Pharmacy Education (2025) 25(4) 1 - 22
https://doi.org/10.46542/pe.2025.254.nms.120

CONFERENCE ABSTRACTS

FIP COPENHAGEN 2025

83rd FIP World Congress of Pharmacy and Pharmaceutical Sciences in Copenhagen, Denmark, 31 August to 3 September 2025

New medicines

Development of a novel Tetrabutylphosphonium-based Perindopril ionic liquid for film-based drug delivery in antihypertensive therapy

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Introduction: Hypertension is among the most prevalent chronic diseases and remains the leading risk factor for the development of other cardiovascular conditions. In Chile, approximately 27% of the population is affected by hypertension, which is similar to global prevalence rates. Perindopril is an angiotensin-converting enzyme (ACE) inhibitor widely used in the treatment of hypertension, heart failure, and coronary artery disease. Initially, the perindopril tert-butylamine salt was developed; however, due to stability concerns, alternative salts, such as perindopril arginine, were introduced to enhance the drug's physicochemical stability and therapeutic efficacy

An additional challenge associated with these salt forms, inherent to their solid-state nature, is polymorphism. Polymorphic transformations during long-term storage can undermine the drug's stability, solubility, and bioavailability. This limitation can be addressed through the development of ionic liquid formulations, which effectively solubilize the drug and eliminate the constraints of the solid state. Moreover, ionic liquids have demonstrated potential to enhance drug permeation, solubility, bioavailability, and storage stability.

The aim of this study was to synthesize a tetrabutylphosphonium-based ionic liquid (IL) of perindopril. Subsequently, the ionic liquid was incorporated into an oral film, with the goal of developing a viable pharmaceutical dosage form.

Method: Perindopril IL was synthesized using a biphasic ethyl acetate-water system, with perindopril erbumine and tetrabutylphosphonium hydroxide as the starting materials. After synthesis, the ionic liquid was isolated through rotary evaporation of the aqueous phase and thoroughly characterized. Subsequently, an oral film incorporating perindopril IL was developed using the film casting technique, with Polyox as the polymer and glycerin as the plasticizer. Comprehensive characterization of the oral dosage form included analyses of its solid-state properties, thickness, disintegration, and dissolution behavior.

Results: Among the findings, the synthesized perindopril IL appeared as a translucent yellowish liquid. Its molecular structure was confirmed through mass spectrometry and proton-phosphorus nuclear magnetic resonance (^{1}H-^{31}P NMR) analysis. The resulting oral films exhibited a thickness of 88 ± 5 micrometers and a drug loading capacity of 2.0 ± 0.3 mg/cm². Differential scanning calorimetry (DSC) revealed an endothermic peak at 154°C for the perindopril erbumine salt, corresponding to its fusion point. In contrast, this thermal event was not observed in the oral film composition, suggesting that the drug does not recrystallize in the polymeric matrix. The perindopril IL-containing film disintegrated in 49 ± 11 seconds in water, and dissolution studies showed 90% drug release in 0.1 N HCl within 10 minutes, highlighting its rapid dissolution properties.

Conclusion: A perindopril IL was successfully synthesized and formulated as an oral film. This ionic liquid offers an alternative to the conventional salt forms typically used in commercial drug products. The oral film successfully incorporated perindopril IL without inducing drug crystallization or encountering challenges related to polymorphism in the solid state. Future research will focus on

optimizing the development of the oral film of perindopril IL, with the goal of creating a new treatment for hypertension.

Assessment of the safety and efficacy of romiplostim in live donor liver transplant recipients: A prospective randomised open label trial

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Introduction: Following live donor liver transplantation (LDLT), thrombocytopenia commonly occurs, with very low platelet counts portending significant morbidity and normalisation of platelet counts foretelling good graft function. Romiplostim, a thrombopoietin receptor agonist used in haematological thrombocytopenic conditions, has not been studied in the context of LDLT. We aimed to evaluate the safety and efficacy of romiplostim in improving thrombocytopenia following LDLT.

Methods: LDLT recipients (>18 years) who had a platelet count <40,000/cmm within the first week following transplantation were block randomised using computer-generated sequences into either the study group (receiving Romiplostim 250 mcg subcutaneously every 3 days until platelet count reached 70,000/cmm) or the control group The primary endpoint was platelet count normalisation time. Secondary endpoints included the incidence of vascular thrombosis, the number of platelet transfusions, the trend in liver parameters, early allograft dysfunction, length of hospital stay, and cost comparison.

Results: Among 58 LDLT patients, 30 were randomised into the Romiplostim (n=15) and control (n=15) groups. The time to achieve a platelet count >70,000 was similar (7 vs. 7 days, p=0.6), but platelet transfusions were significantly lower in the Romiplostim group (1.00 vs. 3.50, p<0.001). Vascular thrombosis and EAD rates were identical (6.6%, p>0.9). The Romiplostim group showed greater reductions in bilirubin (-3.58 vs. -1.24, p=0.008), prothrombin time (-31 vs. -15, p=0.020), and INR (-2.67 vs. -1.39, p=0.017). They also had a shorter hospital stay (20 vs. 29 days, p<0.001) and lower treatment costs (17,423 vs. 48,300, p=0.035)

Conclusion: Romiplostim effectively reduced platelet transfusion requirements in LDLT patients without increasing the risk of vascular thrombosis or early allograft dysfunction. Additionally, it was associated with greater improvements in liver function parameters, shorter hospital stays, and lower treatment costs. These findings suggest that Romiplostim may be a valuable therapeutic option for optimising perioperative management in LDLT.

Design and optimisation of novel benzimidazole hybrid structures capable of simultaneous peroxisome proliferator activated receptor γ (PPARγ) and angiotensin receptor (ATR) modulation

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Introduction: Metabolic Syndrome (MetS) linked to increased risk of diabetes and cardiovascular disease, remains a challenge to manage even with currently used pharmacotherapeutic options. Clinical trials indicate that dual PPARγ/ATR modulators could represent enhanced treatment options. This study aimed to model such molecules in silico using virtual screening (VS) and de novo approaches.

Method: In the VS approach, experimental dual PPARγ/ATR agonist HTR-04 was used as a lead molecule and superimposed in LigandScout® onto the bioactive conformations of telmisartan and olmesartan as identified from PDB crystallographic depositions 3VN2 and 4ZUD respectively. These were used to model a consensus pharmacophore, which was used as a query at the ZincPharmer® database to identify a cohort of Rule of 3-compliant molecules. The binding affinity for modelled protomols (the energetically unsatisfied space at the core of the receptor) for both target receptors was calculated. In the de novo approach, HTR-04 seed fragments were created, docked into both target ligand binding pockets and allowed growth. Lipinski Rule compliant molecules with simultaneous affinity for both targets were consequently obtained.

Results: 2 Lipinski Rule Compliant molecular cohorts with dual PPARγ/ATR affinity.

Conclusion: This study adopted a holistic approach. The molecules derived from VS exhibited diverse molecular structures relative to the lead, and because they were docked into all available space within the target, analogous pharmacological activity to the lead is less probable. However, it is possible that an associated novel pharmacological activity could be identified. In the de novo approach, in which molecular growth was allowed exclusively within the bioactive ligand-binding pocket and was user-directed, the novelty of the molecular cohort was compromised at the expense of predicted bioactivity. For

both cohorts, the most promising structures will be further optimised.

Drug design and optimisation at the Pregnane X receptor

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Introduction: Drug-drug interactions, prevalent in elderly patients with polypharmacy, reduce pharmacotherapeutic efficacy or cause adverse effects, which may lead to morbidity and hospitalisation. The discovery that antagonism of the Pregnane X Receptor (PXR) reduced these interactions made this receptor a highly important target for drug design. This study used GSK002, a high affinity PXR antagonist as a lead molecule to design novel structures capable of PXR modulation, through virtual screening (VS) and de novo techniques.

Method: A GSK002 pharmacophore obtained from PDB crystallographic deposition 7RIU was modelled in LigandScout®. This was used as a query at the online ZINCPharmer® database to screen for hits, which were filtered for lead-likeness and for Lipinski-compliance. The hits were docked into the PXR protomol, generated in SYBYL-X®. The hits' toxicity class was identified in ProTox®. The hits with the highest affinity and lowest toxicity were identified and ranked by affinity for the protomol. Seed structures based on a modelled 2D-topology map describing the interactions of GSK002 with the target receptor were created in Sybyl-X®. These were allowed de novo growth within the PXR ligand binding pocket whose structure was modelled in LigBuilder®. The molecular cohort was filtered for Lipinski rule compliance and ranked by ligand-binding affinity.

Results: Each adopted approach yielded a Lipinski Rule compliant molecular cohort. VS yielded 291 molecules and the de novo approach yielded 200 novel structures.

Conclusion: The reduction of drug-drug interactions could have a significantly positive impact in scenarios of polypharmacy which is typical in ageing populations. This study contributes through the elaboration of novel structures of predicted high affinity for the PXR, and which being Lipinski Rule compliant are predisposed to oral bioavailability. The optimal molecules selected for further development from each modelled cohort also have predicted acceptable toxicity profiles.

Drug design and optimisation at the IL-1 Receptor-Associated Kinase (IRAK)

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Introduction: IRAK4 activation is crucial in the pathophysiology of rheumatoid arthritis and progression of tumour growth. Four experimental IRAK4 inhibitors are currently undergoing phase 1 or 2 clinical trials including Emavusertib (CA4948). Although no IRAK4 inhibitors are currently available, successful antagonism of this target is very attractive from a drug design perspective. This study aimed to use the scaffolds of two experimental IRAK4 inhibitors (CA-4948 and FJ9) as lead molecules for modelling novel structures with similar IRAK4 modulation.

Method: The bioactive conformations of the lead molecules CA-4948 and FJ9 were identified from PDB crystallographic depositions 7C2V and 7C2W respectively. In the Virtual Screening (VS), approach, two pharmacophores describing the bioactive conformations of each lead molecule were separately modelled in LigandScout® and read sequentially into ZincPharmer® with Rule of 3 filters for lead-likeness being applied. An IRAK4 protomol describing the vacant space at the core of the IRAK4 receptor was modelled in SYBYL-X® and used as a docking perimeter for the hit structures identified from ZincPharmer®. The affinity of the hit structures for the protomol was calculated and the 3 optimal structures were identified. In the de novo approach, seed structures were modelled based on the critical interactions forged between the lead structures and the IRAK4 receptor. The bioactive ligand binding pocket of the IRAK4 receptor was modelled in LigBuilderv_1.3® and the seed fragments were allowed growth within it. These molecules were ranked in terms of physicochemical characteristics (Lipinski Rule compliance) and affinity. The optimal structures were identified.

Results: Two groups of molecules emanating from VS (n = 43) and de novo design (n=200) respectively were obtained. These molecules all had high target affinity and were lead-like in the case of the VS cohort and Lipinski Rule compliant in the case of the de novo designed structures.

Conclusion: IRAK4 inhibition is desirable given its role in pathologies including rheumatoid arthritis and malignant disease. This study adopted complementary approaches- VS and de novo design in an attempt to ensure novelty (VS) and bioactivity (de novo design). The molecules with the most promising combinations of physicochemical attributes and affinity for the target from each approach will be further developed.

