12. Drug Discovery and Development

12.001 New insights in the mode of action of erythrinian alkaloids: electrophysiological studies. Gelfuso EA[1], Galan D[2], Peigneur S[2], Lebbe E[2], Pereira AMS[1], Beleboni RO[1], Tytgat J[2]
[1]UNAERP – Biotecnologia, [2]Universidade de Leuven – Toxicologia & Farmacologia

Introduction: Flowerings of plant *Erythrina mulungu* Mart. ex Benth. (Equisetopsida C. Agardh – Fabaceae Lindl) are widely used in folk medicine thanks to their anticonvulsant and anxiolytic properties. Previous studies have attributed those properties to the presence of erythrinian alkaloids (erythravine, 11-α-hydroxyerythravine and erythrartine) in hydro-alcoholic extracts from flowers of the plant[a]. In this way, electrophysiological experiments unraveling the mode of action of these alkaloids have become interesting for a better understanding of their anxiolytic and anticonvulsant activities. The aim of this study was to investigate the potential action of the alkaloids on voltage-gated sodium and potassium channels, as well as on nicotinic acetylcholine receptors using the voltageclamp technique. **Methods**: mRNAs of $Na_V1.3$ and $Na_V1.6$ sodium channels, $K_V1.1$, $K_V1.2$, $K_V1.4$, $K_V4.2$, and $K_V10.1$ potassium channels and α7, α4β2 and α1β1δε nicotinic receptors were injected in *Xenopus laevis* oocytes obtained from a partial ovariectomy. Two-electrode voltage-clamp recordings were performed at room temperature. Whole-cell currents were recorded 1–4 days after injection. **Results**: Erythravine, 11-α-hydroxyerythravine and erythrartine did not produce modifications in the activation and/or inactivation of Na_v and K_v channels, even at the highest concentration of 10 μM. Among the tested alkaloids, only erythravine was able to inhibit the α7, α4β2 and α1β1δε nicotinic receptors, however, at different levels of specificity. Conclusion: The results suggest that small differences in the chemical structures of erythrinian alkaloids, especially for type and position of chemical radicals along A and C rings, produce differences in the affinity of tested alkaloids for α7, α4β2 and α1β1δε nicotinic receptors, at least considering the tested range of concentrations. Indeed, those differences previously have proven to influence the anticonvulsant and anxiolytic activities of tested alkaloids in *in vivo* experiments[a,b]. At least partially, the inhibition of nicotinic receptors caused by erythravine could explain the anxiolytic properties of this alkaloid as well as of *E. mulungu* flowerings. References: a. Faggion, S.A., et al. Epilepsy & Behav. v.20, p. 441, 2011; b. Santos, D.R., et al. Epilepsy Behav. v.23, p. 205, 2012. **Acknowledgements**: E.G., A.P. and R.B. are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Universidade de Ribeirão Preto (UNAERP) for Financial Support.

12.002 Design, synthesis and characterization of novel analogs of Bradykinin. Rodriguez DY, Costa-Neto CM, Parreiras-e-Silva LT, Oliveira EB FMRP-USP – Bioquímica

Introduction: Bradykinin (BK) is a nonapeptide (RPPGFSPFR) generated by the partial hydrolysis of kininogens by plasma and tissue kallikreins. BK is a selective agonist for the B2 kinin receptor, which is a member of the G protein-coupled receptor (GPCR) family [1,2]. The B2 receptor is involved in many pathological and physiological processes, such as pain, inflammation, cardiovascular and renal diseases [2, 3-5]. Considering that the modifications in the C-terminus of BK can affect the functional responses, we designed and synthesized a series of BK analogs to characterize and study their pharmacological properties. **Methods**: The peptides were synthesized by solid phase synthesis [6] using the F-moc strategy [7] and were purified by HPLC using a C-8 reverse phase column. Competitive binding assays were performed using ^{3}H-BK for 16h at 4ºC as previously reported [8]. The Bioluminescence Resonance Energy Transfer (BRET) assays to evaluate G protein activation and β-arrestins recruitment were performed with cells in suspension at 37ºC[9]. The BRET signals were determined as the ratio of the light emitted by acceptors over donors. **Results**: Seven novel BK analogs were synthesized, named BK01 to BK07. Analogs BK01 and BK02 showed a balanced pharmacological profile concerning to G protein and β-arrestin activation pathways. Analogs BK03 and BK04 displayed a biased profile towards Gq protein activation, while analogs BK05 and BK06 showed biased profile towards β-arrestin 2 recruitment. Analog BK07, which presented only one amino acid difference than analog BK04, showed an antagonistic behavior. **Conclusions**: It was observed that modifications in positions 5, 7 and 8 of the BK sequence could significantly modify the behavior of peptide agonists in B2 receptor-dependent signaling pathways. The synthesized analogs were be categorized in 4 groups: balanced agonists, G protein-biased agonists, β-arrestin biased agonists, and antagonists. We are currently planning ex vivo and in vivo studies with these analogs to better evaluate their full functional responses and possible applications. **References**: 1. D. J. Campbell, Clin Exp Pharmacol P, (28), 1060, 2001 2. L. M. Leeb-Lundberg, Pharmacol Rev, (57), 27, 2005 3. F. Marceau, Pharmacol Rev, (50), 357, 1998 4. F. J. Haddy, Handbook Exp Pharmac (25), 362, 1980 5. P. Needleman, Proc Nat Aca Sci, (72), 2063, 1975 6. R. B. Merrifield, J. Am. Chem. Soc., (85), 2149, 1963 7. G. B. Fields, Int. J. Pept. Protein Res., (35), 161, 1990; 7. R. I. Reis, Regul. Peptides, (140), 32, 2007 G.A. Santos, Front. Pharmacol., (6), 131, 2015 **Financial support** and acknowledgments: This study was supported by FAPESP (Sao Paulo State Research Foundation grant 2012/20148-0), CNPq (Brazilian National Research Council), and CAPES (Coordination for the Improvement of Higher Education Personnel).

12.003 Computational modeling approach of polymeric nanoparticles as platelet antiaggregants carriers. Matus MF[1], Palomo I[1], Vilos C[2] [1]University of Talca – Laboratory of Hematology and Immunology, Department of Clinical Biochemistry and Immunohematology, Faculty of Health Sciences, [2]University Andres Bello – Laboratory of Nanomedicine and Targeted Delivery, CIMIS-Faculty of Medicine, CBIB-Faculty of Biological Sciences

Introduction: In recent years, the use of drug delivery systems based on polymeric nanoparticles has generated innovative therapeutic strategies for infection and immune diseases, as well as cancer therapy[1,2]. Polylactic acid (PLA) is one of the most commonly used polymers for the synthesis of nanoparticles[3], PLA nanoparticles conjugated with hydrophilic molecules like polyethyleneglycol (PEG) presents improved blood circulation, clearance, biocompatibility, and less cytotoxicity[4]. The current high prevalence of cardiovascular diseases (CVD) and the vast application of PEGilated nanoparticles propose an excellent opportunity to develop novel therapeutic approaches for CVD. In this work, we developed a computational plan to understanding the structural and physiochemical properties that establish the association of cilostazol and adenosine 5'-monophosphate (AMP), both antiaggregant compounds, loaded into PLA nanoparticles as novel nanosystem for CVD. **Methods:** A combination of Molecular Dynamics (MD) simulations and Docking techniques were employed to model and predict nanoparticle-drug interactions. All-atom models of PLA nanoparticles were pre-optimized before running MD simulations using the ReaxFF (reactive force field)[5] with LAMMPS software[6]. The 3D structures of the drugs were sketched with Maestro software (Maestro, Version 9.0, Schrödinger, LLC, New York, NY, 2007). Blind Docking was performed using AutoDock4[7] and AutoDock Vina[8] software. The calculated binding energies of polymer-drug complexes were correlated with maximum drug loading determined experimentally. **Results:** Cilostazol structure allows a better alignment with the PLA unit than AMP for all docking calculations. AutoDock Vina predicted the strongest drug-polymer affinity in all cases, compared with AutoDock4. Cilostazol presented the highest affinity to PLA core, which was consistent with logP values. Cilostazol is the most hydrophobic drug, then the affinity with the PLA could be expected higher. Further, a simple correlation between binding energy and experimentally determined drug loading is not sufficient to rank drugs, but a more exhaustive analysis is required, such as physical interactions and the orientation of drugs in the core cavities. **Conclusion**: The structural characterization in silico of polymers-drugs provides a comprehensive understanding of the factors that contribute to nanoparticle formation and drug loading of nanocarriers based on polymeric nanoparticles. This approach represents an innovative strategy to evaluate the drug encapsulation of several antiplatelet drugs into PLA nanoparticles. References 1. Bae, Y., Adv. Drug Deliv. Rev., 61, 768, 2009. 2. Avgoustakis, K., Curr. Drug Deliv., 1, 321, 2004. 3. Xiao, R. Z., Int. J. Nanomedicine, 5, 1057, 2010. 4. Gref, R., Protein Delivery, 167, 2002. 5. Aktulga, H. M., Parallel Comput., 38, 245, 2012. 6. Plimpton, S., J. Comput. Phys., 117, 1, 1995. 7. Morris, G. M., J. Comput. Chem., 19, 1639, 1998. 8. Trott, O., J. Comput. Chem., 31, 455, 2010. **Financial support**: This study was supported by CONICYT-PCHA/Doctorado Nacional/2014-21140225.

