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PP002

RHODOMYRTONE: FROM BASIC RESEARCH TO PHARMACEUTICAL APPLICATIONS

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Rhodomyrtone, a natural compound isolated from *Rhodomyrtus tomentosa* (Aiton) Hassk demonstrated profound antibacterial activity against acne-causing organisms. Of main advantage, rhodomyrtone could effectively prevent biofilm formation and kill mature biofilms. Rhodomyrtone caused reduction in staphyloxanthin and increase in susceptibility of *S. aureus* to H_2O_2 and singlet oxygen killing. Consequently, the survival ability of the treated organisms in human blood decreased due to less carotenoid pigments to act as antioxidant scavengers. Rhodomyrtone may be acting via effects on DnaK resulting in many additional effects on the bacterial virulence. Furthermore, the compound effectively prevented alterations including increase in thio-barbituric acid reactive substances and decrease in glutathione, superoxide dismutase, catalase, and glutathione peroxidase in blood, liver, and kidney in mice administered carbon tetrachloride and maintained the antioxidant status indicating that it can serve as a potent antioxidant. Taken together, these findings indicate that rhodomyrtone possesses antibacterial, anti-infective, as well as anti-oxidant activities, which make it an interesting candidate drug. Rhodomyrtone at 128xMIC did not produce toxic effect on human erythrocytes. The data provide sufficient evidence to support therapeutic uses of rhodomyrtone. A novel liposomal encapsulated product, Rhodomyrtone AcneClear, was further developed. The formulation was clearly demonstrated to be more effective than azelaic acid, benzoyl peroxide, and clindamycin, most commonly prescribed topical drugs. Minimal inhibitory concentration that inhibits 90% growth (MIC_{90}) of *Propionibacterium acnes*, an etiologic agent of acnes, was 0.5 μg/mL. MICs and minimal bactericidal concentrations (MBCs) for multidrug-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, associated microorganisms in acnes-affected area, ranged from 1-2 and 4-8 μg/mL, respectively. Time-kill study indicated that the bacterial numbers treated with 4xMIC rhodomyrtone were reduced by 99-99.99% within 3-24 hours. In addition to infection control, uses in human volunteers clinically demonstrated good healing activity and skin whitening. All subjects showed no signs of irritation or long-term undesirable side effects.

PP003

EVALUATION OF MODIFIED RELEASE FORMULATIONS OF A NEW 1,3-DISUBSTITUTED AMINOADAMANTANE DERIVATIVE WITH TUBERCULOCIDAL ACTIVITY

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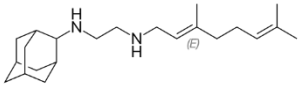
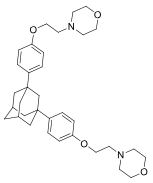
Introduction: Tuberculosis, an infectious disease usually caused by the bacteria *Mycobacterium tuberculosis* (TB), has been present in humans since ancient times. Once rare in developed countries, tuberculosis infections began increasing in 1985, partly because of the emergence of HIV. Many strains of tuberculosis resist to the drugs mostly used to treat the disease and as a result new drugs and regimens are urgently needed that can shorten the required duration of tuberculosis treatment. N-Adamantan-2-yl-N'-[(E)-3,7-dimethyl-octa2,6-dienyl]ethane-1,2-diamine (SQ109, Fig. 1), is a drug candidate that is active against both drug-susceptible and drug-resistant TB strains and affects cell wall synthesis [1]. Based on these findings, it was recently decided to extend our ongoing research on the chemistry and pharmacology of aminoadamantane analogues [2] by synthesizing and evaluating an analogous derivative of SQ109, compound **1** (Fig. 1), which showed a noteworthy tuberculocidal activity. *Albeit* the fact that the lipophilicity of this compound is higher than that of SQ109, it is, however, within the allowed limits for oral administration. Therefore, it was intriguing to probe its oral absorption profile, because this information is of paramount importance for future *in vivo* studies. To this end, dissolution studies were conducted and the preliminary results are presented herein.



SQ109



1

Figure 1: Structures of SQ109 and compound 1

Materials and Methods: The dissolution experiments involved flat tablets (10 mm diameter, 200 mg weight and 6-9 Kp hardness), the release of which, in gastric and intestinal simulated fluids, was determined spectrophotometrically at λ_{max}=223 nm. The % dissolution *versus* time data were fitted to the most common equations used in dissolution testing. Comparison indices like the f_1 and f_2 were also used.

Results: The results obtained reveal that in general, and irrespectively of the relevant quantities of polivinylpyrrolidone (PVP)/sodium alginate used (5 formulations), the new compound is released faster in the acidic medium, relative to the intestinal simulated fluid. Similarly, but to a much lesser extent, this trend was noticed and in the case of HPMC 15K/sodium alginate matrix tablets. This differentiation in the observed release profile is probably due to the much higher hydrophilicity of PVP, especially in the acidic environment, compared to HPMC. However, in all cases, the *in vitro* release profile of the new compound **1**, resembles the respective *in vivo* release pattern of SQ109. Successful fitting results were obtained for each utilized model.

Conclusions: A new potent tuberculocidal compound, albeit being lipophilic, showed a satisfactory controlled release profile, comparable to SQ109. Taking into account that there is an urgent need for effective and side effects devoid antitubercular agents, information about its oral absorption profile will be very useful in future *in vivo* studies.

Literature Reference

[1] Jia L, *et al*. Br J Pharmacol 2005; 144: 80–7.

[2] Papanastasiou I, *et al*. J Med Chem 2008; 51(5): 1496-1500.

PP004

MODIFIED RELEASE STUDIES FROM MATRIX TABLETS OF A NEW 2,2-DISUBSTITUTED AMINOADAMANTANE DERIVATIVE WITH ANTITUBERCULORAR ACTIVITY

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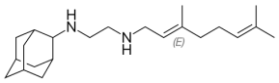
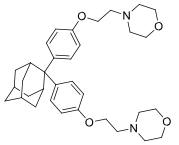
Introduction: Tuberculosis is a disease caused by a bacterium called *Mycobacterium tuberculosis* (TB). The bacteria usually attack the lungs, but TB bacteria can attack any part of the body such as the kidney, spine, and brain. If not treated properly, TB disease can be fatal. Many strains of tuberculosis resist the drugs most used to treat the disease and as a result new chemotherapeutics, devoid of serious side effects are urgently needed. *N*-Adamantan-2-yl-*N*'-[(*E*)-3,7-dimethyl-octa-2,6-dienyl]ethane-1,2-diamine (SQ109, Fig. 1), is a new TB drug candidate with a novel mechanism of action that was safe and well tolerated in Phase I and early Phase II clinical trials [1]. In view of these very interesting findings, we decided to design and synthesize a new aminoadamantane analogue, compound **2** (Fig. 1), bearing in its skeleton the key pharmacophoric moieties of SQ109, along with structural features, which we have found in the past to enhance the so called drug-like character [2]. As the new compound showed a noteworthy tuberculocidal action of the new compound prompted us to investigate its oral absorption profile, because this information is of paramount importance for future *in vivo* studies. To this end, dissolution studies were conducted and the preliminary results are presented herein.



SQ109



2

Figure 1: Structures of SQ109 and compound **2**

Materials and Methods: The dissolution experiments involved flat tablets (10 mm diameter, 200 mg weight and 6-9 kp hardness), the release of which, in gastric and intestinal simulated fluids, was determined spectrophotometrically at λ_{max}=245 nm. **Results:** The dissolution results revealed that the release profile of the HPMC K15/sodium alginate containing matrix tablets was different to the respective solubilisation pattern, shown by compound **2**, when present in PVP/sodium alginate matrices. In general, the % release of **2** from the latter systems was lower than from the former, especially under acidic conditions. The lower and slower release of the new agent from PVP/sodium alginate containing tablets can be possibly attributed to an increase in diffusional path length for the drug, which in turn may be due to the slower erosion rate of the rubbery layer of PVP compared to the respective rate observed in HPMC matrices.

Conclusions: A new potent antitubercular agent, *albeit* being much more lipophilic than SQ109, showed a controlled release profile, comparable to SQ109. Taking into account that the currently used medicines have limited efficacy against the rising threat of drug-resistant TB, have significant side effects, and must be given in combinations of four to six drugs for at least 6 months for drug-sensitive TB and up to 24 months for drug-resistant TB, new drug TB treatment with less frequent dosing and improved patient compliance, is very important.

Literature References:

[1] Jia L, *et al*. Br J Pharmacol 2005; 144: 80–7.

[2] Papanastasiou I, *et al*. J Med Chem 2008; 51(5): 1496-1500.

PP005

DEVELOPMENT OF HPLC-HILIC-METHOD FOR QUALITY CONTROL OF ESTRADIOL HEMIHYDRATE

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Purpose: For analysis of 17β-Estradiol the most often appliyed method is HPLC at reversed phases and UV-detection [1]. Through HPLC with hydrophilic interaction (HILIC) is achieved 10 times increasing of sensitivity in comparison with RP-HPLC [2]. The aim of current study was the development and validation of HPLC-HILIC-method for quality control of Estradiol hemihydate in combined drug products used in therapy of osteoporosis.

Materials and Methods: Reference substance Estradiol hemihydrate, acetonitrile for HPLC, ultra-pure water were used. Isocratic HPLC-HILIC with: column Spherisorb Amino, mobile phase: acetonitrile: water = 55: 45 v/v, flow rate: 2 ml/min., UV-detection at λ = 230 nm was appliyed.
From stock standard solution of Estradiol hemihydrate (1.0 mg/ml in acetonitrile) by dilution of 30 µl, 50 µl, 100 µl, 200 µl, 300 µl, 400 µl 10.0 ml with mobile phase were prepared solutions with concentration: 3.10^{-6} g/ml, 5.10^{-6} g/ml, 1.10^{-5} g/ml, 2.10^{-5} g/ml, 3.10^{-5} g/ml, 4.10^{-5} g/ml.

Results: Linear regression analysis was performed. Linearity accordance between the concentration and spot area in range: 3.10^{-6} g/ml ÷ 4.10^{-5} g/ml was proved by the regression equation: y = 2699.x – 2308, $R^2 = 0.9999$; LOD = 8.10^{-7} g/ml; LOQ = 8.10^{-6} g/ml.
For the estimation of parameter accuracy the recovery was presented by R [%] ± RSD [%]: 99.5 % ± 1.42%. For precision all data for the obtained quantity of Estradiol hemihydrate correspond to confidence interval: 1.96 mg ÷ 2.02 mg (SD = 0.03, RSD = 1.51%).

Conclusions: HPLC-HILIC-method with UV-detection was validated in terms of analytical parameters specificity, linearity, LOD, LOQ, accuracy, precision (repeatability) and is appropriate for quality control of Estradiol hemhydrate in commercially available tablets.

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PP006

QUALITY CONTROL OF CITALOPRAM AND VENLAFAXINE – PATENT STATUS AND DEVELOPMENT OF ANALYTICAL PROCEDURES

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The procedure for grant of a European patent can be launched directly or through the European Patent Office or by national patent organization and subsequent filling of the European application. Because patents are granted and issued by individual national patent offices, the first phase before the European grant is conducting a search for patent purity, and demand for European, national and regional level for the presence of a patent for the claimed process, product, process or other innovation. Antidepressants are included in the top ten therapeutic classes of drugs distributed in the international market. Patent protection of antidepressants is poorly studied and any further clarification of the matter is important for revealing the mechanisms of this process and determines the factors that affect the patent activity of companies. In this sense, it must be dependent on the analytical procedures. The study of patent policy will help to clarify the impact of the chemical structure and characterization of the substances on patents. Increasing use of antidepressants is due to the raising frequency of depressions and related diseases as a consequence of urbanization and stressful way of life. Another important factor for the increased use of antidepressants is the breakthrough in the treatment of depression after the discovery of inhibitors of the reuptake of mediators responsible for numerous clinical effects of depression - serotonin, norepinephrine, dopamine, and others. With regard to this, research and development of reliable analytical methods for self-determination, as well as determination in mixtures, in chemical and biological samples, is essential for providing fast and reliable information to the institutions of the type of substance, its concentration, the presence of metabolites and other chemical characteristics. Research teams in this field work in several directions - creation of analytical methods, their validation in accordance with European pharmacopoeia to expand their application to more than one substance and identification of metabolites.

Subject of our work are antidepressants Venlafaxine, which belongs to the new structural class of drugs SNRIs and Citalopram (RS)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitril) which is an antidepressant drug of SSRI class. It has been used to treat major depressions, anxiety and panic disorders and it is prescribed off-label for a number of anxiety conditions.

PP007

SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS OF CILOSTAZOL: DEVELOPMENT, IN VITRO AND IN VIVO EVALUATION

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Purpose: The current investigation was aimed to improve the solubility of poorly soluble drug, cilostazol (CLZ) for oral and intravenous application.
Material and Methods: Self-nanoemulsifying drug delivery system (SNEDDS) composed of Capryol 90 as an oil phase, Cremophor EL as a surfactant, and Transcutol HP as a co-surfactant in a ratio of 19.8:30.5:49.7 by weight was formulated. The components for SNEDDS were identified by solubility studies, and pseudo-ternary phase diagrams were plotted to identify the efficient self-emulsification regions. Assessment of bioavailability was performed using rabbit model.
Results: The optimum formula was able to solubilize CLZ 2000 times higher than its solubility in water. This formula was able to form grade "A" nanoemulsion when diluted with water, resulted in emulsification time of 50±1.1s, particle size of 14.3nm, PDI of 0.5 and % transmittance was 97.40%±0.65. It showed excellent in vitro dissolution of 93.1% and 81.5% after 5min in 0.3% sodium lauryl sulphate solution and phosphate buffer pH 6.4, respectively when compared with the marketed tablet formulation and drug suspension as the tablets showed only 44.3% and 9.9% while CLZ suspension showed 33.9% and 8.8%, respectively. In vivo study revealed significant increase in bioavailability of CLZ in rabbits to 3.94 fold compared with the marketed tablet formulation after oral administration. This formula could be sterilized by autoclaving for intravenous administration with a 1.12 fold increase in bioavailability compared with its oral administration.
Conclusions: Our study illustrated the potential use of SNEDDS of poorly soluble CLZ orally and parenterally for acute cases of myocardial and cerebral infarction.
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1) "Development of pH-sensitive self-nanoemulsifying drug delivery systems for acid-labile lipophilic drugs". Chem Phys Lipids. 2016 Feb 26;196:81-88. doi: 10.1016/j.chemphyslip.2016.02.00.
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PP008

TWO IN ONE AGAINST BREAST CANCER: OVERVIEW AND CHARACTERIZATION OF A [DOCETAXEL-TRASTUZUMAB] IMMUNOLIPOSOME

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This project aims at developing an innovative entity against breast cancer, which combines stealth liposomal docetaxel grafted with trastuzumab on its external surface called Antibody NanoConjugated (ANC). This new formulation is expected to exhibit a higher efficacy while reducing treatment-related toxicities due to its specificity towards malignant breast cells.

Our goal is to encapsulate docetaxel in a stealth single-unilamellar-vesicle (SUV) liposome by using a Thin Film Method and an Extrusion technique. The anchoring of trastuzumab on the external surface is done through a PEG linker, so as to yield a new entity called ANC. Two types of compositions were studied for this work: one using DOTAP and a more traditional one using choline and glycerol. They were then compared in specific aspects: size (Light Scattering), morphology (electronic microscopy), encapsulation rate (HPLC), grafting rate (ELISA or flow cytometry) and stability studies.

For the last few months we have been focusing on optimizing our entities in order to be the most efficient and synthesize a steady, 150 nm nanocarrier, with a minimum encapsulation rate of 80%.

With those characteristics, this ANC is expected to exhibit a more specific pharmacokinetic profile of distribution and delivery towards HER2+ breast cancer cells through both enhanced permeability retention (EPR) effect and active HER2 targeting through trastuzumab. Consequently, higher efficacy and better tolerance should ultimately be achieved in patients with breast cancer.