Drug design and optimisation at the dihydroorotate dehydrogenase (DHODH) receptor using the teriflunomide scaffold as a lead

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Introduction: Studies in mice show that dihydroorotate dehydrogenase (DHODH) inhibition is a novel route for the treatment of epilepsy. Given that currently available pharmacotherapy provides only marginal control of the condition, DHODH inhibition has become important from a drug design perspective. Teriflunomide, used in multiple sclerosis, has been identified as a potent DHODH inhibitor with poor intra-cerebral penetration due to high polarity. This study consequently aimed to use the teriflunomide scaffold as a lead to identify DHODH inhibitors with higher non-polar characteristics, thereby predisposing them to greater intracerebral penetration.

Method: This study adopted two parallel approaches- virtual screening (VS) and de novo design. The VS approach involved the modelling of an average (consensus) pharmacophore in LigandScout®. This was modelled through the fusion of two DHODH inhibitors, teriflunomide and its biaryl analogue identified from PDB cystallographic depositions 1D3H and 3U2O respectively. The consensus pharmacophore was submitted to ZincPharmer® for VS and resulting the Rule of 3 compliant hits were docked into a DHODH protomol modelled in Sybyl-X®. The hits were ranked in order of affinity for the protomol. In the de novo design approach, the ligand binding pocket of the DHODH receptor was modelled in LigBuilder® and seed structures based on the teriflunomide scaffold were created. Molecular growth of these seeds was allowed at predesignated special hydrogen atoms in the ligand binding pocket and the hits obtained were ranked in order of affinity, Lipinski Rule compliance and pharmacophoric similarities. The highest-ranking molecules from each cohort were selected.

Results: Two Lipinski Rule compliant molecular cohorts (derived from VS (n = 299) and de novo design (n = 442)) based on the teriflunomide scaffold were obtained. Of the VS derived cohort ZINC13637466 had the highest affinity for the protomol and was more non-polar (clogP=3.16) than teriflunomide.

Conclusion: This holistic approach to drug design produced two cohorts of molecules, which have an affinity to the target protomol (VS) and ligand binding pocket (de novo design), respectively. The molecular cohort obtained through VS is structurally and possibly pharmacologically more innovative than that derived from de novo design, which, however, since it was carried out at the locus of the target associated with bioactivity, is more likely to yield molecules which are analogous to the lead teriflunomide. The optimal structures from both cohorts will be further investigated using molecular dynamics simulations.

Drug design and optimisation of the Fibroblast Growth Factor Receptor Type 4(FGFR4)

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Introduction: Colorectal cancer remains one of the most aggressive and treatment-resistant cancers. Chemo-resistance is one of the greatest limitations to the efficacy of existing pharmacotherapy. Literature identified the FGFR4 receptor and its ligand FGF19 as drivers for cancer growth and chemoresistance. FGR4 inhibition could therefore result in improvement in tumour prognosis. Infigratinib, a potent FGFR inhibitor, has the potential to overcome chemoresistance and selected as lead molecule for this study which aimed is to identify high affinity FGFR4 modulators by probing the FGFR4 LBP from an atomic perspective and to model Lipinski Rule compliant molecular analogs using Virtual Screening (VS) and de novo design.

Method: For VS a consensus pharmacophore was generated in LigandScout® by superimposing 2 PDB crystallographic depositions; 4QQC and 4R6V, with infigratinib. Using ZINCPharmer® Lipinski Rule compliant hits were identified, docked into a modelled-promotol of the target and ranked according to binding affinity. In the de novo design phase, 2D ligand:protein contact maps between the small molecule and the FGFR4_LBP were modelled. Critical interactions identified leading to the creation of seed structures in LigBuilder®. The seed were allowed to grow within LBP, at predesignated sites (H.spc). The hits were ranked according to affinity, Lipinski Rule compliance and pharmacophoric similarity, with the highest ranked molecule being selected.

Results: 518 structurally diverse Lipinski-Rule compliant molecules were identified through VS. De novo design led to the generation of 154 Lipinski-Rule compliant novel structures.

Conclusion: Analysis of the highest affinity structures identified via VS showed that all 4 molecules can bind to FGFR4. The highest affinity structure had a low predicted LD50, consequently the molecule with the 2nd highest binding affinity with a lower toxicity profile was selected. The molecules identified through de novo design had superior physicochemical characteristics and high affinity for the target. The optimal structures will be validated using Molecular Dynamics Simulation studies.

Evaluation of the anti-inflammatory and immunomodulatory effects of adipose-derived mesenchymal stromal/stem cells using imiquimod-induced psoriasis-like dermatitis model mice

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Introduction: Adipose-derived mesenchymal stromal/stem cells (ASCs) and their secreted products have successfully alleviated the severity of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis. These clinical outcomes suggest that ASCs have potential for anti-inflammatory and immunomodulation. Although the efficacy of ASC transplantation for psoriasis is reportedly related to an increase in the regulatory T/T helper (Th) 17 cell ratio, decreased levels of interleukin (IL)-17, tumor necrosis factor (TNF)-α and reactive oxygen species (ROS), its mechanisms have not been fully elucidated. Furthermore, the efficacy of treatment with ASCs and their secreted products have varied among each study. This study aimed to investigate the efficacy and mechanisms of ASC transplantation in inflammatory diseases using an imiquimod-induced psoriasis-like dermatitis model.

Methods: To obtain ASCs, inguinal white adipose tissue was harvested from male C57BL/6J mouse, and stromal vascular fractions from the tissue were cultured. ASCs (2.0×10^6 cells) were subcutaneously injected into the dorsal skin of mice at the day before first imiquimod application. After the topical application of 63–65 mg of 5% imiquimod cream for five consecutive days, objective severity scores, cytokine gene expression levels including IL-23/Th17 axis, IL-1 family, and antimicrobial peptides, and neutrophil infiltration grade were determined to evaluate the efficacy of ASC treatment.

Results: ASCs ameliorated imiquimod-induced epidermal thickening and neutrophil infiltration in skin, although the treatment had no effects on skin erythema and scaling. ASCs suppressed the mRNA expressions of IL-23/Th17 cytokines (Il17a, Il17f, Il22, Il23), inflammatory cytokines (Tnfa, Il6) and Cxcl5, and antimicrobial peptides (S100a7, Lipocalin-2) in imiquimod-applied skin. Among IL-1 family cytokines, ASCs suppressed the mRNA expression of Il1b, but not the expressions of Il1f6 (IL-36α) and Il1f9 (IL-36γ). Additionally, the mRNA expression of Nlrp3, an inflammasome marker, was not suppressed by ASCs transplantation.

Conclusion: ASC transplantation suppressed the activation of IL-23/Th17 axis and broad range of inflammatory mediators in imiquimod-applied skin. However, the treatment had only a modest effect on the psoriasis-like dermatitis of this model. Proteases in the inflammasome and neutrophils activate IL-36 cytokines, which contribute to the initiation and progression of inflammatory diseases such as psoriasis vulgaris and generalized pustular psoriasis. To improve the efficacy of ASC-based treatment, further studies are needed to develop optimal culture conditions for priming ASCs and suppressing inflammasome and neutrophil activation. We are currently attempting cell-based experiments to determine the optimal culture conditions under which ASCs can effectively suppress neutrophil activation including ROS production and neutrophil extracellular trap release.

Design of novel molecules targeting breast cancer stem cells

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Introduction: Breast cancer is the most common type of cancer in our country and in the world, especially in women. Today, FDA-approved drugs are used for breast cancer treatment. However, studies have shown that cancer stem cells are resistant to many of these drugs. Molecular docking is a widely used method in structure-based drug design (SBDD). The main goal of molecular docking is to understand and predict molecular recognition, both structurally finding possible binding modes and energetically predicting binding affinity.

Method: In this study, the crystal structures of the receptor proteins (PDB: 7L1X), for which docking processes will be performed, were obtained in PDB format from the Protein Data Bank (PDB) website (http://www.rcsb.org/pdb). The structures and SMILES codes of FDA-approved molecules used in breast cancer treatment were retrieved from the ZINC15 database (https://zinc15.docking.org/). Docking studies were conducted using the Autodock Vina 1.1.2 software. Chimera UCSF 1.17.3 was employed to prepare the ligands for docking. The BIOVIA Discovery Studio Visualizer program was utilized to visualize the ligands and proteins in 3D and to examine the ligand-receptor interactions through the docking results. The ADME profile analysis of the molecules used in molecular docking was carried out using the SwissAdme program.

Result: Olaparib exhibits two hydrogen bonding interactions with active site residues. The –C=O functionality and the aromatic ring were displaying H-bond with TYR50 (2.37 Å), SER51 (2.78 Å) amino acids. It has halogen interactions and eight hydrophobic interactions including π-π, alkyl and π-alkyl interactions with TYR50, LYS49, LEU178, HIS160 residues. It has also an electrostatic π-anion interaction with ASP175 residue.

Conclusion: A novel molecule has been designed for the treatment of breast cancer, based on the drug Olaparib. This new molecule is considered a promising drug candidate for the effective treatment of breast cancer. Overall, the current findings highlight the potential of multiple drug molecules for breast cancer; however, further validation through laboratory experiments is required.

The biological active principles of fruit wine and its ability to prevent cell damage caused by free radicals

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Background: Peaches contain many different biological active ingredients that have a positive effect on health. Fresh peaches are available during few months, so they can be consumed all year round only processed as juice, compote or jam. The processing of peaches in the above-mentioned forms reduces the content of thermolabile compounds (such as phenolic compounds). The solution to this problem could lie in processing the peach into wine, a process that does not require high temperatures. This is one of the reasons why the wines produced are a rich source of phenolic compounds.

Purpose: The aim of this study was to investigate the phenolic profile and activity of fruit wines on enzymatic systems in vitro and the level of lipid peroxidation.

Method: The microvinification process was used to produce fruit wines under various controlled conditions. The wine samples were produced with and without the addition of sugar and enzymatic preparations. Fermentation was carried out using two different yeasts. The UPLC TQ-MS/MS system was used to evaluate the phenolic profile. Lipid peroxidation (malondialdehyde (MDA) levels) and the activity of the antioxidant protective enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were investigated on the isolated rat synaptosomes. Synaptosomes were isolated from the brain of Wistar albino rats.

Results: Phenolic acids and flavonoids were detected in peach wine after UPLC TQ-MS/MS analysis. Chlorogenic acid was the predominant compound with a content of 117.71 to 145.31 µg/ml. Among the other phenolic acids, p-coumaric acid (1.27-2.85 µg/ml), protocatehuic acid (9.27-18.31 µg/ml), gallic acid (12.34-20.27 µg/ml) and caffeic acid (3.27-8.55 µg/ml) were detected. The following flavonoids were detected in the peach wines: Catehin (4.27-12.31 µg/ml), Epicatehin (27.17-45.32 µg/ml) and Quercetin (12.37-25.41 µg/ml). The above phenolic compounds affected the degree of lipid peroxidation and the activity of enzymes in the isolated rat synaptosomes in which oxidative stress was experimentally induced by hydrogen peroxide. The values for MDA were in the range (3.51-3.93 nmol/mg), while SOD activity was in the range (3.87-4.25U/mg). The activity of GPx was in the range (0.0185-0.0207 U/mg), as was that of CAT (0.037-0.058 U/mg). Wines produced with added sugar and enzymatic preparation showed a higher content of quantified phenolic compounds, lower values for MDA content and a higher activity of SOD, GPx and CAT.

