeding or aging of *Mpst*$^{-/-}$ mice results in an enhanced fatty liver phenotype. So, the current study aimed to enlighten the role of endogenously produced H_2S by CTH and MPST enzymes in the pathogenesis of MASLD. **Methods:** Young *Cth/Mpst* knockout (*Cth/Mpst*$^{-/-}$) mice, fed a normal diet were sacrificed, liver tissues were isolated and used for histological studies, protein expression analysis and RNA sequencing. Glucose, pyruvate and insulin tolerance tests were performed after fasting in wild type (WT) and *Cth/Mpst*$^{-/-}$ mice. For *in vitro* procedures, HepG2 cells were cultured in the presence or absence of CTH and MPST pharmacological inhibitors (propargylglycine, PAG, 1mM and IMST-3 50μM) or with siRNA (10 μM) of *Cth* and *Mpst* genes for 48 hours. Lipid accumulation was determined by Oil RedO. To restore H_2S levels, the SG1002 donor was added in chow diet (80mg/kg/day) for 4 weeks. **Results:** *Cth/Mpst*$^{-/-}$ mice had increased liver mass caused by enhanced hepatic lipid accumulation under normal feeding conditions. In addition, decreased insulin, glucose and pyruvate sensitivity was observed in CTH/MPST-deficient mice. *In vitro* experiment revealed that pharmacological inhibition of CTH and MPST enzymes and the silencing of *Cth* and *Mpst* genes induced lipid accumulation in hepatocytes. Transcriptome analysis revealed significant upregulation of cholesterol biosynthesis and SREBP-related genes in the liver of *Cth/Mpst*$^{-/-}$ mice. Transcription factor enrichment analysis of differentially expressed genes between two genotypes, revealed a major impact of LXR, RXR and PPARA in the observed phenotype. Sulfide donor (SG1002) treatment attenuated the fatty liver disease of CTH/MPST-deficient mice. **Conclusion:** Our findings underline the importance of endogenously produced H_2S in the pathogenesis of MASLD and introduce the *Cth/Mpst*$^{-/-}$ mouse as a new animal model of early onset hepatic steatosis.

Poster 4

3-mercaptopyruvate sulfurtransferase (MPST) is required for the cardioprotective action of thiosulfate

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Introduction: Acute myocardial infarction (AMI) is responsible for millions of fatalities worldwide annually. Recent preclinical studies have highlighted the cardioprotective effect of sodium thiosulfate (STS) in myocardial ischemia/reperfusion injury [1]. Thiosulfate, a clinically used drug, is a breakdown product of the signaling molecule hydrogen sulfide (H_2S) but has also been shown to generate H_2S. 3-mercaptopyruvate sulfurtransferase (MPST), is a H_2S-producing enzyme with strong expression in the myocardium capable of converting thiosulfate to H_2S *in vitro* in the presence of reducing agents or hypoxic conditions [2,3]. In this study, we aimed to investigate whether STS-induced cardioprotection depends on MPST. **Methods:** 8-week-old C57BL/6J WT and *Mpst*$^{-/-}$ mice (n=5/group) anesthetized with ketamine/xylazine intraperitoneally were subjected to 30 min occlusion of the left anterior descending coronary artery followed by 120 min reperfusion. STS (45,6 mg/kg) was dissolved in water for injection and administered intravenously 10 min prior to reperfusion. In different groups of animals, the H_2S donors Na_2S: 1 μmol/kg, GYY4137: 25 μmol/kg and AP39: 0,25 μmol/kg were similarly administered. Infarct size was estimated by 2,3,5-triphenyltetrazolium staining, while the area at risk was calculated using Evans blue. **Results:** WT mice exhibited an infarct size of 37,65±2,31% (infarcted area/area at risk). Administration of STS to WT mice significantly reduced the infarcted area (12,21±3,57%). STS's efficacy in reducing infarct size was comparable to other H_2S donors; infarct size after administration of the fast H_2S releasing donor Na_2S was 14,03±2,69%, while GYY-4137 a slowly releasing H_2S donor reduced infarct size to 16,16±1,47%. The mitochondrial donor AP39 attenuated infarct to 9,66±4,43%. No differences were observed in the area at risk/all among groups. Consistent with previous reports from our group, genetic ablation of *Mpst* attenuated myocardial injury (27,03±3,06%). STS administration to Mpst$^{-/-}$ mice did not further reduce infarct size (26,18±1,78%) compared to vehicle-treated Mpst$^{-/-}$ Control group (mean±SD, one-way ANOVA, significance $p<0.05$, Tukey's post hoc). **Conclusion:** We conclude that MPST is required for the cardioprotective effect of STS in myocardial ischemia/reperfusion injury, possibly through H_2S generation.

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Poster 5

Preliminary evaluation of new 1*H*-pyrazolo[3,4-*c*]pyridin-7(6*H*)-one derivatives as direct soluble Guanylyl Cyclase (sGC) stimulators

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Background: The function of Vascular Smooth Muscle Cells (VSMC) and their role in pathophysiology are controlled by the NO/sGC/cGMP axis. Therefore, compounds which are able to directly stimulate VSMC sGC in pathological states characterized by dysfunction of this axis, could be promising therapeutics in cardiovascular diseases. **Aim:** The Aim of the present *in vitro* work, conducted in the A7r5 VSMC line, was to characterize pharmacologically four newly synthesized 1*H*-pyrazolo[3,4-*c*]pyridin-7(6*H*)-one derivatives, rationally designed as direct "stimulators" of the reduced, heme-dependent form of sGC, in order to a) derive insights into desirable structural characteristics as sGC stimulators and b) select the most promising of them for additional bioactivity characterization. **Methods:** We evaluated the ability of these molecules to elevate cGMP ± the NO donor Sodium Nitroprusside (SNP) [by ELISA kit] and to elicit downstream VASP phosphorylation at Ser_{239} [by western blotting]. In order to make comparative evaluations, two different cGMP generation protocols were conducted: 1) We tested increasing concentrations of compounds (0.3, 3 & 30 μM) in combination with a stable concentration of SNP (30 μM) and determined their EC_{50}, 2) Conversely, by combining increasing concentrations of SNP (0.1, 1 & 10 μM) with a given concentration of each compound (30 μM), we tested the ability of each to synergize with low SNP (i.e. NO) levels. Results from evaluation of four such compounds are presented. Statistical comparison between treatments was done by Student's t-test, on data from 2 independent experiments, each done in duplicates. **Results:** The new compounds were not able to induce cGMP production by themselves, but all of them synergized with SNP to maximally elevate A7r5 cGMP levels by >40-fold (n=4), comparably with the potent sGC stimulator BAY 41-2770 (10 μM), thus confirming that they act as direct sGC "stimulators". Of the molecules tested, NM-17 seems to exhibit the best stimulator profile because, combined with SNP, it induces higher maximal production of cGMP than the others, displays the lowest value of EC_{50} in this synergy and is also able to strongly potentiate the effect of the lowest SNP concentration. All compounds also elicited characteristic cGMP-dependent phosphorylation of the protein VASP at Ser_{239}, synergistically with SNP. **Conclusions:** All four new, rationally designed 1*H*-pyrazolo[3,4-*c*]pyridin-7(6*H*)-one derivatives possess the tell-tale features of direct sGC stimulators, with a profile that sets them apart from reported such molecules (e.g. BAY compounds), since they have no cGMP-elevating effects by themselves but strongly synergize with low NO concentrations. The two best compounds of this series will be further studied for their ability to elicit mouse aortic ring relaxation *in vitro* and to reduce blood pressure in mice *in vivo*.