12.004 Comparison of LDT5, a multi-target lead compound for the treatment of benign prostatic hyperplasia, and tamsulosin binding at the D_3 and 5-HT_{1A} receptors. Quaresma BMCS[1,2], Figueiredo CDM[1], Silva ACS[3], Romeiro LAS[4], Silva CLM[1], Noël F[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UFRJ – Farmacologia e Química Medicinal, [3]IFRJ – Farmacologia Bioquímica e Molecular, [4]UnB – Ciências Farmacêuticas

Introduction: Benign prostatic hyperplasia (BPH), characterized by the progressive increase in prostate volume, is the major cause of lower urinary tract symptoms (LUTS) in the elderly. Current treatment is mainly based on monotherapy with alpha1A adrenoceptor antagonists such as tamsulosin. The ejaculation problems reported with this drug could be due to a high affinity for the D3 and 5-HT1A receptors involved in the central control of ejaculation, as reported by some authors. We recently reported that LDT5 is a multitarget lead compound designed for reduction of prostate contraction through alpha1A adrenoceptor antagonism and prostate enlargement through blockage of alpha1D and 5-HT1A receptors (Nascimento-Viana et al. J Pharmacol Exp Ther 356:212, 2016). We also reported that LDT5, but not tamsulosin, was able to block the phenylephrine- and 5-HT-induced proliferation of BPH cells in culture. The objective of this work was to test the affinity of LDT5 at the D3 receptor and to check the affinity of tamsulosin and of other four alpha1A blockers approved for BPH treatment at the 5-HT1A receptor. **Methods:** For the dopaminergic receptors, we used a membrane preparation from rat striatum (D2 receptor) or cells transfected with the human D3 receptor in competitive binding assays with [^{3}H]-YM-09151-2 as the radioligand. For the 5-HT1A receptors, we used a membrane preparation from rat hippocampus either with an agonist ([^{3}H]-8-OHDPAT) or an antagonist ([^{3}H]-p-MPPF) radioligand for determination of affinity and intrinsic activity through the Ki ratio method (Noël et al., J. Pharmacol Toxicol **Methods** 70:12, 2014). **Results:** LDT5 and tamsulosin have similar affinities for the D3 receptor (Ki=30.7 and 15.7 nM, respectively) and for the D2 receptor (Ki=85 and 110 nM, respectively). These values are at least 170 times higher than the Ki values for the α1A adrenoceptor (Ki=0.18 and 0.08 nM, for LDT5 and tamsulosin, respectively). LDT5, tamsulosin (Nascimento-Viana et al. J Pharmacol Exp Ther 356:212, 2016) and silodosin bind with high affinity to the 5-HT1A receptors (Ki =4.9, 2.3 and 5.2 nM, respectively), i.e. with Ki values only 10-30 times higher than their Ki´s for binding to the alpha1A adrenoceptors. LDT5 and silodosin appear as 5-HT1A receptor antagonists (Ki ratio ~1) whereas tamsulosin would be a very weak partial agonist (Ki ratio ~5). **Conclusion:** 1. LDT5 shares with tamsulosin a relative selectivity towards the main target for the BPH treatment (α1A adrenoceptor) when compared to the off-target D2 and D3 receptors. As a consequence, we cannot predict an improved safety profile for LDT5 with respect to ejaculation problems, at least based on the hypothesis involving the D3 receptors. 2. LDT5 and tamsulosin bind with similar high affinity to the 5-HT1A receptors and are expected to behave as antagonist (LDT5) or very weak partial agonist (tamsulosin). Current findings do not explain the observed difference between LDT5 and tamsulosin for blocking cellular proliferation induced by 5-HT. **Financial support**: CNPq, CAPES; **Ethical Committee**: Process DFBCICB021

12.005 Synthesis and pharmacological screening of pyridopyrimidines as new effective inhibitors of cyclic nucleotide synthesis. Zaminelli T, De Nucci G FCM-Unicamp – Farmacologia

The increased levels of cyclic nucleotides (cGMP and cAMP) in enterocytes trigger intracellular mechanisms of ion and fluid secretion into the lumen causing secretory diarrhea. Twelve novel pyridopyrimidines derived from 5-(3-fluoro-5-trifluoromethylphenyl)-1,3-dimethyl-5,11-dihydro-1H-indeno[2,1:5,6]pyrido[2,3-d]pyrimidine-2,4,6-trione were synthesized and evaluated on intracellular cyclic nucleotides accumulation. All the compounds inhibited the accumulation of cGMP (17-94%) stimulated by STa toxin in T84 cells and cAMP (35-89%) in Jurkat cells stimulated by forskolin, none of the compounds induced changes on the cyclic nucleotides basal levels. The compounds exhibited no effect on vascular tonus of pre-contracted aortic rings when compared to amlodipine. The metabolic activity and viability were not affected in T84 cells when evaluated by MTT and LDH assay respectively. Thus, these novel pyridopyrimidine derivatives represent promising tools for treatment of diarrhea and other diseases. **Keywords:** Pyridopyrimidines, Diarrhea, Cyclic Nucleotides. **Acknowledgment.** Tiago Zaminelli is thankful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support (process 2013/15525-1).

12.006 New family of antibacterials, ubiquinone analogues, with activity against clinical isolate of *Staphylococcus aureus* **and** *Enterococcus* **spp. multiresistant** Campanini-Salinas J[1], Andrades-Lagos J[1], Hinojosa N[1], Alarcon P[2], Gonzalez-Rocha G[3], Vásquez-Velásquez D[4] [1]Universidad de Chile – Laboratorio de Desarrollo de Fármacos, Facultad de Ciencias Químicas y Farmacéuticas, [2]Instituto de Salud Pública de Chile – Gram-Positive coccus Laboratory, [3]Universidad de Concepción – Laboratorio de investigación en agentes antibacterianos, Facultad de Ciencias Biológicas, [4]Universidad de Chile – Laboratorio de Desarrollo de Fármacos, Facultad de Ciencias Químicas y Farmacéuticas

Introduction: Our laboratory has worked on the development of rationally designed pyrimidoisoquinolinequinones compounds from the structure ubiquinone, with the aim to interfere in bacterial energy metabolism, specifically in electron transport chain between I - III y II - III complexes. Previous results have shown that the compounds obtained have an interesting profile of activity against gram-positive bacteria of clinical importance. The series of synthesized compounds, highlighted 2 in particular: DFUCh-O5 and DFUCh-P4. **Methods:** For potential therapeutic application, MIC_{90} [1] and MBC_{90} [2] was determinated according to standards of CLSI, in a heterogeneous bacterial population, composed of clinical isolates, *Staphylococcus aureus* and *Enterococcus* spp. Multi-resistants, isolated from sterile sites (blood, bone, etc.) and different hospital sources. The toxicity of the compounds on human and murine cells was evaluated by MTT method and activity of the compounds in combination with linezolid, vancomycin and daptomycin, determining the index of FIC (Fractional Inhibitory Concentration). Finally, it was evaluated the presence of post antibiotic effect [3] and efectiveness *in vivo* it was studied, using a model infection in *Galleria mellonella* larvae. **Results**: MIC_{90} values for DFUCh-O5 and DFUCh-P4 are 2 and 4 *u*g/mL for multi-resistants *S Aureus* and 4 and 4 *u*g/mL for multi-resistants *Enterococcus* spp. MBC_{90} values for DFUCh-O5 and DFUCh-P4 are 4 and 4 *u*g/mL for multi-resistants *S Aureus* and 4 and 8 *u*g/mL for multi-resistants *Enterococcus* spp. The viability of the cell lines tested at concentrations around the MIC of each compound was not affected. Additive effects of the antibacterial activity were observed in association of derivates with Linezolid. No post antibiotic effect was observed. The results of model infection in G*alleria mellonella* larvae will shown in the congress. **Conclusion:** DFUCh-O5 and DFUCh-P4 are good candidates for further development as antibacterial agents. [1] *CLSI*. **Methods** for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically Approved Standard, CLSI Document M7-A9, ninth edition. (2012), ISBN1-56238-783-9. [2] RD, Steigbigel RT, Davis HT, Chapman SW: Method of reliable determination of minimal lethal antibiotic concentrations. Antimicrobial Agents and Chemotherapy 1980; 18(5):699-708 [3] Craig WA GS: Antibiotics in laboratory medicine: Postantibiotic effect. , 4th ed edn. Baltimore: Williams & Wilkins; 1996 **Acknowledgements:** J. Campanini and J. Andrades thank CONICYT for financial support “Beca Doctorado Nacional grant 21130643 and 21130628”, respectively. H.Pessoa thanks FONDECYT grant 1130347. Technology under patent application **Nº 3780-2015.**INAPI.Chile.

12.007 Synthesis, antibacterial activity and structure-activity relationship study of functional analogues of ubiquinone. Andrades J[1], Campanini J[1], Poblete F[1], Gutierrez C[1], Pessoa H[2], Vásquez D[1] [1]Universidad de Chile – Drug Development Laboratory, Faculty of Chemical and Pharmaceutical Sciences, [2]Universidad de Chile – Reaction Mechanisms Laboratory, Faculty of Chemical and Pharmaceutical Sciences

Introduction: Antimicrobial resistance is a growing problem affecting the effective treatment of infections caused by bacteria, reducing the efficacy of antibacterial agents increasing the morbidity and mortality of patients [1]. Thus, there is an important demand for the discovery and development of new classes of antibiotics with novel chemical structure to add to our current arsenal in order to help for overcoming drug resistance and improving the antimicrobial potency [2]. Besides, structure-activity relationship studies of new agents are an important tool for the development and choice of leader compounds. Consequently, an interesting target is ubiquinone. This performs functions in bacteria such as: the transfer of electrons in the electron transport chain, the regulation of reactive oxygen species generation and virulence factors and resistance. This multifunctionality makes to ubiquinone an attractive target because its intervention with ubiquinone analogues could be alter multiple metabolic processes, triggering irreversible events that will lead to bacterial death. **Methods**: First, the core like ubiquinone were synthesized from commercially available precursors, using one-pot synthesis [3]. Secondly, thiophenol and aniline derivatives were added to ubiquinone core through Michael additions. Its purification was performed by column chromatography with silice gel and the structures were confirmed by RMN spectral. The minimum inhibitory concentration (MIC) values of the tested compounds were performed by microbroth dilution method, according to CLSI protocols, against methicillin-resistant Staphylococcus aureus ATCC 43300, methicillin-sensitive Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Pseudomonas aeruginosa ATCC 27853 and Eschericha coli ATCC 25922 [4]. **Results**: The analogues of ubiquinone exhibit antibacterial activity with MICs values between 32 µg/mL and 0.5 µg/mL in Gram positive strains. No activity was observed for Gram negative strains evaluated at the concentrations tested. The structure-activity relationship indicate:

- Substituents hydrophobic improve the antibacterial activity.
- A lipophilic chain in analogues of ubiquinone core improve the antibacterial activity.
- The addition of carbons between thiol and aromatic ring decreased the antibacterial activity.
- Double addition in the core like ubiquinone generate inactive compounds.
- Some aniline derivatives have the highest antibacterial activity.