Conclusion: Peach wine is a rich source of phenolic acids and flavonoids, which have a positive effect on the human organism. It is also important to emphasize other biologically active compounds that were not quantified in the fruit wines and that were also responsible for the reduction of MDA levels and the higher activity of SOD, CAT and GPx. The properties of peach wines related to the reduction of MDA levels and the activation of antioxidant protective enzymes could potentially be used against free radicals and to prevent oxidative stress.

The phenolic potential and antioxidant properties of newly created red grape variety in Serbia

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Background: Red wine is rich in phenolic compounds that act as antioxidants and neutralize free radicals that cause oxidative stress and cell damage and can contribute to aging, chronic diseases and cancer. Their antioxidant properties help to reduce inflammation and protect the body. The content of polyphenols in red wine can be influenced by both pre-fermentation and fermentation conditions.

Purpose: The aim of this study was to investigate the potential of the newly bred red grape variety Vožd as a source of polyphenols and consequently its antioxidant properties in wine.

Methods: The wine samples were obtained after the three maceration periods (5, 14 and 21 days). Alcoholic fermentation of the Vožd grape variety took place with the yeast strain BDX (Lallemand, Canada). During mash fermentation, the grapes were sulphurized with $K_2S_2O_5$ at a

rate of 10 g/100 kg. The total phenolic content was determined using the Folin-Ciocalteu method and expressed in mg GAE/L. Antioxidant activity was measured as anti-DPPH radical activity (% of inhibition, IC_{50}).

Results: The highest total phenolic content was determined on the 21st day of maceration and amounted to 1365.0 mg GAE/L, while the other samples had slightly lower values. In the wine with a maceration period of 5 days, the total phenolic content was 1205.0 mg GAE/L and in the wines with a maceration period of 14 days it was 1170.0 mg GAE/L. The wine macerated for 14 days showed the highest anti-DPPH radical activity of 8.55%, while the wine with the shortest maceration time showed the lowest radical activity.

Conclusion: The results show that a longer maceration of the Vožd variety leads to a higher total phenolic content, with the wine macerated for 14 days having the highest anti-DPPH activity. However, the Vožd wine with the shortest maceration time showed the lowest radical activity.

Synthesis of metformin complex with yttrium

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Introduction: Metformin is an oral antidiabetic drug belonging to the biguanide group. In addition to its antidiabetic effects, metformin is considered to have antimicrobial, antifungal, anti-inflammatory and antitumor effects. Due to its structure and the presence of two cis-positioned imino groups, metformin can form complexes with transition metals. These transition metal complexes with metformin may exhibit improved pharmacokinetic properties and biological activity compared to metformin, thereby enhancing its efficacy and therapeutic potential.

Purpose: The aim of this study was to synthesize and characterize complex of metformin with Ytrium (Y^{3+}).

Methods: The complex was prepared according to the procedure: 3 mmol of metformin hydrochlorid was dissolved in 25 ml methanol then mixed with 25 ml of methanolic solution of 1 mmol metal nitrate of Ytrium (Y^{3+}). A mixture of mole ratio of 1:3 (Y(NO3)3xH2O: metformin), at pH adjusted to 8–9 (i.e. by adding 1 M methanolic ammonia solution) was heated under reflux and continuous stirring at 60–70 ºC for about 2 h. The mixtures were left overnight until precipitation occurred. The synthesized complex was characterised using: Fourier-Transform Infrared Spectroscopy (FTIR), spectrofluorimetry and UV spectroscopy. Electronic spectra of complexes were recorded in DMSO and DMF.

Results: The change of the intensity of the stretching vibration bands of −C=N at 1634 cm-1 and vibration bands observed at 1384 cm-1 confirm that the Y (III) metformin complex has been synthesized. The synthesized Y (III) metformin complex exhibits maximum absorption at 256 nm in DMSO and 265 nm in DMF. The free metformin ligand is excited by the absorption bands at 276 nm and 336 nm which attributed to π-π* electronic transitions. The fluorescence emission spectra of Y (III) metformin complex in DMSO exhibit absorbtion band at 270 and emission band at 309 while in DMF the absorption and emission bands occur at 280 nm and 307 nm.

Conclusion: By combining the performed techniques with others such as elemental analysis, NMR, and X-ray diffraction the structural formula of the compound can be confirmed, allowing the evaluation of various biological activities.

Transforming evidence into practice: Bisacodyl's dual approach to constipation treatment

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Introduction: Bisacodyl, a stimulant laxative, is widely used for relieving constipation. To elucidate the mode of action of bisacodyl, Magnetic Resonance Imaging was used to define the mechanisms underlying the laxative effect of bisacodyl in two independent studies.

Method: In these two randomised, double-blind, placebo-controlled, cross-over studies, participants meeting Rome IV criteria for functional constipation and self-medicating with occasional laxatives <4 times in the previous month, were randomly assigned to receive either bisacodyl or placebo. A single dose of 5 mg bisacodyl and a repeated daily dose of 5 mg for 3 days were given to evaluate changes in gastrointestinal responses. Primary endpoint was ascending colon water content (ACWC) difference as assessed by T1AC AUC300–450 minutes. Secondary endpoints included small bowel water content (SBWC), whole gut transit time (WGTT), colonic volumes, stool frequency, and consistency using Bristol Stool Form Score.

Results: Participants in both studies were predominantly middle-aged women, (age, mean (SD) 50.0 and 53.5 (16.3) years gender). Fourteen were randomised to single dose and 15 to repeated dose study. No significant difference was observed in ACWC compared to placebo (P = 0.58) after single dose. However, after repeated doses, the water content was

62% higher with bisacodyl (P = 0.02). The single dose significantly increased ACWC over six hours, which was not observed with placebo while baseline ACWC was already elevated in repeated dose. There was no significant difference in SBWC between bisacodyl and placebo for the 0–150 and 150–450 minutes period after single dose (P = 0.22 and 0.29, respectively). Repeated doses showed significant mean differences in SBWC for both AUC0-150 minutes and AUC150–450 minutes (P = 0.027 and 0.018). Both single and repeated doses of bisacodyl accelerated WGTT, reducing it from 63-22 hours after single dose (P < 0.001) and from 57.4-15.6 hours after 3 doses (P = 0.049). Rectosigmoid colon volume increased with bisacodyl (P = 0.03). Ascending colon volume reduced from 150-450 minutes with bisacodyl (P = 0.03). While both single and repeated doses accelerated whole gut transit and reduced time to first defecation, only repeated doses increased the frequency of bowel movements (P = 0.006) and reduced stool consistency (P = 0.015). The treatments were well tolerated with only minor symptoms like cramps, gas, and bloating.

Conclusion: Bisacodyl 5 mg (one Dulcolax® tablet) effectively normalises gut transit and reduces time to first defecation without altering stool consistency. When counselling patients, pharmacists should highlight that taking it thrice enhances efficacy by increasing intestinal water content, improving stool frequency and consistency. Bisacodyl stimulates natural gut motility without changing underlying physiology, which normalizes 24 hours after discontinuation. When guiding patients through constipation treatment options, clinical evidence suggests that initiating therapy with a 5 mg dose might be preferable to 10 mg in many cases, though individual assessment remains essential for determining the optimal starting dose. Patient education on these evidence-based recommendations is crucial for optimal management, with pharmacists playing a key role in guiding appropriate treatment and dosage selection.

FXR agonist for post liver transplantation cholestasis: A randomized open-labelled study-preliminary results

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Background: Following live donor liver transplantation (LDLT), intra-hepatic cholestasis may occur for a multitude of reasons, such as reperfusion injury, infections, vasculobiliary complications, or rejection. This study compares the effectiveness of the farnesoid-X-receptor agonist obeticholic acid (OCA) with ursodeoxycholic acid (UDCA) in ameliorating post-transplant intra-hepatic cholestasis and improving graft survival.

Methodology: In this, randomized controlled trial, we assigned all patients following LDLT to receive either 5 mg OCA once- daily or 300 mg UDCA 3 times daily from postoperative day 3 to one year. Exclusion criteria included ABO incompatibility, acute liver failure, failure to consent to the study and pediatric population. The primary goal was a 15% reduction in alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) levels from baseline one-month post-transplant to the 3rd,6th, and 12th months. Secondary endpoints included quantifying cellular-level biochemical indices like total plasma bile acids, bile salt export pump, transforming growth factor -β, Cytokeratin -18, Serum autotaxin, fibroblast growth factor-19; assessing rejection rates, incidence of biliary complications, adverse events, mortality, and quality of life (using CLDQ)

Results: Following the analysis of 83 patients for the primary endpoint, there was a 15% reduction in ALP and GGT. ALP reduction was significantly greater in the OCA group compared to UDCA [17 (43%), 8 (21%) (p = 0.036)] from 6 to 12 months. From 1 to 3 months, 15% GGT reduction occurred in 80% of OCA compared to 62% in UDCA (p=062).Total plasma bile acid concentration at one month was significantly lower in the OCA group compared to UDCA [170 (108, 263) vs 226 (193, 317), p=0.012]. No significant differences between the two groups were observed between the remaining secondary endpoints, such as cellular markers, the incidence of biliary complications, rejection rates, early allograft dysfunction, and mortality, between the two groups. The plasma LDL cholesterol was significantly higher at 3 months (121 (102-170) versus 107 (89-133) p=0.027) and 6 months (130 (102-156) versus 117 (91-130) p+0.043) in the OCA group.

Conclusion: In this randomized trial on OCA versus UDCA following LDLT, preliminary results suggest significantly more ALP reduction and lower total plasma bile acids in the OCA group compared to UDCA with better quality of life score.

Quinoline-naphthohydroquinone hybrid compounds: synthesis and biological evaluation

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Background: Quinolines and naphthohydroquinones are found in natural products such as quinine and alkannin, respectively. These structures have shown important bioactivity such as anticancer, antibacterial, antifungal, and antimalarial. Some examples bioactive compounds currently in use for the treatment of such diseases are: Ciprofloxacin, Lenvatinib, Mefloquine – quinoline derivatives and Doxorubicin- naphthoquinone derivatives. Both moieties

continue to be an important subject in Medicinal Chemistry and the focus of many pharmacological studies. Among their many bioactive properties, such structures have also shown to display antioxidant activity. Compounds that present antioxidant activity can scavenge reactive oxygen species and prevent oxidative stress, which is related to the development of cancer. Treatment resistance and consequent loss of efficacy have led to the search of new bioactive compounds, with these cores, aiming to improve therapeutic indices. One strategy to develop new bioactive compounds is molecular hybridization or conjugation. This allows the combination of various bioactive chemical structures in one single molecule with the aim of developing new compounds with improved properties.

Purpose: In this project it was aimed the synthesis of new hybrid-type compounds with a quinoline and a naphthohydroquinone moieties. The obtained hybrids were then tested for their antioxidant and anticancer properties.