Poster 6

Deciphering the off targets of sodium glucose co-transporter 2 inhibitors to provide insights into their cardioprotective mechanism

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+ We dedicate this work to the memory of Professor Ioanna Andreadou, whose scientific legacy and pioneering contributions to the field of SGLT2 inhibitors continue to inspire us. We remain profoundly grateful for her vision, and lasting impact on cardiovascular research.

Background and aims: Sodium-glucose co-transporter 2 (SGLT2) inhibitors are the newest class of antidiabetic drugs that act by inhibiting SGLT2 in the proximal tubules of the kidneys, leading to increased urinary glucose excretion[1]. Landmark clinical studies have demonstrated that the SGLT2 inhibitors, empagliflozin and dapagliflozin, improve cardiovascular outcomes in heart failure patients across the spectrum of left ventricular ejection fraction, independently of the presence of diabetes[2,3]. Preclinical studies on acute myocardial infarction suggest that empagliflozin exerts cardioprotective effects independent of SGLT2 inhibition[1,4]. The aim of this study was to identify the off-targets of SGLT2 inhibitors that could contribute to the activation of cardioprotective pathways beyond SGLT2 inhibition, using chemoproteomics-based target deconvolution techniques[5]. **Methods**: Sepharose beads were mesylated and reacted to O-desethyl dapagliflozin, the common pharmacophore of empagliflozin and dapagliflozin, to achieve covalent immobilization. C57Bl6 mice (n=6) were humanely sacrificed, the kidneys and heart were harvested, and the tissues were pulverized for Igepal-based lysis buffer protein extraction. Sepharose beads with immobilized O-desethyl-dapagliflozin (the so called "tailored affinity matrix") were incubated with the tissue lysates in the presence of increasing concentrations of empagliflozin in as many competition pulldowns experiments. The proteins bound to beads were digested and eluted for mass-spectrometry based bottom-up proteomics readout. CurveCurator, an open-source software providing reliable dose-response characteristics based on a recalibrated F-statistic, was employed to extract dose-response curves[6]. **Results**: We identified off-target proteins that both bind to the immobilized common pharmacophore of SGLT2 inhibitors and to empaglifozin. The most promising off-targets of empagliflozin determined by our study were: phosphodiesterase-4 (PDE4) which is the major enzyme responsible for the hydrolysis of cyclic adenosine monophosphate and possesses putative anti-inflammatory properties (EC_{50}=104.5nM), alpha-synuclein Gene (SNCA) (EC_{50}=39.6nM) and peroxisomal L-bifunctional enzyme (EHHADH) which is part of the classical fatty acid β-oxidation pathway (EC_{50}=140.9nM). **Conclusions**: We successfully immobilized O-desmethyl-dapagliflozin to produce suitable tailored affinity matrices for target deconvolution and conducted competitive pull-down experiments. The identification of PDE4 as a potential target highlights a novel mechanism through which

SGLT2 inhibitors may exert beneficial effects in the context of heart failure and ischemic heart disease, where altered cAMP signaling are critical components of disease progression.

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Poster 7

Peripheral dysregulation of mTOR and mRNA splicing pathways in first episode of psychosis (FEP) patients

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The first episode of psychosis (FEP) is the first manifestation of psychotic symptoms and often marks the diagnosis of schizophrenia. Signaling by the protein kinase mTOR, a master regulator of cellular growth, metabolism, and protein synthesis, has been highlighted as a prominent pathophysiological mechanism of many neurodevelopmental disorders including schizophrenia. In previous studies, we identified an antipsychotic drug-resistant mTOR signaling dysregulation in peripheral blood mononuclear cells (PBMCs) of FEP patients. The aim of the present study was to further investigate mTOR downstream signaling and the related whole proteome differences in control and FEP PBMCs before and after antipsychotic drug treatment. FEP PBMCs exhibit reduced phosphorylation of ribosomal protein S6, a key mTORC1 effector via S6 kinase (S6K). To determine whether mTORC1 signaling was globally impaired, we assessed phosphorylation of 4EBPs—direct substrates of mTORC1—and found no significant differences between FEP and control PBMCs. Interestingly, the strong correlation typically seen between p-S6 and p-4EBP in control PBMCs was absent in FEP samples, suggesting a selective disruption of mTORC1 downstream signaling at the S6K level. This altered S6K activity was confirmed via ex vivo pharmacological inhibition experiments. Since mTORC1 and S6K1 control protein synthesis, ribosome biogenesis and mRNA translation pathways we further investigated the changes in the FEP PBMC proteome. Mass spectrometry identified 215 differentially expressed proteins in FEP PBMCs. Functional clustering and bioinformatics analyses revealed dysregulation in RNA splicing and processing pathways. Among these, six differentially expressed RNA-binding proteins previously linked to schizophrenia were all found to be downregulated in FEP PBMCs; some of which normalized after antipsychotic treatment. Our results corroborate previous findings in peripheral and post-mortem CNS tissues and underscore the role of mTOR signaling and RNA processing in schizophrenia. Further analyses will likely reveal novel potential biomarkers and inform predictive tools for treatment response and disease progression in the future.

Poster 8

Ο ρόλος των microRNAs στην αναζήτηση των βιολογικών μονοπατιών που μεσολαβούν τις πλειοτροπικές δράσεις των SSRIs