Conclusion: our results indicate that new ubiquinone analogues display antibacterial activity against Gram positive strains and the knowledge about their structure-activity relationship will be a guide for the rational design and develop of new derivatives. 1.WHO: ANTIMICROBIAL RESISTANCE Global Report on surveillance [ONLINE]; 2014. 2.Kong, Bioor Med Chem 24, 1376, 2016. 3.Valderrama. Tetrahedron Lett. 49, 703, 2008. *4.CLSI*. CLSI Document M7-A9, ninth edition. 2012. **Acknowledgements**: J. Andrades and J. Campanini thank CONICYT for financial support “Beca Doctorado Nacional grant 21130628 and 21130643”, respectively. H.Pessoa thanks FONDECYT grant 1130347. Technology under patent application Nº 3780-2015.

12.008 Development and validation of analytical method by HPLC for determination of caspofungin in formulations. de Paula DCC, Garcia GM, Lima MSR, Silva JES, Leite EA, Grabe-Guimarães A

Introduction: The incidence of fungal infections has increased considerably. The resistance to conventional antifungal agents and their toxicity are some factors that suggest the development of new formulations to treat systemic fungal infections. The determination of the concentration of the drug during the development of new formulations is an essential feature. The objective of this study was to develop and validate an analytical method using high performance liquid chromatography with fluorescence detection (HPLC-FLU) to quantify in formulations. **Methods:** All solvents used were HPLC grade and were purchased from Tedia (Brazil) and caspofungin from Sigma-Aldrich (Brazil). The HPLC system employed consisted of a Water Alliance e2695 separation module with FLU detection (Waters 2475), using a Waters C18-RP column (250 mm × 4.6 mm) protected by a security guard C18 column (2 mm × 4.6 mm, 5 µm). The gradient mobile phase was consisted of acetonitrile (A) and water (B). The samples were eluted with 25 % of A during 1 min, increasing to 55 % during 1 min and returning to 25 % in 0.5 min. The solvents were pumped at 1 ml/min. The analysis was performed at 35ºC and the volume injection was 50 µl. The column eluent was monitored at excitation 224 nm and emission of 304 nm. **Results:** It was evaluated limit of detection (LOD) and limit of quantification (LOQ), linearity, accuracy and precision, and all parameters were in accordance with International Conference on Harmonization (ICH) guidelines (ICH, 2005, 1–13). The linearity was over 20-150 µg/ml, and the slopes, intercepts and the coefficients of determination were found to be 165297 (a), 9215603 (b), 0.9985 (r^2), respectively. The LOD was 10 µg/ml and the LOQ was 20 µg/ml. Caspofungin was detected with retention time of 5 min, approximately. Different methods reported in the literature were tested using acid acetonitrile or methanol, but only the condition described herein was efficient to quantify caspofungin using a reverse phase C18 column. **Conclusion**: A linear, accurate, and precise gradient reverse-phase HPLC-FLU method was described for the determination of caspofungin. This new method will be applied to quantify the caspofungin in new formulations. **Acknowledgments:** This work was funded by Rede Mineira de Nanobiotecnologia-FAPEMIG and UFOP.

12.009 Studies on the bioadhesion and safety of a nanocarrier for intraductal delivery of drugs for chemoprevention and treatment of breast cancer. Migotto A, Carvalho VFM, Lemos DP, Depieri LV, Bentley MVLB, Lopes LB

Introduction: Considering that the majority of breast cancer begins in the lining of the ducts, providing local intervention into the ducts could target potential cancer cells locally while reducing adverse effects resulting from systemic drug exposure. In the face of these facts, this study aims at assessing the safety and bioadhesive properties of novelsurface-modifiednanocarriers for increased retention in the ducts. **Methods**: Nanoemulsions were prepared by probe sonicating the surfactant polysorbate 80, with the oil and aqueous phases. The nanodroplets surface was modified with chitosan, and subsequently subjected to characterization of size, zeta potential and rheological properties. The bioadhesionproperties of the nanoemulsion were studied by assessing changes on size and zeta potential after incubation with a mucindispersion for 30 min under stirring (150 rpm) at 37□C. To study the relative formulation safety, the viability of 3T3 fibroblasts plated in 96-well plates (6,000 cells/well) and treated for 12 h with the nanoemulsion, PBS (negative control) or sodium lauryl sulfate (SLS, a moderate-to-severe irritant) at 1-500 µg/mL was assessed by MTS. **Results**: Surface modification of the nanoemulsion with chitosan increased the droplet size from 33.2□0.8 to 46.3□0.7 nm, imparted a positive charge in the system (zeta potential increased from -2.9 to +13.6 mV), and promoted a 10-fold increase in the dispersion viscosity, although it did not modify the rheological behavior (Newtonian). Incubation of the chitosan-modified nanoemulsion with mucinincreased the size from 47.7□0.8 to 58.7□5.4 and inverted the zeta potential (from +33.2 to -30 and -12.7 mV), indicating an interaction between the nanocarrier and mucin. Cell viability was 1.6-2.2-fold higher after treatment with the nanoemulsion compared to SLS up to 100 µg/mL, demonstrating that the formulation is less cytotoxic and safer. **Conclusions**: Taken together, these results suggest that chitosan-modified nanoemulsion displayedsuitable size, charge and rheological properties for injection. Interaction with mucincould potentiallyfavor adhesion of the nanodropletson mammary ducts and improve local retention. These properties, combined with the higher safety of the nanoemulsion compared to the moderate-to-severe irritant SLS at the same concentrations, suggest the potential usefulness of the formulation for intraductal drug delivery. Acknowledgements: This study was supported by FAPESP (grant#2013/16617-7). A. Migotto is the recipient of a FAPESP fellowship (2015/23976-9).

12.010 *In silico* **study of biological activity and lipophilicity for quinazolines proposed as EGFR inhibitors.** Fernandes GS, Pereira BMP, Antunes JE UFJF

Introduction: *In silico* pharmacokinetics studies can aid the search for molecules with potential ability to be drug candidates. The main physicochemical property of a molecule capable of changing its pharmacotherapeutic profile includes the partition coefficient, which expresses the relative lipophilicity of the molecule[1, 2]. Numerous studies of structure-activity relationships involving many series of quinazoline derivatives have led to advances in power, specificity and the pharmacokinetics properties of these inhibitors.[3] For instance, three drugs have been approved and have been marketed for the treatment of lung cancer cells[4]. In addition, several reversible and irreversible inhibitors of epidermal growth factor receptor inhibitors (EGFR) tyrosine kinase are currently being investigated[5]. The literature described that 69 quinazoline molecules were synthesized and the respective half maximum inhibitory concentrations (IC_{50}) were obtained[6]. In this study, through *in silico* pharmacokinetics studies, quinazoline candidates for EGFR were computationally analyzed to contribute to the prediction of new drug candidates. **Methods**: A bilinear parabolic model was built to investigate the druglikeness by correlating the corresponding lipophilicities (*Log P)*, with the optimal biological activity in terms of *pIC*50 values. Structural characteristics leading to improved pharmacokinetics parameters were then analyzed. **Results**: In the literature, compound 56 exhibited the lowest *IC*50 and, therefore, it had the highest ability to inhibitthe EGFR. In the present work, the most potent inhibitor 56 is not calculated to be the most promising drug candidate, since it is out of the parabolic model obtained due to a *Log P* above 5, which is not within the expected optimum range. **Conclusion**: This work is an example of computational prediction that an experimentally, highly active EGFR inhibitor can be unsuccessful as drug candidate because of pitfalls in pharmacokinetics parameters[7]. Therefore, computational methods and the rational design of new drugs provide useful tools for synthesis of promising drug candidates, thus saving time and costs during drug development. **References**: [1] Thomas, G. (2012) *Química Medicinal: uma introdução*. Guanabara Koogan, São Paulo. [2] Barreiro, E.J. (2008) *As Bases Moleculares da Ação dos Fármacos*. 2th Edition, C.A.M, Porto Alegre. [3] Levitzki, A. (2003). *Accounts of Chemical Research*, 36, 462-469. [4] Bonomi, P. (2003) *Investigational Drugs*, 12, 1395-1401. [5] Rahman, M.U., Rathore, A., Siddiqui, A.A., Parveen, G. and Yar, M.S. (2014). *Journal of Enzyme Inhibitionand Medicinal Chemistry*, 29, 733-743. [6]Bridges, A.J., Zhou, H., Cody, D.R., Rewcastle, G.W., McMichael, A., Showalter, H.D., Fry, D.W., Kraker, A.J. andDenny, W.A.T. (1996). *Journal of Medicinal Chemistry*, 39, 267-276.[7] Fernades, G.S., Pereira, M.B.M., Antunes, J.E. *Open Jour. Med. Chem.* (2015), 5, 106-115. **Acknowledgements:** The authors are thankful to Program to Support Publishing (PROPESQ) of Federal University of Juiz de Fora - UFJF and FAPEMIG. **Financial Support:** 1. PROPESQ of Federal University of Juiz de Fora – UFJF; 2. FAPEMIG. RESEARCH APPROVAL BY THE HAREC? No.

12.011 Evaluation of Neuroprotective Effect of a New Anticholinesterasic Drug in Mice Submitted to Intra-hippocampal Injection of Amyloid-B 1-40 Oliveira LR, Bellozi PMQ, Junior WOC, Campos AC, Machado RP, Viegas C, Oliveira ACP