Method: The quinoline structures were synthesized in a one-pot Doebner type reaction and the naphthohydroquinone unit was synthesized from myrcene and p-benzoquinone. Hybrid compounds were obtained by joining both previously obtained moieties through several linkers with ester bonds using α,ω-dibromo-reagents. Antioxidant activity was measured using the colorimetric assay DPPH. Anticancer activity was tested on HT-29 and H460 cancer cell lines using the MTT method.

Results: Quinolines were successfully obtained using the Doebner type reaction under mild conditions, in good yields (64 to 85 %). After obtaining the naphthohydroquinone moiety, this was conjugated by an ester bond with α,ω-dibromo-reagents, aliphatic or aromatic, achieving the hybrid precursors in yields ranging from 50 to 60 %. These precursors were then conjugated by another ester bond with the previously synthesised quinolines, obtaining 6 different hybrid compounds (yields between 16 to 46 %). Antioxidant assays performed with the hybrid compounds and its precursors showed a better antioxidant capacity than their quinoline precursors. Anticancer activity against HT-29 and H460 cancer cell lines showed that quinolines were not cytotoxic at concentration < 100 µM. On the other hand, naphthohydroquinone precursors presented cytotoxicity with < 100 µM. Most hybrid compounds were cytotoxic in HT-29 and H460 cell lines, being two of them more cytotoxic than its precursors on both tested cell lines.

Conclusions: The proposed quinoline-naphthohydroquinone hybrid compounds were successfully obtained. Antioxidant and anticancer assays for the hybrid compounds demonstrated an improvement in these activities when compared with their quinoline precursors and in some cases also with respect to their naphthohydroquinone precursor. The obtained results in this project show the hybrid compounds as possible anticancer drug candidates.

Comparative analysis of essential oils from solidago species in the flora of the Republic of Moldova

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Background: Essential oils constitute a significant group of biologically active compounds that have applications across diverse fields, including medicine, agriculture, food industry, and cosmetics. In recent years, research on essential oils derived from Solidago species has increased considerably due to their pharmacotherapeutic potential and broad spectrum of pharmacological effects, such as antibacterial, antifungal, antiviral, sedative, and cytotoxic properties. Purpose: This study aims to compare the chemical composition of essential oils extracted from Solidago virgaurea L. and Solidago canadensis L. collected from the Scientific-Practical Center for Medicinal Plants at Nicolae Testemitanu State University of Medicine and Pharmacy in the Republic of Moldova.

Method: Essential oils were extracted by hydro-distillation with a NeoClevenger extractor from fresh aerial parts (stems, leaves, and inflorescences) collected at full bloom and analyzed using gas chromatography-mass spectrometry (GC-MS) with an Agilent Series GC-MS system.

Results: Based on the mass spectral analysis, 51 chemical components were identified in the essential oil of S. canadensis, and 37 components in S. virgaurea, respectively. The dominant compound found in the essential oil of S. virgaurea was α-pinene (28.8%), followed by β-caryophyllene (9.96%), β-elemene (8.29%), germacrene D (7.49%), β-myrcene (6.2%), caryophyllene oxide (4.24%), limonene (3.06%), and α-humulene (3.01%). In contrast, the main component in S. canadensis essential oil was limonene (22.81%), followed by germacrene D (19.73%), α-pinene (19.03%), β-caryophyllene (6.53%), β-myrcene (4.75%), β-ylangene (4.4%), and bornyl acetate (3.81%).

The findings highlight a notable similarity in the chemical composition of the essential oils of both Solidago species, particularly the presence of α-pinene as a major component, with a higher concentration in S. virgaurea. Moreover, monoterpenes and sesquiterpenes were identified as the dominant hydrocarbon groups in the essential oils of both

species. A key difference is the high limonene content in the essential oil of S. canadensis, contrasting with its presence as a minor component in S. virgaurea.

Conclusion: This study provides a valuable foundation for further research into the therapeutic potential of volatile oils from Solidago species in the flora of the Republic of Moldova, as their properties remain largely unexplored to date.
Acknowledgement
This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS-UEFISCDI, project number PN-IV-P8-8.3-ROMD-2023-0307, within PNCDI IV.

Mechanism of lipid metabolism improvement by Lycium and Lycium-based polyherbal supplement polysaccharides in apolipoprotein E-deficient mice

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Introduction: Dyslipidaemia is a major cause of atherosclerotic diseases, which are prevalent in Japan. Peroxisome proliferator-activated receptors (PPARα and PPARγ) are factors involved in lipid metabolism, and it has been reported that PPARγ activates fatty acid metabolism, influencing T cell activity. NFATc is known as a T cell activation factor, while cytotoxic T lymphocyte antigen (CTLA-4) is recognised as an inhibitory factor. On the other hand, LLA, a polyherbal supplement primarily composed of Lycium, has been reported to have immune-activating effects, and Lycium polysaccharides, the main component of Lycium, have been shown to activate T cell activity. Our previous studies have clarified that Lycium polysaccharides and LLA polysaccharides have a cholesterol-lowering effect. The present study aims to elucidate the mechanism of lipid metabolism improvement in the liver.

Method: Polysaccharides were extracted and purified from Lycium extract and LLA extract. In animal experiments, normal mice (BALB/c, male, 6 weeks old) were given standard food and water, while apolipoprotein E-deficient mice were given a high-fat diet (0.2% cholesterol) along with either water, Lycium extract, LLA, Lycium polysaccharides, or LLA polysaccharides for 7 weeks. Serum and liver tissues were collected and frozen. For serum, the HDL levels were evaluated. For liver tissue, lipids were first extracted, and the lipid content was assessed. Next, total protein was extracted, and SOD activity and GSH levels were evaluated. Additionally, the expression of PPARα, PPARγ, NFATc1, NFATc2, and CTLA-4 was evaluated by Western blot analysis.

Results:

1. HDL levels in the serum showed a significant decrease in the Apoe-/- water-treated group compared to normal mice, with a tendency for increased HDL in the Lycium polysaccharide-treated group.
2. Total cholesterol levels in the liver tissue significantly increased in the Apoe-/- water-treated group, but showed a tendency to decrease with Lycium extract treatment.
3. GSH levels in the liver tissue significantly increased in the Apoe-/- water-treated group, but no significant changes were observed in the sample-treated groups. SOD activity did not show any effects in any of the treatment groups.
4. Western blot analysis of PPARγ expression showed a significant decrease in the Apoe-/- water-treated group, with a significant increase in the lycium extract and LLA polysaccharide-treated groups, and a tendency for increase in the Lycium polysaccharide-treated group. PPARα, CTLA-4, and NFATc1/2 showed no significant effects in any of the treatment groups.

Conclusion: Based on the above results, it is suggested that Lycium extract and polysaccharides improve cholesterol abnormalities in the liver by increasing the activity of PPARγ, rather than enhancing antioxidant activity through GSH and SOD. However, no association was found between T cell activation and lipid metabolism improvement due to changes in the expression of NFATc or CTLA-4. As a future direction, it is necessary to investigate the effects on the expression of sterol regulatory element-binding proteins (SREBP), transcription factors that promote fatty acid and cholesterol synthesis, as well as the impact on the activity of M2 macrophages, whose differentiation is promoted by PPARγ, to further elucidate the mechanism behind the lipid-improving effects of Lycium polysaccharides.

Non-Enzymatic microbial approach for curing Refsum's Enzymatic disease

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Background: Refsum's disease is a disease characterized by the lacked presence of phytanol-coenzyme A hydroxylase, a vital enzyme necessary for the metabolism of the dairy and meats toxin phytanic acid. Due to the lack of this enzyme Refsum's patients suffer most commonly both visual and auditory manifestations, which in a short number of years leads to cardiovascular asthenia and death.

For many genetic disorders the most approachable treatments are symptomatic relief medications, mental aid, and in some cases like Refsum's disease blood transfusions. These sorts of treatments are very common due to their cost

effectiveness and accessibility, but they are temporary treatments that exhibit very short therapeutic effects.

Objective: This study is approaching a less common yet less expensive treatments that have a high chance of giving the desired effects. Microorganisms of all sorts have been known of their inclusion in several treatments. This inclusion is due to their composition of secondary metabolites like violacein from Bacillus species that holds inhibitory effects on breast cancer, a genetic disease. These secondary metabolites hold unique biological properties that in the appropriate growth conditions have shown several times their effect as antitoxins, antitumor, antibacterial, and antiviral. They also posses several activities that are widely similar to the human body like Prodigiosin in streptomyces species known for its neuroprotective and anti-inflammatory effects.

Results: Experimental trials are undergoing extracting a metabolite that would aid in mimicking the action of phytanol-coenzyme A. Several strains of bacteria are included in these trials such as Esheriace coli, Actinobacteria, Streptomyces, and Bacillus. These strains were carefully chosen based on previous successful extractions that were included in drugs treating diseases such as food and chemical poisoning, antibiotic resistance infections, GIT disorders, and even Parkinson's disease.

Conclusion: The aim of this study is to highlight future novel approaches in treating Refsum's disease with an equally powerful natural metabolite. After comparing the enzyme with several pigments, a few have shown great potential. Synthetic approaches for years have failed to come close to a definitive lifelong treatment, thus the most sensible way to find a cure is to scavenge for metabolites in organisms widely known for their vast toxic and therapeutic effects.

Novel Triazole linked thymol-thienopyrimidine hybrids: In vitro and in silico studies

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Background Information: Cancer remains a leading cause of death, with increasing incidence and mortality. By 2030, cancer cases are expected to reach 21 million annually. The need for novel agents with improved efficacy and fewer side effects is urgent. Natural products play a crucial role in drug discovery, particularly in oncology. Hybrid molecules, which integrate different pharmacophores, have emerged as promising drug candidates.

Purpose: This study aims to focus on novel hybrid derivatives containing a thymol-triazole-thienopyrimidine moiety, which may enhance anticancer activity by targeting multiple pathways.

Method: Eleven novel hybrid compounds were synthesized via Huisgen 1,3-dipolar cycloaddition, followed by condensation with corresponding aldehydes and characterized using NMR, HRMS and IR spectroscopy. Cytotoxicity was evaluated on MCF7 breast cancer and HUVEC normal cells using the MTT assay to determine IC50 values. In silico studies were also conducted to evaluate the potential inhibition mechanism on HER2, EGFR, VEGFR1, and VEGFR2 proteins. Additionally, predicted ADME properties were calculated.

Results: Docking studies indicated reasonable interactions, particularly with VEGFR2 and HER-2, with some compounds showing favorable binding scores. Molecular dynamics simulations confirmed the stability of ligand-protein complexes through a 250 ns simulation period. Cytotoxicity results demonstrated selective inhibition of MCF7 cells, with minimal toxicity on HUVEC cells. In silico ADME predictions indicated favorable pharmacokinetic properties, supporting their potential for further development.

Conclusion: The synthesized hybrid compounds exhibited significant anticancer activity in vitro and strong molecular interactions with cancer-related proteins. This study highlights the potential of monoterpene-1,2,3-triazole-thenopyrimidine derivatives anticancer agents. Future work will expand biological evaluations, optimize molecular structures for enhanced efficacy, and investigate additional cancer cell lines.