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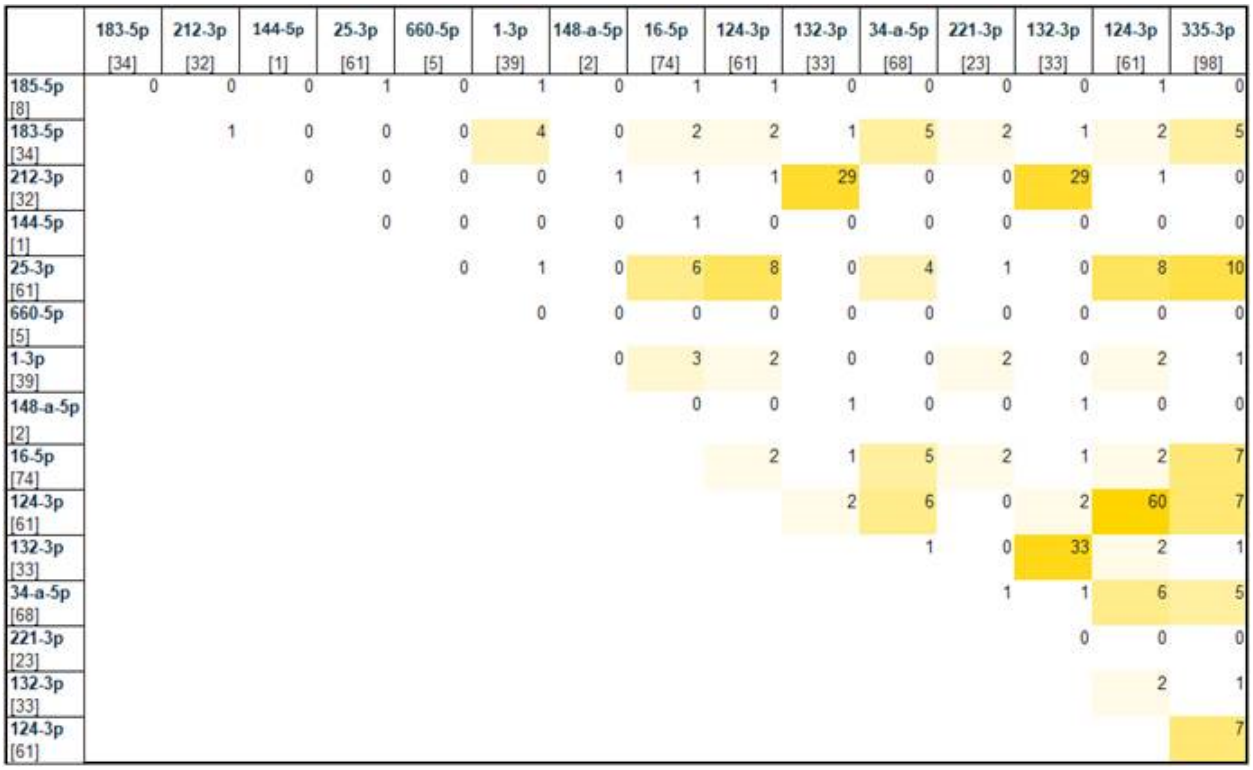
Εισαγωγή: Οι εκλεκτικοί αναστολείς επαναπρόσληψης σεροτονίνης (SSRIs) αποτελούν την κύρια αγωγή στη θεραπεία καταθλιπτικών συνδρόμων και αγχωδών διαταραχών. Οι πιθανές πλειοτροπικές δράσεις των SSRIs και οι εμπλεκόμενοι μηχανισμοί έχουν προσελκύσει έντονο ερευνητικό ενδιαφέρον. Τα microRNAs είναι μικρά μη-κωδικά μόρια RNA που έχουν σημαντικό ρόλο στη ρύθμιση της έκφρασης των γονιδίων. Η μελέτη της επίδρασης των SSRIs στην έκφραση των microRNAs μπορεί να αναδείξει βιολογικά μονοπάτια που μεσολαβούν πλειοτροπικές δράσεις των SSRIs. **Σκοπός:** Σκοπός της εργασίας είναι μέσω των αλλαγών έκφρασης των microRNAs κατά τη χορήγηση SSRIs να εντοπιστούν ποια γονίδια και βιολογικά μονοπάτια υπό- ή υπερ-λειτουργούν με αποτέλεσμα την πιθανή πρόβλεψη πλειοτροπικών δράσεων των SSRIs. **Μεθοδολογία:** Αναζητήθηκαν δημοσιευμένες μελέτες στην PubMed που αναφέρουν σημαντικές αλλαγές στην έκφραση microRNAs ύστερα από χορήγηση SSRIs. Από τη βάση miRDB, καταγράφηκαν τα γονίδια-στόχοι των microRNAs με συγγένεια πρόσδεσης >98% και ακολούθως καταγράφηκαν τα προβλεπόμενα βιολογικά/σηματοδοτικά μονοπάτια στα οποία συμμετέχει το κάθε γονίδιο με χρήση της βάσης δεδομένων STRING. Έγινε σύγκριση των μονοπατιών που συμμετέχει κάθε microRNA και αναζητήθηκαν τομές μεταξύ αυτών με χρήση του ελεύθερα διαθέσιμου εργαλείου *Multiple List Comparator*. **Αποτελέσματα:** Στην ανάλυση συμπεριλήφθηκαν 8 μελέτες. Εντοπίστηκαν 16 microRNAs των οποίων η έκφραση αλλάζει κατά τη χορήγηση SSRIs (miR -1, -16, -25-3p, -34a-5p, -124, -132, -144-5p, -148-5p, -183, -185-5p, -212, -221-3p, -335, -451a, -660-5p, -1202). Το πλήθος των κοινών μονοπατιών στα οποία συμμετέχουν τα συγκεκριμένα microRNAs φαίνεται στην Εικόνα 1. Ανάμεσα σε αυτά περιλαμβάνονται μονοπάτια που εμπλέκονται στην καρκινογένεση (HSA-2644607: Loss of Function of FBXW7 in Cancer and NOTCH1 Signaling), τη φλεγμονή (HSA-449147: Signaling by Interleukins) και τη νευροδιαβίβαση (HSA-181429: Serotonin Neurotransmitter Release Cycle). **Συμπεράσματα:** Η μελέτη της επίδρασης των SSRIs στην έκφραση των microRNAs μπορεί να συμβάλλει στην ταυτοποίηση βιολογικών και σηματοδοτικών μονοπατιών που μεσολαβούν των θεραπευτικών και πλειοτροπικών δράσεων των φαρμάκων αυτών.



	183-5p [34]	212-3p [32]	144-5p [1]	25-3p [61]	660-5p [5]	1-3p [39]	148-a-5p [2]	16-5p [74]	124-3p [61]	132-3p [33]	34-a-5p [68]	221-3p [23]	132-3p [33]	124-3p [61]	335-3p [98]
185-5p [8]	0	0	0	1	0	1	0	1	1	0	0	0	0	1	0
183-5p [34]		1	0	0	0	4	0	2	2	1	5	2	1	2	5
212-3p [32]			0	0	0	0	1	1	1	29	0	0	29	1	0
144-5p [1]				0	0	0	0	1	0	0	0	0	0	0	0
25-3p [61]					0	1	0	6	8	0	4	1	0	8	10
660-5p [5]						0	0	0	0	0	0	0	0	0	0
1-3p [39]							0	3	2	0	0	2	0	2	1
148-a-5p [2]								0	0	1	0	0	1	0	0
16-5p [74]									2	1	5	2	1	2	7
124-3p [61]										2	6	0	2	60	7
132-3p [33]											1	0	33	2	1
34-a-5p [68]												1	1	6	5
221-3p [23]													0	0	0
132-3p [33]														2	1
124-3p [61]															7

Εικόνα 1. Διαγραμματική απεικόνιση πλήθους κοινών βιολογικών μονοπατιών των διαφόρων microRNAs. Δημιουργήθηκε με χρήση του *Multiple List Comparator (https://molbiotools.com/listcompare.php).*

Poster 9

In silico analysis of the perturbations in metabolic fluxes in a murine model of prion diseases

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Prion diseases are caused by PrPSc, the abnormally folded isoform of the prion protein (PrPC). PrPSc molecules form aggregates, which lead to neurodegeneration and eventually death, after a long incubation period. Although evaluation of the metabolic changes occurring throughout this incubation period could unveil mechanisms of disease progression and neuronal loss, identify potential diagnostic biomarkers and therapeutic targets, and provide insights into the pathogenesis of other neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, very few metabolomic studies have been conducted in the context of prion diseases to date. In this study, we utilize RNA-sequencing data from the hippocampi of prion-infected and healthy control mice at different disease stages, to uncover and characterize through *in silico* analysis disease-associated metabolic perturbations. To this end, we initially performed a Pathway Enrichment Analysis that revealed a correlation between the extent of metabolic changes and disease progression. Specific metabolic pathways were dysregulated in prion disease murine models, such as the Cholesterol Biosynthesis pathway, which was significantly enriched in mice approaching end-stage disease. We aim at further mapping and understanding metabolic shifts in prion disease by examining metabolic flow rates, thus implementing a fluxomics approach. Fluxomics, a novel field of systems biology, integrates information from genomics, transcriptomics, proteomics and metabolomics to quantify metabolic flow rates (fluxes) through the metabolic pathways, revealing metabolism as a dynamic process influenced by genomic and/or environmental changes. We will estimate metabolic fluxes by *single-cell Flux Estimation Analysis (scFEA),* a computational model that employs multilayered neural networks to model the nonlinear dependency between gene expression and metabolic fluxes.