Literature References:

Immordino et al. Journal of controlled Release 2003.

Kirpotin et al. Methods in Enzymology 2012.

PP009

EFFECTS OF NEWLY SYNTHESIZED N-PYROLYLCARBOXYLIC ACID HYDRAZONES, WITH ESTABLISHED ANTI-TUBERCULAR ACTIVITY, ON ISOLATED RAT HEPATOCYTES

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Medical University-Sofia, Bulgaria

Purpose: The purpose of this study is to investigate the effects of newly synthesized hydrazones of substituted N-pyrolylcarboxylic acids, with determined anti-tubercular activity on isolated rat hepatocytes.
Material and Methods: The analyzed compounds were divided into two groups, depending on their structural characteristics: **Group I** including **11a**, **11b**, **11c**, **11x**, **11p**, **11l**, **11o**, **11e** and **Group II** including **11**, **11d**, **11n**, **11m**, **11i**, **11h**, **11g**. The hepatocytes were obtained by two-stepped, collagenase perfusion and were incubated with 50 μM concentration of the corresponding compound. The lactate dehydrogenase leakage (LDH) and production of malondialdehyde (MDA) were measured, as some of the biomarkers, characterizing the functional and metabolic status of isolated hepatocytes. The applied methodology is described elsewhere [1].
Results: All compounds, administered alone, reveal statistically significant cytotoxic effects on isolated rat hepatocytes, when compared to the control (non-treated rats). For structures in **Group I**, **11l** was found to show lowest cytotoxic effect, increasing the LDH leakage and MDA production by 28 % and 21 %, respectively. In the same group **11x** is outlined with highest cytotoxic effect, with increscent of the LDH leakage and production of MDA by 48 % and 52 %, respectively. For compounds in **Group II** lowest cytotoxic effect was established for **11**, which increases the LDH leakage and MDA production by 21 % and 15 %, respectively. Highest cytotoxic effect for compounds from this group was found for **11d**, which increases the LDH leakage and MDA production by 40 % and 48 %, respectively. All results were determined as compared to the control (non-treated hepatocytes).
Conclusion: Based on the obtained results after comparison was found, that on isolated rat hepatocytes the compounds included in **Group II** are less cytotoxic, then those included in **Group I**. We consider this result to be based on the presence of an additional pyrrol heterocycle in the structure of compounds, included in **Group II**.

Literature Reference:
[1]. Georgieva M., Kondeva-Burdina M., Mitkov J., Tzankova V., Momekov G., Zlatkov Al. Determination of the Antiproliferative Activity of New Theobromine Derivatives and Evaluation of Their In Vitro Hepatotoxic Effects. Anti-Cancer Agents in Medicinal Chemistry, 2016, 16, in press.

PP010

SPECTRAL ANALYSIS AND STRUCTURAL ELUCIDATION OF SYNTHESIZED PYRROLE HYDRAZONE DERIVATIVE WITH ANTITUBERCULAR ACTIVITY THROUGH QUANTUM CHEMICAL CALCULATIONS

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Purpose: The objective of this work is to perform a detailed calculation of the molecular structure of one pyrrole based hydrazone with proved tuberculostatic activity (compound 1, ethyl 5-(4-bromophenyl)-2-methyl-1-(3-oxo-3-(2-(2-oxoindolin-3-ylidene)hydrazinyl)methyl) -1H-pyrrole-3-carboxylate, as well as to predict its infrared (IR) ultraviolet (UV–vis) and NMR spectra, and some other electronic parameters by using a quantum chemical calculations.
Material and Methods: UV spectra were recorded on a Hewlet Packard 8452A Diode Array Spectrophotometer in ethanol at concentration 10^{-5} mol/L. The IR spectra 400–4000 cm^{-1} were recorded on a Nicolet iS10 FTIR Spectrometer in KBr pellets. The ^{1}H- and ^{13}C-NMR spectra were registered on a Bruker Spectrospin WM250 spectrometer using DMSO-d_6 as solvent and chemical shifts were expressed as *δ* values in ppm against TMS as an internal standard. The energetically preferred ground state geometry was optimized by PM3/RHF method using Polak–Ribiere algorithm at RMS gradient 0.005 kcal.Å.mol^{-1}.^{1}H and $^{1}3C$ chemical shifts were calculated with the GIAO method, using corresponding TMS shielding calculated at B3LYP/6-31G* and B3LYP/6-311+ G** theoretical levels. Electronic spectra were calculated with time-dependent DFT (B3LYP/6-31G**) method for a singlet state and for a half-singlet/half-triplet state (''50–50'' – calculating singlet and triplet states in one calculation) in gas phase and in ethanol, as well as ZINDO/S calculations of the singlet electronic transitions. All the DFT and NDO calculations were performed on Hyper Chem 7.50 software. Linear correlation analyses were carried out using Origin 6.1 program.
Results: Correlations between experimental chemical shifts and GIAO calculated isotropic shielding constants of hydrogen and carbon atoms, as obtained from the studied compound were established in order to assess the performance of NMR spectral calculation. DFT/B3LYP level of theory with 6-31G** and 6-311+G** basis sets were used for geometry optimisation and spectral calculations. Linear regressions for hydrogen atoms shielding constants yield very symilar values for r^2 (0.7319 for first basis set and 0.7923 for the second one). The results for carbon atoms shielding constants are better (0.9753 for first basis set and 0.9749 for the second one) due to the influence of the solvent on N-H protons. The obtained *b* values differ statistically significant from zero, which means that the used linear model is adequate and correctly describes the dependence between the experimental and the calculated values of the corresponding chemical shifts. The main descrepancies between theoretical and experimental IR spectra are in νN-H due to possibility of polyassosciation and intermolecular or intramolecular hydrogen bonding in the experimental spectra performed in solid state (KBr pellets) in compare to a gas-phase in theoretical spectra. The differences between theoretical and experimental UV spectra are probably due to vibration effects and H-bonding with the solvent molecules.
Conclusions: The calculated IR and UV spectra are consistent with the experimentally determined values and correspond with the proposed chemical structure. Considering the adjacent values of the computed and experimental chemical shifts, it is obvious that for further evaluation of the NMR spectral calculation both basis sets may be used. All the calculated properties could be used for future QSAR analysis.

PP011

SYNTHESIS, CARACHTERIZATION AND EVALUTATION OF THE PROPERTIES OF TWO DIFFERENT HOLLOW NANOCONTAINERS FOR DRUG DELIVERY

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Introduction: Two different hollow stimuli-sensitive nanocontainers for drug delivery were synthetized, characterized and evaluated as stimuli-responsive systems. The first drug delivery system is made of one single pH-sensitive shell, whereas the second one consists of three different shells, each one responsible for a different sensitivity toward a specific stimulus, namely pH, temperature and reductive environments.
Material and methods: The two hollow drug delivery systems were produced respectively by a threestep process and a five-step process, the first two of which were in common. The first step was the synthesis of non-crosslinked poly-methacrylic acid (PMAA) core through distillation-precipitation polymerization. Afterwards, the first pH-sensitive shell was obtained by a second distillationprecipitation polymerization process, resulting in a core/shell structure. The removal of the noncrosslinked core gave the first system, the pH-sensitive hollow nanocontainers. To obtain the second system, before the core removal procedure, two other distillation-precipitation polymerization processes were carried out to obtain respectively the temperature- and redox-sensitive shells. The systems were characterized by FT-IR, SEM and TEM. The anticancer drug daunorubicin (DNR) was selected as a model drug for the study of the loading and release behavior of the nanocontainers.
Results: The loading capacity of the three-stimuli sensitive nanocontainers was found to be two times higher than the pH-sensitive one (80% and 40%). Furthermore, the three-shell system presented a better drug release profile, releasing almost 100% of the encapsulated drug in acidic environment and only 10% at physiological pH. On the contrary, the first system released 60% at acidic pH values and roughly 30% in physiological conditions, showing pH-sensitivity but an unsatisfactory drug release profile.
Conclusions: Two different polymeric nanocarriers were synthesized, characterized and tested as stimuli-responsive systems. The three-shell system showed a very good pH-sensitivity and its ability of responding to reductive environments makes it a very promising drug delivery system.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N. 642023 (Click Gene).

PP012

CHEMICAL COMPOSITION OF DIFFERENT EXTRACTS FROM WILD AND CULTIVATED VARIETIES OF *VALERIANA OFFICINALIS* ANALYSED BY GC AND GC/MS

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Purpose: To examine the accumulation of the major components of various *Valeriana officinalis* extracts in order to make an appropriate selection of the most favourable conditions to yield a larger quantity of the extracted substances.

Experimental

Materials: The essential oil was isolated from dried valerian roots with the use of the following methods: extraction with ethanol; gas-extraction with CO_2 and butane. Concrete and absolute extracts gathered from valerian roots also was used.

Method: Analysis of the essential oil in the extracts was effectuated using gas chromatography/mass spectrometry to elucidate the composition of the essential oil and other volatile components and the quantity in each extract.

Results: The identity of the components was assigned by comparison of their retention time, relative to C_9-C_{32} n-alkanes, and mass spectra with corresponding data from reference compounds and taken from the literature. The concentration of the components was calculated from the GC peak areas, using the normalization method.

Conclusions: The identified in the essential oils and the other volatile components isolated on the different methods show that the content of these compounds strongly differs from one another.

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PP013

DESIGN AND DEVELOPMENT OF HYBRID LIPID/BLOCK COPOLYMER NANOSTRUCTURES: PHYSICOCHEMICAL, MORPHOLOGICAL AND IN VITRO EVALUATION

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Introduction – Purpose: Mixed drug nanocarriers comprised of lipids and an amphiphilic non-ionic block copolymer are constructed and studied in terms of their structure, properties and biological behavior. Mixed drug nanocarriers are composed by different in nature materials and exhibit new functionalities and properties.
Materials and Methods: We have utilized two lipids, L-a-phosphatidylcholine, hydrogenated (Soy) (HSPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and a poly(oligoethylene glycol acrylate)-b-poly(lauryl acrylate) (POEGA-PLA) block copolymer, at different molar ratios, in aqueous media. Light scattering, differential scanning calorimetry (DSC) and imaging techniques (cryo-TEM, AFM) were employed in order to elucidate the structure and properties of the nanostructures, as well as the cooperativity between the components.
Results: DSC experiments showed considerable interaction of the block copolymer with the lipid bilayers and suggested an inhomogeneous distribution of the copolymer chains and lateral phase separation of the components. Vesicle formation was observed in most cases by cryo-TEM with a mixed membrane exhibiting kinks, in accordance to DSC data. The results from the *in vitro* screening in cells showed low toxicity of the majority of the chimeric vesicles especially at the highest molar ratio of the polymeric guest, even at high concentrations of the nanosystems. The normal hepatic histology and the absence of lesions from in vivo experiments indicate the low toxicity of the chimeric vesicles.
Conclusions: The prepared DPPC/HSPC: POEGA-PLA mixed vesicles could be used as drug delivery platforms and a starting point for pharmaceutical applications in order to develop nanomedicines with added value for delivery and release toxic anticancer drugs.

PP014

CONTROLLED DRUG RELEASE FROM PH-RESPONSIVE POLYMER-GRAFTED PHOSPHOLIPID BILAYERS

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Introduction – Purpose: A new era of stimuli-responsive medical practices has emerged, in which pH-responsive liposomes figure prominently. This study investigates the characteristics of chimeric liposomes,emerging from the mixing of the pH-sensitive polymer $C_{12}H_{25}$-PAA, (poly(acrylic acid) with a hydrophobic end group), and the phospholipid L-a-phosphatidylcholine, hydrogenated (Soy) (HSPC), with respect to biomimicry and functionality.
Materials and Methods: PAA is a macromolecule, classified as pH-sensitive polymer, whose pH-sensitivity is attributed to its regulative –COOH groups, which are protonated under acidic pH (pKa~4.2). Our concern was to fully characterize, in a biophysical and thermodynamic manner, the mixed nanoassemblies arising from the combination of the two biomaterials. At first, we quantified the physicochemical characteristics and physical stability of the prepared chimeric nanosystems, with Dynamic and Electrophoretic Light Scattering. Then, we studied their thermotropic behavior, through measurement of thermodynamicparameters, using Differential Scanning Calorimetry (DSC). Finally, the loading and release of indomethacin (IND) were evaluated, as well as the physicochemical properties and stability of the nanocarriers incorporating it.
Results: Particle mean size and polydispersity varied, according to the reconstitution medium, while the pH-sensitivity of the designed formulations was confirmed, through the differentiation of all their studied properties in adequately acidic environment. Moreover, all but two nanosystems exhibited sufficient physical stability, at least for the study period. Increased polymer ratio led to diminished drug encapsulation and retarded, but more complete drug release. Notably, acidic pH accelerated the later, highlighting that pH-responsiveness can be a "physiological switch" for controlled drug release. As expected, thermodynamical findings are in line with physicochemical results and also explain the loading and release profiles of IND.
Conclusions: The utilization of these pH-responsive chimeric advanced Drug Delivery nano Systems in targeted drug delivery relies entirely on the biophysics and thermodynamics interplay between such nanoconstructs and the physiological membranes and environment within living organisms.

PP015

THE PHYSIOCHEMICAL CHARACTERISTICS OF COSMECEUTICAL VEHICLE IN COSMETIC PRODUCT SERIES FOR ACNE (ACNOFIX®): INNOVATION AND EFFICACY

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Introduction – Purpose: Salicylic acid has many functions and may be used in many types of cosmetics and personal care products including anti-acne properties. The purpose of this investigation is to study the physicochemical characteristics of different formulations of salicylic acid which are used as anti-acne cosmetic products (foam, cream, lotion and wipes, commercialized under the trade name ACNOFIX®, by InterMed). In these formulations, innovative excipients are used in order to achieve high-quality products for consumers.
Materials and Methods: These innovative excipients offer advantages to the cosmeceutical vesicles. Dynamic and Electrophoretic Light Scattering Techniques were used in order to determine the size distribution and the zeta potential of the colloidal particles of these formulations. Contin and NNLS were used in order to evaluate the size and the size distribution of colloidal particles of the final formulations.
Results: These studies demonstrated that the colloidal particles of lotion and cream are in the micrometer range and of the foam and wipes (bulk liquid) in nanoscale, with adequate size distribution with respect to the nature of the product particles. The negative zeta potential of the particles of the cream and lotion justifies sufficiently the physicochemical stability of the final cosmeceutical formulation.
Conclusions: Finally, the physicochemical characteristics, in combination with the active compounds, prove the superior quality the products ACNOFIX®. Finally, the existence of supramolecular structures, support the reliance on the successful transportation of actives in the skin.

PP016

IN SILICO DESIGN AND SYNTHESIS OF NOVEL QUINOLINONE CARBOXAMIDES AS POTENTIAL CYCLOOXYGENASE-2 INHIBITORS

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The aim of this work is to identify putative cyclooxygenase-2 (COX-2) enzyme inhibitors through virtual screening of in-house chemical libraries of heterocyclic compounds. Molecular design and synthesis of novel compounds with promising inhibitory activity complemented the present study.

The crystal structure of COX-2 from mus musculus was used as a template for the manipulation and comparison of the human COX-2 using the programming language python. It appeared that the two structures were similar, especially at the active site of the enzyme thus they share the same functionality. Thus the crystallized structure was used for docking studies. The validation of the docking procedure was performed with Maestro (Schrödinger) Glide standard (SP) and extra (XP) precision calculations.