Factorial design applied in the stability analysis of epidermal substitutes for diabetic ulcer treatment.

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Background: In Mexico, 400,000 people with diabetes suffer from foot ulcers, with traditional treatments proving ineffective and often leading to amputations. Regenerative medicine has developed skin substitutes that enhance wound healing; however, their acceptance faces challenges due to stringent storage conditions. Cryopreservation is a viable alternative, though it may cause tissue damage due to ice formation and osmotic imbalance. This study evaluated

different cryopreservation conditions to optimize the viability of skin substitutes as Class III cellular therapy devices, with potential applications in the treatment of diabetic ulcers. Purpose: To assess the stability of epidermal substitutes under different cryopreservation conditions to determine short-term viability maintenance in the development of a Class III cellular therapy device.

Method: Four cryopreservation conditions were evaluated in 25 mm diameter skin substitutes stored at -80°C for 1, 3, and 6 months. Samples were thawed at 37°C, and cell viability was assessed using MTS assay 4 hours post-thawing. Based on the best results, a factorial experiment was designed with three main factors: (1) Freezing method: vitrification, one-step (1°C/min), two-step (-20°C/5h, -80°C), and three-step (4°C/2h, -80°C/24h, -135°C); (2) Storage temperature: -80°C and -135°C; (3) Cryoprotectants (CPA 1 and CPA 2): tested at high (1) and low (-1) levels in 24 runs with three replicates. HaCaT cell monolayers at 100% confluence and >95% viability was used and stored for one month. Viability percentage (MTS assay) was the measured response. Statistical optimisation was applied to identify the optimal combination for maximised viability. The model was validated by storing skin substitutes under these optimised conditions and assessing stability over 1, 3, and 6 months.

Results: After cryopreserving skin substitutes for 1, 3, and 6 months, results indicated that condition 4 achieved the highest viability (66% at 6 months). Based on these findings, new combinations were designed to optimise cryopreservation. Statistical analysis demonstrated that all evaluated factors significantly influenced cell viability ($p<0.05$, $R^2=99\%$). The optimal condition was identified as vitrification at -80°C with CPA 1 and CPA 2 at high levels, achieving a 92 ± 3.42% viability in HaCaT cells. Under this improved protocol, stored skin substitutes-maintained viabilities of 89%, 81%, and 74% at 1, 3, and 6 months, respectively, demonstrating significant stability improvements.

Conclusions: Vitrification at -80°C with CPA 1 and CPA 2 at high levels is the optimal storage condition for epidermal substitutes. This protocol ensures short-term viability, supporting its application in developing Class III cellular therapy devices for the treatment of diabetic ulcers.

Lactococcus lactis carrying staphylococcal protein A as a new weapon to fight against Methicillin Resistant Staphylococcus aureus skin and soft tissue infections

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Introduction: Staphylococcus aureus is the most prominent cause of skin and soft tissue infections (SSTI) worldwide and the associated mortality is a major threat to public health given the incidence of methicillin-resistant S. aureus (MRSA). Moreover, the predominant S. aureus isolates involved in SSTIs produce multiple toxins that cause tissue damage and impair proper abscess formation, leading to bacterial dissemination. It has been demonstrated that the pro-inflammatory properties of the staphylococcal protein A (SpA) are critical for neutrophil recruitment, proper organisation of skin abscesses and accelerated wound healing. This project aimed to determine the feasibility of local administration of heat-killed Lactococcus lactis SpA as an immunomodulator and inducer of wound healing in a mouse model of MRSA cutaneous infection.

Methods: Groups of healthy mice or challenged with S. aureus (USA300 LAC or ST30), were treated intradermally with heat-killed L. lactis SpA (SpA-encoding vector) or L. lactis CV (control) at two sites in the healthy skin surrounding the lesion, 24 hours post-challenge. Mast cell activation (toluidine blue stain), pro-inflammatory cytokines and chemokines (ELISA) were assessed. Lesion size was measured on days 1, 3, and 7, and histological analyses (haematoxylin-eosin and Masson's trichrome staining) were performed 6 days post-treatment.

Results: Histological analysis revealed that mice inoculated with L. lactis SpA maintained intact skin structures, similar to controls (L. lactis CV and PBS), without inflammatory damage. By day 6, TNF-α and IL-6 levels were elevated in L. lactis SpA-treated mice compared with PBS controls, but similar to L. lactis CV-treated animals. Additionally, L. lactis SpA induced a significant increase in mature mast cells. These findings highlight the immunostimulatory potential and safety of L. lactis SpA. To determine the effect of L. lactis SpA-treatment on S. aureus skin infection, groups of mice were subcutaneously inoculated with S. aureus. L. lactis SpA-treated mice showed a significant increase in the number of

mast cells in the lesions compared with untreated groups 24 hours post-treatment. Moreover, the L. lactis SpA-treated group exhibited a reduction in lesion size on days 3 and 7 post-challenge, unlike L. lactis CV-treated or untreated animals.

In all experimental groups at day 7 post-challenge, lesions showed extensive necrosis of the epidermis and dermis, characteristic of S. aureus USA300 LAC infections, associated with their high toxin production. However, histopathological analysis revealed that mice treated with L. lactis SpA exhibited better-contained cutaneous lesions with reduced depth than the other groups. Moreover, bacterial clusters were surrounded by pyocytes in the L. lactis SpA-treated group, whereas in control groups, lesions exhibited bacterial colonies throughout. Additionally, in L. lactis SpA-treated animals, a fibrous tissue capsule surrounding the lesion was observed. The effects of L. lactis SpA treatment were verified in mice challenged with a clinically relevant MRSA isolate from Argentina (S. aureus ST30).

Conclusion: These results suggest that L. lactis SpA administration in S. aureus-induced skin lesions could reduce lesion size and promote abscess formation, which limits bacterial dissemination and tissue damage, crucial factors in improving treatment efficacy.

Anti-neuroinflammatory effects and mechanism of exosome-like nanoparticles derived from Atractylodes lancea rhizome

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Background: Exosome-like nanoparticles (ELNs) mediate interspecific intercellular communication and regulate gene expression. ELNs derived from Atractylodes lancea rhizome (ALR-ELNs), a traditional herbal medicine, exhibit potential anti-neuroinflammatory effects on microglia. However, the mechanisms and associated signaling pathways remain unclear.

Purpose: This study aimed to isolate and characterize ALR-ELNs and investigate their anti-inflammatory effects and molecular mechanisms in lipopolysaccharide (LPS)-stimulated BV-2 microglial cells.

Methods: ALR-ELNs were isolated and purified from dried Atractylodes lancea rhizomes using differential centrifugation. Transmission electron microscopy was used to analyze their morphology, and RNA sequencing was performed to identify the microRNAs (miRNAs) present in ALR-ELNs. The uptake of ALR-ELNs by BV-2 microglial cells was confirmed using fluorescence microscopy. The anti-inflammatory effects of ALR-ELNs on LPS-stimulated BV-2 microglial cells were evaluated by mRNA and protein expression analyses. Furthermore, RNA sequencing and an Ingenuity Pathway Analysis (IPA) were performed to elucidate the mechanisms underlying the regulation of pro- and anti-inflammatory factors.

Results: BV-2 cells efficiently internalized ALR-ELNs that contained three potential anti-inflammatory miRNAs: ath-miR166f, ath-miR162a-5p, and ath-miR162b-5p. Pretreatment of BV-2 cells with ALR-ELNs significantly reduced production of the LPS-induced pro-inflammatory factors nitric oxide, interleukin (IL)-1β, IL-6, and tumor necrosis factor-α. Additionally, the mRNA expression levels of the pro-inflammatory genes, IL-1β, IL-6, inducible nitric oxide synthase, C-C motif chemokine ligand 2 (CCL2), and C-X-C motif chemokine ligand 10, were significantly decreased. Conversely, the expression of anti-inflammatory genes, including heme oxygenase 1, interferon regulatory factor 7, CCL12, and immune-responsive gene 1, was significantly upregulated after ALR-ELN treatment. RNA sequencing revealed that 651 genes were downregulated and 1204 were upregulated in ALR-ELN-treated LPS-stimulated BV-2 cells. The IPA indicated that ALR-ELNs modulated inflammation through pathogen-associated signaling, and a network analysis highlighted the role of toll-like receptor (TLR) signaling in suppressing inflammation. Notably, the real-time quantitative polymerase chain reaction confirmed that ALR-ELNs significantly downregulated TLR4 mRNA expression.

Conclusion: ALR-ELNs exhibit potent anti-inflammatory effects on microglial cells by suppressing TLR4 expression and modulating key inflammatory pathways. Identifying the active components of ALR-ELNs that are responsible for TLR4 suppression may contribute to the development of novel therapeutics for neuroinflammatory diseases.

Construction and efficacy of an Ad5/casp3 adenoviral vector to induce apoptosis in MCF-7 cells as a potential advanced medicinal product for breast cancer

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Introduction: Cancer is a term used to define a set of diseases in which cells are characterized by unlimited potential to replicate, resistance to apoptosis, immune evasion and metabolic reprogramming. The study of apoptosis resistance has led to the establishment of new therapeutic targets. The MCF-7 cell line is an example of an in vitro model of breast cancer where the caspase-3 gene is not expressed correctly. Therefore, this cell line will be used to evaluate the restoration of the function of this protein by transgenesis. Thus, the aim of this work is the molecular construction of a first-generation adenovirus expressing caspase-3 (Ad5-Casp3) in the MCF-7 breast cancer cell to induce programmed cell death, as a potential biopharmaceutical for gene therapy. Purpose: Construction and evaluation of an adenoviral vector with caspase-3 to induce apoptosis in MCF-7 cells, with potential use as a biopharmaceutical in the treatment of breast cancer.

Methods. The first-generation adenoviral vector Ad5-Casp3 was designed by cloning the Casp3 gene into the Adeno-X™ expression system. Production and purification of Ad5-Casp3 in HEK293 cells cultured in serum-free EXCELL293 medium was optimized by supplementation with a caspase inhibitor. Viral titles were determined by end-point dilution. The identity of Ad5/Casp3 was confirmed by PCR, monitoring the presence of the Casp3 transgene and the absence of the region. Vector efficacy was performed in MCF-7 cell cultures as a function of the effective concentration 50 (EC50), apoptosis induction was determined by trypan blue cell viability assays, DNA fragmentation analysis, flow cytometry with Annexin V assay and cell cycle analysis. The morphology of the transduced cultures was analyzed by epifluorescence microscopy using acridine orange/propidium iodide (AO/IP) differential staining.

Results. The adenoviral vector Ad5-Casp3 had the ability to induce apoptosis in transduced MCF-7 cell cultures. The expression of caspase-3 was confirmed by RT-PCR. The caspase inhibitor enhanced Ad5-Casp3 vector production in HEK293 cell cultures by preventing apoptosis and increasing viral titters from 3.9×10^{7} PFU/mL to up to 1.67×10^{10} PFU/mL. Vector efficacy was determined as a function of effective concentration 50 in MCF-7 cell culture with a value of EC50= 22 virus/cell 120 h post-transduction. In addition, a significant reduction in cell viability was observed, the morphology observed by epifluorescence microscopy (AO/IP staining) is consistent with programmed cell death process, DNA fragmentation consistent with endonuclease activation, the increase of cells in sub-G1 and the Annexin V assay confirmed the generation of an apoptotic process.