Poster 10

Utilization of Knowledge Graphs for Drug Safety Purposes

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Aim Pharmacovigilance (PV) is vital for ensuring medication safety, especially as reports of adverse drug reactions (ADRs) increase. This study explores the use of Knowledge Graphs (KGs) in PV signal analysis, focusing on OpenPVSignal, a semantic framework aimed at enhancing detection, enrichment, and interpretation of safety signals. **Methods** OpenPVSignal is a KG model built on Semantic Web standards and Linked Data principles. It was developed using 101 PV signal report instances from VigiBase, modeled in OWL/RDF syntax. Two real-world PV signals were selected to enrich the KG: (1) Desloratadine/Loratadine – Weight increase in children, and (2) Rosuvastatin and Ticagrelor – Rhabdomyolysis. Data integration was performed in Protégé, with quality control to ensure accuracy. **Results** The enriched KG enabled improved visualization of complex relationships between drugs, ADRs, and patient-specific factors. For the pediatric signal, structured data highlighted key challenges in assessing ADRs in children, where clinical trial data are scarce. The two suspected drugs, Desloratadine and Loratadine, were linked in KG with their respective indications for use, mechanism of action, ICSRs, free-text reporting elements etc. Users can filter data by age, medication, or ADR, offering a more efficient alternative to manual review of signal reports. In the DDI signal, the KG demonstrated potential to improve drug safety monitoring and support clinical decision-making. Rosuvastatin and Ticagrelor were linked similarly, including their interaction relationship and mechanisms. This KG provides the ability to view the entire medication regimen from an ICSR in contrast to widely known drug interaction checkers. **Conclusions** KGs offer promising support for PV research and practice. Clinically enriched graphs enhance data accessibility and interpretation, supporting more accurate signal detection. Future work will explore automation and machine learning to expand their utility.

Poster 11

Η φαρμακοκινητική της καφεΐνης: πιλοτική μελέτη σε υγιείς εθελοντές

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Εισαγωγή: Η μεγαλύτερη κατανάλωση καφέ παγκοσμίως παρατηρείται στην Ευρώπη. Εκτιμάται ότι στην Ελλάδα καταναλώνονται περί τα 4,52 kg καφέ ανά κεφαλή ετησίως. Αν και η κατανάλωση καφεΐνης θεωρείται ασφαλής, η υπέρμετρη χρήση της μπορεί να έχει επιπτώσεις στην υγεία. **Σκοπός:** Ο προσδιορισμός των φαρμακοκινητικών παραμέτρων της καφεΐνης (1,3,7 τριμεθυλοξανθίνη, 137X) και της παραξανθίνης (1,7 διμεθυλοξανθίνη, 17X) σε Έλληνες υγιείς εθελοντές. **Μέθοδοι:** Σε διασταυρούμενη μελέτη ανοιχτής ετικέτας, 6 υγιείς εθελοντές (4 άνδρες και 2 γυναίκες) κατανάλωσαν κάψουλα καφεΐνης σε δόσεις 150 και 300 mg μετά από 12ωρη αποχή από ποτά και τρόφιμα περιέχοντα καφεΐνη. Δείγματα αίματος συλλέχθηκαν στις 0,5, 1, 2, 4, 8, 12, και 24 ώρες μετά την κατανάλωση καφεΐνης. Τα δείγματα αναλύθηκαν με Υγρή Χρωματογραφία Ανάστροφης Φάσης (RP-HPLC) για τον προσδιορισμό των συγκεντρώσεων των 137X και 17X. Ο υπολογισμός των κινητικών παραμέτρων βασίσθηκε σε κινητική πρώτης τάξης ενός διαμερίσματος χρησιμοποιώντας το PKSolver (Microsoft Excel add-in program). **Αποτελέσματα:** Οι τιμές C_{max} (μg/mL) για την 137X (4,32±1,69) και την 17X (1,46±0,51) μετά από κατανάλωση 150mg καφεΐνης ήταν στατιστικώς σημαντικά μικρότερο από τις αντίστοιχες τιμές (7,11±2,42 και 2,75±1,02, αντίστοιχα; $p<0,05$) μετά από κατανάλωση 300mg καφεΐνης. Παρομοίως, οι τιμές $AUC_{0-\infty}$ (mg×mL-1×h-1) για την 137X (28,3±11,19) και 17X (27,1±13,5) μετά από κατανάλωση 150mg καφεΐνης, ήταν στατιστικά σημαντικά χαμηλότερες σε σύγκριση με τις αντίστοιχες τιμές $AUC_{0-\infty}$ (55,4±24,6 and 52,2±24,8, αντίστοιχα; $p<0,05$) μετά από κατανάλωση 300mg καφεΐνης. Οι τιμές T_{max} (h) δεν διέφεραν σημαντικά μεταξύ των δύο δοκιμών (137X: 1,17±0,41 και 1,33±0,52; 17X: 4,00±2,19 και 5,00±2,45 για 150 και 300 mg, αντίστοιχα). Τέλος, οι τιμές $T_{1/2}$ (h) ήταν παρόμοιες μεταξύ των δύο δοκιμών για αμφότερες την 137X (4,40±1,69 και 5,18±1,88; $p>0,05$) και την 17X (9,12±5,23 και 10,8±4,87; $p>0,05$). **Συμπεράσματα:** Αναπτύχθηκε αξιόπιστη και απλή χρωματογραφική μέθοδος HPLC για τον προσδιορισμό των φαρμακοκινητικών παραμέτρων της καφεΐνης σε υγιείς εθελοντές. Οι τιμές των παραμέτρων που υπολογίσθηκαν στην παρούσα μελέτη συνάδουν με αυτές που αναφέρονται στη διεθνή βιβλιογραφία σε άλλες εθνότητες. Τα αποτελέσματα της παρούσας μελέτης μπορούν να χρησιμοποιηθούν σε κλινικά πρωτόκολλα σχετικά με την κατάχρηση ή τοξικότητα στην καφεΐνη.