The quinolinone analogue 4-hydroxy-N-(2-hydroxyethyl)-2-oxo-1-phenyl-1,2-dihydroquinoline-3-carboxamide (**I**) had the best docking score as well as critical interactions with crucial aminoacids of the active site, among all the studied molecules. As a result, 41 new molecules, possessing structural modifications of the lead compound were designed in order to discover a molecule with potentially better binding characteristics and score. The computations were performed with SP and XP docking methods as well as the method of Induced Fit Docking (IFD). The quinolinone carboxamide analogues bearing N-phenyl or N-benzyl substituents presented the best docking scores and were subsequently synthesized in order to be studied *in vitro* for their COX-2 inhibitory activity.

PP017

A COMPARATIVE RP−HPLC ANALYSIS OF DIFFERENT BATCHES OF TENOFOVIR DISOPROXIL FUMARATE TABLETS

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Introduction: Tenofovir disoproxil fumarate is a white to off white crystalline powder. It is a salt of bis (isopropyloxycarbonyloxymethyl ester of (*R*)-9-(2 phosphonomethoxypropyl) adenine with fumaric acid. It is soluble in water:methanol (1:1) with empirical formula $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$ having molecular weight of 635.5. Currently it is used as an anti-HIV agent. It comes under the category of nucleoside and nucleotide reverse transcriptase inhibitors. The aim of this study was to establish pharmaceutical equivalence among of five batches of Tenofovir disoproxil fumarate tablets (245 mg) purchased from pharmacies in Bulgaria.

Material and Methods: The drug samples were subjected to weight variation test, dissolution tests and quantity determination following acceptable and official protocols. The Tenofovir disoproxil fumarate content was determined using high performance liquid chromatography method. HPLC analysis was performed by isocratic elution with a flow rate 1.0 ml/min. A HPLC system (SHIMADZU Corporation, LC-20 AD quaternary pump) with an auto sampler, Shimadzu DGU-20A5 vacuum degasser and a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solutions Software. Separation was carried out at 30 °C, using LiChrosorb® RP-18 (250 x 4.6 mm) column packed with octadecylsilyl silica gel 5 µm. The mobile phase containing acetonitrile and buffer solution (pH=6) (9:11 v/v) was applied due to good symmetrical peak. The detector was set at 260 nm. Injection volume was selected to be 10 µl which gave a good peak area. The retention time for Tenofovir disoproxil fumarate was found to be 4.94 min.

Results: The quality control parameters: weight variation test, dissolution test and quantity determination were carried out according to USP. Weight variation of the tablets proved statistically that all of the tablets were in accordance to the required limits that is not more than ±5% deviation. All batches of Tenofovir disoproxil fumarate showed more than 80% drug release within 45 minutes. Every batch tablets contain the amount of drug substance equivalent to its label amount.

Conclusion: The study suggests that the tested batches of Tenofovir disoproxil fumarate were in accordance to the specifications for quality control.

PP018

TRANS PHOSPHOLIPID-CONTAINING LIPOSOMES: PROMISING DRUG DELIVERY SYSTEM AND SYNERGIC STRATEGY FOR CANCER THERAPY

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Introduction: Phospholipids are natural amphipathic molecules that have been successfully used for biocompatible and ameliorated delivery of drugs now reaching the clinical use. The natural phospholipid structures have two hydrophobic fatty acids chains and, when unsaturated moieties are present, they display the cis double bond geometry. Trans phospholipids are geometrical isomers of the natural lipids that can change membrane properties and lipid signaling [1]. The formation of trans fatty acids in membrane phospholipids have been recently reported under the effect of the antitumoral drug bleomycin, thus advancing the hypothesis of a participation of the lipid transformations for a reinforced toxic effect toward tumoral cells [2].
Results of trans phospholipid-containing liposomes are presented, reporting their different properties compared to the cis analogues. At the same time, a new perspective for antitumoral strategy can be also envisaged, because the trans geometry of phospholipids can alter the tumoral cell growth and eventually realize an innovative synergy with the drug effect.
Material and methods: Phosphatidylcholine and phosphatidylethanolamine liposomes with mean diameter of about 100 nm were prepared by lipid film hydration, followed by extrusion. Different percentages of trans-isomers were obtained by the isomerization reaction starting from the cis isomers, and the new liposomal compositions were characterized by Thin Layer Chromatography and Gas Chromatography. Stability of the new trans-liposome formulations was tested by Dynamic Light Scattering (DLS) and Atomic Force Microscopy (AFM). Encapsulation of Rhodamine B as drug model was performed by dialysis technique, quantified by UV spectrophotometer and optimized in conditions to ensure the most significant yield.
Results: Preliminary DLS results indicated trans-liposomes to be more stable than cis-liposomes when exposed to different temperature conditions and biological mediums. Data recorded by AFM showed trans-liposomes to be more rigid than cis-analogues but comparable Rhodamine B encapsulation efficiency was achieved.
Conclusions: Trans-liposomes showed promising properties as drug carriers. Further investigations are ongoing on the drug release mechanism as well as on the cytotoxicity effects associated to the double bond isomerism in normal and tumoral cells.
Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N. 642023 (ClickGene).
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PP019

SYNTHESIS OF NEW HYBRID QUINOLINONE-CHALCONES AND PYRAZOLINE ANALOGUES WITH COMBINED ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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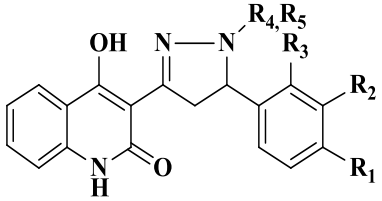
Purpose: The present work aims at the synthesis of new bioactive hybrid quinolinone-chalcones, as well as their chemical modification to form heterocyclic pyrazolines analogues. The new pyrazolines were studied for potential antioxidant and anti-inflammatory activity.

Material and Methods: The synthesis of the desired compounds was performed by condensing α,β-unsaturated carbonyl compounds (quinolinone-chalcones) with hydrazine derivatives. Initially, 3-acetyl-4-hydroxy-2-quinolinone and various aromatic aldehydes reacted via an aldol condensation to produce the corresponding quinolinone-chalcones. The pyrazoline analogues were synthesized by heating the corresponding chalcone with hydrazine derivatives in acetic acid.



R1=OH, OCH_3
R2=OCH_3
R3=CH_3

R4= phenyl
R5= 4-chlorophenyl,4-cyannophenyl, 1(3,4)dimethylphenyl,4-methoxyphenyl 4-benzyloxyphenyl

Results: Thirteen novel compounds were synthesized in total, bearing a variety of substituents on the aromatic ring system of the pyrazoline and the ring B of the chalcone moiety. The new derivatives were tested for their antioxidant activity in vitro based on the stable free radical scavenging capacity DPPH, showing remarkable results. Furthermore, the molecules were tested for the inhibition of lipid peroxidation of linoleic acid induced by AAPH radical, and for their ability to inhibit soybean lipoxygenase, as an indication of their anti-inflammatory activity.

The identification of the structure of these novel compounds was performed by NMR spectroscopy.

Conclusions: In the present study, a series of pyrazoline analogues were synthesized and evaluated for their antioxidant and anti-inflammatory activity. The results of the in vitro assays indicate that these new compounds possess interesting bioactivity and merit further investigation.

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PP020

QUANTIFYING DRUG RELEASE KINETICS USING MONTE CARLO SIMULATIONS

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Purpose: Modeling and quantifying drug release profiles from simple or composite spherical devices, as well as from slabs with either flat or rough surfaces, when diffusion is the dominant release mechanism.
Material and Methods: Numerical calculations using Monte Carlo simulations [1] and analytical solutions of the diffusion equation.
Results and Conclusions:
Release curves are accurately described by the stretched exponential (Weibull) function [2,3]:

$$\frac{M_t}{M_\infty} = 1 - \exp\left[-(t/\tau)^b\right] \qquad \textbf{(1)}$$

where M_t is the amount of drug released at time t, M_∞ is the total amount of drug, and τ, b are the two parameters describing the analytical release profile of Eq. (1).

For simple spheres, or composite spheres consisting of an inner core and an outer shell, fitting of the numerically obtained release profiles with Eq. (1) provides the values of the parameters τ and b in each case. The dependence of the two stretched exponential parameters on the device characteristics is investigated and simple analytical relations are provided [2].
Drug release form slabs with flat or irregular (non-planar) surfaces, presenting non-uniform thickness, is also considered. The dependence of the release profiles on the degree of roughness and the corresponding variation of the parameters τ and b is presented [3].
Acknowledgement: This work has been co-financed by the European Union (ESF) and Greek national funds (NSRF) through the THALES project MACOMSYS.

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PP021

ENCAPSULATION OF TYROSOL IN POLY(LACTIC)ACID NANOPARTICLES

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Purpose: The aim of the current work the encapsulation of the natural antioxidant tyrosol in poly(lactic) acid (PLA) nanoparticles in order to efficiently protect its physicochemical and antioxidant properties as well as to modify its bioavailability.
Material and Methods: Derived from olive leaf and olive oil, tyrosol presents remarkable biological properties such as cardioprotective, antioxidant, antibacterial and anti-inflammatory. Polylactic acid (PLA) is an aliphatic polyester with adjusted hydrolyzability and it is commonly used in several clinical trial as a biodegradable and biocompatible polymer. Tyrosol was encapsulated in PLA nanoparticles according to the emulsification-solvent evaporation technique. [1] PLA and tyrosol were dissolved in a specific organic solvent and then the organic solution was injected in an aqueous solution of PVA 1% w/v and the emulsion was performed using ultrasonication. The organic phase was evaporated in vacuo and the nanoparticles were recovered with centrifugation. Different complementary techniques were used to characterize the loaded PLA NPs and as to their size, encapsulation efficiency (EE %), structure, thermal properties and morphology. These techniques include dynamic light scattering (DLS), zeta potential measurements, UV-Vis spectrometry, Fourier transform infrared spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA) and Atomic Force Microscopy (AFM).
Results: Tyrosol was efficiently encapsulated in PLA nanoparticles. The size of nanoparticles was 200±0.65 nm, the polydispersity index was extremely good, 0.05, as well the zeta potential, -30±0.83 mV. The EE ranged from 45% to 60%.
Conclusions: Encapsulation of bioactive compounds in nanoparticles using biodegradable polymers as matrices is a technique with extremely interesting potential for applications in biomedicine and controlled drug delivery and release. Tyrosol has been successfully encapsulated in PLA nanoparticles with excellent physicochemical characteristics. Evaluation of the antioxidant activity of the encapsulated tyrosol is currently underway.
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PP022

DESIGN AND SYNTHESIS OF NEW 2,3-DISUBSTITUTED-QUINAZOLIN-4(3*H*)-ONE ANALOGUESAS LIPOXYGENASE INHIBITORS

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Purpose: Lipoxygenases (LOX) are iron-containing enzymes widely distributed in plants and animals. In humans LOX plays a key role in the biosynthesis of leukotrienes, the proinflammatory mediators mainly released from myeloid cells. Thus, inhibitors of lipoxygenases have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases, but their therapeutic potential has now been expanded to certain types of cancer and cardiovascular diseases. The aim of the present work is the in silico design and synthesis of 2, 3-disubstituted-quinazolin-4(3H)-one analogues as inhibitors of the enzyme lipoxygenase (LOX).
Materials and methods: Preliminary *in silico* molecular docking studies were conducted in order to identify structural motifs in the quinazolinone scaffold that can lead to LOX inhibition. Taking the results of these studies into consideration, a series of quinazolinone analogues was synthesized by reacting an appropriately substituted benzoxazinone with amine and hydrazine derivatives under microwave irradiation conditions. The structure of the new compounds was identified using NMR spectroscopy. The ability of the compounds to inhibit the activity of soybean lipoxygenase was evaluated in vitro.
Results and discussion: The majority of the synthesized 2, 3-disubstituted-quinazolin-4(3H)-ones exhibited very promising LOX inhibitory activity ranging from IC_{50} 12.2 to 43.1μM. The best activity was shown by the compound derived by the reaction between 6,7-dimethoxybenzoxazinone and tyramine. The results indicate that the privileged scaffold of quinazolinone can be further exploited to develop potential anti-inflammatory agents.

PP023

ENCAPSULATION OF GRAPEFRUIT ESSENTIAL OIL IN BIODEGRADAPLE POLY(LACTIC ACID) (PLA) NANOPARTICLES

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Purpose: The aim of the present study is the encapsulation of the grapefruit essential oil (EO) in poly(lactic acid) (PLA) nanoparticles, in order to maintain its quality and properties, the characterization of the formed nanoparticles, as well as the directly determination of the encapsulation efficacy.
Material and Methods: Grapefruit EO was encapsulated in PLA nanoparticles using the emulsification solvent evaporation technique, which forms simple emulsions [1]. The PLA and Grapefruit EO solutions were prepared in organic solvents and then were poured into an aqueous solution of polyvinyl alcohol (PVA) surfactant (1% w/v) for the formation of the nanoparticle suspension. The organic solvent was evaporated in vacuo from the nanoparticle suspension and the formed nanoparticles were collected by ultracentrifugation and were freeze dried. Their size, polydispersity index, and ζ-potential were determined by the Dynamic Light Scattering (DLS) method, while the encapsulation efficiency (EE) was determined directly, using UV-Vis spectroscopy. Further evidence for the nanoparticle formation and their physicochemical characteristics were provided by diverse methods, such as FT-IR spectroscopy, Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) and Scanning Electron Microscopy (SEM).
Results: According to the FT-IR, DSC and TGA results, the encapsulation of the grapefruit EO in PLA nanoparticles was successful. The size of the formed nanoparticles ranged from 206nm to 229nm, while their polydispersity index ranged from 0.070 to 0.082, and their ζ-potential from -15.77mV to -22.80mV. Furthermore, the EE% was approximately 60%.
Conclusions: Encapsulation of grapefruit EO in PLA nanoparticles provided nanoparticles with satisfactory physicochemical characteristics (nanoscale size, uniform size dispersion and stability in suspension), as well as very good EE.

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PP024

PHOTOPROTECTIVE CHARACTERISTICS OF NEW PHENOLIC ESTERS

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Purpose: The current work aims at the design and synthesis of new phenolic esters, which will combine photoprotective and antioxidant activity so as to provide protection against solar irradiation, oxidative damages in DNA and skin cancer.
Materials and methods: The molecules synthesized in this study are esters of selected phenolic acids and anthranilic acid with 2-(3,4-dimethoxyphenyl)ethanol, which is a lipophilic analogue of the well-known natural antioxidant hydroxytyrosol.
The synthesis of new esters involves structural modifications on the molecular framework in order to investigate their effect on their Sun Protection Factor (SPF) as long as their antioxidant activity. In order to determine the SPF, an in vitro study was conducted using the spectrophotometer SPF 290S Optometrics LLC. All the new compounds were structurally elucidated using Nuclear Magnetic Resonance (NMR) spectroscopy.
Results and Discussion: The new phenolic esters were effectively synthesized and structurally characterized through NMR spectroscopy. SPF evaluation showed that one of the synthesized molecules has comparable photoprotective activity to one of the most commonly employed commercial UV filter (OctylMethoxyCinnamate, OMC). In vivo evaluation of the photoprotective activity of the new compounds is currently underway.