Conclusion: The production of an Ad5/Casp3 adenoviral vector was successful and the results indicate that the use of a caspase inhibitor significantly improves Ad5-Casp3 vector production in HEK293 cells, increasing viral titles and optimizing its availability for functional studies. Evaluation in MCF-7 cells demonstrated that Ad5-Casp3 is effective in inducing apoptosis, as evidenced by caspase-3 expression, reduced cell viability, DNA fragmentation and Annexin V analysis. In addition, morphology observed by epifluorescence microscopy showed characteristic changes of apoptosis, such as nuclear condensation and fragmentation. These observations support the potential of Ad5-Casp3 as a biopharmaceutical for gene therapy in breast cancer.

An efficient model to optimize the production of cart cells in Mexico: An example for assume such a technology in non-developed countries

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Introduction: In Mexico, Acute Lymphoblastic Leukemia (ALL) is the most prevalent type of cancer in pediatric patients and the leading cause of cancer-related mortality in this population, posing a major public health challenge. While conventional treatments such as chemotherapy and radiation are effective for newly diagnosed cases, the prognosis drastically worsens in relapse or refractory cases. Over the past two decades, innovative therapies have emerged, showing promising results. Among them, Chimeric Antigen Receptor T-cell (CAR-T) therapy has gained prominence for treating relapse/refractory leukemia in pediatric patients. This gene therapy involves isolating autologous T cells from the patient, genetically modifying them with a lentiviral vector to express a specific CAR and enhancing their ability to recognize specific antigens (e.g., CD19 in B-cell ALL). However, CAR-T therapy faces significant challenges, including high costs (USD 375,000–475,000) and long production times (2–4 weeks), which limit accessibility in developing countries like Mexico, particularly for critically ill patients. To address these limitations, ongoing research aims to optimize manufacturing processes to reduce costs and production times.

This project proposes a viable CAR-T cell production strategy, focusing on the lentiviral transduction and cell expansion stages, using an immortalized T-cell line as a proof of concept. This approach has the potential to advance the accessibility of CAR-T cell therapy for pediatric leukemia treatment in Mexico.

Methodology: A second-generation CAR-T cell model was developed targeting the CD19 antigen using a lentiviral vector to transduce an immortalized T-cell line (Jurkat E6-1). The identity of the transduced cells was confirmed through endpoint PCR and qPCR. Subsequently, the cells were characterized and enriched using flow cytometry. To optimize cell expansion, various strategies were evaluated, including different culture media, operational modes (batch, fed-batch, and perfusion), and stirring conditions. These strategies were assessed based on key parameters such as maximum cell density, doubling time, and the maintenance of CAR/CD19 expression without alteration.

Results: The lentiviral transduction achieved an initial efficiency of 3.0%, making the enrichment step essential to obtain a population of 98–99% CAR-T positive cells. The enriched cells were then characterized, confirming that 60% of the clones expressed functional receptors capable of docking with the CD19 antigen. These cells were subsequently used in expansion strategies, achieving maximum cell densities of 9 cel/mL, 11.8x106 cel/mL and 3.5x107 cel/mL in batch mode, fed-batch, and perfusion mode within two weeks. All experiments were conducted using a specific growth medium without fetal bovine serum, maintaining an unaltered doubling time of 21–23 hours. Importantly, CAR/CD19 expression remained stable across all operational modes.

Conclusions: A successful model was developed a CAR-T cell using a lentiviral vector in an immortalized T-cell line. Furthermore, a suitable and efficient expansion strategy was established, and it has the potential to reduce production costs and time, making CAR-T cell therapy more accessible both financially and logistically.

CANnabidiol impact on challenging behaviour in adults with Intellectual disability with Lennox Gastaut and Dravet syndrome (CANABID-LD): A naturalistic case-control study

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Background information: Intellectual disability (ID) is a condition characterised by deficits in both intellectual and adaptive functioning, with onset during the developmental period. Dravet syndrome and Lennox-Gastaut syndrome are both epileptic syndromes associated with co-occurring ID.

Challenging behaviour (CB) is described as culturally abnormal behaviour that places the physical safety of the person or others at risk, or will lead to limited access to ordinary community facilities. It is common in people with ID, with significant CB present in about 3.8-10% of adults with ID. People with CB are often exposed to restrictive practices such as limited access to activities of daily life, seclusion, and polypharmacy, without a strong evidence base.

Currently, there is a lack of effective pharmacological treatments for CB although cannabidiol is a licenced treatment option for pharmaco-resistant epilepsy in people with LGS or Dravet.

Purpose: The aim is to evaluate the impact of Cannabidiol on CB prescribed in patients with LGS or DS and co-occurring ID for pharmco-resistant seizures.

Method: Work Package 1 is an observational study. All patients with LGS/DS meeting inclusion criteria will be invited to join the study. The outcome will be the change in the Aberrant Behaviour Checklist-Irritability subscale between baseline, 3-months and six-month follow-up and its association with cannabidiol dose. A mixed effects repeated measure model, with adjustment for baseline score and recruitment site, will be used.

Sixty patient-participants will be recruited over 12 months. A sample size of 52 will allow us to detect an effect size of 0.5, based on 80% power and 5% significance; thus, allowing for 15% loss to follow-up, we require 60 participants. The study will be conducted across ten UK based centres each recruiting 5-7 participants.

Work Package 2 is an embedded qualitative study. Semi-structured interviews with up to 30 people (10 participants, 10 family carers, 10 clinicians) or until data saturation is reached will explore acceptability including the perceived impact on seizures and behaviour.

An economic evaluation will develop and test methods for assessing cost-effectiveness. Resource use will be collected by healthcare professionals delivering the intervention. Data on the utilisation of health and social care services, and on wider societal resource use, will be collected. The Euroqol-5D-5L (EQ-5D-5L) will measure health-related quality of life. Quality-Adjusted Life-Year (QALY) weights will be estimated in line with current National Institute for Health and Care Excellence (NICE) guidance.

Results: The results from this observation study will inform the potential role of Cannabidiol. If there is a signal from the trial, and the intervention is acceptable and cost-effective then a full scale randomised-controlled trial (RCT) is the planned next step. Such a trial could establish whether, or not, Cannabidiol is effective for these difficult to treat conditions.

Conclusion: Developing clinically effective and cost-effective treatments for CB for patients with ID and LGS or DS and co-occurring pharmaco-resistant seizures can improve outcomes and quality of life.

Funders: This study is being supported by an unrestricted investigator-sponsored research grant from Jazz Pharmaceuticals.

Early positive signals for new treatments of primary hypercholesterolemia and mixed dyslipidemia: Bempedoic acid and inclisiran in Spain

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Introduction: Primary hypercholesterolemia and mixed dyslipidemia are common conditions that often require lipid-lowering therapies. New treatments such as bempedoic acid and inclisiran have recently been approved in combination with diet, with statins, or with other lipid-lowering drugs in patients with hypercholesterolemia, mixed dyslipidemia, atherosclerosis, statin intolerance, or when these are contraindicated. Both represent two innovative strategies in lipid management, with alternative mechanisms of action. Bempedoic acid, an ATP citrate lyase inhibitor reduces hepatic cholesterol synthesis upstream of statins, reducing muscle-related adverse effects. Inclisiran, on the other hand, enhances LDL receptor recycling and lowers LDL-c levels in a two-yearly dosing regimen, offering long-term lipid control, and improving adherence and therapy results. Given their novel mechanisms, it is essential to establish their safety profiles through continuous pharmacovigilance. This study aims to identify early positive signals for these treatments, contributing to post-marketing surveillance and optimizing patient care.

Method: A data mining study was performed following the Bayesian methodology applied by the Uppsala Monitoring Center belonging to WHO for searching positive signals in databases of suspected adverse drug reaction reports. Data of all reported adverse events for the chemical subgroup 'Other lipid modifying agents' (C10AX) and the combination of bempedoic acid and ezetimibe (C10BA10, combination of two active ingredients of subgroup C10AX) from the Spanish Agency of Medicines and Medical Devices repository were analyzed. This database follows MedDRA terminology, and data were extracted in an aggregated format based on Preferred Terms (PTs). As a control, it was analyzed suspected adverse events reported for ezetimibe (ATC: C10AX09). The main outcome measures were early positive signals of adverse drug reaction candidates to be analyzed in specific clinical trials or post-marketing studies. For the statistical analysis, a validated adaptation of the data mining Bayesian Confidence Propagation Neural Network (BCPNN) methodology extended to the multiple comparison setting was applied. The threshold of the estimator false discovery rate (FDR) equal to or lower than 0.050 implies a positive signal for the active ingredient and a candidate to follow up. In this adaptation, each pair of active ingredient-suspected adverse event is compared with all the pairs reported for its specific ATC subgroups.

Results: A clear signal was observed for the pair ezetimibe-myalgia (FDR<0.001), a well-documented adverse effect, serving as a control to validate the methodology. Positive signals obtained and not reported in the Summaries of Product Characteristics for bempedoic acid -as monotherapy treatment- were arthralgia (FDR=0.006), abdominal pain upper (FDR=0.032), and abdominal discomfort (FDR=0.043), signals and adverse events also reported in combination with ezetimibe. Early positive signal of toxic skin eruption (0.048) appears for inclisiran.

Conclusions: This study identifies new potential safety signals for bempedoic acid to elucidate if they also appear in monotherapy strategy; and a toxic condition of skin eruption for inclisiran more severe than those reported as mild and frequent. These signals should be further explored to confirm their clinical relevance and potential impact on patient care.

Evaluation of the immunoprotective power of a Multiple Antigenic Peptide against Aah II toxin of Androctonus australis hector scorpion

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Scorpion envenoming (SE) is a public health problem in developing countries. In Algeria, the population exposed to the risk of SE was estimated at 86.45% in 2019. Thus, the development of a vaccine to protect the exposed population against scorpion toxins would be a major advance in the fight against this disease.

This work aimed to evaluate the immunoprotective effect of a Multiple Antigenic Peptide against the Aah II toxin of Androctonus australis hector scorpion, the most dangerous scorpion species in Algeria. The immunogen MAP1Aah2 was designed and tested accordingly. This molecule contains a B epitope, derived from Aah II toxin, linked by a spacer to a universal T epitope, derived from the tetanus toxin.

The results showed that MAP1Aah2 was non-toxic despite the fact that its sequence was derived from Aah II toxin. The immunoenzymatic assay revealed that the 3 immunization regimens tested generated specific anti-MAP1Aah2 antibodies and cross-reacted with the toxin. Mice immunized with this immunogen were partially protected against mortality caused by challenge doses of 2 and 3 LD50 of the toxin. The survival rate and developed symptoms varied depending on the adjuvant and the challenge dose used. In the in vitro neutralization test, the immune sera of mice having received the immunogen with incomplete Freund's adjuvant neutralized a challenge dose of 2 LD50.