Introduction: Alzheimer's disease (AD) is the most common neurodegenerative disease nowadays, with its prevalence increasing due to increased human life span. The main hallmark of AD is memory impairment, often accompanied by disorientation and loss of cognitive and intellectual functions. Important molecular features of AD, that trigger the disease changes, are accumulation of amyloid-β peptide and hyperphosphorylation of tau protein. These characteristics lead to neuroinflammation and neuronal death, especially of cholinergic neurons. However, the initial cause of the disease is still controversial and the available treatments are not able to reverse the condition or prevent its progress. The main pharmacological treatment for AD consists of the anticholinesterasic drugs rivastigmine, donepezil and galantamine. Thus, the aim of this study was to investigate the effects of the new anticholinesterasic drug PQM67, developed at the Federal University of Alfenas, Brazil. **Methods:** The compound was administered intraperitoneally in C57Bl/6 mice, 10-12 weeks old. Sixty minutes after the injection, animals underwent stereotaxic surgery for the administration of 400 pmol of amyloid-β 1-40 or PBS, in a volume of 0,5 µL, through intrahippocampal injection (Coordinates AP = -1.9 mm; LL = -1.5 mm; DV = -2.3 mm, relative to bregma, according to Paxinos atlas). Seven days after the surgery, animals were submitted to Object Recognition Task (ORT) followed by intracardiac perfusion to obtain hippocampal sections. Animals were divided into groups (1) PBS + vehicle; (2) amyloid-β 1-40 + vehicle; (3) amyloid-β 1-40 + PQM67 17.77 mg/ Kg; (4) amyloid-β 1-40 + PQM67 35.54 mg/ Kg. The hippocampal sections were stained with Iba-1 for detecting microgliosis, which was evaluated by fluorescence intensity in pixels/ μm^2. Data were analyzed by one-way ANOVA followed by Newman-Keuls post-hoc test. **Results:** In the ORT test, the amyloid-β 1-40 + vehicle group showed a decrease in recognition of the new object when compared to the PBS + vehicle group. However, PQM67 (35.54 mg/ Kg) prevented the cognitive impairment induced by amyloid-β 1-40, although a lower dose (17.77 mg/ Kg) did not prevent the effect. In CA1 region of hippocampus, it was observed an increased microgliosis induced by amyloid-β, which was prevented by PQM67. In both CA3 and dentate gyrus regions, there was a tendency towards an increase in microgliosis induced by the peptide, and the compound could reduce this tendency in CA3. **Conclusions:** The compound PQM67 demonstrated an ability to reverse the cognitive impairment generated by amyloid-β 1-40 peptide, in the ORT, and prevented microgliosis induced by the peptide. Thus, further studies should be done in order to investigate its mechanisms and if it has a therapeutic potential. **Acknowledgments:** FAPEMIG (Protocol numbers PPM-00372-13 and CEX-PPM-00241-15), and CNPq (Protocol numbers 479254/2013-3, 454088/2014-0) for financial support and fellowships. **Approval:** All procedures were approved by Institutional Ethics Committee protocol nº 39/2012.

12.012 Favorable toxicological profile for a novel series of anti-tuberculosis quinoloxyacetamide-based compounds. Danesi GM[1,2], Sperotto ND[3,4], Erig TC[5], Machado P[4], Pissinati K[4], Campos MM[6,2], Basso LA[4], Rodrigues-Junior V[4], Santiago DS[4] [1]PUCRS – Faculdade de Medicina, [2]INTOX-PUCRS, [3]INCT-TB-CPBMF- PUCRS), [4]INCT-TB-CPBMF-PUCRS, [5]INTOX-PUCRS, [6]PUCRS – Odontologia

Tuberculosis (TB) is a multisystem infectious disease caused by *Mycobacterium tuberculosis (Mtb),* affecting the lungs and other organs. It has a great impact in public health systems, especially in developing countries, such as Brazil (World Health Association, Fact Sheet n°104, 2016). The managing of this disease is based on a combination of different drugs, requiring long periods of treatment, being associated to many adverse effects. These facts importantly contribute to the patient's non-compliance to the treatment (C. Robert Horsburgh, N Engl J Med, 373, 2149, 2015). Moreover, data has shown increasing rates of bacterial strains resistance to the main drugs used to treat TB, such as rifampicin and isoniazid (World Health Association, Fact Sheet n°104, 2016). Thus, there is a need to develop new drugs to efficiently treat TB. In order to develop new drugs, different aspects should be considered such as toxicological studies, which are essential in the pre-clinical phases of drug discovery process (J.P. Hughes1, Brit Jour Pharm, 162, 1239, 2011). Our research group recently evaluated the efficacy of a series of quinoloxyacetamide-based compounds on M*tb*-infected macrophages, with minimal inhibitory concentration (MIC) values ranging from 0.05 to 0.2 µM (Pissinati et al., ACS Med Chem Lett, 7, 235, 2016). The present study was aimed to assess the *in vitro* toxicity of these molecules in different cell lines. For this purpose, we used the following cell lines: HaCat (human keratinocyte cells), Vero (kidney epithelial cells derived from African Green Monkey), and RAW 264.7 (murine macrophage-like cells). The cells were maintained at 37°C, in an atmosphere of 5% CO_2, in *Dulbecco's Modified Eagle's Medium* (DMEM), supplemented with 10% of Fetal Bovine Serum (FBS). The cells were treated for 72 h with eleven different compounds at the concentration of 10 µM (10-fold higher than MIC values), diluted in 2% dimethylsulfoxide (DMSO). The cell viability was evaluated by using the MTT assay. The experiments were performed in triplicates, and the tests were repeated at least three times for each cell line. The results indicate that, after 72 h of treatment with the different compounds, the cellular viability ranged from 86.2 to 106.7%, for HaCat cell line; 69.8 to 96.9%, for Vero cell line; and 82.9 to 113.2%, for RAW 264.7 cell line. The results were calculated considering the DMSO-treated cells as 100 % of cell viability. Our study revealed a satisfactory profile of safety for all the tested compounds. None of the molecules displayed reductions of cell viability higher than 50%, suggesting that IC_{50} (i.e. the concentration required to inhibit 50% of cell viability) is higher than 10 µM. It is tempting to suggest that such substances are prominent candidates for the development of new anti-TB drugs. **Financial Support**: CNPq, CAPES

12.013 Leishmanicidal activity of new 2-N,N'-dialkylamino-1,4-naphthoquinone derivatives. Silva KCJ[1], Santos JM[1], Araujo MV[1], David CC[2], Oliveira LAPL[1], Silva TMS[2], Camara CA[2], Moreira MSA[1] [1]UFAL- Ciências Biológicas e da Saúde, [2]UFRPE

Introduction: Leishmaniasis is an anthropozoonosis considered a public health problem. Currently, the available therapeutic arsenal for this disease is limited. It also presents high toxicity and acquired resistance by the parasite that leads to an increase in treatment failure, pointing to the needing of the development of new leishmanicidal drugs. **Methods**: Two Leishmania strains were used in the present study: *Leishmania amazonensis* and *Leishmania chagasi*. The compounds toxicity to host cells (peritoneal macrophages) (protocol no. 2015.01) and to promastigote forms was evaluated through MTT assay. Furthermore, the leishmanicidal activity was determined by evaluating both the infected macrophage number as well as the amastigote number in an amount of 100 macrophages amount. **Results:** The results revealed that only the compounds 1d, 1h, 1i,1k, and pentamidine showed toxic effects to host cells. Other compounds showed no deleterious effect to the host cell. At the evaluation of the direct activity on *L. amazonensis* promastigotes, he compounds 1d, 1h, and 1k, which showed maximal effect (ME) against *L. amazonensis* promastigotes exceeding 50%, with ME of 51 ± 0.1%, 73.8 ± 7.3%, and 67.6 ± 7.9%, and IC_{50} of 97.7 ± 0.3 µM, 40.2 ± 6.8 µM, and 46.5 ± 9.0 µM, respectively. The leishmanicidal activity against *L. chagasi* promastigotes evidentiated that derivatives 1d, 1e, 1f, 1h, 1k, and 1n had significant activity with ME of 94.6 ± 6.3%, 89.7 ± 4.9%, 72.7 ± 5.0%, 95.9 ± 5.8%, 79.5 ± 0.8%, and 74.7 ± 2.9%, and IC_{50} of 28.3 ± 1.4 µM, 44.5 ± 2.5 µM, 65.8 ± 3.0 µM, 25.7 ± 3.8 µM, 39 ± 8.0 µM, and 55.0 ± 3.5 µM. Based on all these results, it was calculated the selectivity index (SI) of compounds. Thus, when comparing the SI, the compounds did not show selectivity for *L. amazonensis.* However, pentamidine showed selectivity for *L. chagasi* 13 times more active and compounds 1e, 1f, and 1n are probably selective for *L. chagasi*. The results of the evaluation of leishmanicidal activity against intracellular forms of *L. chagasi* revealed that 1a, 1b, 1c, 1d, 1h, 1i, 1k, and 1m presented statistically activity, with ME of 87.7 ± 1.8%, 66.9 ± 1.2%, 92.5 ± 0.5%, 100 ± 0.0%, 100 ± 0.0%, 100 ± 0.0, 100% ± 0.0, and 71.8 ± 5.2% and IC_{50} of 26.5 ± 1.5 µM, 49.3 ± 10.8 µM, 57.3 ± 7.3 µM, 38.7 ± 3.5 µM, 51 ± 0.3 µM, 19 ± 6.4 µM, 43.5 ± 3.0 µM, and 6.5 ± 0.5 µM, respectively. **Conclusion:** Treatment with derivatives 1d, 1h, and 1k had pronounced leishmanicidal activity against *L. amazonensis* promastigotes, and treatment with 1d, 1e, 1f, 1h, 1k, and 1n had significant leishmanicidal activity against *L.chagasi* promastigotes. In the *L. chagasi* amastigotes, the derivatives 1a, 1b, 1c, and 1m showed significant activity, without deleterious effects to the host cell. These data show the derivatives as promising compounds for designing new prototypes of antileishmanial drugs. **Financial support :** UFAL, FAPEAL, CNPq e CAPES.

12.014 Acetylcholinesterase inhibition and anti-amnesic effects of new dual compounds candidates for Alzheimer's disease treatment. Souza INO[1], dos Santos FP[1], da Silva FMR[1], Viegas Junior C[2], Castro NG[1], Neves G[1] [1]UFRJ, [2]Unifal

Alzheimer's disease (AD) is a neurodegenerative condition characterized by a progressive and severe compromise of cognition. It is considered the most common type of dementia and affects about 50-60% of the world elderly population. Since it's a disabling disease, it presents strong socioeconomic consequences. Acetylcholinesterase inhibitors (such as donepezil) enhance the central cholinergic function therefore being the most used pharmacological strategy for minimizing AD symptoms. However, these drugs have considerable side effects making it difficult to scale up the dosage or to achieve appropriate drug compliance. Therefore, the search for new, more selective and favorable substances for AD treatment is justified. Among the compounds under study, curcumin has shown promising anti-inflammatory, antioxidant and neuroprotective properties. With such objective in mind, a series of innovative compounds with potential acetylcholinesterase inhibitory activity, anti-inflammatory and neuroprotective effects was planned through molecular hybridization of donepezil's pharmacofore and the curcumin molecule. This rational approach aimed to design new compounds able to simultaneously induce the symptomatic relief of cholinergic therapy and suppress the chronic neuroinflammation present in AD, possibly modifying the neurodegenerative process. Three novel compounds were synthesized, characterized and purified. The main objective of this work was to characterize the cholinergic actions of said compounds using *in vitro* and *in vivo* assays. First, the cholinesterase inhibitory activity of the compounds were assessed *in vitro* as well as PQM-130's mechanism of action using variants of the Ellman's assay. Then, PQM-130's ability to block scopolamine-induced cholinergic amnesia (1 mg/kg i.p.) in the Y maze paradigm was investigated. Adult male Swiss mice (CECAL/Fiocruz) were used. The compound was administered at 100 or 30 □mol/kg *per os.* PQM-130 presented a half maximal inhibitory concentration (IC_{50}) of 440 nM for acetylcholinesterase, PQM-131, 54.72 µM and PQM-132, 44.71 µM. It was characterized as a non-competitive inhibitor of acetylcholinesterase. In mice, PQM 130 wasn't able to block the cholinergic amnesia in the Y maze paradigm in the doses of 100 or 30 □mol/kg *per os*. It didn't induce any change in animals memory *per se* nor in the animals locomotion profile. PQM 130 showed a promissing in vitro profile with submicromolar IC_{50} and non-competitive inhibition. Additional experiments in different dosage regimens and paradigms, like the novel object recognition task, are underway in order to further characterize its *in vivo* neuropharmacological profile. We thank the financial support provided by CNPq, FAPERJ and CAPES. All animal use protocols were approved by the Comission for the Ethical Use of Animals (CEUA-UFRJ, protocol number DFBCICB053).