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Poster 12

Preliminary findings on the effect of an *LRP8* polymorphism on symptom severity and response to antipsychotic medication of schizophrenic patients

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Introduction-aim: The low-density lipoprotein receptor related protein 8 (LRP8, ApoE2R) serves a dual role as a Reelin receptor and that of Selenoprotein P (SELENOP), the latter mediating cellular selenium uptake. The receptor is abundantly expressed in the brain as opposed to most other tissues and has been suggested as a schizophrenia susceptibility gene in the past. A SNP in the 3′UTR of *LRP8* (rs5177) has been reported to affect gene expression in various brain tissues and has been associated with schizophrenia, anxiety disorders, and bipolar disorder in some studies. We aimed at examining the effect of rs5177 on symptom severity and response to antipsychotic drug treatment, in a group of patients suffering from schizophrenia-spectrum disorders, under naturalistic conditions. **Materials and methods:** Sixty-nine patients diagnosed with schizophreniform and other psychotic disorders, from April 2017 to July 2019, in the 2nd Psychiatric Clinic of the Aristotle University of Thessaloniki School of Medicine, were included in this study. Assessment of psychopathology was conducted using the Positive and Negative Syndrome Scale (PANSS). A four-week follow-up period was used to evaluate treatment response. Genotyping was with an in-house PCR-RFLP method. **Results:** A tendency for heterozygotes to display higher total PANSS values at baseline was observed, which was significant at the level of general psychopathology subscale ($p = 0.016$). A similar tendency was observed for the decrease in PANSS values following one month of treatment with antipsychotic medication, which was apparently unaffected by the use of typical vs. atypical drugs. **Conclusions:** The rs5177 polymorphism may affect symptom severity and response of patients with schizophrenia-spectrum disorders to antipsychotic medication.

Poster 13

Ο συνδυασμός διαζεπάμης και rTMS σε μοντέλο επιληψίας στην κυτταρική σειρά SH-SY5Y

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Υπόβαθρο: Η επιληψία, μια πάθηση του εγκεφάλου, επηρεάζει εκατομμύρια ανθρώπους σε όλο τον κόσμο. Παρά την ύπαρξη μεγάλου αριθμού αντιεπιληπτικών φαρμάκων με ποικίλους μηχανισμούς δράσης, πολλοί ασθενείς δεν επιτυγχάνουν τον πλήρη έλεγχο των συμπτωμάτων τους, κάτι που κάνει επιτακτική την ανάγκη για περισσότερες στρατηγικές αντιμετώπισης. Αυτές μπορούν να περιλαμβάνουν μη φαρμακολογικές προσεγγίσεις ή πιθανώς τον συνδυασμό φαρμακολογικών και μη φαρμακολογικών μεθόδων. **Σκοπός:** Στην παρούσα in vitro μελέτη, προσπαθήσαμε να διευκρινίσουμε και να ποσοτικοποιήσουμε τις αλλαγές στα ενδοκυτταρικά επίπεδα ασβεστίου σε κυτταροκαλλιέργειες όπου εφαρμόστηκαν διαζεπάμη και rTMS είτε μεμονωμένα είτε συνδυαστικά. **Μέθοδοι:** Χρησιμοποιώντας τη διαφοροποιημένη κυτταρική σειρά νευροβλαστώματος ανθρώπινης προέλευσης SH-SY5Y, διερευνήσαμε τις αλλαγές στα ενδοκυτταρικά επίπεδα ασβεστίου υπό την επίδραση χαμηλής συχνότητας rTMS (1Hz), διαζεπάμης (14μM), ή του συνδυασμού τους. Για την απεικόνιση του ασβεστίου χρησιμοποιήθηκε ο ευαίσθητος στο ασβέστιο φθορίζων δείκτης fluo-4 AM, ενώ η νευρωνική διέγερση επιτεύχθηκε με τη χρήση χλωριούχου καλίου. Ο λόγος %ΔF/F χρησιμοποιήθηκε για την κανονικοποίηση των επιπέδων φθορισμού καθ' όλη τη διάρκεια της μελέτης. **Αποτελέσματα:** Η υψηλότερη μέση αύξηση της έντασης φθορισμού (%ΔF/F = 24,80) παρατηρήθηκε στις καλλιέργειες control, ακολουθούμενες από τις καλλιέργειες όπου έγινε εφαρμογή του rTMS (%ΔF/F = 16,96) και τις καλλιέργειες όπου έγινε προσθήκη της διαζεπάμης (%ΔF/F = 11,46). Η χαμηλότερη μέση τιμή έντασης φθορισμού (%ΔF/F = -0,44) παρατηρήθηκε όταν η διαζεπάμη χρησιμοποιήθηκε συνδυαστικά με το rTMS. Η post hoc ανάλυση αξιολόγησε τις διαφορές ανά ζεύγη, δείχνοντας στατιστικά σημαντική διαφορά μεταξύ της ομάδας ελέγχου και όλων των άλλων ομάδων. Επιπλέον, παρατηρήθηκαν στατιστικά σημαντικά αποτελέσματα μεταξύ του rTMS ή της διαζεπάμης και του συνδυασμού τους, αλλά όχι μεταξύ τους. **Συμπέρασμα:** Η διαζεπάμη και το rTMS, όταν χρησιμοποιήθηκαν συνδυαστικά, παρουσίασαν την χαμηλότερη τιμή φθορισμού στα ενδοκυτταρικά επίπεδα ασβεστίου σε σύγκριση με το control, ενώ κάθε ένα από αυτά, όταν χρησιμοποιήθηκε μεμονωμένα, εμφάνισε ένα μικρότερο αλλά ισχυρό αποτέλεσμα. Συμπερασματικά τόσο η διαζεπάμη όσο και το rTMS χαμηλής συχνότητας φαίνεται να έχουν την ικανότητα να ελέγχουν την επιληπτική δραστηριότητα in vitro, ενώ ο συνδυασμός τους φαίνεται να είναι ο πιο αποτελεσματικός.

Poster 14

In Vitro Profiling of Novel Anticancer Compounds Targeting Prostate and Breast Cancer Cells: Cytotoxicity and Cellular Bioavailability Potential

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Introduction: The development of new anticancer drugs is a key objective in cancer research. A promising strategy is based on the design and testing of de novo small-molecule compounds, which, compared to larger, more complex structures (e.g., antibody drugs), can efficiently enter cells and act on intracellular targets. Prostate and breast cancer remain among the most common types worldwide, with remaining challenges when it comes to efficient treatments, emphasizing the need for continuous therapeutic innovation. In this study, PC3 and MCF7 cell lines were used as in vitro models of prostate and breast cancer, respectively. The aim of this study was to investigate the anticancer potential of a series of de novo synthesized analogues. Cytotoxicity was evaluated in these models, while HUVEC, human umbilical vein endothelial cells, were included to assess effects on healthy cells. To further characterize the compounds, cellular uptake studies were performed in PC3 cells to explore whether the level of intracellular accumulation of selected compounds contributes to their antiproliferative activity. **Methods:** Two lead compounds previously published[1] by this study's participants and 38 novel structural analogues were screened in PC3 and MCF7 cells using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, MTT assay. Cells were seeded at 5.000 cells/well, incubated for 24 h, treated with compounds, and assessed after 72 h. This colorimetric assay measures cytotoxicity by quantifying the reduction of MTT reagent to insoluble formazan crystals by metabolically active cells. The most active candidates were selected for full IC_{50} profiling. The same MTT protocol was also applied in HUVEC cells to assess their effect on healthy tissue, providing important information on dose tolerance. To evaluate intracellular accumulation, PC3 cells were seeded in 24-well plates at a density of 200.000 cells per well and treated with 5 μM of the lead compound and then in the same protocol with its most potent analogue. At multiple time points (1,4,8, and 24 hours), cells were washed twice with cold PBS to remove extracellular compounds, then collected and lysed with a cold 3:2 acetonitrile/water solution. Intracellular content was extracted using cold acetonitrile, followed by centrifugation, solvent evaporation, and reconstitution in mobile phase prior to injection. Samples were analyzed by Liquid Chromatography–Tandem Mass Spectrometry using a method developed with a C18 reverse-phase column and triple quadrupole mass spectrometry equipped with electrospray ionization in positive mode (ESI+) to quantify the compounds of interest. Calibration curves were prepared in control cell lysates, and unknown samples were quantified using their corresponding curves. When analyzing the lead compound, the most potent analogue was used as the internal standard, and vice versa. This reciprocal setup provided consistent and reliable quantification due to the high degree of structural resemblance between the two compounds. **Results:** The 40 small-molecule analogues showed a wide range of effects, from minimal to strong antiproliferative activity. Among them, MG218 and MG408 emerged as the most potent, with both outperforming their structural lead. MG218 displayed IC_{50} values of 4.8±0.9 μM in PC3 cells and 3.9±0.9 μM in MCF7 cells, while MG408 showed 4.0±0.4 μM and 3.3±0.3 μM,