Hence, the concept of using peptide dendrimers, based on linear epitopes of scorpion toxins, as immunogens against the parent toxin was established. However, the protective properties of the tested immunogen require further optimizations.

A molecular docking and quantitative structure binding relationship study of sesquiterpene lactones as inhibitors of n. gonorrhoeae penicillin-binding protein 2 (PBP2h041) enzyme

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Introduction: Neisseria gonorrhoeae has developed resistance to drugs that are commonly used for its treatment and management. Very recently the World Health Organization (WHO) listed N. gonorrhoeae's drug resistance as a problem that requires new drug developments. As part of research efforts to solve this problem xerantholide a sesquiterpene lactone (SqLs) from Pechuel-loeschea leubnitziae was found to be active towards gonorrhea. This project explores the mechanism by which sesquiterpene lactones may elicit antibacterial effect on N. gonorrhoeae through inhibition of PBP2H041 which is an essential enzyme in the bacteria.

Aim: This study aimed to investigate the binding of sesquiterpene lactones to PBP2H041 as a means of inhibiting the enzyme and to develop quantitative models in which binding affinity is related to the physicochemical properties of the sesquiterpene lactones.

Method: The geometry of one hundred and seven (107) conformers from eight three (83) SqLs were fully optimized using the density functional theory (DFT) in the Spartan programme. To determine the binding patterns of sesquiterpene lactones to PBP2H041 enzyme, the binding affinities and interactions were computed using molecular docking approach as implemented on AutoDock Vina programme.

Results: The lowest binding affinity computed ranged from -7.1 to -6.9 kcal/mol for ten analogues and they were lower than the binding affinity of the lead compound Xerantholide which is -6.8 kcal/mol and also lower than that of Azithromycin (-5.3 kcal/mol), Erythromycin (-5.7 kcal/mol) and identical to that of Tetracycline (-7.1 kcal/mol).

Conclusion: The results obtained in this study provide a basis to investigate the in-vitro PBP2 inhibition potential of sesquiterpene lactones analogues. This study provides an alternative treatment modality option that can be considered for implemention of antibacterial multi-modal genorrhaea treatment and/or management with an addative or synergistic therapeutic effect.

Comparative analysis of polyphenols in fruits and non-edible parts of aronia melanocarpa (MICHX.) Elliot from the republic of Moldova

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Background: Aronia melanocarpa (Michx.) Elliot, commonly known as black chokeberry and a member of the Rosaceae family, has been widely recognized as a fruit-bearing plant with substantial pharmacological potential. This species serves as a biofactory of biologically active compounds, including polyphenols, vitamins, macro- and microelements, and fibers. These constituents contribute to its diverse pharmacological effects, which include antioxidant, anti-inflammatory, anticancer, hepatoprotective, antimicrobial, and antifungal properties.

Purpose: The present aims to conduct a comparative analysis of the fruits and non-edible parts (frequently regarded as waste) of A. melanocarpa, Nero variety, focusing on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA).

Method: The plant material used in this research was sourced from the collection of the Scientific-Practical Center for Medicinal Plants at Nicolae Testimitanu State University of Medicine and Pharmacy, Republic of Moldova. Hydro-alcoholic extracts were prepared from dried fruits and various non-edible plant parts, including one- and three-year-old twigs and their bark, leaves (collected at distinct phenological stages: pre-flowering, flowering, and fruit ripening), and flowers. The TPC was determined using the Folin-Ciocalteu method and expressed as gallic acid equivalents (mg GAE/g). The TFC was quantified spectrophotometrically and expressed as quercetin equivalents (mg QE/g). Antioxidant activity was evaluated using the DPPH radical scavenging assay, calibrated with Trolox, and expressed as mg TE/g.

Results: The results revealed that the TPC ranged from 30.21 to 135.12 mg GAE/g among the analyzed samples. The highest TPC was observed in the three-year-old twigs (134.44 mg GAE/g), followed closely by the one-year-old twigs (126.74 mg GAE/g). Bark samples exhibited high TPC values as well (121.92 mg GAE/g for three-year-old twigs and 105.72

mg GAE/g for one-year-old twigs), approximately 1.5 times greater than that of the fruits. Conversely, flowers exhibited the lowest TPC (30.21 mg GAE/g). A strong positive correlation was noted between TPC and AA, with the highest antioxidant potential recorded in the twigs and their bark. The TFC of the analyzed extracts ranged from 3.53 mg QE/g (bark of three-year-old twigs) to 18.29 mg QE/g (leaves collected during the fruit maturation phase).

Conclusion: These findings underscore the potential of A. melanocarpa non-edible parts as valuable sources of bioactive compounds. The one- and three-year-old twigs and their bark are particularly rich in polyphenols with high antioxidant activity, while leaves collected during fruit maturation are notably abundant in flavonoids. Utilizing these plant parts, particularly at the end of the vegetation period, ensures sustainable resource use without compromising the plant's physiology or development. These results lay the foundation for further exploration of A. melanocarpa as a sustainable source of raw materials for pharmaceutical, cosmetic, and food industries. Future research should focus on optimizing extraction techniques and investigating practical applications of these bioactive plant products.

Acknowledgement: This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS-UEFISCDI, project number PN-IV-P8-8.3-ROMD-2023-0307, within PNCDI IV.

Evaluation of antiplasmodial and cytotoxic activity of harmisinins, harmine-artemisinin hybrids

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Introduction: Malaria, an infectious disease caused by Plasmodium parasites, affected 263 million people and claimed 597 000 lives in 2023, remaining significant global health threat due to high mortality rates and drug resistance challenges. Therefore, finding of effective new agents is of great interest. Molecular hybridization strategy is a valuable tool for developing new agents and may overcome some drawbacks of the combined therapy. Harmine, an alkaloid of the β-carboline type, and artemisinin, a natural sesquiterpene lactone, possess a wide range of biological properties. It has been shown that harmine is a selective inhibitor of P. falciparum heat shock protein 90 (PfHsp90) and has extensively been investigated due to its antiplasmodial activity. On the other hand, artemisinin and its derivatives have been used as one of the most important antimalarial drugs with proven mechanisms of action against P. falciparum.

Method: Synthesis of harmisinins required synthesis of their building blocks which were all obtained in multiple-step reactions. Triazole-type harmisinins were prepared by Cu(I)-catalyzed cycloaddition from artesunate-based azide and β-carboline-based alkynes or dihydroartemisinin based alkyne and β-carboline-based azides. Amide-type harmisinins were obtained by coupling reaction of artesunate or different dihydroartemisinin-based carboxylic acids with β-carboline-based amines. The activity of the compounds against the erythrocytic stage of the Plasmodium life cycle was evaluated in vitro against two strains of Plasmodium falciparum, chloroquine-sensitive (Pf3D7) and chloroquine resistant (PfDd2) strains, employing a previously described method. Cytotoxicity against human liver hepatocellular carcinoma cell line (HepG2) was evaluated using the neutral red assay with minor modifications.

Results: To this end, we have synthesized five series of harmisinins, harmine–artemisinin hybrids of triazole and amide type, and investigated their antiplasmodial activity. Harmisinins exerted remarkable activity against both tested strains, higher than the parent compound harmine (more than two orders of magnitude higher). The experiments revealed that 12 harmisinins display activities in single-digit nanomolar range against both strains. The activity of other harmine-artemisinin hybrids was in low nanomolar concentrations. The activity against Pf3D7 strain was comparable with that of chloroquine while harmisinins showed an order of magnitude higher activity against PfDd2 strain. Screening of the cytotoxic activity of harmisinins against HepG2 was performed to evaluate their selectivity against Plasmodium over mammalian cells. All compounds exhibited selective activity against P. falciparum in comparison to HepG2 cell line. Remarkably, most harmisinins showed more than 4 orders of magnitude higher activity against the parasite than against mammalian cells (SI > 10000).

Conclusion: Five series of novel hybrid molecules comprising harmine and artemisinin scaffolds have been synthesized. Their antiplasmodial activity as well as cytotoxicity against HepG2 cells have been evaluated. All compounds exerted remarkable activities against both Pf3D7 and PfDd2 strains as well as high selectivity towards P. falciparum. In our future experiments, we will focus on the elucidation of harmisinins mechanisms of action

In-silico and in-vitro investigation of the molecular mechanisms of antiviral compounds for potentially inhibiting Dengue Virus NS2B-NS3 protease.

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The recent outlook of Dengue viral infection as a global public health concern, coupled with the reportage of resistance and lack of anti-dengue drugs, calls for a concerted effort to find new leads. The study combined In-silico and in vitro approaches to identify novel potential synthetic small-molecule inhibitors targeting the DENV-2 NS2B-NS3 protease.

The NS2B-NS3 protease enzyme in the dengue virus transmission pathway is required for the replication of the virus within the host cell. The lack of NS2B-NS3 protease homologue in the human host and its conserved nature among all dengue viruses make it a viable target for future anti-dengue drugs.

Initially, six inhibitors of dengue NS2B-NS3 protease with IC50 < 10 µM were used to generate a pharmacophore model with a score of 0.9143 using LigandScout. The validated model was used to screen a synthetic library of 65,345 compounds obtained from ChemDiv. Thirty compounds with pharmacophore fit scores above 55 were docked against the modelled three-dimensional structure of NS2B-NS3 protease using AutoDock Vina.

Consequently, nine compounds with binding energies ranging from−7.5 to −8.7 kcal/mol were identified as potential hit molecules. Three compounds comprising STOCK6S-06667, STOCK6S-65928, and STOCK6S-65450 with respective binding energies of −8.7, −8.2, and −8.0 kcal/mol, were selected as plausible lead molecules.

Molecular dynamics simulation studies and molecular mechanics Poisson–Boltzmann surface area calculations showed that the residues Asp75 and His51 were critical for ligand binding. The compounds were also predicted to have anti-dengue activity with reasonable pharmacological and toxicity profiles.

When the anti-dengue activity of the three hits was evaluated in vitro against the dengue protease, mean half-maximal inhibitory concentrations (IC50) of 21.9 ± 1.5 µM (STOCK6S-5928), 23.5 ± 1.1 µM (STOCK6S-06667), and 118.3 ± 5.8 µM (STOCK6S-65450) were obtained.

Furthermore, STOCK6S-5928 and STOCK6S-06667 inhibited the growth of dengue virus, with IC50 of 14.3 ± 2.0 µM and 18.1 ± 1.4 µM, respectively. The identified compounds could be optimized to develop potent anti-dengue therapeutic agents.

The findings of this study provide a promising lead for the development of anti-dengue drugs. Further optimization and in vivo evaluation of these compounds are necessary to determine their potential as therapeutic agents. Additionally, the study highlights the importance of targeting the NS2B-NS3 protease in the development of anti-dengue drugs.

In conclusion, this study demonstrates the potential of combining In-silico and in vitro approaches to identify novel inhibitors of the DENV-2 NS2B-NS3 protease. The identified compounds provide a promising starting point for the development of anti-dengue drugs, and further research is warranted to explore their therapeutic potential.

Future studies will focus on optimizing the lead compounds through structural modifications and evaluating their efficacy in vivo. Additionally, the study's findings will be used to design and synthesize new compounds with improved potency and selectivity. The ultimate goal is to develop effective anti-dengue drugs that can combat this devastating disease.