12.015 Multifunctional nanoemulsions improve cytotoxicity and skin co-localization of antitumor agents. Carvalho VFM[1], de Lemos DP[1], Zanoni TB[2], Maria-Engler SS[2], Costa-Lotufo LV[1], Lopes LB[1] [1]ICB-USP – Farmacologia, [2]FCF-USP – Análises Clínicas e Toxicológicas

Introduction: Considering the high incidence of skin cancer, and the unavailability of self-administered strategies capable of localizing drugs in the tumors while avoiding systemic exposure and adverse effects, we propose the topical use of multifunctional nanoemulsions to improve the cutaneous co-localization and cytotoxicity of the chemotherapeutic agents paclitaxel and C6 ceramide. **Methods:** Nanoemulsions (NE) were prepared by sonicating the surfactant (Tween 80), oil (tributyrin:oleic acid:miglyol) and aqueous phases, and the formulation was subsequently characterized for droplet size, zeta potential and rheological characteristics. To evaluate whether drugs co-encapsulation in the nanocarrier improved cytotoxicity, the viability of human melanoma cells (SK MEL 19) plated at 10.000 cell/well in 96 well plates was assessed using MTT after treatment with the unloaded NE, or NE containing ceramide, paclitaxel or both drugs for 24 h. The nanoemulsion safety was evaluated by assessing the viability and histological characteristics of reconstructed human epidermis after topical application in comparison to sodium lauryl sulfate (considered an irritant). Co-localization of the drugs in the skin layers was assessed *in vitro* in porcine skin using fluorescence microscopy after topical treatment for 8 h. **Results:** The NE displayed size of 55 □ 0,8 nm, slightly negative zeta potential (-6,12 □ 0,24 mV), and Newtonian behavior, with a viscosity value of 0.031 □0.05 mPa.s. These characteristics were not affected by drug incorporation. The concentrations of paclitaxel and C6 ceramide necessary to reduce cell viability to 50% decreased approximately 6- and 15-fold, respectively, after incorporation of each drug individually in the NE compared to solutions in DMSO. A further lowering of these concentrations by 4-fold was observed when the drugs were co-encapsulated in the nanoemulsion compared to their use separately, suggesting the potential benefit of the nanocarrier as well as drug combination to improve efficacy. The viability of reconstructed human epidermis was 91 and 28% after exposure to the NE and the positive control, respectively, suggesting that NE is safe for topical application. An increased localization of the drugs in the viable layers of epidermis was observed after topical administration of the NE compared to drug solutions. **Conclusions:** Our results support the benefit of the nanocarrier to improve the cytotoxicity of each drug individually against cancer cells compared to solutions. Co-encapsulation of the drugs in the nanocarrier further improved formulation cytotoxicity. These results, combined with the improved penetration of the drugs in the epidermis and NE safety, as demonstrated by the high viability of bioengineered skin after topical NE application, support the potential benefit of the selected strategy for topical treatment of skin tumors. **Acknowledgements:** This study was supported by FAPESP (grants 2013/16617-7 and 2014/24400-0). V. Carvalho received a CAPES Fellowship.

12.016 Swelling of microemulsions and *in vivo* transition into nanostructured gels for sustained drug release. Santos RA[1], Ribeiros PF[1], de Lemos DP[2], Steiner A[3] Lopes LR[1 1]ICB-USP – Farmacologia, [2]ICB-USP, [3]ICB-USP – Imunologia

Purpose: Significant efforts have been made to develop formulations capable of sustaining the release of drugs used in the treatment of cronic disease, but simple formulations that can be easily and self-administered, and cause minimal local reactions are still needed. This study focuses on the characterization of the swelling properties of fluid microemulsions that can swell and transition into nanostructured hexagonal phase gels upon water uptake in vivo as a platform for sustained drug release. **Methods**: Microemulsions were prepared using monoolein or vitamin E TPGS (TPGS) as structure-forming surfactants in combination with mono or tricaprylin as oil phase and water. Propylene glycol was used as viscosity modifier. Rheological properties were evaluated using a R/S plus controlled stress rheometer with shear rates from 1-100 s^{-1}. The swelling kinetics of monoolein and TPGS-based microemulsions were studied by weighing the microemulsions exposed to water to assess uptake during 48 h. In vivo phase transformation was assessed after subcutaneous injection of 100 µL of the selected monoolein-based microemulsion in mice. In vivo release of Alexa fluor from the gel was assessed using an in vivo bioimaging system. **Results**: Both microemulsions displayed rheological behavior consistent with Newtonian systems; uptake of 20% or more of water led to formation of hexagonal phase gels with pseudoplastic behavior. Water uptake by both microemulsions followed second-order kinetics independently on the type of structure-forming surfactant and oil phase used, although the maximum amount of water absorbed by TPGS-based systems was lower, and the system transitioned into a micellar dispersion over time. The monoolein-based system was selected for further evaluation based on its ability to form gels that resisted dilution. Gels formed in vivo approximately 24 h after subcutaneous administration of the monoolein-based microemulsion, and persisted locally for over 10 days providing slow release of Alexa fluor. **Conclusions**: Monoolein and vitamin E TPGS gave rise to microemulsions capable of forming hexagonal phase gels upon water uptake, but only monoolein-based gels resisted dilution. Subcutaneous administration of the monoolein-based microemulsion resulted in hexagonal phase formation and slow release of a fluorescent probe for over 10 days, supporting the use of this strategy as a platform for sustained drug release. Acknowledgements**:** The authors are grateful to CNPq (grant#443549-2014-1) for Financial Support: Protocol for animal use approved by the Institute of Biomedical Sciences Institucional Animal Care Use Committee (protocol number 72, p.20, book 3)

12.017 *In vitro* **activity of a chalcone (LZ46) AGAINST** *Candida albicans***: microdiluition, fungicidal activity and time-kill curve studies.** Lima WG, Andrade JT, Sousa CDF, Santos FRS, Villar JAFP, Araújo MGF, Souza ACS, Ferreira JMS UFSJ-Centro-Oeste

Introduction: *Candida albicans* is the most common species of fungal pathogens in humans and can lead to a wide range of severe complications including hemorrhage and disseminate infections[1]. Limited number of currently available antifungal agents and the emergence of resistant strains impose a considerable clinical challenge for the treatment of candidiasis[2]. Thus, it is urgent the search for new molecules with therapeutic properties against *C. albicans*[2,3]. *The chalcones are intermediates for the biosynthesis of flavonoids and isoflavonoids and their backbones have been associated with pharmacological activities such antitumor, antiviral, anti-inflammatory and antimicrobial*[4]. Therefore, this work aims to evaluate the antifungal potential of a chalcone (LZ46) against *C. albi*cans ATCC 10231. **Methods:** Minimal inhibitory concentrations (MIC) were determined by microdilution method in Sabouraud Dextrose Broth (SDB) employing concentrations ranging from 1000µg/ml to 3.9µg/ml[5]. Minimum fungicidal concentration (MFC) was found by subculturing 10 µl of each well on Sabouraud Dextrose Agar (SDA)[5]. Time-kill assay was performed according to Foersters and coworkers (2011)[6] using the concentrations of 1- and 2-folds the MIC and the times 0, 2, 4, 6, 8, 10, 12, 24, 36 and 48 hours. The antifungal ketoconazole and dimethylsulfoxide (diluent of compounds) were included in all assays as positive and negative controls, respectively. **Results:** The compound LZ46 showed both, MIC e CFM, of 15.6 µg/ml against *C. albicans*. In time-kill assay, no growth was observed at 2×MIC after 6 hours and at 1×MIC after 10 hours. The ketoconazole showed MIC of 125 µg/ml and CFM > 1000 µg/ml. In time-kill assay, growth of *C. albicans* was observed in all concentrations of the antifungal compound and range of times tested. The negative control with dimethylsulfoxides showed no toxicity on fungal cells, validating the experimental conditions. **Conclusion:** These results reveal the chalcone LZ46 has an important fungicidal activity against *C. albicans*. According to Wong (2014)[3], compounds that exhibit fungicidal activity are strong candidates for clinical use, making chalcone LZ46 is a potential antifungal drug candidate. **Acknowledgements:** UFSJ, FAPEMIG (postgraduate scholarship), CAPES and CNPq (financial support). **References:** [1]MAYER FL *et al.* Virulence, v.4, p.119, 2013. [2]SANGLARD D, Front. Med. (Lausanne), v.3, p.1, 2016. [3]WONG. J. Pept. Sci, v.14, p.152-164, 2014. [4]MAHAPATRA DK, BHARTI SK, Life Sci., v.148, p.154, 2016. [5]Clinical and Laboratory Standards Institute (CLSI). Pennsylvania, USA, 2003. [6]FOERSTERS *et al*., Front. Microbiol., v.6, p.1, 2015.