respectively. Both compounds demonstrated steep, well-defined dose–response curves. In HUVEC cells, MG408 showed increased cytotoxicity, indicating poor selectivity (cancer versus healthy cells). In contrast, MG218 exhibited a mean EC_{50} of 8.8 μM, suggesting reduced toxicity in healthy cells and a narrow but promising therapeutic window of approximately two-fold between effective and toxic concentrations. Additional experiments are underway to further support these findings. Due to its overall profile, MG218 was selected for cellular uptake analysis. Time-dependent accumulation in PC3 cells showed 31% at 1 h, increasing to about 100% at 24 h. The lead compound, MG74, followed a similar trend but with slightly slower early uptake. These results suggest efficient intracellular accumulation of MG218, which may prove to be a significant asset towards its anticancer activity in animal studies. **Conclusion:** This in vitro pharmacological evaluation was crucial for understanding the behavior and properties of the novel class of presented compounds. It provides a foundation for identifying promising candidates with solid anticancer effects and supports their progression toward pre-clinical development. MG218 demonstrated a strong anticancer profile, with potent activity in prostate and breast cancer cell lines, reduced toxicity in healthy endothelial cells, and efficient time-dependent cellular uptake. An in vivo pharmacokinetic study in mice will provide key data on its biological behavior and guide the next steps in its preclinical development.

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Poster 15

cMet activation as a mechanism of resistance to angiogenesis inhibitors

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Targeting angiogenesis is a therapeutic approach used for treating several different types of cancer that rely on this process to grow and metastasize. However, the emergence of resistance to the existing anti-angiogenic drugs that mainly target the vascular endothelial growth factor (VEGF) / VEGF receptor 2 (VEGFR2) system makes it necessary to elucidate the mechanisms involved and find new targets for developing novel therapeutic approaches. Such targets include $\alpha_v\beta_3$ integrin, cell surface nucleolin (NCL), and the protein tyrosine phosphatase receptor zeta 1 (PTPRZ1) that interact with each other and/or with VEGFR2 and play important roles in regulating angiogenesis. Inhibitors of $\alpha_v\beta_3$ integrin and cell surface NCL have been developed and entered clinical trials for different types of angiogenesis-dependent cancers; however, they have not yet made it to the clinic despite their encouraging preclinical data. This study shows that pharmacological inhibitors of these targets abolish endothelial cell migration induced by $VEGFA_{165}$ or pleiotrophin but enhance cMet activation through their effect on β_3 integrin phosphorylation or levels. Downstream of cMet, mTORC1 and protein synthesis are also activated, and this may explain, at least partially, their clinical failure. It also suggests that cMet activation may be a mechanism of resistance to angiogenesis inhibitors targeting $\alpha_v\beta_3$ integrin, cell surface NCL, and PTPRZ1, and proposes their combined use with cMet inhibitors.

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Poster 16

Targeting the 'Undruggable' MYC: Development of PROTACs and Molecular Glues for the Treatment of Cancer

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Despite advancements in cancer therapy, targeting the c-MYC oncoprotein remains a formidable challenge due to its intrinsically disordered structure and lack of defined binding pockets, classifying it as “undruggable”. While significant efforts have been made to develop small molecule inhibitors targeting c-MYC (Stellas et al., 2014; Truica et al., 2021), none have yet advanced to clinical application. In this context, PRoteolysis-TArgeting Chimeras (PROTACs) have emerged as a promising strategy to overcome the challenges posed by such elusive targets. PROTACs are bifunctional molecules that recruit the ubiquitin-proteasome system by bringing a protein of interest (POI) into proximity with an E3 ligase, leading to its ubiquitination and subsequent degradation. Recent findings from our group (Siokatas et al 2024) demonstrate that selected MYC-targeting PROTACs exhibit significant antiproliferative effects in prostate and breast cancer cell lines, with IC50 values in the range of 10–20 μM. These compounds induced dose-dependent degradation of MYC (DC50 ~10 μM), which was shown to be proteasome-mediated, as treatment with the proteasome inhibitor MG-132 effectively rescued MYC levels. A negative control, an epimer of the lead degrader, was synthesized to confirm that degradation occurs via E3 ligase-mediated ubiquitination. In this work, we further explore the mechanism of action of our lead degraders, including their time-dependent effects, and assess their applicability to challenging cancers such as triple-negative breast cancer (TNBC) cells. Additionally, LC-MS/MS assays were developed to quantify PROTAC levels in biological fluids, revealing detectable systemic exposure in mice and ~20% cellular uptake in vitro, supporting favorable pharmacokinetic and permeability profiles. Importantly, our degraders are safe in animal models and are free from some of the "unwanted" alternative outcomes beyond protein degradation, observed with MYC-targeting PROTACs developed by Boyd et al. (2025). Their compounds led to degradation of full-length MYC (~55 kDa) but also generated truncated MYC (~45 kDa) that sustains an oncogenic proliferative state, resulting the lack of anti-proliferative effects. In contrast, our degraders do not produce truncated MYC, and their ability to degrade MYC correlates well with their anti-proliferative activity. We are currently developing MYC degraders with reduced molecular weight (~750 g/mol) in order to improve their drug-like properties including solubility, membrane permeability, and overall bioavailability. Preliminary data indicate that these compounds retain their ability to degrade MYC (60% relative MYC protein expression after 24 hours of treatment at 10 μM). Furthermore, these compounds inhibit cellular proliferation with IC50 values around 10 μM.