PP025

VERIFICATION OF WEIBULL KINETICS THROUGH THE BEHAVIOR NEAR THERELEASE BOUNDARY

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Purpose: Investigation of the evolution of the fraction of drug molecules that are sufficiently close to the release boundary, in order to check the validity of the assumption underlying the theoretical derivation of a stretched exponential (Weibull) release kinetics [1].
Methods: The Diffusion-controlled drug release from slabs and spheres is considered. Both analytical results and Monte Carlo simulations are used to calculate the evolution of diffusive drug particles.
Results: Both analytical and Monte Carlo simulations data show ainverse power-law time dependence of the fraction of diffusive drug particles near the boundary, after an initial short time, followed by saturation [2]. The power-law dependence starts early during the process, at around 1% of the release and lasts up to at least 80% of the release. The obtained results indicate an agreement between the values of the powerlaw exponent, m, and the Weibull exponent, b, as predicted from the relation b=1-m [1].
Conclusions: The fraction of drug molecules near to an exit, as a function of time, follows an inverse power-law in a substantial part of the release problem, justifying an approximate description of the release kinetics through a stretched exponential function.
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PP026

A COMPARISON OF THE PHARMACOKINETICS OF INHALED SALMETEROL IN HEALTHY AND ASTHMATIC SUBJECTS: A POPULATION PHARMACOKINETIC ANALYSIS

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Purpose: To apply population pharmacokinetic (PK) modeling in order to describe the concentration-time (C-t) profile of salmeterol in healthy subjects and asthmatic patients following administration of two dry powder inhalers (DPIs).
Materials and methods: Salmeterol plasma C-t data were obtained from two different bioequivalence (BE) studies: i) a single dose 2x2 study in 60 healthy subjects with co-administration of activated charcoal and ii) a single dose 2x4 study in 48 asthmatic patients in the absence of activated charcoal. Non-linear mixed-effect modeling was applied to the obtained C-t datasets. Pharmacokinetic models able to describe the C-t data in the two populations were developed. Many residual error models were tested, whereas age, gender, body weight, and height were explored as potential covariates. The entire computational work was implemented in Monolix v.4.3.
Results: A two-compartment disposition model with very rapid absorption kinetics (like IV bolus) was found to describe successfully the C-t profiles of salmeterol in the healthy subjects. The application of a combined (additive and proportional) error model led to the optimum performance. Gender was found a significant covariate on salmeterol clearance. For the asthmatic patients of the second study, the absence of activated charcoal allowed the parallel lung and gastrointestinal absorption of salmeterol. In this case, a two-compartment disposition model, with first order absorption from the gastrointestinal tract and very rapid absorption kinetics from the lungs, was found to characterize the C-t data. A proportional error model led to the best results. Gender was found a significant covariate on salmeterol clearance and body weight on volume of distribution of the central compartment. Similar PK estimates were obtained from the two models. No difference in the performances of the two tested DPIs was observed in either population.
Conclusions: Two population pharmacokinetic models describing successfully the C-t profile of salmeterol in healthy and asthmatic subjects were developed and compared. Men were found to exhibit higher clearance of the drug than women in both treated populations.

PP027

IN VITRO SKIN PERMEATION STUDY OF CANDESARTAN USING EXPERIMENTAL DESIGN TECHNIQUES

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Purpose: The in-vitro skin permeation study of Candesartan, an antihypertensive drug, from transdermal gel formulations.

Materials and Methods: The in vitro skin permeation of the drug from saturated transdermal gels was examined using modified Franz diffusion cells and epidermal membrane, taken from full thickness hairless mouse skin by the heat separation technique. On the ground of appropriate preliminary experiments, a 24 full factorial screening design was executed, where the effect of four factors at two levels on the cumulative amount Q (μg/cm2) of drug permeated at 24, 48 and 72 hours was investigated. The drug concentration in the receptor fluid was determined using HPLC. Moreover, the possibility of ion pair formation between Triethanolamine and Candesartan was thoroughly investigated as a strategy for improving further skin permeation of the active, employing NMR spectroscopy for its verification.

Results: The selected factors for the factorial design were as follows: the drug's chemical form (Candesartan cilexetil ester or Candesartan, obtained by alkaline hydrolysis), the concentration of Triethanolamine as a counter ion1 for the active (0 or 5% v/v), the concentration of Cineole as permeation enhancer2 (0 or 10% v/v) and the type of co-solvent in the vehicle (Glycerol or Glycerol formal). The ANOVA revealed statistically significant effects and thus it was possible to identify the important factors and their interactions in regard to the selected responses. 1H-NMR spectroscopy experiments provided clear evidence of complex formation between Candesartan and Triethanolamine.

Conclusions: Based on the factorial design results, an optimum composition for a Candesartan transdermal gel was justified as a candidate formulation for the next phase of the skin permeation experiments.

NMR results confirmed the initial hypothesis of ion pair formation between Candesartan and Triethanolamine, which is currently under a more detailed examination using Mass Spectrometry.

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PP028

NOVEL INDOLE-FLUTIMIDE HETEROCYCLES WITH ACTIVITY AGAINST INFLUENZA PA ENDONUCLEASE AND HEPATITIS C VIRUS

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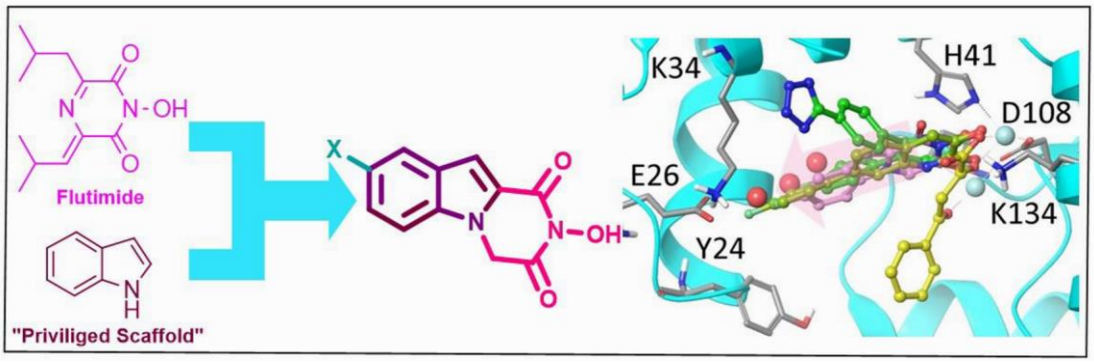
Influenza viruses cause considerable morbidity and mortality, whether in the context of annual epidemics, sporadic pandemics, or outbreaks of avian influenza virus. For hepatitis C virus (HCV), an estimated 170 million people are chronically infected worldwide. These individuals are at high risk of developing progressive liver injury or hepatocellular carcinoma. Since the efficacy of currently approved antiviral drugs is threatened by emerging viral resistance and the cost remains high, new antiviral drugs are still required.
By utilizing a structure-based approach, novel substituted indole-flutimide heterocyclic derivatives (1,2-annulated indole-diketopiperazines) were rationally designed, synthesized and evaluated as influenza PA endonuclease and HCV NS5B polymerase inhibitors. The compounds were also tested for their antiviral effect against HCV and cytotoxicity.
All N-hydroxyimides were potent PA endonuclease inhibitors while displaying low cytotoxicity. The novel unsubstituted indole-flutimide heterocyclic derivative proved to be the most active analogue, while the most favorable indole substitution was fluorine at position 8. The chloro-derivative showed additional potent anti-HCV activity and exhibited remarkable selectivity (>19). In accordance with the SAR data, removal of the hydroxyl group from the imidic nitrogen caused a complete loss of activity against influenza PA endonuclease as well as HCV.
Our findings suggest that the novel pyrazino[1,2-*a*]indole-1,3(2*H*,4*H*)-dione framework that we have developed, following mild and experimentally convenient protocols, offers a promising motif for further construction of new analogues with optimized antiviral properties through appropriate substitution on the diketopiperazine or indole ring nuclei.



PP029

DRY POWDERS OF HYBRID LIPID-POLYMER MICROPARTICLES FOR PULMONARY DELIVERY OF BUDESONIDE

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Purpose: To develop inhalable microparticles containing budesonide for pulmonary delivery.
Materials and Methods:Lipid/polymer hybrid microparticles comprised of different ratios of chitosan, dipalmitoylphosphatidylcholine, polyvinyl alcohol, L-leucine and lactose were prepared by the spray drying method. The microparticles were characterized by scanning electron microscopy (SEM), infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). The stability of the microparticles was assessed by means of **ζ**-potential studies. The mucoadhesive properties of the Rhodamine-B loaded microparticles was assessed by monitoring the transport of the dye across artificial mucus. The aerolization properties were assessed by the Cascade Andersen Impactor whereas the pharmacological evaluation (cell proliferation) of the microparticles was tested in human epithelial lung cells from adenocarcinoma (Calu-3). The integrity and functionality of the cell barriers were demonstrated by measurement of trans-epithelial electrical resistance (TEER).The release of budesonide (BUD) from the microparticles was recorded in simulated lung fluid.
Results: Budesonide efficiency in spray dried hybrid microparticles was 98-99%. SEM studies revealed spherical microparticles whereas IR identified the presence of excipients and DSC studies indicated the amorphous state of BUD. All compositions possessed high ζ-potential values due to the presence of chitosan. A reduction to these values recorded in the mucin-including media, indicated the interaction of hybrid microparticles with mucin. Mucus permeation experiments shown that the particles with the highest amount of chitosan were able to diffuse more easily. Fine particle fraction was 23-65% and mass mean aerodynamic diameter was 3.9-6.5μm whereas 92-100% of BUD was released in simulated lung fluid, following the Korsmeyer-Peppas kinetics. No specific cytotoxicity was observed and TEER studies shown that application of these formulations didn't induce the transient opening of tight junctions.
Conclusions:The results obtained from this study highlight the potential of these hybrid lipid/polymer microparticles for the pulmonary delivery of anti-asthmatic agents .

PP030

PERMEATION ENHANCEMENT OF MACROMOLECULES ACROSS HUMAN SKIN *IN VITRO* BY A MICRONEEDLE DEVICE (DERMAROLLER) AND TERPENES

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2. *University of Patras, Greece*

Purpose: To investigate the permeation of a model hydrophilic macromolecule [fluorescein isothiocyanate – dextran 4 kDa (FD-4)] across full thickness human skin *in vitro* by means of physical (microneedle device; dermarollers with different needle length) and chemical enhancers (sesquiterpenes).
Materials and Methods: *In vitro* studies were conducted using vertical Franz diffusion cells followed by tape stripping in an attempt to examine the disposition of FD-4 on and within the skin. Possible degradation of FD-4 across skin tissue was evaluated by size exclusion chromatography (SEC).Visualization studies [scanning electron microscopy (SEM) and light microscopy] were further employed to assess skin integrity in the presence of the enhancers. Biophysical changes of human stratum corneum (SC) in the presence of the sesquiterpenes was monitored by means of Fourier transformation infrared spectrometry (FT-IR) and differential scanning calorimetry (DSC).
Results: The transport of FD-4 was greatly enhanced as a function of needle length; of FD-4 10.67 (1mm) and 23.44 (1.5 mm) fold compared to plain model compound. Lower enhancement values were obtained in the presence of sesquiterpenes farnesol and its two derivatives enhanced namely; 3.34, 2.86 and 3.48-fold compared to control. SEC studies revealed that FD-4 is transported across skin intact. SEM studies shown a reduction of diameter of skin pores upon application of the dermarollers as a function of time. Distribution profiles in the stratum corneum suggest that FD-4 can rapidly enter in the deeper layers in the presence of physical and chemical enhancers. Histological analysis shown that the use of longer microneedles and farnesol disorganized the dermis, whereas the derivatives caused detachment of the stratum corneum from the epidermis. Biophysical studies shown that the presence of sesquiterpenes induce reversible changes to the structure of stratum corneum.
Conclusions: Both approaches significantly increased the transport of FD-4 across human skin holding promise for drug delivery purposes.

PP031

PHARMACOLOGICAL ASSESSEMENT OF HIERARCHICAL ZEOLITES BEA, NaX-FAU AND ZSM-5 FOR ORAL DELIVERY OF POORLY SOLUBLE DRUGS

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Purpose: To pharmacologically investigate the cytocompatibility of different types of mesoporous zeolites on Caco-2 cell cultures for oral drug delivery applications.
Materials and Methods: Mesoporous zeolites BEA, NaX-FAU and ZSM-5 of different framework structures were evaluated by means of ζ-potential, scanning electron microscopy (SEM) and N_2 physicosorption studies. The cytocompatibility of the siliceous zeolitic particles was assessed in the human colon carcinoma cell line (Caco-2) by measuring cell viability (as determined by the tetrazolium reduction) using the MTT assay and the Annexin V-propidium iodide staining method to assess cell death induced by programmed cell death (apoptosis) activation by flow cytometry analysis in a concentration- and time-dependant manner. Fluorescence microscopy was used to evaluate intracellular localization of the zeolitic particles loaded with the lipophilic fluorescent dye, Nile Red.
Results: Zeolites possessing a net negative charge demonstrated large specific surface areas (m^2/g) and different microporosity. The effect of zeolitic particles on the viability of Caco-2 monolayers showed no cytotoxicity after short-term incubation (3 hr and 24 hr) and in a wide concentration range (0.1-8.0 mg/mL). The cellular uptake and subcellular localization of zeolitic particles was further assessed by differential interference contrast microscopy (DIC) and fluorescence microscopy. The data obtained have shown that the zeolitic particles have been internalized by Caco-2 cells forming aggregates. When the nuclei were stained with DAPI, aggregates of zeolitic particles could be clearly seen located nearby the nuclei.
Conclusions: The results obtained have indicated that these zeolitic particles present the potential to be used as carriers for oral drug delivery.

PP032

DESIGN AND SYNTHESIS OF HYBRIDS OF STAUROSPORINE AND EVODIAMINE WITH POSSIBLE ANTICANCER ACTIVITY

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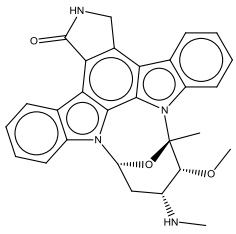
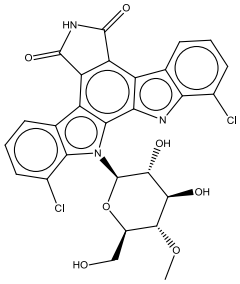
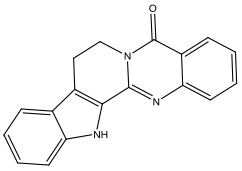
Cancer is one of the major problems these days and many researchers have been inspired to synthesize drugs, based on natural compounds with intended anticancer activity. Some well-known compounds, secluded from plants with such properties are Staurosporine and Rebeccamycin, as well as Rutaecarpine and Evodiamine and their analogues. Staurosporine and Rebeccamycin are indolocarbazole antibiotics and the interest focuses on their broad spectrum antitumor activity. Despite their structural similarity, the two compounds exert their biological activity with completely distinct pathways. Rebeccamycin and its analogues stabilize the DNA-topoisomerase cleavable complex. On the other hand, Staurosporine is one of the strongest known inhibitors of protein kinases. In addition, indoloquinazoline compounds, Rutaecarpine and Evodiamine possess anti-cancer activities both in vitro and in vivo by inhibiting proliferation, invasion and metastasis of a variety of tumor cell lines. The main goal of this project is to design and synthesize novel hybrids based on Staurosporine/Rebeccamycin and Rutaecarpine/Evodiamine with structural modifications in order to evaluate the effect of them on topoisomerases' and/or protein kinases' inhibition, as well as on the tumor cell growth inhibition.