12.018 Conformation Analysis of HIV-1 Wild-Type Protease Bound and Unbound to Nelfinavir Inhibitor Holanda LHC[1,2], Pinheiro GLM[2,3], Gomes GC[2,4], Lameira J[2], Sousa MS[1]
[1]UFPA – Biologia Molecular, Núcleo de Medicina Tropical [2]UFPA – Planejamento e Desenvolvimento de Fármacos, [3]UFPA-Marajó – Faculdade de Ciências Naturais, [4]ICB-UFPA

Introduction: HIV, is a retrovirus that has highly virulent glycoproteins invading the T-CD4+ lymphocyte through their CCR4 and CXCR5 receptors, that 36.7 million people living with HIV. The life cycle is mediated by enzymes protease (PR), reverse transcriptase (TR) and integrase (IN). The PR is present at the final stage of the biological cycle of the virus causing the cleavage of polyproteins gag and pol, which then will mature virus making it infective, terminating this step is an important pharmacological target for the treatment of HIV infection. In this work we have studied the conformational dynamics of the PR enzyme complexed and non-complexed with the Nelfinavir inhibitor (NFV). **Methods:** Molecular dynamics (MD) simulations and Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) analysis were performed to analyze the flexibility of the HIV-1 PR (PDB code: 1OHR). The systems were placed in a octahedral box of TIP3P water molecules. All simulations were carried out using the AMBER 12. **Results:** A total of 40 nanoseconds (ns) of molecular dynamics simulation were performed for the HIV-1 PR in complex with NFV and in free form. Results about structure were visualized in Pymol Program and together with RMSD and RMSF analyses to confirm the flexibility loss of HIV-1 PR with NFV like are demonstrated in literature, NFV keeps the HIV-1 PR in a closed conformation, and influence of the I50 residue open the flap and cause changes in HIV-1 PR dynamics. The MD results suggested that protease has different conformations, when the enzyme is in bound and unbound to NFV. **Conclusion:** The understanding about conformation and flap flexibility of HIV-1 PR is important for a drug design of new compounds against HIV. We are now calculating the binding free energy of NFV-PR and we are also exploring the biological activity change of ligands upon mutation in HIV-1 PR that affect the binding between NFV and HIV-1 PR and causes changes in conformation of enzyme mainly in flap flexibility in residue I50. **References:** UNAIDS. Epidemiological status. 2015. Brito, A. M. Fármacos recentes usados para o tratamento da infecção pelo HIV-1: enfuvirtina, marovic, raltegravir e etravirina. Rev. de Ciências Farmac. Bás. e Avan. 159-168. v 32. 2011. Broder CC. Chemokine receptors and HIV. J Leukoc Biol, 62: 20–29. 1997. Case, d. A., T. E. The Amber Biomolecular simulation programs. J Comput Chem 26(16); 1668-1668. 2005 Cramer, C. J. Essential Computational Chemistry Theories and Models. UK, British Library. 2004 Roe, D. R. T. E. “PTRAJ and CPPTRAJ: Software for Processind and Analysis of Molecular Dynamics Trajectory Data.” J of Chem The and Comp. 9 (7), pp 3084–3095. 2013. **Financial support**: CNPq **Acknowledgments**: The author gratefully acknowledge all members of this study.

12.019 Putative microsomal prostaglandin E synthase-1 (mPGES-1) inhibitors identified by virtual screening show in vivo antipyretic activity Froes TQ[1], Castilho MS[1], Melo MCC[2], de Souza GEP[2], Soares DM[1] [1]UFBA – Medicamentos, [2]FCFRP-USP

Introdution: Fever is one of the most common causes for medical consultation worldwide (TEAGLE, Arch. Dis. Child., 99, 701, 2014). However, currently available antipyretic drugs have serious toxic side effects that limit their use (AL-SAEED, Oman Med J, 26385, 2011). In order to overcome this dilemma, mPGES-1 inhibition has been pursued (KOROTKOVA, Front Pharmacol, 1, 1, 2011). Herein we report novel putative mPGES-1 inhibitors that were identified by virtual-screening and evaluated in vivo and in vitro. **Methods:** GALAHAD software (SYBYLX 2.0) was employed to build pharmacophore models with the ability to differentiate true inhibitors (128) from decoys (6.528) and enlighten the structure-activity relationships for known mPGES-1 inhibitors. The best model was employed to selected 18 molecules for in vivo evaluation. Rats were given (i.v.) different doses (0.1 mg kg^{-1}, 0.3 mg kg^{-1}, 1.0 mg kg^{-1}) of each molecule 30 min before i.p. injection of LPS (50µg·kg^{-1}). Rectal temperature was measured by tele-thermometry during 6h each 30 minutes. Central activity was determined by i.c.v. pretreatment with 1.0 µg/rat of the compounds. Hypothalamic PGE_2 concentration was determined by ELISA. The inhibitory effect on COX-2 activity was determined by colorimetric assay kit. All biological results are expressed as mean ± standard error from the mean basal body temperature (°C) at different intervals. **Results:** The best pharmacophore model (4 hydrophobic, 2 H-bond acceptor, 2H-bond donor centers) was used for virtual screening. All 18 acquired molecules were assayed in murine fever assay using LPS as the pyrogenic stimulus, 33% of them have shown antipyretic activity ($p<0.05$) when given intravenously (1.0 mg kg^{-1}) ($p<0,05$). Among them: compound 1 inhibits 82.18% of the febrile response (1.0 mg/kg *i.v.*) if peripherally injected, but completely abolishes the fever if injected within the brain (1µg/rat *i.c.v*). Besides, its biological action onset is similar to Celecoxib and CAY10526 (COX-2 selective and mPGES-1 gene expression inhibitor, respectively) ones; compound 2 reduces 58.91% of the febrile response when given peripherally (1.0 mg/kg i.v.) but shows improved pharmacologic profile (95.75% inhibition) when injected within the brain (1µg/rat i.c.v). In contrast to compound 1, 2 has a late biological action onset (4h) that is compatible with direct inhibition of PGE_2 production. In parallel, these molecules showed no significant COX-2 inhibition at the highest dose. Visual analysis of bioactive molecules overlaid on the pharmacophore model suggests that substitution pattern in the aromatic ring nearby the triazole ring of compound 2 and its analogs plays a crucial role towards biological activity: *para* substituents reduce anti-pyretic activity, probably due to steric clash. On the other hand, the lack of activity in a compound 1 analog seems to be a consequence of pharmacokinetic properties (ester hydrolysis). **Conclusion:** Our results suggest that the most potent molecule can be considered a suitable lead compound for antipyretic drug development. **Financial support**: FAPESB. Ethical committee of Veterinary Medicine School - UFBA (Nº18/2013).

12.020 The quinoxaline-derived chalcone N9 displays potential antiproliferative effects in breast cancer cells. Erig TC[1], Mielcke TR[1], Mascarello A[2], Chiaradia LD[2], Nunes RJ[2], Basso LA[1], Campos MM[1] [1]PUCRS, [2]UFSC

Introduction: Breast cancer is highly prevalent in women worldwide, presenting elevated mortality rates. Triple-negative and HER2-positive tumors exhibit poor prognosis, showing invasive growth and metastasis potential (Hida et al., Breast Cancer Res Treat. DOI10.1007/s10549-016-3848-2, 2016). A recent publication demonstrated marked *in vitro* and *in vivo* antiproliferative effects for the quinoxaline-derived chalcone N9 in gliomas (Loch-Neckel et al., Eur J Med Chem. 90: 93, 2015). The present study investigated the *in vitro* effects of the N9 quinoxaline-derived chalcone on breast cancer cells, when tested alone or in combination with classical chemotherapeutic drugs. **Methods:** The human breast cancer cell lines, MDA-MB-231 (triple-negative) and SKBR-3 (HER-2 positive), were seeded in 96-well plates to determine the cell viability by MTT assay. As a first approach, the cells were exposed to different concentrations of N9 (0.5, 1, 2.5, 5 and 10 µg/ml), for 24, 48 and 72 h. In a separate experimental set, the compound N9 (2.5 µg/ml) was tested in combination with subthreshold concentrations of clinically available anticancer drugs. The following agents were tested combined to N9: cisplatin (2 µg/ml), 5-fluorouracil (6.5 µg/ml), cyclophosphamide (1.4 µg/ml), doxorubicin (1.7 µg/ml), docetaxel (0.02 and 0.5 µg/ml), and trastuzumab (3 µg/ml), at 48 h. The cell death profile was assessed by using Flow cytometry (annexin V/propidium iodide). Nuclear morphometric analysis was carried out by staining with the fluorescent dye DAPI. **Results:** The compound N9 induced cell death with double-staining characteristics, indicating the occurrence of apoptosis and necrosis. This chalcone induced concentration and time-dependent reduction of the viability in both tested cell lines. The maximal inhibitions were obtained at the concentration of five µg/ml, at 72 h, corresponding to 72 ± 1 % and 77 ± 4 %, for MDA-MB-231 and SKBR-3, respectively. For MDA-MB-231 cells, N9 (2.5 µg/ml) produced an inhibitory rate of 56 ± 6 %, whereas cisplatin (7.5 µg/ml), 5-fluorouracil (6.5 g/ml), and cyclophosphamide (1.4 µg/ml) produced inhibitions of 49 ± 5 %, 30 ± 11 % and 22 ± 13 %, at 48 h. In the combination protocols, the percentages of inhibition were 74 ± 3 %, 67 ± 8 % and 68 ± 6 %, for N9+cisplatin, N9+5-fluorouracil and N9+ cyclophosphamide, respectively. For SKBR-3, none of the tested combinations displayed additive effects. Qualitative DAPI analysis extended cell viability data, demonstrating a decrease of nuclear aberrations for the combination protocols. **Conclusions:** The incubation of quinoxaline-derived chalcone N9 demonstrated promising antitumor effects, according to assessment of the two tested cell lines, with cell death via mixed necrosis/apoptosis. Of note, N9 presented superior antiproliferative effects when combined to reference chemotherapeutic drugs, namely cisplatin, 5-fluorouracil and cyclophosphamide. Further experiments are in progress to assess the mechanisms involved in the antiproliferative effects of this chalcone. **Financial Support:** CNPq, CAPES, PUCRS.