SNP-6: A novel multi-mechanistic therapy for MASH with evidence from preclinical and phase 2a study

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) encompasses a wide spectrum, ranging from simple fatty liver to metabolic dysfunction-associated steatohepatitis (MASH), hepatic fibrosis, and ultimately progressing to cirrhosis and hepatocellular carcinoma (HCC). With a global prevalence of approximately 25%, MASLD has become a significant health concern. MASH represents the active stage of the disease, necessitating pharmacological intervention, particularly when necroptotic damage and fibrotic progression are present. Given the complexity and heterogeneous nature of MASH, there is an urgent need for effective therapeutic strategies that address comorbidities,

inflammation, and fibrosis. Although only one drug has been approved so far, its efficacy remains limited. Therefore, the development of novel drug candidates with improved efficacy and a well-tolerated safety profile remains a critical priority.

Purpose This study aims to evaluate the effects of SNP-630MS, the active metabolites of SNP-6, on MASH and explore its underlying mechanisms in combating MASLD.

Method: [In vivo murine MASH model] Male C57BL/6 mice were fed with a high-fat diet (HFD) for 21 weeks and then, SNP-630-MS was administered orally while continuing the HFD for an additional 12 weeks. [Phase 2a clinical trial] The efficacy, tolerability and safety of SNP-630-MS were evaluated in 35 MASH patients. 17 patients were enrolled in the first arm, receiving 2 tablets of SNP-630-MS, assigned as high dose group and 18 patients were receiving 1 tablet of SNP-630-MS, assigned as low dose group. The primary and secondary endpoints were the changes in serum aminotransferase (ALT) level and liver steatosis, inflammation and fibrosis (CCL4, CCL5, Caspase 3 and FibroScan) from baseline to week 12.

Results: SNP-630-MS treatment lowered ALT levels, a key marker of liver inflammation, by limiting intrahepatic CD4 and CD8 T cell accumulation and suppressing inflammation-related gene expression in MASH mice. It also alleviated HFD-induced liver steatosis, decreased hepatic triglycerides, and improved the plasma lipid profile through the modulation of lipid metabolism-related genes. Additionally, SNP-630-MS reduced the expression of fibrotic genes, including Col1a1, Col3a1, and Timp1, suggesting its potential anti-fibrotic effects. Consistent with preclinical findings, patients treated with SNP-630-MS showed a significant reduction in ALT levels at week 12 compared to baseline, with no reports of severe adverse events. Notably, this ALT reduction exceeded that observed with most other MASH drug candidates. Furthermore, SNP-630-MS exhibited antifibrotic potential, as indicated by a significant decline in fibrogenesis-related biomarkers such as CCL4, CCL5, and caspase 3. Subgroup analysis using FibroScan measurements further supported its efficacy in ameliorating liver fibrosis.

Conclusion: SNP-630-MS showed excellent tolerability in mice and patients with MASH. SNP-630-MS demonstrated favorable results in mice. Efficacy analyses indicated that SNP-630-MS improved liver steatosis and injury in patients with MASH, suggesting that SNP-630-MS are promising therapeutic options for MASH. Larger scale clinical trials remain warranted to assess the efficacy and safety of SNP-630-MS in MASH.

Machine learning-based screening and identification of antimalarial compounds

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Introduction: Malaria remains a critical global health burden, with 247 million cases and 619,000 deaths reported in 2023 alone [1], disproportionately affecting resource-limited regions like sub-Saharan Africa. The traditional drug discovery pipeline faces two fundamental constraints in low-resource settings: prohibitive costs of high-throughput screening and computational bottlenecks in virtual screening workflows of large-scale datasets.

Purpose: In this work, we aim to design a Machine Learning (ML)-driven framework to identify prospective active antimalarials during virtual screening and also compare different machine learning architectures to optimize predictive performance.

Methodology: Using the open-source CHEMBL Legacy malaria dataset, a subset containing potency and IC50 data was selected. The dataset was partitioned using the LoHi splitter to ensure chemically diverse training and test sets. We evaluated four molecular representation types: fingerprints, descriptors, graph-based, and SMILES across different ML architectures. Traditional ML models (e.g., Random Forest, XGBoost, Adaboost) were trained on fingerprints and descriptors, while deep learning models (Graph Attention Networks [GAT], Graph Convolution Networks [GCN], Message Passing Neural Networks [MPNN]) were applied to graph representations. Additionally, a transformer-based model (ChemBerta) was trained on SMILES representations. Model performance was assessed using accuracy, precision, recall, and ROC_AUC.

Result: Descriptors outperformed other molecular representations in terms of predictive performance, with Random Forest and Adaboost achieving the highest accuracy and precision. Among fingerprint-based models, Random Forest and Adaboost consistently performed well, while Multi-Layer Perceptron achieved the best recall. For graph-based models, GCN exhibited the highest recall, whereas GAT demonstrated the best accuracy and precision. ChemBerta showed strong accuracy and precision but exhibited weak recall, indicating potential limitations in identifying active compounds.

Conclusion: This research highlights the potential of ML in virtual screening for antimalarial drug discovery. The findings suggest that simpler molecular representations (descriptors) and traditional ML models (Random Forest) can outperform more complex architectures (Graph-based and Transformer models) in this context. However, further improvements in

precision, recall, and ROC_AUC are necessary for ML models to be fully integrated into drug discovery pipelines.

Sugar-conjugated dithiocarbamates: A novel strategy to overcome chemoresistance in breast cancer

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Introduction: Breast cancer remains a leading cause of cancer-related mortality worldwide, with triple-negative breast cancer (TNBC) being one of the most aggressive subtypes. Chemotherapy remains the primary treatment; however, resistance to cytotoxic agents, including paclitaxel, significantly limits therapeutic success and contributes to treatment failure. Therefore, novel therapeutic strategies are urgently required to improve treatment efficacy and overcome drug resistance.

Disulfiram (DS), a licensed anti-alcoholism drug, has demonstrated potent anticancer activity by forming a copper-chelating complex through its active metabolite, diethyldithiocarbamate (DDC). This complex generates reactive oxygen species (ROS), leading to oxidative stress-induced apoptosis in cancer cells. However, the clinical application of DS is hindered by rapid metabolism, poor solubility, and instability in physiological conditions. To address these limitations, this study investigates the development of sugar-conjugated dithiocarbamate derivatives with improved stability and efficacy for the treatment of chemoresistant breast cancer.

Method: Two sugar-functionalised dithiocarbamate derivatives, N, N-dimethyl 2-deoxy-D-glucopyranosyl dithiocarbamate (2DG-DDC) and its acetylated derivative (Ac-2DG-DDC), were synthesised and characterised using Fourier-transform infrared spectroscopy, nuclear magnetic resonance, and electrospray ionisation mass spectrometry. These derivatives were designed to enhance the biological stability of DDC while preserving its copper-chelating ability.

The copper-binding capacity of 2DG-DDC and Ac-2DG-DDC was assessed via reaction with copper chloride ($CuCl_2$), followed by mass spectrometric analysis. The cytotoxicity of these compounds was evaluated using MTT and ATP assays on breast cancer cell lines, including MDA-MB-231 (wild-type) and its paclitaxel-resistant variant (MDA-MB-231PAC10). Mechanistic studies included apoptosis detection via flow cytometry, intracellular ROS quantification, and assessment of autophagy markers through Western blot analysis.

In vivo studies were conducted to assess the pharmacokinetic stability of 2DG-DDC in the bloodstream and its therapeutic efficacy in murine breast cancer models. Tumour growth inhibition and survival rates were monitored in treated animals compared to control groups.

Results: Both 2DG-DDC and Ac-2DG-DDC efficiently chelated Cu^{2+}, forming stable $Cu(DDC)_2$ complexes. These sugar-functionalised derivatives exhibited significantly enhanced cytotoxicity in both wild-type and chemoresistant breast cancer cells compared to unmodified dithiocarbamates, with lower IC50 values indicating improved potency. Flow cytometry revealed a substantial increase in apoptotic cell populations following treatment with 2DG-DDC/Cu, correlating with elevated ROS levels. Mechanistic studies confirmed the activation of endoplasmic reticulum stress pathways and autophagy, contributing to cancer cell death.

The use of autophagy inhibitors partially rescued cell viability, confirming autophagy as a key mechanism of cytotoxicity. In vivo, 2DG-DDC demonstrated prolonged circulation stability compared to its unmodified counterpart, reducing rapid clearance and improving bioavailability. Significant tumour volume reductions and increased survival rates were observed in treated animals compared to controls.

Conclusion: This study demonstrates that sugar conjugation enhances the biological stability and therapeutic potential of dithiocarbamates. The sugar-functionalised derivatives, in combination with copper, effectively induce apoptosis and autophagy in chemoresistant breast cancer cells, overcoming key resistance mechanisms. The in vivo findings highlight the strong therapeutic potential of these compounds for breast cancer treatment. Further research is warranted to optimise the formulation and assess clinical feasibility.

Desmodium gangeticum restores testicular health and enhances reproductive outcomes in multi-generational and age-related stress models

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Introduction: This study extends previous research on the fertility-enhancing properties of Desmodium gangeticum (DG) root extracts in immobilization-induced stress (SIMB) in male Wistar rats. DG roots were extracted using n-hexane (HEDG), chloroform (CEDG), and water (AEDG), with HEDG and AEDG evaluated at 125 and 250 mg/kg doses over 28 days. Parameters such as gonadosomatic index, semen quality, hormonal levels, oxidative stress, and testicular histopathology were assessed. HEDG exhibited significant aphrodisiac activity, improving sperm parameters, testosterone levels, and testicular histology. In silico studies highlighted Gangetin, a pterocarpan, with superior binding to

PDE5 compared to tadalafil, supported by molecular dynamics and Swiss ADME data. The observed effects were linked to antioxidant and anti-inflammatory properties without supporting the experimental data. Further, the study was carried out only in the current generation. The present study describes the effects of HEDG against SIMB in age-related male Wistar rats model on fertility in a multi-generational (F0-F1-F2) approach by considering various molecular aspects.

Methods: HEDG was tested in 28-week-old male Wistar rats and in a multi-generational (F0-F1-F2) study using 12-week-old rats subjected to 6 weeks of SIMB. Parameters including sexual behavior, hormonal levels, testicular histopathology, sperm-related parameters, and spermatogenesis-associated gene expression (PRM1, PRM2) were evaluated, alongside pregnancy outcomes on litter size, and offspring development.

Results: HEDG treatment enhanced spermatogenesis, sperm count, motility, and morphology while reducing oxidative stress markers and upregulating PRM1 expression. Testosterone levels and Leydig and Sertoli cell health improved, supported by restored testicular architecture confirmed by histopathological analysis. Multi-generational studies showed no adverse effects on F1 and F2 generations, with healthy litters exhibiting normal growth, sexual maturity, and reproductive behavior. HEDG-treated groups had larger litter sizes, normal birth weights, and better sperm quality compared to negative controls.

Conclusion: This comprehensive evaluation supports HEDG as a safe and effective agent for mitigating stress-induced infertility and improving male reproductive health across generations.