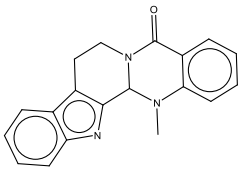
Staurosporine



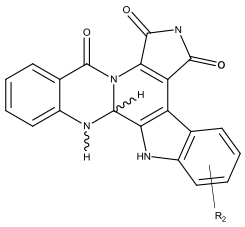
Rebaccamycin

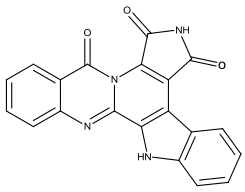


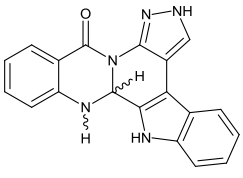
Rutaecarpine

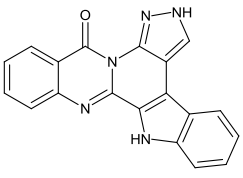


Evodiamine









New Compounds

PP033

DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL AZA-ACRIDINE DERIVATIVES WITH ACTIVITY AGAINST TOPOISOMERASES

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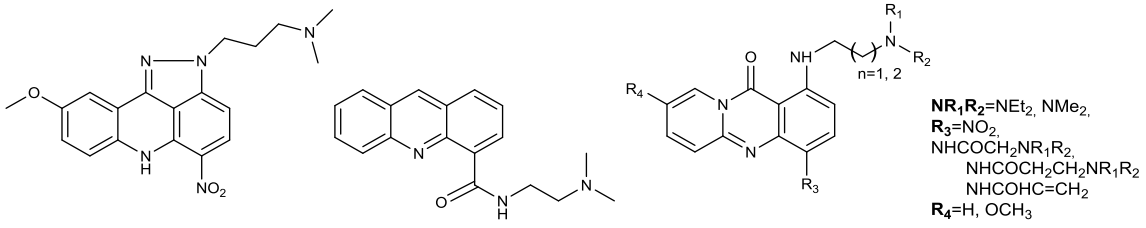
Purpose: Topoisomerases are ezymes which resolve topological problems that are created during the functions of replication, transcription and chromatin condensation of DNA. They act by creating a transient enzyme-DNA complex. The topoisomerase inhibitors act by stabilizing this complex, making the enzyme nonfunctional leading thus at apoptosis of tumor cells. Among different classes of topoisomerase inhibitors, PZA (Pyrazinamide) and DACA (N-(2-dimethylamino)ethyl)acridine-4-carboxamide), have been extensively studied and were also used as lead compounds for the development of structurally related analogues. Much effort has been devoted to the modification of the chromophore in order to optimize its characteristics, regarding severe side effects and solubility under physiological conditions, resulting in the development of a number of aminosubstituted acridine derivatives. Prompted by the above mentioned studies we present here the synthesis of some novel aza-acridone aminosubstituted compounds. Our aim is to understand the structure–activity relationships regarding the impact of the fused nitrogen in the acridone core, the impact of the nitro, the aminosubstituted side chain and the a,β unsaturated group. We also aim to study if the presence of a methoxy group would increase the stability, something that was found after molecular calculations.
Materials and methods: With the use of 2,6 dichloro benzoic acid as starting material after several reactions we synthesised the following products type I.



Results: All the compounds were tested for their anticancer activity as topoisomerase inhibitors. Among them some showed significant activity against topoisomerase I while some others showed activity against topoisomerase II. It is also important to mention that some compounds showed activity against TDP1 (Tyrosil DNA-Phosphodiesterase-1).
Conclusions: The nitro and the aminosubstitution in the aza-acridine core resulted in activity against topoisomerases.
Literature Reference: 2 Zhang B., Li, X., Li, B., Gao C., Jiang Y. Acridine and its derivatives: a patent review (2009-2013). *Expert Opin. Ther. Patents*, **2014**, *24*, 652-653.

PP034

ENGINEERED CELL VESICLES FOR DRUG DELIVERY

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Purpose: To isolate, characterize and evaluate engineered cells as vesicles for Drug delivery.
Materials and methods: Engineered cell vesicles (CVs) (encapsulating FITC-dextran or Calcein as an aqueous space probe), were prepared following isolation of Human Embryonic Kidney (HEK) cells and B16 melanoma cells. The cell material was engineered to prepare vesicles of nanosize which encapsulate a fluorescent aqueous compartment probe, such as FITC-dextran or calcein, by applying a number of consecutive freeze-thawing cycles. The CVs produced were characterized for their size distribution and surface charge by DLS, and for their FITC-dextran content by measuring Fluorescent Intensity (FI). The lipid concentration was measured by the Stewart assay. Calcein integrity of HEK and B16 CVs in absence and presence of serum proteins was evaluated, as a method to evaluate their applicability for Drug Delivery applications, by measuring the latency of entrapped Calcein during incubation in buffer or FCS at 37^0C, for 24h. Finally, the cytotoxicity of the two CV-types towards B16 and HEK cells after 4h of incubation of 200 and 400 nmoles of lipid (from each formulation)/10^6 cells, was measured by the MTT assay.
Results: CV mean hydrodynamic diameters were between 200-300nm with negative charge (-15mV). The retention of calcein in CVs, was stable during 8h of incubation in buffer and FCS, however there was a gradual release of the vesicle-entrapped dye between 8 and 24h. Interestingly, there was no difference of CV integrity in buffer and in presence of proteins (FCS). The cytotoxicity results indicated that both CV types are non-toxic towards the cells evaluated.
Conclusions: The CVs produced are nano-sized, encapsulate good amounts of calcein and FITC, are non-cytotoxic and are considerably stable in presence of proteins, which prove that such engineered cellular vesicles have potential for drug delivery applications.
Literature References:
[1]E. Markoutsa, et al., ***EJPB***, 77: 2, 265-274, 2011
[2] S. Jang, et al., ***ACS Nano***, 7:9, 7698-7710, 2013

PP035

POPULATION PHARMACOKINETIC MODELING APPLIED TO THE PLASMA CONCENTRATION-TIME DATA OF DONEPEZIL

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Purpose: The purpose of this study is: a) to investigate the pharmacokinetics (PK) of donepezil using population models and b) to evaluate the impact of some crucial demographic characteristics like body weight, height, age, BMI and gender on its PKs.
Materials and methods: This population pharmacokinetic study was performed on the data of 30 healthy volunteers, after receiving a single dose of donepezil 10 mg. Plasma concentration (C) – time (t) data were obtained from a two-sequence, two-period, crossover (2 × 2) bioequivalence (BE) study of two donepezil formulations (test and reference). A variety of structural (one- and two-compartment) and residual error (additive, multiplying and combined) models were tested to fit the C–t data. A number of important factors were also investigated such as the initial values of the parameters and the effect of covariates. 'Treatment' (i.e., test or reference) of the BE study was considered as covariate. Demographic data (body weight, height, gender, age and BMI) were evaluated for their impact on the PK parameters of donepezil. Several goodness-of-fit criteria were utilized to find the best model. The latter included statistical and graphic diagnostic tools such as −2LL (Log-Likelihood) function, Akaike and Bayesian information criteria, predicted–observed plots, individual weighted residuals (IWRES) *versus* the individual predicted (IPRED) concentrations values, and VPC plots etc. The entire analysis was performed within MONOLIX v.4.3.3.
Results: The population PK analysis showed that donepezil data were best described by a two-compartment model with first-order absorption and elimination kinetics. An additive error model led to the optimum performance. The estimated mean first order absorption rate constant was equal to 0.30 h^{-1}, the mean apparent clearance was 11.3 L/h and the mean apparent inter-compartmental clearance was 50 L/h. The apparent volume of distribution of the central compartment was 259 L and that of the peripheral compartment equal to 603 L. Body weight and sex were found to affect significantly volume of distribution of the central compartment and clearance. As body weight increases, the volume of distribution in the central compartment and clearance of the drug also rise. Also it was observed that the women of the study exhibited greater volume of distribution of the central compartment and clearance than men.
Conclusions: A two-compartment model, with first-order absorption and elimination kinetics, was found to describe the C–t data of donepezil. Body weight and sex were found to influence significantly the volume of distribution of the central compartment and the clearance.

PP036

IDENTIFYING RELATIONSHIPS BETWEEN THE PHARMACOKINETIC PROPERTIES OF DRUGS AND TWO CLASSIFICATION SYSTEMS, BCS AND BDDCS

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Purpose: The purpose of this study was to investigate the relationships between pharmacokinetic (PK) parameters and their biopharmaceutical classification in the Biopharmaceutics Classification System (BCS) and the Biopharmaceutics Drug Disposition Classification System (BDDCS).
Materials and methods: The PK parameters for a diverse set of 318 drugs used in therapeutics were collected. The PK data included elimination half-life ($t_{1/2}$), the bioavailable fraction of dose (F), the % portion of urinary excretion (UE) and metabolism (MET), the fraction of drug bound to plasma proteins (f_b), as well as clearance (CL), apparent volume of drug distribution (V_{ap}) and their two 'fractal analogues', the so-called fractal clearance (CL_f) and fractal volume of distribution (v_f). The BCS and BDDCS classes for as many as possible drugs were found from a literature search. For the aforementioned dataset of PK parameters, the BCS and BDDCS grouping were available for a number of 131 drugs. The investigation was based on descriptive statistics, scatter plots, and the application of principal component analysis (PCA). The entire computational work was performed within MATLAB 2015a.
Results: The descriptive statistical criteria did not reveal any significant trend in the PK parameters among the different BCS and/or BDDCS classes. Visual observation of the two-dimensional scatter plots did not also show a tendency of the PK parameters. The only exception was f_b, where it was observed that classes II and IV of BCS consist of drugs with relatively high f_b values. The PCA applied to the dataset of 131 drugs and the entire set descriptors resulted in a model with one principal component that accounted for more than the 99% of the total variability. The PK parameters, which mainly contributed to the variability of this model, were CL_f, v_f, F, UE, and $t_{1/2}$. In the PCA score plot, using the first two principal components, the BCS and BDDCS were not placed far away, but they were relatively close one to another. It is noteworthy that MET was placed between the BCS and BDDCS factors, which implies that MET is strongly related to both of these two classification systems.
Conclusions: This study is the first attempt on identifying possible relationships between the PK parameters for a diverse set of drugs and the BCS/BDDCS classifications. Pharmacokinetic parameters like CL_f, v_f, F, UE, and $t_{1/2}$ contributed mainly to the variability of the PCA model. The % fraction of drug metabolism was found to exhibit a performance similar to the BCS/BDDCS grouping.

PP037

DISCOVERY OF TUMOR NECROSIS FACTOR SMALL MOLECULE INHIBITORS AND SOLVENT SELECTION FOR BIOASSAYS

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[2] *Institute de Biologie Structurale (IBS), Grenoble, France*
[3] *Centre for Research and Technology of Thessaly (CERETETH), Volos, Greece*
[4] *Agricultural University of Athens, Athens, Greece*
[5] *Pro-ACTINA SA, Athens, Greece*
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Introduction: Tumor Necrosis Factor (TNF), is a trimeric cytokine which has been associated with the inflammatory response to tissue injury and possesses a key role in Rheumatoid Arthritis pathogenesis. SPD304 is a toxic TNF inhibitor[1] which cannot be utilized as a drug but only as a basis to discover small molecules that disrupt trimer formation. A series of SPD304 analogues was designed and synthesized in order to eliminate toxicity and enhance activity towards TNF inhibition[2].

Purpose: The main purpose of this study is the discovery of new TNF inhibitors and enhancement of aqueous solubility of insoluble candidates in compatibility with the fluorescence binding assay used for the measurement of dissociation constant (Kd).

Materials and methods:

a) Drug design. *In silico* identification of novel drug compounds was based on two approaches: i) structure based drug design using the 3D structure of TNF and ii) design of more potent and less toxic SPD304 analogues.
b) Drug synthesis. A series of SPD304 analogues were in house synthesized while novel candidates discovered by *in silico* approaches were commercially available.
c) Solubility measurement and enhancement. Cosolvent addition was investigated in order to enhance aqueous solubility. A direct UV method was used for measuring.
d) Kd measurement. A fluorescence binding assay was used for the evaluation of the inhibitory activity of compounds. A few trials with Isothermal Titration Calorimetry (ITC) were also made.
e) Crystallization. Crystallization trials took place with TNF and some of the hit drug compounds.

Results: DMSO and PEG3350 were used as cosolvents in fluorescence, ITC and crystallization assays. Kd of 39 compounds as well as the compatibility of 6 cosolvents with TNF were studied with fluorescence spectroscopy. Solubility of SPD304 in these cosolvents was measured with direct UV.

Conclusions: DMSO and PEG3350 can be used for solubility enhancement without interfering with fluorescence assay. DMSO is more suitable for ITC measurements and crystallization while PEG3350 increases the background noise in ITC and causes phase separation in crystallization. Last but not least, incorporation of electron withdrawing moieties can further improve the inhibitory activity of the SPD304 derivatives[2] and result in compounds with lower toxicity and higher activity than SPD304.

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PP038

DERMATOPHARMACOKINETICS OF [^{14}C]-a-TOCOPHEROL AND a-TOCOPHEROL IN HAIRLESS MOUSE SKIN IN VIVO

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Purpose: To determine a-tocopherol's, this most important compound of vitamin E, absorption capacity into the skin.

Material and Methods

A 5% of radiolabeled [^{14}C] and non adiolabeled a-tocopherol was applied onto the backs of SKH-1 hairless mice, in vivo for 0,5 to 4 hours. The skin was washed, the animals killed and from a punch biopsy, stratum corneum, epidermis, dermis and subcutis were cryosectioned. In the case of the [^{14}C]-a-tocopherol the penetration into the stratum corneum was taken by 10 tape strippings, the biopsy was obtained on stripped skin and the radioactivity was evaluated after digestion of the tissue. In the case of a-tocopherol the slices were homogenized, extracted and measured by HPLC analysis with electrochemical detection (EC).

Results: From the first application time (30 minutes) an appreciable amount of a-tocopherol was detected within stratum corneum, epidermis, dermis and subcutis. Upper stratum corneum contains 100 times more tocopherol than the lower one. Stratum corneum penetration achieves its maximum already from 30 minutes. a-Tocopherol show much more affinity for stratum corneum, epidermis and papillary dermis. Important concentrations of a-tocopherol were obtained in all skin compartments after topical application. Differences were observed using the radioactivity and HPLC-EC method.

Conclusions: a-Tocopherol penetrates rapidly in vivo into the skin and a constant flux of absorption seems to be quickly established. Its higher affinity seems to be for the more lipophilic stratum corneum. Topical application of a-tocopherol could protect all skin compartments by its important penetration. Even though high sensitives, differences were obtained on the evaluation of skin absorption between the two methods.

PP039

ANALYSIS OF THE CONTROLLED DRUG RELEASE (CDR) FROM BIOPOLYMER NANOPARTICLES DURING THE INITIAL BURST USING A NOVEL MODELING METHOD

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In the initial stage of the controlled release of a drug from a nanoparticle into a medium a phenomenon referred to as burst can occur. During burst a large amount of drug is released over a small period of time. Apart from the loss in the overall CDR time of actuation, high initial drug release rates can result into toxic drug levels, which would not be attained otherwise. The initial burst has been studied in the past, but with little success in elucidating the mechanisms that control the phenomenon.
In this contribution, a mathematical model is established to investigate how experimental conditions and nanoparticle formulations impact on the initial burst release. Experimental conditions, nanoparticle formulations and drug release profiles were extracted from publications for drugPLGA or -PLGA/PEG carriers and a database was created. Subsequently, statistical methods were utilized to analyze the data and a model was developed that can predict the burst release based on experimental conditions and nanoparticle formulations. Good agreement between model predictions and experimental burst data was obtained. Further analysis revealed that a clear augmentation in the intensity of the burst is obtained when PEG is bound to PLGA. It also seems that an increase in the burst release occurs for greater carrier particles, i.e. going from 5E1 nanometers towards microparticles.
The increased understanding of the burst release can in future be used to manipulate the system more rationally e.g. to reduce the intensity of the burst release.

Biography:
Cristiana de Azevedo has completed her MSc from the Faculty of Engineering, University do Porto (Portugal). She currently accomplishes her PhD at the University Nova de Lisboa (Portugal) and is a visiting researcher at Newcastle University (UK). She is the co-founder of HybPAT, a company that provides software for a more efficient implementation of the PAT initiative in biopharma.