12.021 Pre-clinical evaluation of new encapsulated places anesthetic formulations with liposomes ionic gradient and internal transmembrane gradient Carvalho CR, Papine J, Couto V USF

The Ropivacaine (RVC) has been widely used in clinical practice to possess a safer pharmacological profile compared to local used anesthetics (LA). However, the CVR has a relatively short duration of action, due to its rapid transfer and redistribution of the injection site, trying to reduce this redistribution and increase its duration of sustained release systems ("drug delivery systems"), such as liposomes with or without ion gradient, have been studied. Thus, the anesthetic activity research is of fundamental importance to confirm the effectiveness of these carrier systems, enabling greater knowledge of the effectiveness of these RVC new formulations and also the observation is this new system of "drug-delivery" has biological effects of higher intensity and duration than the free drug, which will confirm the future clinical use of these formulations. This project has been approved by the Ethics Committee on Animal Experimentation of Campinas State University - Unicamp (Protocol 4003-1). This study has evaluated the effectiveness of two new formulations of liposomes being the first of multivesicular (LMVV) with internal pH 5.5 and sodium citrate with RVC 0.75% (F1) and 2% (F3) and the second Univesiculares (LUV) with the associated internal pH 5.5 containing ionic gradient LMVV ammonium sulfate (external pH 7.4) with 0.75% RVC (F2) and 2% (F4) compared with 0.75% RVC (F5) in aqueous solution and 2% (F6) in aqueous solution. For the realization of preclinical tests have been used adult Wistar rats, male and weighing between 300 and 350 g, the animals were distributed randomly and received one of the six proposed treatments (F1 to F6) via the sciatic block or intrathecal injection . also they have used two control groups which received the formulations without the RVC (C1 LMVV pH 5.5 + citrate and C2 LMVV sulfate pH 7.4 + 5.5 LUV) in two-way proposals. Intrathecal injection (20 uL) was performed in the space between the L5 and L6 vertebrae and sciatic nerve blockade (0.4 mL) was carried out according to the method described by Leszczynska & Kau (1992). After administration of the formulations was rated motor and sensory block. The motor block evaluation was performed according these score values: 0 (normal use of the hind limbs), 1 (inability to fully flex the hind limbs) and 2 (inability to use hindlimb). The evaluation was made to a total recovery of animal movements, the sensory block was determined by paw withdrawal threshold of the animals against the mechanical stimulus. For the tests we have used a analgesymeter (Ugo Basile, Italy), which generates a gradual increase in force (in grams). The end point of analgesia was established when there was no statistical difference between test and control groups. A maximum value was (cut off) of 350 grams to prevent legs injuries and excessive stimulation of nociceptors. The data were analyzed with Tukey - Kramer Test (Parametric ANOVA) with 5% significance level. The sensory block test showed that the formulations with liposomal anesthetic had higher activity in relation to the free RVC (F5 and F6) ($p < 0.001$). In two administration routes, other control formulations showed no anesthetic action in relation to the motor block, the formulations showed no statistically significant differences compared to the free RVC 0.75 and 2%. For both sciatic nerve blockade and the intrathecal block were not observed given the Results: We may conclude that the liposomes use with ionic gradient increased the duration of the RVC anesthetic activity, was not observed statistical differences in motor block compared with the free ropivacaine. Keywords: ropivacaine, Local Anesthetics, liposomes. **Financial support** FAPESP (process no. 2014 / 14457-5). * Scholarship Master (CAPES / PROSUP)

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12.022 Polymer blending systems as strategies for nerve regeneration: biocompatibility evaluation. Nicoletti NF[1], Amaral MEA[2], Valente CA[3], Basso NR[3], Campo MM[1], Silva JLB[4] [1] PUCRS – Medicina e Ciências da Saúde, [2]PUCRS –Biologia Celular e Molecular,[3] PUCRS – Química, [4]PUCRS – Medicina

Introduction: Peripheral nerve injury is a serious health concern and a challenge to surgeons. Loss of sensory and motor function, pain and discomfort has functional consequences, driving marked social and psychological impacts (Bushnell BD, *J Hand Surg Am*. 33:1081-7, 2008). Polymer blending systems have been employed to develop suitable scaffolds with specific properties for tissue engineering applications (Subramanian A., *J Biomed Sci*. 16:108. 2009). Nanofibrous nerve conduits, such as poly (D, L-lactide- co-glycolide) and poly (ε-caprolactone) (PCL/PLGA), have been used in a variety of regeneration approaches. This study aimed at investigating the biocompatibility of PCL/PLGA blends in cultured cells. **Methods:** The effects of PCL/PLGA films were assessed in the following cell lineages: VERO (kidney epithelial cells African green monkey), RAW 264.7 (mouse macrophage-like cells), HaCat (human keratinocytes) or MRC-5 and FGH (human fetal lung and gingival fibroblast-like cells, respectively). PCL/PLGA blends were selected and tested at the following proportions: PCL 100%, PCL90%/PLGA10%, PCL80%/PLGA20%, PCL70%/PLGA30%, and PLGA100%. A silicone film was used as positive control of toxicity. The polymers were incubated by direct contact with the cells, in a size corresponding to 6 cm^2/ mL, as recommended by ISO 10993-5. The different cell lines were seeded at $5x10^3$ cells/well in DMEM/10% FBS, in 96-well plates. The cell viability (MTT assay), and the nuclear morphological alterations (DAPI staining) were evaluated after 24 h of exposure to polymers. **Results:** PCL 100% and PCL90%/PLGA10% displayed significant inhibitory effects on the proliferation of VERO, RAW 264.7 and HaCat cells. The maximal anti-proliferative effects of PCL 100% were observed in VERO cells (49 ± 6%), whereas the maximal reduction of cell viability with PCL90/PLGA10 was seen in RAW 264.7 cells (37± 6%). The evaluation of nuclear morphological features by DAPI staining, clearly demonstrated signs of nuclear fragmentation and blebbing, confirming the cytotoxic effects of PCL 100% and PCL90%/PLGA10%. However, these polymer blends did not significantly alter the viability of MRC-5 and FGH fibroblasts. Strikingly, PCL70%/30% blend did not display any cytotoxicity in all tested cell lines. In addition, the incubation of PCL70%/30% films induced cell migration toward the parallel grooves of the biomaterial. **Conclusion:** The *in vitro* results indicated that PCL/PLGA blend in a proportion of 70:30 did not display cytotoxic effects, besides inducing favorable cell migration toward the biomaterial parallel grooves. Based on this data, this material might represent a promising device for regenerative medicine applications. Further studies are being carried out to assess the *in vivo* effects of this polymer blend in peripheral nerve regeneration. **Financial Support:** PRPPG/PUCRS, CAPES-AUX-PE, CNPq and FINEP/PUCRSINFRA #01.11.0014-00.

12.023 Anti-inflammatory activity of the synthetic compound 1-nitro-2-phenylethene (NPA). Sugimoto MA[1], Silva MJA[2], Brito LF[1], Vago JP[1], Borges RS[3], Silva EL[2], Sousa LP[1]
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Inflammation is a reaction of the host to infectious or sterile tissue damage and has the physiological purpose of restoring tissue homeostasis(Medzhitov, 2010). However, uncontrolled or unresolved inflammation can lead to tissue damage, giving rise to a plethora of chronic inflammatory diseases, including metabolic syndromes and autoimmunity pathologies with eventual loss of organ function. Nitrostyrene compounds consist of an aromatic ring linked to a chain of the nitro group (NO2). These compounds are known to have several biological activities such as anti-apoptotic, anti-platelet and anti-microbial, however, little research has been carried out regarding the anti-inflammatory activity of these compounds. Thereby, the aim of this study was to evaluate the anti-inflammatory activity of the nitrostyrene 1-nitro-2-phenylethene (NPA) using in vitro and in vivo assays. At first, we evaluated the capacity of NPA to interfere with the viability of human monocyte-like cell line THP-1 differentiated into macrophages. To this end, THP-1 monocytes were differentiated into macrophages with phorbol myristate acetate (PMA) and incubated with different concentrations of NPA (1-20 µg/ml) for 7h followed by MTT colorimetric assay. Then, the anti-inflammatory activity of NPA was evaluated by measuring TNF-α produced by THP-1 cells stimulated with lipopolysaccharide (LPS, 100 ng/ml). Dexamethasone (Dexa) was employed as positive control (20 µg/ml). The anti-inflammatory effect of NPA was subsequently evaluated in vivo using a model of acute pleurisy induced by LPS (Vago et al., 2012). To this purpose, BALB/c mice were treated with an intraperitoneal (i.p.) injection of NPA (0.9 mg/Kg) or Dexa (2mg/kg), as a control, and 1h later they were challenged with an intrapleural (i.pl.) injection of LPS or PBS. After 7h of challenge the animals were sacrificed and the cells were collected and processed for differential cell counts and western blot analysis. In vitro tests with THP-1 suggested that NPA at 10 and 20µg/ml produce a cytotoxic effect (viability under 70%). Conversely, NPA in concentrations less than or equal to 5µg/ml showed no significant reduction in viability after 7h of treatment. At nontoxic concentrations NPA significantly inhibited TNF-α production by THP-1 cells in a dose-dependent manner. The drug reduced TNF-α production by 29.7 ± 2.3% when tested at 1 μg/ml and by 66.6 ± 4.4% at 5 μg/ml indicating a potency similar to the positive control Dexa which inhibited TNF-α production by 75.7 ± 3.7%. Notably, such an effect was associated to decrease of the prosurvival ERK (evaluated by ERK phosphorylation). Treatment of mice with NPA decreased neutrophil numbers into the pleural cavity after 7h of challenge (PBS, 0.3 ± 0.3; LPS, 20.1 ± 7.8; NPA, 10.8 ± 4.4; Dexa, 9.9 ± 2.1; number of neutrophils x 105/ cavity). Interestingly, such an effect was associated to decrease of the prosurvival pathways NF-kB (evaluated by IkB-α phosphorylation) and phospho-ERK. Taken together the results suggest a potential anti-inflammatory activity for NPA as observed by in vitro and in vivo tests. Financial Support: CNPq, PRPq-UFMG, FAPEMIG and CAPES. Research approval Animal Ethical Committee: (CETEA/UFMG Protocol number 83/2015). **Reference:** MEDZHITOV, R. Cell, v. 140, n. 6, p. 771-6, Mar 19 2010. VAGO, J. P. JLB, v. 92, p.1-10, Aug 2012.