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Poster 17

Μελέτη της αντικαρκινικής δράσης παραγώγων της σκλαρεόλης στον ανθρώπινο παγκρεατικό καρκίνο

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Εισαγωγή: Ο παγκρεατικός καρκίνος χαρακτηρίζεται από μεγάλη επιθετικότητα, υψηλά ποσοστά θνησιμότητας και μικρή ποικιλία θεραπευτικών προσεγγίσεων, καθώς είναι ανθεκτικός στις περισσότερες από τις υπάρχουσες θεραπείες. Γι αυτό το λόγο καθοριστικό ρόλο παίζει η ανακάλυψη νέων αντικαρκινικών φαρμάκων και αποτελεσματικότερων θεραπειών. Η σκλαρεόλη είναι μια φυσική ουσία που βρίσκεται σε πολλά φυτά και με βάση έρευνες έχει βρεθεί ότι παρουσιάζει αντικαρκινικές ιδιότητες, αφού προωθεί την απόπτωση και αναστέλλει τον κυτταρικό πολλαπλασιασμό. Στη συγκεκριμένη εργασία μελετήθηκε η αντιπολλαπλασιαστική δράση ημισυνθετικών παραγώγων της σκλαρεόλης, σε δύο κυτταρικές σειρές ανθρώπινου παγκρεατικού καρκίνου, τις PANC-1 (εγκαθιδρυμένη κυτταρική σειρά) και EANM–att021013 (πρωτογενής κυτταρική σειρά). **Υλικά και μέθοδοι:** Για τον έλεγχο της επίδρασης των ουσιών στον κυτταρικό πολλαπλασιασμό χρησιμοποιήθηκε η μέθοδος κυτταροτοξικότητας SRB. Για την αναστολή της ικανότητας των κυττάρων να μεταναστεύουν πραγματοποιήθηκε το wound healing ενώ για τη δυνατότητα δημιουργίας αποικιών, αντιπροσωπευτική των πιο επιθετικών κυττάρων, πραγματοποιήθηκαν πειράματα αναστολής της δημιουργίας κλώνων (clonogenic). Οι ουσίες που μελετήθηκαν ήταν έξι ημισυνθετικά παράγωγα με βάση τη σκλαρεόλη και την ένωση της 1,2,4-τριαζόλο [1,5-a]πυριμιδίνη(TNT396, TNT403, TNT404, TNT397, TNT405, TNT406), σε διάφορες συγκεντρώσεις από 100 έως 0,1μM. Οι συγκεκριμένες ουσίες επιλέχθηκαν ως οι πιο δραστικές μετά από πρωταρχικά πειράματα του Εργαστηρίου Φαρμακολογίας Τμήματος Ιατρικής του Πανεπιστημίου Θεσσαλίας σε διάφορες καρκινικές κυτταρικές σειρές. Οι ουσίες συντέθηκαν από το Πανεπιστήμιο του Βελιγραδίου (University of Belgrade – Faculty of Chemistry and Institute for Biological Research "Siniša Stanković" – National Institute of the Republic of Serbia). **Αποτελέσματα:** Τα αποτελέσματα έδειξαν ότι όλα τα παράγωγα της σκλαρεόλης είχαν ισχυρή ανασταλτική δράση στην κυτταρική ανάπτυξη των καρκινικών κυττάρων, με διαφορετική ωστόσο ισχύ το καθένα. Στις πειραματικές συνθήκες που επιλέχθηκαν η ουσία TNT404 είχε την ισχυρότερη αντιπολλαπλασιαστική δράση στα PANC-1(GI50 = 0,3μM). Επιπλέον η ένωση αυτή έδειξε ισχυρή αναστολή της μετανάστευσης των κυττάρων σε όλες τις συγκεντρώσεις (0,6, 0,3 και 0,1μM) η οποία φάνηκε να είναι χρονο-και δοσοεξαρτώμενη. Τέλος, έδειξε ιδιαιτέρα σημαντική ικανότητα αναστολής της δημιουργίας κλώνων στα PANC-1 σε όλες τις συγκεντρώσεις (0,6, 0,3 και 0,1μM) οι οποίες ελέγχθηκαν. **Συμπερασματικά,** τα έξι ημισυνθετικα παράγωγα της σκλαρεόλης τα οποία ελέγχθηκαν για πρώτη φορά για την *in vitro* αντικαρκινική τους δράση έδειξαν σε παγκρεατικό καρκίνο ιδιαίτερη δραστικότητα στις συνθήκες που ελέγχθηκαν με την ένωση TNT404 να είναι η περισσότερο δραστική. Περαιτέρω μελέτες για την λεπτομερέστερη μελέτη του μηχανισμού δράσης τους βρίσκονται σε εξέλιξη.

Poster 18

Αναστολείς κινασών CDK4/6. Μηχανισμός δράσης και μηχανισμοί αντίστασης στη θεραπεία

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Εισαγωγή: Οι CDK4/6 (κυκλινο - εξαρτώμενες "4" και "6") κινάσες, αποτελούν μέρος μιας ομάδας ενζύμων, βασικούς ρυθμιστές - ελεγκτές της ορθής συνοχής και εξέλιξης του κυτταρικού κύκλου, αποκαλούμενοι συχνά ως *«μοριακά φρένα»*. Η απορρύθμιση της δράσης των CDK4/6 μπορεί να οδηγήσει σε ανεξέλεγκτο κυτταρικό πολλαπλασιασμό και καρκινογένεση. Ακολουθεί συνοπτική παράθεση του μηχανισμού λειτουργίας των CDK4/6 αναστολέων, καθώς και πρόσφατη βιβλιογραφία, σχετικά με την αντίσταση στη δράση τους. ***Συζήτηση***: Οι CDK4/6 αναστολείς των κυκλινο - εξαρτώμενων κινασών "4" και "6" (CDK4/6 Inhibitors) θεωρούνται από τις αποτελεσματικότερες θεραπευτικές επιλογές, τόσο έναντι του μεταστατικού καρκίνου του μαστού (θετικού στον υποδοχέα οιστρογόνων, καθώς και του "HER_2 {-}" Ca μαστού), όσο και ως επικουρική θεραπεία στον πρώιμο καρκίνο του μαστού, βάσει των πλέον πρόσφατων μελετών. Στην Ελλάδα, οι ακόλουθοι τρεις αναστολείς συναντούν ευρεία εφαρμογή: Η *Παλμποσικλίμπη (Palbociclib®),* η *Αμπεμασικλίμπη (Abemaciclib®)* και η *Ριμποσικλίμπη (Ribociclib®)*. Η κύρια δράση τους εστιάζει στην αναστολή της λειτουργίας των CDK4/6 κινασών, και συγκεκριμένα στην αναστολή δράσης της φωσφορυλίωσης της πρωτεΐνης του *ρετινοβλαστώματος (pRb)*. Ως αποτέλεσμα, παρεμποδίζεται η απελευθέρωση του παράγοντα μεταγραφής "E_2F", ο οποίος φυσιολογικά προωθεί τη μεταγραφή των σχετικών γονιδίων με τον κυτταρικό κύκλο, και κατ' επέκταση τη μετάβαση από τη φάση "G_1" στη φάση "S". Οι μηχανισμοί που εκδηλώνουν αντίσταση έναντι των θεραπευτικών σχημάτων, είτε εγγενούς χαρακτήρα *("intrinsic")* είτε επίκτητου (*"acquired"*), δεν είναι πλήρως κατανοητοί. Οι κυριότεροι από αυτούς, οι οποίοι συγκεντρώνουν και το μεγαλύτερο ενδιαφέρον μελέτης, εστιάζουν στην απώλεια έκφρασης της *πρωτεΐνης pRb*, στις μεταλλάξεις της οδού του *PI3K* (συμπεριλαμβανομένων των *PTEN/PDK1*), στην υπερέκφραση της *CDK6* και της cyclin E_2/E_1, καθώς και στις μεταλλάξεις των υποδοχέων *ERBB2* και *FGFR2*. ***Συμπεράσματα***: Η μελέτη και δράση των CDK4/6 αναστολέων αποτελεί μια σημαντική, διαχρονική θεραπευτική πρόκληση. Η κατανόηση των μηχανισμών αντίστασης μπορεί να οδηγήσει σε νέες στρατηγικές αντιμετώπισης των ασθενών, μέσω εφαρμογής νέων θεραπευτικών πλάνων, είτε κατόπιν συνδυασμού αυτών, οδηγώντας στα βέλτιστα θεραπευτικά αποτελέσματα.