PP040

DESIGN OF LIPOIC- OR IBUPROFEN-SUBSTITUTED MORPHOLINE DERIVATIVES YIELDING IMPROVED ANTIOXIDANT, ANTI-INFLAMMATORY AND HYPOLIPIDEMIC ACTIVITY

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Purpose: The design, synthesis and evaluation of new derivatives as multi-target agents against atherosclerosis/diabetes II.
Material and Methods: The new multi-target compounds were synthesized by reaction of 2,2hydroxy-biphenyl-morpholine with the appropriate alcohol of lipoic, ibuprofen or adamantane, while their structures were confirmed by ^{1}H-NMR and ^{13}C-NMR. Antioxidant activity was evaluated in vitro. These derivatives were further evaluated in two different in vivo protocols. The hypercholesterolaemic mouse model and a long-term hyperlipidaemic/diabetes II mouse model. In the first case, hypercholesterolaemia was induced in mice by administration of tyloxapol, while after 24h, plasma cholesterol and triglyceride levels, as well as total antioxidant capacity were measured. In the second case, hyperlipidaemia/diabetes II was induced by a 50-day high-fat diet and a 5 days low-dose of streptozotocin. In the end, lipidemic indices, plasma total antioxidant capacity and glucose levels were determined.
Results: The new derivatives reduced plasma total cholesterol (40%), LDL (45%) and triglycerides levels (70%). They also increased 2-fold the antiatherogenic index, plasma total antioxidant capacity up to 40% and reduced glucose levels up to 40%.
Conclusions: The results of these in vivo experiments confirm the increased antioxidant and antihyperlipidaemic activity of the designed multi-target derivatives.
Consequently these derivatives may be considered as potentially useful agents in the treatment of multifactorial diseases such as atherosclerosis, diabetes and the metabolic syndrome.
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PP041

NOVEL SUBSTITUTION OF BIPHENYL MORPHOLINE DERIVATIVES LEADS TO MORE ACTIVE ANTIINFLAMMATORY/ANTIOXIDANT AND ANTIHYPERLIPIDEMIC AGENTS

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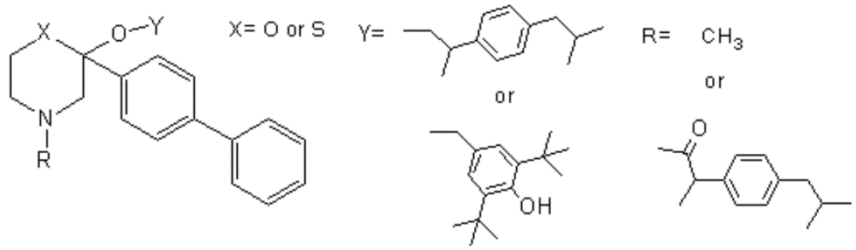
Purpose: The design of more potent antioxidant/anti-inflammatory derivatives of morpholine/thiomorpholine as a means to enhance their activity range for therapeutic intervention in multifactorial disorders such as atherosclerosis/diabetes II.

Materials and Methods: Three new derivatives were designed to combine structural features shown below and were synthesized according to known methodology[1] .Their structures were verified by ^{1}H-NMR and ^{13}C-NMR.



The new molecules were pharmacologically evaluated in vitro in two separate assays, interaction with the free radical of DPPH and inhibition of lipid peroxidation of rat microsomal membranes. Their anti-inflammatory activity was evaluated in vitro via inhibition of lipoxygenase.
Their in vivo activity was evaluated in a hypercholesterolaemia mouse model (tyloxapol induced) in which plasma total cholesterol, triglyceride levels and total antioxidant capacity were measured 24h after a single dose of the new compounds. Further, an inflammation mouse model (carrageenan-induced paw edema) was used for estimation of their anti-inflammatory activity in vivo.1, 2, 3.

Results: The two thio/morpholine derivatives exhibited very significant protection against lipid peroxidation (IC_{50}=1.7 μM). One of them interacted with the free radical of DPPH (IC_{50}=186 μM). All the three molecules inhibited lipoxygonase enzyme with IC_{50} values ranging from 22 μM to 140 μM. The new derivatives reduced plasma total cholesterol up to 42% and triglyceride levels up to 70%. They also increased the plasma total antioxidant capacity up to 75% and reduced inflammation (edema) up to 56%.

Conclusions: The results show the increased antioxidant, antiinflammatory and antihyperlipidaemic activity of the designed new derivatives. Thus, they may be considered as interesting agents for the treatment of multifactorial diseases such as atherosclerosis, diabetes and metabolic syndrome.

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[2] A. Matralis, A. Kourounakis *J. Med. Chem. 2014, 57, 2568*
[3]A. Kourounakis et al. / *Bioorg. Med. Chem. 2010, 18, 7402*

PP042

ENHANCING ANTIOXIDANT & INTRODUCTING ANTIINFLAMMATORY ACTIVITY BY STRUCTURAL MODIFICATION OF KNOWN ANTIOXIDANTS

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Purpose: The design of more potent antioxidant/anti-inflammatory derivatives of known antioxidants (such as resveratrol, caffeic and cinnamic acid) as a means to expand their activity profile for therapeutic intervention in multifactorial disorders such as atherosclerosis/diabetes II.
Materials and Methods: The new compounds were synthesized by esterification reactions in the presence of DCC and their structures were confirmed by ^{1}H-NMR and ^{13}C-NMR. Antioxidant activity was evaluated in vitro using two different assays, scavenging the free radical of DPPH and protection against rat liver microsomal lipid peroxidation[1]. Inhibition of inflammation-related enzyme lipoxygenase was also evaluated[2]. The derivatives were further evaluated in two different in vivo protocols[2,3]; a hypercholesterolaemia mouse model (tyloxapol induced) where plasma cholesterol and triglyceride levels, as well as total antioxidant capacity were measured, and an inflammation mouse model (carrageenan-induced paw edema) where edema inhibition was measured, in all cases after the administration of a single dose of the new derivative.
Results: Most of the compounds present enhanced activity, i.e. lower values of IC_{50}, compared to the reference compounds, indicating a more efficient interaction with the DPPH free radical. In the lipid peroxidation assay, IC_{50} values ranged between 0.6 μM and 46 μM, exhibiting in most cases an improved antioxidant profile compared to reference compounds.The same holds for lipoxygenase inhibition, with IC_{50} values ranging from 170 μM to 0.09 μM. Further, the new derivatives reduced plasma total cholesterol up to 36%, and triglyceride levels up to 61%. They also increased the plasma total antioxidant capacity up to 62%. Finally, inflammation levels were reduced up to 45%.
Conclusions: The results confirm the increased antioxidant activity of the designed new derivatives, as well as the introduction of new pharmacologically relevant activities such as antiinflammatory and antihyperlipidaemic properties. This wider activity profile may be of potential use for therapeutic intervention in multifactorial disorders such as atherosclerosis, diabetes and neurodegeneration.

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PP043

INVESTIGATING A COMMON BINDING MODE OF NEW HIGHLY POTENT SQUALENE SYNTHASE INHIBITORS

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Purpose: Inhibition of squalene synthase (SQS), an enzyme participating in the cholesterol biosynthetic pathway, is of therapeutic interest. Several substituted morpholine derivatives have been designed and proven to have interesting such activity.[1,2,3,4] The aim of this work is the study of the binding mode of a few selected derivatives with measured high inhibitory activity on SQS in order to derive a binding hypothesis and to further the discovery of new actives.
Material and Methods: Docking of the ligands was carried out using MOE 2014 software.[5] A new crystal structure complex of hSQS including three Mg^{2+} ions in the enzyme's active site (PBD: *3WEG)* was used.[6] The final protein structure model used resulted from minimization with the LigX tool (MOE). The sites occupied by the two substrate molecules (FsPP) were appointed as the Binding Pocket. A Rigid Docking Protocol was followed and the ligands' placement was done by use of Triangle Matcher methodology. Poses were minimized, rescored and clustered based on a common scaffold clustering methodology.[7,8] Subsequently, the clusters were evaluated based on the number of included poses, consensus scoring[9] values (combining three different Force field-based scoring functions), and interaction fingerprints with the binding site residues. Finally, predominant clusters were selected and used to reveal the most probable binding modes of the compounds studied.
Results: The binding hypothesis extracted from the explicit docking studies, consensus scoring and common scaffold clustering of the active compounds resulted in deriving probable common binding modes for the investigated compounds. More specifically, ligands participate in specific interactions with ARG77 (a residue much involved in the catalytic process of the enzyme) as well as with surrounding residues, also belonging to the flexible AB-flap. Polyaromatic substituents tend to be accommodated in hydrophobic cavities S1 and S2, hypothesized to stabilize the unsaturated chains of the natural substrate molecules (FPP). Mg^{2+} interactions with these ligands seems limited.
Conclusions: The combination of docking and clustering of poses seems promising in order to elucidate interactions of inhibitors in the large active site of this enzyme.

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PP044

DEVELOPMENT AND VALIDATION OF AN HPLC-FLUORESCENCE METHOD FOR THE DETERMINATION OF ANIDULAFUNGIN AND MICAFUNGIN IN HUMAN PLASMA: APPLICATION TO A PHARMACOKINETIC STUDY

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Objectives: To develop and validate RP-HPLC-RF method for the quantitative determination of anidulafungin or micafungin in plasma samples for application in human pharmacokinetic study, in ICU patients with different type of fungal infections.
Materials and methods: 100 μL of sample were spiked with 40 μL of IS and vortex-mixed for 5sec. The plasma samples were extracted with the addition of 500 μL 0.1% TFA in ACN. After centrifugation for 5 min, at 8000 rcf and at 0°C, 20 μL of the anidulafungin-supernatant are collected, mixed with 80 μL of MeOH and injected (5 μL) into the HPLC system. In case of micafungin, the clear supernatant was injected (10 μL) directly, with no further dilution. A gradient profile was used for the chromatographic separation of the analyte and the IS. The analytical column was Phenomenex Gemini, 5u, C_6-phenyl, 150 x 4.6 mm, with 110Å particle size, protected by a Security Guard System. Mobile phase was consisted from either 0.1% TFA in H_2O or 0.1% TFA in ACN. The running time was 20 minutes and the wavelengths of excitation and emission were set at 273 nm and 464 nm. The process validation was based on the official guidance for bioanalytical process validation, proposed from FDA and EMEA.
Results: The calibration curve was linear for both anidulafungin and micafungin over the concentration range of 0.06-28.8 μg/mL and 0.13-28.3 μg/mL, respectively. The retention time of micafungin was at 7.4 min and anidulafungin eluted at 9.2 min. All the validation parameters were within the acceptable limits. The intra- and inter-day accuracy and precision were less than 5%, for both echinocandins and demonstrated that this method is accurate and precise. The method was used for the quantification of patients' plasma samples, in order to conduct a pharmacokinetic study.
Conclusions: A simple, selective and reproducible HPLC-fluorescence method for plasma concentration measurement of anidulafungin and micafungin has been developed. The method was successfully applied to a pharmacokinetic study enabling the development of a population pharmacokinetic model for both echinocandines.

PP045

POPULATION PHARMACOKINETIC ANALYSIS OF MICAFUNGIN IN PATIENTS WITH DIFFERENT TYPES OF FUNGAL INFECTIONS

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Objectives: To develop a PopPK model for micafungin (MCF) based on the concentration vs time data obtained from critically ill patients.
Methods: 27 critically ill patients hospitalized in ATTIKON (AGHA) and HIPPOKRATEION (HGHA) General Hospitals of Athens treated with 100 mg micafungin intravenously once daily with a median (range) age of 60.7 (31-83) years and body mass index (BMI) of 26.6 (21.5-46.9) kg/m^2 were enrolled in the study. At AGHA, blood samples were withdrawn on day 1, 3, 6, 12 and 24 or last day of therapy at the following time points: 0h (prior to the start of infusion), 60 min after the start of infusion (i.e. at the end of infusion) and 3, 6, 12 and 24 hours after the start of infusion. At HGHA, one blood sample was withdrawn prior to drug administration at days 1,3 and 5 while at the day 7 samples were collected before and 0, 1, 3, 6 12 and 24h after infusion. Micafungin concentrations were measured with a validated HPLC-fluorescence plasma assay method. Population PK data analysis was performed using NONMEM software to describe the time course of MCF in plasma. The effect of covariates including demographic characteristics, laboratory indexes, combined medication, were screened for their influence on PK parameters.
Results: A two-compartment (2-CMT) PK model was found to best describe the concentration vs time data of MCF. The basic model was parameterized as clearance Cl=1.06 L/h, central volume of distribution V1=12.2 L, inter-compartmental clearance Q=3.03 L/h and volume of distribution of the peripheral compartment V2=9.00 L. The inter-individual variability expressed as percentage coefficient of variation (CV %) was 35.6%, 30.3%, 28.7%, 24.2 for Cl, V1, Q and V2, respectively. The application of a proportional residual error model led to better performance. Further analysis for the detection of the effect of various covariates on MCF PK parameters is in process.
Conclusions: The derived model described adequately the concentration-time data of MCF. Further analysis is in process to detect possible effect of various covariates on MCF PK parameters. The model is intended to serve as a prior information for the individualization of MCF levels in Greek hospitals. The model will be enriched with more patients till the end of the study, further elucidating the effect of various covariates on PK parameters.

PP046

THE IMPACT OF SAFFRON AQUEOUS EXTRACT ADMINISTRATION ON ATHEROSCLEROSIS DEVELOPMENT IN AN EXPERIMENTAL ANIMAL MODEL

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Purpose: Saffron is an antioxidant herbal derivative containing significant components (crocin, safranal) with potential anti-atherosclerotic properties[1]. Objective of the study it to assess the effects of saffron on the development and stability of atherosclerotic lesions in diabetic apolipoprotein-E deficient (Apo-E$^{-/-}$) mice.

Materials and methods: 40 8-week old male Apo-E$^{-/-}$ mice were fed western-type diabetogenic diet for 12 weeks and then randomized to receive either saffron 30 mg/kg (saffron group 1, SF1, n=10), 60mg/kg (SF2, n=10), 90 mg/kg (SF3, n=10) or equivalent volume of water for injection (control group, CO, n=10) for 4 weeks. After this period, all mice underwent a glucose tolerance test and the respective area under the concentration *vs* time curve (AUC) was calculated. After euthanasia, lipid and glucose profile in blood samples were assayed, while in aortic tissue specimen the mean plaque area, the relative plaque content of elastin and collagen and the thickness of fibrous cap were measured.

Results: The mean plaque area tended to be smaller in all SF groups than CO-group. Notably, saffron treatment seemed to promote plaque stability due to high concentration of elastin and collagen in atherosclerotic lesions derived from saffron-treated mice in conjunction with thicker fibrous caps and reduced internal lamina ruptures (IEL) per mm of arterial girth. Finally, high saffron dose (90 mg/kg) significantly ameliorated glucose levels in comparison to control group.

Conclusions: Saffron exerted anti-diabetic and anti-atherosclerotic effects and promoted plaque stability in Apo-E$^{-/-}$ mice in a dose-dependent manner.

Literature reference: 1. Giaccio M., Crocetin from saffron: an active component of an ancient spice. *Crit Rev Food Sci Nutr.* 2004; 44(3):155-72.