12.024 Cytotoxic potential of synthetics chalcones-sulfonamides. Moura AF[1], Araújo AJ[1,2], Barret FS[1], Castro MRC[3], Perez CN[3], Pessoa CO[4], Moraes MO[1] [1]UFC – Fisiologia e Farmacologia, [2]UFPI – Curso de Medicina, [3]UFG – Química, [4]Fiocruz

Introduction: Modern medicinal chemistry has tried to develop new drugs based on knowledge of the diseases pathophysiology, the study of biochemical pathways and selection of molecular targets through the integration between molecular design, organic synthesis and biological evaluation to identify new compounds with optimized biological activity (ANDRICOPULO *et al*., 2009). Chalcone is an important secondary plant metabolite which shows an array of pharmacological properties. New chalcones have been developed from the insertion of organic groups as substituents of aromatic rings of these compounds. Sulfonamides comprise a group of synthetic antibiotics used in the treatment of infectious diseases caused by micro-organisms. Recent studies showed activities of chalcones-sulfonamides against some diseases such as leishmaniasis, malaria, and malignant tumors (LAWRENCE *et al.*, 2001). Therefore, the chalcones-sulfonamides synthesis is an attractive to the development of new drugs. Thus, the aim of this work was to evaluate the cytotoxic effect of synthetic chalcones-sulfonamides in tumoral cell lines. **Methods:** Four similar synthetics chalcones-sulfonamides were tested against three cancer cell lines, HCT-116 (colon), SF-295 (brain) and PC-3 (prostate); and one non tumoral cell line, L-929 (murine fibroblast), using the MTT assay, after 72 hours of incubation. Cell growth was quantified by the ability of living cells to reduce MTT to a blue formazan product. **Results:** The compound **63Ce** showed no cytotoxic effect with IC_{50} values greater than 52 µM. The compounds **99**, **55** and **185** showed antiproliferative effects against all tested tumoral cell lines. The compound **99** showed similar IC_{50} values between all tested tumoral cells with IC_{50} values ranged from 3.65 to 5.76 µM in HCT-116 and SF-295, respectively. When tested in L-929, this value was 10.1 µM. The compound **55** showed antitumor effect to PC-3 and HCT-116 cells, with IC_{50} values of 10.14 and 13.85 µM, respectively, whereas, the IC_{50} values to SF-295 and L-929 was similar, 30.65 and 34.23 µM. The compound **185** presented a marked effect to HCT-116 cells, with IC_{50} value of 5.59 µM, whereas IC_{50} values were similar between the others tested cell lines, with IC_{50} values ranged from 24.09 to 29.52 µM in L-929 and PC-3, respectively. Changing the position of the sulfonamide, possibly reduces the activity of chalcone-sulfonamide in PC3, SF-295 and L-929, like observed in **185**. However, IC_{50} value in HCT-116 it was similar to **99**. Changing the position of the sulfonamide and nitro (NO_2) radical, possibly reduces the activity of chalcone-sulfonamide in all tested cell lines, like observed in **55**, compared to **185** and **99**. **Conclusion:** Structural modifications, like sulfonamide and nitro radical position, can modify molecules antitumor effects. **Supported by:** CNPq, PRONEX, CAPES, FAPEG and FUNCAP. **References:** ANDRICOPULO, A.D. et al. **Curr. Top. Med. Chem**., v.9, p.771, 2009; LAWRENCE, N.J. et al. J. Comb. Chem., v. 3, p. 421, 2001.

12.025 Anticancer and antimicrobial activity of essential oil from *Pilocarpus microphyllus* **leaves.** Marinho-Filho JDB, Araújo AJ, Mendes MGA, Costa KRL, Barbosa MS, Cruz J, Lima-Neto JS, Véras LMC UFPI

Introduction: The *Pilocarpus* gender (Rutacea family), also known as "Jaborandi", presents about 13 species and 78 varieties which occur in all neotropicals regions. Its economic value is significant as food, ornaments and medicinal uses such as diuretic, diaphoretic, digestive, sialogogue. The search for new substances with biological activity, antimicrobial and anticancer, led the study with essential oil *Pilocarpus*. **Methods:** The analysis of the essential oil components was performed by system GCMS-QP2010 SE, AOC-5000 (Shimadzu). The constituents were identified by comparison of the mass spectra obtained with Wiley 229 libraries and bibliography. The antimicrobial activity was evaluated by broth microdilution method in 96-well plates according to CLSI 2009 for these bacterias: *Streptococcus mutans* ATCC 25175, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 e *Streptococcus sobrinus* ATCC 27351. The cytotoxicity of the essential oil was tested against K-562 (leukaemias), PC-3 (prostate), SF-295 (brain), and HCT-116 (colon) human cancer cell lines using MTT assay after 72 hours. **Results:** The essential oil from *Pilocarpus microphyllus* displayed moderate cytotoxicity against cancer cell lines, showing IC_{50} values in the range of 19.1 (14.1 – 26.0) to 28.7 (18.0 – 45.7) µg/mL in K562 and PC3, respectively. However, there was no antimicrobial activity in all tested strains. Three major compounds were identified as γ-cadinene, transcaryophyllene and tridecanone. These compounds may be related to the effects seen in tumor cell lines. **Conclusion:** In conclusion, the essential oil from the leaves of *Pilocarpus microphyllus* and its constituent, could explain, at least in part, the ethnopharmacological use of this plant in the treatment of cancer. **Financial Support:** CNPq, CAPES, FAPEPI and FINEP.

12.026 The effect of small molecules on skin regeneration. Horinouchi CDS[1,2], Oostendorp C[2], van den Bogaard EH[3], Schalkwijk J[3], van Kuppevelt TH[2], Daamen WF[2] [1]CAPES Foundation, [2]Radboud University Medical Center – Bioquímica, [3]Radboud University Medical Center – Dermatology

Introduction: The field of regenerative medicine has gained interest in chemical small molecules which are able to modulate the fate of cells while being less complex and unstable than biological drugs[1]. In this study three small molecules were evaluated as potential healing agents using cultured skin cells. The molecules were SUN11602, a fibroblast growth factor 2 signaling mimetic; ONO-1301, a prostacyclin agonist; and purmorphamine, a sonic hedgehog signaling mimetic. **Methods**: Keratinocytes or fibroblasts were exposed to SUN11602 (10-200 µM), ONO-1301 (0.1-30 µM) or purmorphamine (0.005-1 µM) in different experimental set ups in order to evaluate cell viability, proliferation, differentiation and migration. Cytotoxicity was assessed in HaCaT cells and NIH/3T3 fibroblasts after 24 hours of compound exposure by Alamar blue viability assay. For proliferation analysis, stablished cell lines and primary human skin fibroblasts were exposed to the small molecules for up to 120 hours and cell viability was assessed as an indication of proliferation. In addition, the proliferation and differentiation of primary human fibroblasts and keratinocytes were assessed by gene expression analysis of VEGF, MMP1, Ptch1, Cyclin D1, Ki67, CK14, TGM1, involucrin, collagen I and elastin using qPCR. Scratch assay was performed to follow up migration of primary keratinocytes or fibroblasts. **Results**: Small molecule exposure had no effect on cell viability after 24 h of treatment even at high concentrations. SUN11602 did not change fibroblast proliferation even when tested in the FGF hyperresponsive cell line NR6-3T3, similar to published data by Murayama, et al. 2015 [2]. SUN11602 (50 µM), however, reduced expression of type I collagen on primary fibroblasts. On keratinocytes, SUN11602 (100 µM) reduced cell migration and increased the expression of MMP1 (15-fold), VEGF (2-fold), TGM1 (2-fold) and involucrin (3-fold). ONO-1301 (2 µM) increased proliferation of primary fibroblasts while reduced fibroblast migration. ONO-1301 also reduced the expression of Ki67 and Cyclin D1 in primary fibroblasts while expression of MMP1 (3-fold) and Ptch1 (2-fold) was increased. On keratinocytes, ONO-1301 promoted a one-fold increase on MMP1 expression. Purmorphamine (0.5 µM) increased proliferation of primary fibroblasts while it did not affect fibroblast or keratinocyte migration. Purmorphamine increased the expression of CK14 and TGM1 on keratinocytes. On fibroblasts, purmorphamine (0.25 µM) reduced the expression of VEGF, collagen I and elastin. Conclusion: Our results show that while SUN11602 modulates mainly differentiation-related activity on keratinocytes, ONO-1301 seems to influence fibroblast proliferative capacity, and purmorphamine is able to alter both proliferation and differentiation. Additional studies are necessary to delineate the activity of the compounds and predict which wound healing phase would be the perfect target for each small molecule. Support: CAPES foundation (process 11718/13-7). References: 1. Längle, D, ACS Chem. Biol., 9, 57, 2014. 2. Murayama, N, Brain Res., 1594, 71, 2015.

12.027 Zebrafish (*Danio Rerio*) an emerging tool for drug discovery in mood disorders and nicotine addiction. Iturriaga Vasquez P[1], Viscarra F[1], Paillalil P[1], Quiroz G[2], Reyes Parada M[3]
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Zebrafish (*Danio rerio*) has become an emerging model for drug discovery. Its short development time, and the possibility of obtaining large numbers of individuals for experimentation, makes zebrafish an animal model suitable for drug screening. Moreover, they can absorb chemical substances from their tank water. The potential of zebrafish for behavioral studies has been applied using different paradigms associated to complex behavior such as memory, anxiety, stress, addiction and reinforcement properties of drugs of abuse. The novel tank diving test (NTT) and the conditioning place preference (CPP) have been used by different research groups as models for anxiety, stress or addiction in zebrafish. NTT is conceptually similar to the rodent open field test, since it takes advantage of the instinctive behavior of both zebrafish and rats to seek refuge when exposed to an unfamiliar environment. On the other hand, CPP is based on the concept that animals seek addictive drug even in unpleasant environments. In our work, we performed a complete profile of behavior using NTT with different ligands that bind to a) nicotinic receptors (agonists and antagonist) and b) the monoamine transporter of serotonin, dopamine, and norepinephrine. Additionally, we compared the effects of different drugs synthesized by us. In the light of our results we selected one molecule (UFR2709) which elicits an anxiolytic effect in the NTT and we studied the effects of this molecule on the CPP evoked by nicotine. Our results indicate that UFR2709 (a selective antagonist for heteromeric nicotinic acetylcholine receptors) displays an anxiolytic effect on the NTT and reverses the conditioning preference evoked by nicotine in CPP experiments. Thus, UFR2709 has a potential application in mood disorders and nicotine addiction. **References**: Kedikian X, Faillace MP, Bernabeu R. Behavioral and molecular analysis of nicotine-conditioning place preference in zebrafish. PloS One. 8(7):e69453, 2013. Bencan Z, Levin ED. The role of alpha7 and alpha4beta2 nicotinic receptors in the nicotine-induced anxiolytic effect in zebrafish. Physiol. Behav. 95(3):408, 2008. Acknowledgments: Proyecto Fondecyt 1150615 and 1130185. Research Ethical Committee Res. N°:048/2015