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Poster 19

Ανάπτυξη μεθοδολογίας για την απομόνωση και τον χαρακτηρισμό βλεννινών από πτύελα ασθματικών ασθενών

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Εισαγωγή: Οι βλεννίνες, ειδικότερα οι MUC5B & MUC5AC, είναι γλυκοπρωτεΐνες μεγάλου μοριακού βάρους, οι οποίες αποτελούν το κύριο συστατικό της βλέννας των αεραγωγών και διαδραματίζουν πολύ σημαντικό ρόλο στην παθοφυσιολογία του άσθματος. Ωστόσο, τεχνικά εμπόδια στην ανάλυση των βλεννινών, όπως η δυσκολία διαλυτοποίησης, καθαρισμού και διατήρησης της ακεραιότητας των γλυκανών, έχουν περιορίσει την έρευνα σχετικά με τον τρόπο με τον οποίο αυτά τα μόρια συμβάλλουν σε κλινικούς φαινότυπους. **Σκοπός:** Στόχος της παρούσας μελέτης ήταν η ανάπτυξη μιας μεθόδου με στόχο την απομόνωση και το χαρακτηρισμό των βλεννινών από πτύελα ασθματικών ασθενών, καθώς και τον προσδιορισμό της αναλογίας των MUC5B/MUC5AC. **Υλικά και μέθοδοι:** Δείγματα πτυέλου από πέντε ασθενείς, επεξεργάστηκαν με τέσσερα διαφορετικά διαλύματα λύσης (ουρία/γουανιδίνη, RIPA, RIPA/Tris/SDS, Tris/SDS) και έπειτα εφαρμόσθηκε σε αυτά το πρωτόκολλο FASP (προετοιμασία των δειγμάτων υπό βοήθεια φίλτρου), ώστε να ακολουθήσει πρωτεϊνωματική ανάλυση με φασματομετρία μάζας. Επίσης, πραγματοποιήθηκε αποδιατακτικός διαχωρισμός των πρωτεϊνικών δειγμάτων με SDS-PAGE ηλεκτροφόρηση, ώστε να ακολουθήσει ανοσοαποτύπωση κατά western blot για τις βλεννίνεςMUC5AC και MUC5B, αλλά και για την οπτικοποίηση του πρωτεϊνικού περιεχομένου των δειγμάτων. **Αποτελέσματα:** Τα αποτελέσματα της πρωτεϊνωματικής ανάλυσης ταυτοποίησαν τις MUC5AC & MUC5B σε όλα τα δείγματα πτυέλου, μαζί με ένα πλήθος άλλων πρωτεϊνών της πτύελου. Τα δείγματα ουρίας/γουανιδίνης έδωσαν τα καλύτερα αποτελέσματα όσον αφορά τη φασματομετρία μάζας, αλλά δεν είχαν καλή εικόνα διαχωρισμού στη γέλη πολυακρυλαμιδίου. Επίσης, τα αποτελέσματα της ηλεκτροφόρησης και της ανοσοαποτύπωσης κατά western blot ανέδειξαν χαρακτηριστικές ζώνες διαφορετικής έντασης μεταξύ των δειγμάτων. **Συμπεράσματα:** Η παρούσα μελέτη προτείνει μια βελτιστοποιημένη μέθοδο για την απομόνωση των βλεννινών από πτύελα ασθματικών ασθενών, θέτοντας τις βάσεις για την απλοποίηση και τυποποίηση της ανάλυσής τους. Η προσέγγισή μας μπορεί να συμβάλλει στον εντοπισμό νέων βιοδεικτών για τη νόσο, καθώς και στην ανάπτυξη εξατομικευμένων θεραπειών, βασισμένων στις τροποποιήσεις των βλεννινών.

Poster 20

A multi-property study of Lamotrigine-Citric acid co-crystals

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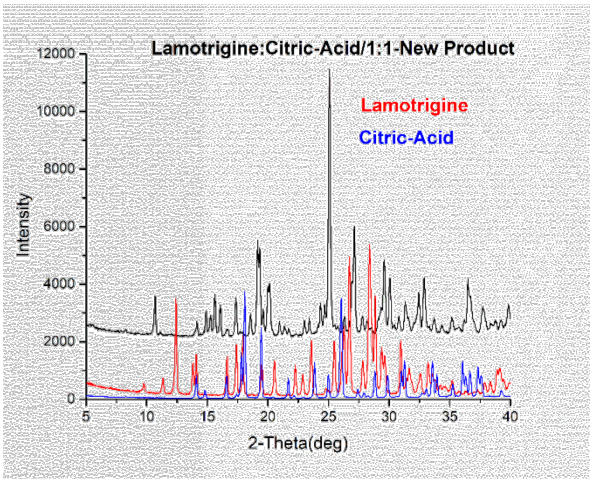
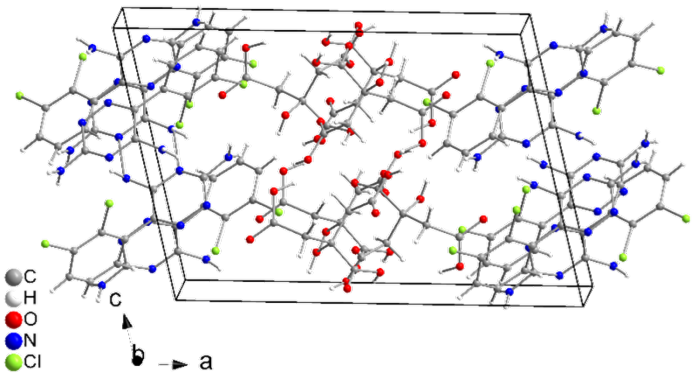
This study presents an in-depth investigation of Lamotrigine–Citric Acid co-crystals to elucidate their structural, thermal, and spectroscopic properties. The co-crystals were synthesized via liquid-assisted grinding, using EtOH as the solvent, and characterized using a combination of experimental techniques. Structural characterization was performed using X-ray powder diffraction (PXRD) studies with a CuKα radiation source, confirming the formation of a new solid phase distinct from the parent compounds. Thermal and spectroscopic analyses provided further insights into the nature of the co-crystal. Thermogravimetric analysis (TGA) was used to assess thermal stability and hydration state. Infrared (IR) and Raman spectroscopy were employed to investigate hydrogen bonding and molecular interactions between Lamotrigine and citric acid within the crystal lattice. Differential scanning calorimetry (DSC) measurements revealed distinct thermal events associated with melting and possible phase transitions. However, further analysis of the DSC and Raman data is ongoing to better understand polymorphism and thermal behavior under various conditions. Complementing the experimental work, quantum chemical calculations were performed to analyze the strength and nature of intermolecular interactions as revealed in previous work [1]. These computational results support the experimental observations and provide a molecular-level understanding of the stabilizing forces within the co-crystal. Taken together, this multi-technique approach not only confirms the successful formation of the Lamotrigine–Citric acid co-crystal but also highlights its potential pharmaceutical advantages in terms of enhanced thermal stability and modified molecular interactions.





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