PP047

CORRELATION OF CYP2C19 GENOTYPE WITH PLASMA VORICONAZOLE CONCENTRATIONS: A PRELIMINARY RETROSPECTIVE STUDY IN GREEK PATIENTS

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Purpose: Plasma concentrations of the triazole second generation antifungal, voriconazole, exhibit wide interindividual variability due to demographic, food or drug interactions, clinical and genetic factors. The effect of *CYP2C19* polymorphisms on voriconazole trough concentrations and adverse effects have been investigated in many studies but some controversy remains[1,2]. The aim of the present study was to develop a RP-HPLC method for the measurement of voriconazole serum concentrations in order to assess their correlation with CYP2C19 genotype in patients on voriconazole therapy.
Material and Methods: A sensitive voriconazole HPLC assay was developed and validated. Voriconazole concentrations were subsequently determined in 57 steady state trough serum (C_{trough}) samples collected from 27 patients (13 female, median ages 47.5(range 6-73) years) treated with voriconazole (IV or per os) prophylactically/ preemptively/ empirically for fungal infections at the University Hospital "Attikon", for at least 5 days. The genotyping of CYP2C19*2 and CYP2C19*3 polymorphisms was performed by PCR-RFLP. Statistical analysis was performed with the SPSS Statistics for Windows software package.
Results: Based on guidelines for method validation, analytical sensitivity of the HPLC method was verified to have a limit of quantification of 0.1815±4.30 μg/ml and a linearity of 0.1815-12.16 μg/ml. The measured concentrations of voriconazole in patient-samples reflected a wide inter-individual variability (range 1.15±19.01 to 16.6±3.69mg/L). The allele frequency of the *1 and *2 variant were found to be 67% (*1/*1, extensive metabolizers), 29% (*1/*2,intermediate metabolizers(IMs)) and 4% (*2/*2, poor metabolizers (PMs)). Notably, only 8.7% of patients with *1/*1 genotype and 22.2% with *1/*2 genotype had C_{trough} voriconazole concentrations outside the therapeutic trough range (1.5 - 6 μg/ml), but concentrations were not significantly different between EMs and PMs ($p = 0.21$) for the same administered dose. A 14% incidence of hepatotoxicity was noted. Voriconazole dose-concentration correlations showed a disproportionate 2.2 times increase in mean Ctrough ($p = 0.088 > 0.05$, CI: 5%) with an increase in the oral dose from 400 to 600mg/day (1.5 times), but a proportional increase during IV administration.
Conclusions: The HPLC-UV method was characterized by precision, sensitivity, repeatability and speed. No association between CYP2C19 genotype and voriconazole concentrations, as well as between voriconazole concentrations and the development of hepatotoxicity, was found in accordance with several prospective studies[1,2]. In this study, CYP2C19 genotype does not appear to account for the variability in voriconazole concentrations between individuals.
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PP048

COLISTIN CONCENTRATIONS *IN BRONCHOALVEOLAR LAVAGE FLUID AND SERUM* FOLLOWING *ADMINISTRATION OF NEBULIZED COLISTIMETHATE SODIUM*

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Purpose: *Colistin, administered in the form of colistimethate sodium (CMS), has been used intravenously for the treatment of ventilator-associated pneumonia (VAP) and ventilator-associated tracheobronchitis (VAT). Studies relating to pulmonary penetration of intravenously administered colistin have shown conflicting outcomes. Treatment with nebulized colistin may be beneficial in terms of decreased toxicities by limiting systemic drug exposure.* The purpose of this study was to determine the levels of colistin in bronchoalveolar lavage fluid and serum after administration via nebulization of the maximum recommended dose of colistimethate sodium (CMS) to *intubated and mechanically ventilated* critically ill patients with *lung infections due to multidrug-resistant (MDR), gram-negative bacteria*.
Methods: Inhaled colistimethate sodium (CMS) was administered at a dose of 2.000.000 IU (international units) every 8 h. Mini bronchoalveolar lavage (BAL) was performed before and at 1, 4, 6 and 8 h, while blood samples were collected before and at 0.5, 1, 2, 4, 6 and 8 h after the first dose. Colistin concentrations in BAL and serum were determined by high performance liquid chromatography.
Results: Our study population included 5 patients, three with pneumonia and two with ventilator-associated tracheobronchitis (VAT). Median ELF (25-75% interquartile range) colistin concentration in epithelial lining fluid (ELF) were 5.0 (1.7-5.6), 16.1 (12.9-17.0), 17.9 (16.1-30.3) and 3.9 (3.6-5.4) μg/ml at 1, 4, 6, and 8 h, respectively, and more than a hundred times higher than those achieved in serum. *Median colistin ELF concentrations were above MIC values and EUCAST MIC breakpoints for A. baumannii (<2 μg/ml)*. Colistin pharmacokinetic parameters/pharmacodynamic parameters in ELF were associated with minimal systematic exposure and less side effects.
Conclusion: Inhalation of CMS was an effective means of delivering CMS and formed colistin to the lungs, as high lung exposure and minimal systemic exposure were achieved in critically ill patients with respiratory infections. This study should be continued in a greater number of patients so as to confirm the above findings.

PP049

A NEW PROCESS FOR THE RECOVERY OF BIOACTIVE INGREDIENTS FROM MASTIC GUM

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Introduction: Mastic gum is an ancient, high-value product obtained from *Pistacia lentiscus* L var Chia and is used in traditional medicine, in perfumery, dentistry and as a spice in Mediterranean cuisine [1]. Due to the high complexity of this material (triterpenes and polymers) the purification of the bioactive compounds is time consuming and requires multi-stage separation procedures [2]. The present work aims to develop a rapid and effective process for the isolation of the main mastic gum constituents.
Material and methods: The separation procedure consists of two main steps. The first step includes the fractionation of mastic gum by using a novel CCC method while in the second step the supercritical fluid chromatography SFC-CO_2 was used to purify the main triterpenic isomers. The CCC experiment started by treating the row material (7 g) in a 300 mL CPE column with the biphasic system *n*-hexane/EtOAc/EtOH/H_2O 8:2:5:5 (v/v/v/v) in pH zone refining mode, continued in step gradient elution mode by passing the lower phases of the same biphasic system in ratios 8:2:7:3, 8:2:8:2 and 8:2:9:1 (v/v/v/v) and completed by extruding the column with *n*-hexane. The chemical composition of each CPE fraction was established by ^{13}C NMR dereplication analysis.
Results: The combination (in the same run) of the pH zone refining and step gradient elution modes led to the rapid and effective fractionation of the bioactive acidic and neutral triterpenes and in the recovery of pure polymer. Further SFC-CO_2 analysis of the enriched CPE fractions by using a chirial column resulted in the purification of the main acidic triterpenic isomers.
Conclusions: This process can be considered as a new approach for the isolation of bioactive compounds from mastic gum while the proposed CCC methodology could be applied for the effective fractionation of numerous complex mixtures of neutral or acidic components.

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PP050

RETENTION TIME IN GRADIENT MODE AND CHROMATOGRAPHIC HYDROPHOBICITY INDEX (CHI). AN EXTENTION FOR IONIZED COMPOUNDS

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An essential connection between drug discovery and drug delivery is the lipophilicity of bioactive compounds, expressed as n-octanol water partition coefficient logP or distribution coefficient logD for neutral and (partially) ionized species respectively. Limitations in the determination of logP or logD by the traditional shaking flask method can be faced by reversed-phase chromatographic techniques, which comprise two different concepts, either the use of isocratic / extrapolated retention factors logk / $logk_w$ or the Chromatographic Hydrophobicity Index CHI derived by gradient elution. The latter correspond to the fraction φo of organic modifier, which leads to equal distribution between mobile and stationary phase and is determined upon construction of calibration equations between the gradient elution time gtR and isocratically measured φo. Up to now CHI indices have been developed only for unionized compounds[1]. In the present study we expanded CHI estimation for ionized acidic drugs using different pH (5.0 and 7.4) and buffer conditions (ammonium acetate and phosphate). In the case of phosphate buffer n-octanol was used as mobile phase additive to obtain conditions, similar to those used in previous investigations of our group for the determination of $logk_w$ values, best simulating $logD_{7.4}$. For pH 5.0 and ammonium acetate buffer, highly reliable CHI indices were obtained which showed excellent correlation with $logD_{5.0}$ (R^2 = 0.9045). The corresponding correlation with $logk_w$ had R^2 = 0.7678. For pH 7,4 and ammonium acetate buffer a curvature in the gtR/ φo relationship was observed for low φo values. The derived CHI values showed an inferior correlation with $logD_{7.4}$, R^2 = 0.723, while the corresponding correlation with isocratically φo values had R^2 = 0.900. Poor correlation was obtained with $logk_w$ values. In contrast, for phosphate buffer and n-octanol as mobile phase additive, $logk_w$ values showed better performance (R^2 = 0.850) than both CHI (R^2 = 0.741) and φo values R^2 = 0.734

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PP051

DEVELOPMENT AND VALIDATION OF A UPLC METHOD FOR QUANTIFYING *TRANS*-CROCIN 4 AND CROCETIN FROM SAFFRON IN PLASMA: A PHARMACOKINETIC STUDY

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Purpose: The scientific purpose of the present project is the development of a novel and fully validated UPLC-UV methodology for the rapid and accurate quantification of TC4 and crocetin in mice plasma after *i.p* administration.
Material and Methods: Saffron is the dried stigmas of the flower *Crocus sativus* L. [Iridaceae]. The main saffron constituents include crocins which are mono- and bis-esters of crocetin with glucose and/or gentiobiose [1]. Several studies indicate that the main carotenoid constituent, *trans*-crocin-4 (TC4) has shown remarkable activity against Alzheimer's disease [2]. Therefore, a novel UPLC-UV methodology was developed and fully validated, according to FDA and EMA guidelines, for the quantification of TC4 and crocetin in mice plasma after *i.p* administration. The separation of the analytes was performed on a C8 Hypersil Gold column with 2.5 min run time, employing the internal standard (IS) methodology.
Results: The results show adequate linearity ($r^2 > 0.996$) over a wide concentration range [0.01−6 μg/mL (n = 10)] with a Lower Limit of Quantification (LLOQ) value of 0.01 and 0.03 μg/mL for crocetin and for TC4 respectively, whereas the aforementioned methodology demonstrated adequate precision, accuracy, sensitivity and selectivity. The method was successfully applied for the determination of crocetin and TC4 in mouse plasma after 50 and 150 mg/kg TC4 *i.p* administration in mice. In details, 42 samples of the two administered groups (both male and female mice), covering a sampling period of 0−240 min were analyzed. The results showed that circulating TC4 levels were found to be statistically different in female and male population. Indicatively, for the female mice [50 mg/kg TC4], the experimentally determined plasma Cmax TC4 levels were 3304 ± 413 ng/mL (T_{max} = 60 min). Interestingly, crocetin was not detected in any plasma sample, although it has been reported that TC4 quickly hydrolyzes to crocetin after *p.o* administration.
Conclusions: Overall, the developed methodology offers important information about the bioavailability of TC4, as well as the differences in TC4 absorption between males and females.

Acknowledgements: Despoina Papasavva is acknowledged for technical assistance in the animal experiments

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PP052

SECONDARY METABOLITES IN HONEY AND THEIR INHIBITORY ACTIVITY ON MATRIX METALLOPROTEINASES

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Purpose: Honey is a viscous, supersaturated sugar solution derived from nectar, gathered and modified by the honeybee, *Apis mellifera*. It has been used as a traditional medicine for centuries by different cultures. Honey possesses antimicrobial properties against a broad range of microbes, and promotes wound healing [1].
Materials and Methods: In the present study, after a first elimination of sugars, the chemical profile of 10 Greek monofloral honeys (e.g. thyme, chestnut, fir honeydew, pine, orange, heather, strawberry tree) was investigated by HPTLC, which permits the automatic, rapid and low cost evaluation of complex herbal mixtures. Furthermore, their radical scavenging activity was tested with the aid of a DPPH assay. Two honey samples, a strawberry tree and a heather honey, were further processed, and major compounds were purified by MPLC. Subsequently, raw honeys and pure compounds were evaluated for their ability to inhibit the matrix metalloproteinases collagenase and elastase.
Results: Unedone was obtained from strawberry tree honey and the flavonoids quercitrin and naringenin from heather honey, while two isomers of abscisic acid (*cis, trans*-ABA and *trans, trans*-ABA) were isolated from both samples. Both raw honey samples (100μg/mL) and pure compounds (100μM) exerted moderate inhibitory activity (≤65%) against elastase and collagenase enzymes. Spectrophotometric methods were used to determine the enzymatic activity.
Conclusions: Elastase and collagenase are implicated in the wound healing process and their overexpression and activation is thought to be involved in the pathogenesis of chronic wounds. Improving basic knowledge in this area ultimately may help clarify the role of honey in the wound healing mechanism and proactively intervene in an effort to prevent normal wounds from becoming chronic [2].

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PP053

GREEK FLORA AS A SOURCE OF NEW ANTI-OXIDANT, ANTI-ELASTASE, ANTI-COLLAGENASE AND ANTI-HYALURONIDASE NATURAL AGENTS

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Purpose: In order to investigate new cosmetic ingredients of natural origin, 50 plant extracts obtained from greek flora were screened for their capacity to scavenge free radicals and inhibit enzymes related to skin ageing. Degradation of the extracellular matrix (ECM) has directly been linked to skin ageing and is correlated to increase in activity of enzymes such as elastase, collagenase and hyaluronidase. As a result elastin, collagen and hyaluronic acid decrease, leading to loss of strength and flexibility in skin and formation of deep wrinkles. Also free radicals induce the activity of these enzymes[1,2].
Materials and Methods: Anti-elastase, anti-collagenase and anti-hyaluronidase activities of 50 samples, consisting of methanol and ethyl acetate extracts of collected plants, were determined using spectrophotometric methods. Radical scavenging activity was determined by the ability of the extracts to scavenge DPPH and ABTS radicals. Moreover bioguided isolation process was performed in order to reveal the active secondary metabolites.
Results: The majority of the samples especially methanolic extracts showed high anti-oxidant capacity, for example *Rosa damascena* extract exhibited IC50 value at 47.6 μg/ml. In anti-elastase assay eight extracts showed more than 50% inhibition at a concentration of 100μg/ml, in anti-collagenase assay fifteen extracts showed more than 60% inhibiton at 100μg/ml and in anti-hyaluronidase assay eight extracts showed more than 80% inhibition at 300μg/ml. Methanolic extracts of *Sedum sediforme* and *Umbilicus horizontalis* aerial parts inhibited elastase and hyaluronidase activity by more than 85%. *Rosa damascena* inhibited 80% of collagenase activity at 100μg/ml.
Conclusions: Free radical scavenging and enzyme inhibitory activities of plant extracts suggest that they can help restore skin elasticity and thereby delay wrinkling process. In the case of *Rosa damascena*, quercetin-3-O-rhamnoside, kaempferol-3-glucopyranoside, phenylethanol were proved to exhibit high anti-collagenase activity.

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PP054

EXPLOITATION OF AGRICULTURAL BY-PRODUCTS FOR THE RECOVERY OF BIOACTIVE COMPOUNDS WITH APPLICATIONS IN COSMETIC INDUSTRY

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Purpose: In the present study, by-products derived from wood (*Pinus nigra*, *Pinus heldreichii*, *Eucalyptus globulus* and *Juniperus phoenicea*) and food-juice industries (*Punica granatum* and *Prunus persica*) were evaluated for their antioxidant and skin whitening activity, as well as for their total phenolic and flavonoid content and the major compounds of the most active extracts were identified.
Material and Methods: The bioassays were carried out in a cell-free system using the 96-well microplate method. For the evaluation of antioxidant activity, DPPH (2,2-diphenyl-1-picryl-hydrazyl) Radical Scavenging Assay and ABTS (2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation Assay were performed. The anti-hyperpigmentation activity was estimated by tyrosinase enzyme assay. Total phenols were determined by Folin-Ciocalteu method, while Aluminum chloride colorimetric method was used for flavonoids determination. Subsequently, the active extracts were investigated by Liquid Chromatography– High Resolution Mass Spectrometry (LC–HRMS) and MS/MS for identification of their major constituents. Analyses were performed on an Accela High Speed LC System hyphenated to a LTQ-Orbitrap XL hybrid mass spectrometer.
Results: Both the DPPH and the ABTS assay revealed strong antioxidant activity for all the extracts of *P. granatum* (up to 90.07 – 93.53 % and 99.51 – 99.65 %) and *E. globulus* (up to 93.27 – 96.12 % and 99.45 – 99.84 %) at a concentration of 200 μg/mL and 100 μg/mL, respectively. Moreover, the aqueous extract of *P. granatum* and all the extracts of *E. globulus* revealed significant anti-tyrosinase activity at a concentration of 300 μg/mL (49.25 %, 49.15 – 50.67 % respectively). The total phenolic content for *E. globulus* and *P. granatum* extracts was particularly high (up to 121.81-124.88 and 124.57-125.62 mg GAE/g), while the ratio total flavonoids/phenolics was 0.069-0.078 and 0.200-0.217, respectively. *E. globulus* extracts were rich in proanthocyanidins and ellagitannins and *P. granatum* extracts were characterized by punicalagins, anthocyanidins, gallic acid and flavonoids.
Conclusions: Overall wood industry and food processing by-products that were investigated are promising source of bioactive compounds and have powerful potential to be exploited in cosmetic industry.

Acknowledgment: This work has been partially funded by GSRT under the "AGROSMETICS" project (1440-BET-2013).

PP055

GREEK FLORA AS A SOURCE FOR THE DISCOVERY OF NOVEL OSTEOPROTECTIVE AGENTS

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Purpose: The aim of this study was to identify and characterize Greek plant extracts and isolated compounds that display significant Selective Estrogen Receptor Modulator (SERM) activity.
Material and Methods: Considering our research experience on natural products with estrogenic activities and current litterature [1, 2], 39 plant species were selected and 75 extracts were prepared using conventional and modern-green techniques. All extracts were biologically evaluated for their ability to promote the differentiation of MC3T3-E1 cells (pre-osteoblasts) to mature osteoblasts, using Alkaline Phosphate (AlkP) as differentiation marker. The bioactive extracts were fractionated by the use of Fast Centrifugal Partition Chromatography (FCPC) and the major constituents were isolated using several chromatographic techniques and structurally elucidated via NMR spectroscopy. The purified compounds were *in vitro* evaluated by cell–based screening assays, concerning their osteoblastogenic ability (MC3T3-E1 cells) and their estrogenic-antiestrogenic properties (Ishikawa, MCF-7 cell lines).
Results: The biological evaluation revealed *Rhamnus* species, *Lupinus albus*, *Psoralea bituminosa*, *Ceratonia siliqua*, *Hippocrepis comosa* and *Cytisus villosus* between the most capable extracts to induce AlkP activity in MC3T3-E1 cells. Among the isolated secondary metabolites, flavonoids such as genistein derivatives, dihydromyricetin, kaempferol, as well as kaempferol derivatives were proved as the most promising bioactive constituents.
Conclusions: Plant extracts of *Rhamnus* species, *Lupinus albus*, *Psoralea bituminosa*, *Ceratonia siliqua*, *Hippocrepis comosa* and *Cytisus villosus* could be promoted as potentially osteoprotective agents, since these extracts, as well as the contained secondary metabolites were capable to induce osteoblastic differentiation.

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PP056

NOVEL COSMECEUTICALS AND FOOD SUPPLEMENTS FROM EXPOITATION OF AROMATIC PLANTS' BY-PRODUCTS

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EXANDAS is the acronym of a research European project which aims to apply emerging and cutting edge technologies in the field of Natural Products in order to fully and efficiently exploit the therapeutic potential of medicinal and aromatic plants processing waste and by-products. The consortium, consisting of six academic and six SMEs partners, will join forces and exchange know-how through an extended secondments scheme to advance Research & Innovation.
Purpose: The scientific concept of **EXANDAS** involves the exploitation of aromatic plants' by-products for the development of novel cosmeceuticals and food supplements.
Materials and Methods: The cornerstone of **EXANDAS** project is the development of novel processes based on eco-friendly technologies for the efficient extraction, purification and transformation of active ingredients, as well as the complete chemical characterization and biological evaluation of produced extracts and pure compounds that can be commercially exploited. Optimization and scaling up of these procedures, as well as formulation using emerging technologies will lead to the development of novel final products. In more details, byproducts and wastes from the industrial exploitation of mastic gum, rose, mountain tea, lavender, geranium and sweet basil will be selected. Improved techniques such as ASE, CPC, MAE etc. will be used to isolate, purify, and structurally characterize the active constituents that will be further investigated with the aim to be exploited commercially. A broad spectrum of bioassays will be incorporated for the evaluation of antioxidant, anti-inflammatory, antimicrobial and anti-aging properties activity of all derived extracts and products. Using the experience of the academic partners in phytochemistry and natural product chemistry, as well as the practical experience of the SMEs in large scale processing of plant material and development of innovative final products, transfer of scientific knowledge, best practices and know-how will take place. The abovementioned objectives will be implemented through an extended and balanced scheme of researchers' exchanges, in both directions and via a mutual scientific project developed on the needs and interests of both industrial and academic sectors, exploiting the existing complimentary expertise.
Conclusions: Overall, the implementation of **EXANDAS** aspires to develop a successful and sustainable international and intersectoral collaboration model, which will contribute to the innovation potential of Europe for the most effective exploitation of natural resources and the development of novel cosmeceuticals and food supplements.

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PP057

EXTRACTION OPTIMIZATION AND *IN VITRO* EVALUATION OF MICROBIAL EXTRACTS FOR THE DISCOVERY OF NOVEL COSMEUCEUTICAL AGENTS

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Purpose: In the frame of the EU project MICROSMETICS, 8 fungi (CF) and 12 actinomycetes (CA) were selected from a collection of >100.000 strains to be further investigated based on their potential anti-ageing and skin-whitening activity [1].
Material and Methods: Different extraction protocols using various solvents and in some cases in combination with an adsorbsion resin were used. In total 61 extracts were generated. The antioxidant activity was evaluated in a cell-free bioassay using the DPPH and ABTS methods. The whitening activity was determined using the tyrosinase assay. Active extracts were further evaluation in cell-based assays for their skin whitening (bleaching) activity (i.e. tyrosinase inhibition) in mouse melanocytes (B16F10 cell line), as well as in normal human skin fibroblasts for their capacity to activate proteostasis ensuring anti-ageing mechanisms, namely the ubiquitin-proteasome and autophagy-lysosomal systems.
Results: All 61 extracts were evaluated for their antioxidant and skin-whitening activity. EtOAc extracts of the strains CA-126574, CA-129255, CA-131129 and CF-223716, and XAD-4 treated extracts of CF-092983 and CA-129531 demonstrated significant anti-tyrosinase activity (≥50%, 300ug/ml) while the CF-223709 extract showed the highest anti-oxidant activity (62.83%, 200ug/ml). The XAD-4 extracts of the strains CA-129247, CA-218259 and CA-126581 revealed both anti-oxidant and anti-tyrosinase activity. The ten most active extracts have been further evaluated for their bleaching activity in the B16F10 mouse melanocytes cell line. The EtOAc extract CA-129255 and the EtOH/H_2O extracts CA-129531 and CA-126581 inhibited significantly the tyrosinase activity (10 μg/ml) while the CA-129247 and CF-223709 extracts showed a greater effect at a lower concentration (1μg/ml); the anti-ageing effect of these compounds on human skin fibroblast is currently under investigation.
Conclusions: Overall the extraction optimization and the bio-evaluation of all extracts led to the selection of the ten most promising extracts. It is anticipated that at least one of those extracts will be excellent candidates for industrial development and can serve as a proof of concept that microbial ingredients can have successful applications in cosmeceutical industry.
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Acknowledgments: *This work has been financially supported by EU under the frame of MICROSMETICS project (FP7-PEOPLE-IAPP 2013, Grant agreement: 612276).*

PP058

A NEW PIPELINE COMBING LC-HRMS AND NMR METABOLOMICS FOR THE DISCOVERY OF BIOACTIVE MOLECULES FROM MARINE ORGANISMS AND MICROORGANISMS

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Purpose: TASCMAR is an EU funded project that aspires to develop new tools and strategies to overcome existing bottlenecks in the bio-discovery and industrial exploitation of novel marine derived bio-molecules with applications in pharmaceuticals, nutraceuticals and cosmeceuticals as anti-aging ingredients. An integrated, holistic technological approach including NMR- and MS-based metabolomics has been applied, in conjunction with bioactivity profiling, as a filtering and a bio-prioritization tool for the selection of the top 10% most promising extracts.
Material and Methods: In the frame of the project, more than 180 organisms (53% Demospongiae, 11% Holothuroidea, 10% Anthozoa, 7.5% Echinoidea, 4% Gastropoda, others < 3%) collected from the Indian Ocean, the Red Sea and Mediterranean, were extracted using DCM/MeOH. Metabolic profiles were recorded by a combining strategy employing UHPLC/Orbitrap-HRMS and NMR (600 MHz). An optimized standard procedure was followed and the derived spectra were analyzed by multivariate analysis (MVA) for the statistical evaluation of the results (PCA, PLS-DA, and OPLS) and the detection of the features responsible for the observed clustering. Additionally, statistical total correlation spectroscopy (STOCSY) and in-house machine learning algorithms were applied to correlate specific spectral features with bio-activity results.
Results: On the level of class both non-supervised (PCA) and supervised analysis (PLS-DA) showed good clustering separation. On the level of order only the supervised analysis resulted in sample discrimination. On the level of genus and species cluster formation was observed only in certain cases, e.g., in the case of *Biemna fortis* or *Clathria reinwardti* samples collected from different spots were closely related, while in the case of *Iotrochota baculifera* samples even from the same spot exhibit high variation.
Conclusions: Overall UHPLC-HRMS and NMR metabolomics approach in combination with bioactivity profiling provide a powerful tool for the prioritization of hits in large high throughput screening drug discovery projects.
Acknowledgments: This work has been financially supported by EU under the frame of TASCMAR project (H2020-BG-03-2014, Grant agreement: 634674).

PP059

INVESTIGATION OF PURSLANE, OLIVE TREE, SANTA YERBA, CHINESE PLUM AND SOY SPROUT FOR THEIR ACTIVITY ON OSTEOBLASTIC DIFFERENTIATION AND BIOGUIDED ISOLATION OF ACTIVE INGREDIENTS

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Purpose: In continuation of our research for discovering plant extracts that can be used against osteoporosis [1, 2], five plant extracts were selected to be investigated for their potential osteoblastogenic ability.
Material and Methods: Five extracts originated from the leaves of Portulaca oleracea (purslane), Olea europea (olive tree), Eriodictyon californicum (santa yerba), Prunus mume (Chinese plum) and from the sprouts of Glycine max (soy sprouts) were generated using as solvent system aqueous ethanol in different percentage and then were fractionated using FCPC technology. The initial extracts, as well as their fractions were evaluated for their ability to induce differentiation of MC3T3-E1 cells (pre-osteoblasts) to mature osteoblasts by monitoring induction of the differentiation marker Alkaline Phosphatase (AlkP). Samples that induced AlkP activity were further tested for their ability to promote mineralization, as assessed by Alizarin red staining of differentiated MC3T3-E1 cells.
Results: From the 5 extracts and 38 fractions that were tested at 30μg/ml, 14 samples up-regulated AlkP activity, 15 appeared to marginally affect the differentiation process, while 14 appeared to negatively impact cell viability. Extracts that induced AlkP expression were further evaluated for their ability to promote mineralization in the same concentration. Twelve samples appeared to promote differentiation and/or mineralization and four of the samples that displayed the most significant effect were then tested in a dose response manner (3, 10, 30, 100, 300 μg/ml). Among them the most promising sample was a fraction of santa yerba, which displayed EC_{50}=10 μg/ml. This fraction was further investigated by LC-HRMS and as only some compounds could be identified, it was further investigated phytochemically using prep HPLC. The isolated compounds were then identified by 1D and 2D NMR as homoeriodictyol, 4'-methyl-eriodictyol, erionic acid A and erionic acid E. All pure compounds were also evaluated for their ability to induce AlkP in MC3T3-E1 cells at 0.1, 1.0 and 10 μM. Among them homoeriodictyol displayed the most significant effect at 10 μM.
Conclusions: From the samples that were investigated *Eriodictyon californicum* (santa yerba) extract and its secondary metabolite "homoeriodictyol" enhance significant the osteoblastic differentiation and could be considered as potentially osteoprotective agents.

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PP060

MALDI TOF IMAGING MASS SPECTROMETRY OF FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUES OF PROSTATE CANCER

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Proteins and peptides expression in cancer tissues have proven their clinical utility as potential biomarkers for the diagnosis and prognosis of a given disease. MALDI mass spectrometry imaging (MALDI-IMS) is a powerful technique for the molecular profiling of tissues through comprehensive visualization of the distribution of biomolecules across tissue sections. In this work we employed MALDI-IMS to analyze formalin-fixed paraffin-embedded (FFPE) prostate cancer tissue samples. Tissue sample preparation was performed using a heat induced antigen retrieval (HIAR) procedure to release proteins and peptides from formalin fixation. Sinapinic acid was used as a matrix for MALDI-TOF analysis. Mass spectra were processed by principal component analysis and 2-D peak distribution tests. PCA analysis revealed a number of mass ion peaks obtained from tumor areas that were distinguishable from non-tumor regions within a given specimen. This study provides evidence in support of the clinical utility of MALDI-IMS to analyze FFPE tissues post antigen retrieval procedure at an in-depth molecular level, which supersedes all other standard histopathologic techniques for diagnostic purposes used in the current practice. Given its ability to extract comprehensive and accurate protein/peptide information from a minimal tissue-size specimen, MALDI-IMS analysis holds promise for enhancing the quality and the number of samples available from cancer patients allowing for personalized decisions in our era of precision medicine.

PP061

ASYMPTOTIC ANALYSIS ON A TARGET MEDIATED DRUG DISPOSITION MODEL: ALGORITHMIC AND TRADITIONAL APPROACHES

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Introduction: A detailed analysis is reported of a multiscale pharmacokinetic-pharmacodynamic Target Mediated Drug Disposition (TMDD) one-compartment model, simulating the interaction of a drug with its target, the binding of the compounds (generation of the complex) and the outcome of their interaction. This analysis aims at identifying means to control the process.
Materials and Methods: The analysis is based on the algorithmic Computational Singular Perturbation (CSP) methodology. The reactions in the model that (i) generate the fast time scales, (ii) generate the constraints in which the system evolves and (iii) drive the system at various phases are identified, with the use of algorithmic CSP tools. These identifications are very important for the improvement of the model and for the discovery of ways to control the evolution of the TMDD process.
Results: The TMDD process evolves in four periods, which can be characterized as fast, slow, fast and slow. The first fast period relates to the generation of the complex, while the first slow period that follows relates to the attainment of a constant concentration of the complex. The second fast period relates to the depletion of the drug and the final slow period relates to the depletion of the complex.
Conclusions: Regarding the qualitative understanding of the process, the present analysis systematizes the findings in the literature and provides some new insights. Among the major findings are those that relate to the adjustment of the period in which the complex is present.

PP062

WHEN A REACTION IN A PHARMACOKINETIC MODEL CONTRIBUTES TO THE CONSUMPTION OF ITS PRODUCT

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Introduction: The dynamics of a multiscale pharmacokinetic-pharmacodynamic Target Mediated Drug Disposition (TMDD) multi-compartment model are studied, in order to explain an interesting feature. In particular, an increase of the reaction rate constant of the reaction that refers to the removal of the drug from the tissue compartment leads to a decreased level of its product (i.e., the drug in the central compartment where it binds with the target), during the period in which the drug is depleted.
Materials and Methods: The TMDD model simulates the interaction of (i) the drug with a biological target, (ii) their binding (formation of the complex) and (iii) the results of such an interaction. The model is analyzed with the Computational Singular Perturbation (CSP) algorithm, which allows for the identification of the fast and slow dynamics and for the understanding of their interaction.
Results: The fast dynamics of the TMDD model create the confines in which the system evolves and the associated processes are in equilibrium. Within these confines the evolution of the system is characterized by the slow dynamics and the processes that generate these time scales are the ones that drive the evolution of the TMDD system. It is shown that in the model that governs the slow evolution of the system, within the generated confines due to the fast dynamics, the action of the reaction that removes the drug from the tissue compartment reverses.
Conclusions: It is shown that the interaction of the fast and slow dynamics of a model might generate unexpected results. Such features can be analyzed only when mathematical models that govern the slow evolution can be constructed.