10. Cancer and Cell Proliferation

10.001

XIAP silencing and p53 superexpression cooperate in glioma cell death. Hütten MO, Silva AO, Lenz G UFRGS

Gliomas are the most common primary brain tumor subtype, and Glioblastoma is the most aggressive and deadly form, which is justified by many alterations in signaling pathways in these tumors. XIAP and p53 proteins are important in apoptotic pathways, and hence represent potential therapeutic targets. XIAP blocks apoptosis through its direct inhibition of caspases 3, 7 and 9 and is overexpressed in many cancers. On the other hand, p53 is a tumor suppressor protein which acts in mechanisms as cell cycle arrest, senescence and apoptosis, and is frequently mutated or deleted in tumors. Finally, it's interesting to investigate other mechanisms activated in parallel with cell death, such as autophagy. Autophagy is a physiologic degradation process in which organelles and proteins are recycled, and it can act as tumor suppressor, maintaining genomic integrity and avoiding tumorigenesis, or it can help cancer cells overcome stressful situations, helping its survival. Therefore, the present study aims to evaluate the effects of XIAP silencing combined with p53 superexpression on autophagy mechanism, proliferation and survival of gliomas. XIAP silenced cells (U87Xi) with p53 overexpression (P +) presented a reduced proliferation when compared with control U87Xi transfected only with GFP and with the wild type control (U87wt) with p53 overexpression. Through autophagosome formation analysis, we observed an increase on autophagic cell numbers on U87Xi cell line compared with U87wt. This was also observed in two other glioma cells lines U373 and A172. On the other hand, p53 overexpression reduced autophagy levels on both U87 cell lines (Xi and wt). AnnexinV/PI assay showed an increase in double positive cells in U87Xi P +, indicating an increase in cell death induction in comparison with the controls. Protein expression analysis showed that, independent of p53 levels, there was a reduction on Bcl-XL levels on U87Xi cell line in comparision with U87wt. With p53 overexpression, pro-apoptotic proteins Bax and PUMA levels were also raised and, besides, there was an increase on caspase-3 levels on U87Xi in relation to U87wt, and an even bigger increase in U87 P + . These data indicate that the combined treatment on apoptotic pathway proteins modulation is effective on reducing proliferation and cell death induction on U87 glioma cell line, representing a promising therapeutic target. Furthermore, there is a possible integration pathway between p53, XIAP and Bcl-XL, since the decrease on Bcl-xL levels on U87Xi cell line facilitated induction of autophagic process, which wasn't seen in U87wt, since this cell line kept higher levels of Bcl-xL. As for p53, superexpression of this protein in both cell lines (U87wt and Xi) decreased autophagy levels, once p52 is capable of inhibiting autophagy and could be directing cells towards activation of antiproliferative mechanisms. Therefore, it is important to consider autophagy induction on glioma cell lines and identify which are the antiproliferative mechanisms and in which way they are initiated when p53 is overexpressed and XIAP silenced. **Support**: CNPq, FAPERGS, Capes.

10.002

***In vitro* effects of the guaraná (*Paullinia cupana*) hydroalcoholic extract in association with different chemotherapeutics used in the treatment of breast cancer.** Azzolin VF, Hertz E, Cadoná F, Machado AK, Barbisan F, Cruz IBM UFSM – Biogenômica

Introduction: Cancer related fatigue (CRF) is a common phenomenon in patients undergoing chemotherapy and radiotherapy representing a negative impact on functional status and quality of life. Some plants present tonic and anti-fatiguing properties, such as guaraná (*Paullinia cupana*). Previous study described positive effect of the guaraná on CRF of breast cancer patients submitted to chemotherapy. Guaraná can improve CRF because this fruit present bioactive molecules as caffeine and catechin. However, the effect of the hydroalcoholic stratum of guaraná consumption during the chemotherapy is unknown. Therefore, the aim of this study was to evaluate if guaraná could change the viability and antiproliferative effect of seven chemotherapeutic agents currently used in the breast cancer treatment using as *in vitro* experimental model MCF-7 breast cancer cell line. **Materials and Methods** MCF-7 cells obtained from ATCC were cultured at 37°C with 5% CO_2 and were expanded by obtaining the optimal amount for the experiments. Cell suspension was placed in each of 96-well plates (10^6 cells/well). Next, cell cultures were treated with different guaraná hydroalcoholic extract concentrations (1, 5 and 10 µg/mL) with and without association with several chemotherapics (10uM gemcitabine, vinorelbine and methotrexate, 2uM 5-fluorouracil, 50uM paclitaxel, 200nM doxorubicin, and 5mM cyclophosphamide). Next the cells were incubated for 24 and 72 hours at 37°C in humidified atmosphere with 5% CO_2. After 24 and 72 hours of incubation, the cell viability and proliferation percentage was investigated through of MTT Assay (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide). **Results and discussion** As expected, all chemical drugs tested here decreased significantly the MCF-7 cell viability as well as cell proliferation ($p<0.01$). The main results showed an antiproliferative effect of the guaraná isolated at 5 and 10 µg/mL. Different of the results found after 24 hours of exposition, the guaraná plus all chemotherapic drugs showed a strong antiproliferative effect (≥ 40 % of cell inhibition) on MCF -7 cells after 72 hour exposition of gemcitabine, vinorelbine and methotrexate, 5-fluorouracil, paclitaxel, doxorubicin, and cyclophosphamide. Because chemotherapy drugs were affected by the guaraná addition most improve the antiproliferative activity, the therapeutic use of guaraná against CRF seems did not affect negatively the chemotherapy. **Conclusion** Therefore, the results showed that: guaraná, regardless of chemotherapeutic, presents antitumor activity in MCF-7 cells; the effect of the guaraná on the viability of the MCF-7 cells exposed to chemotherapy was heterogeneous and dependent on the type of drug, guaraná potentiated the antiproliferative effect of all tested chemotherapeutic. However, *in vitro* protocols present methodological limitations that need to be considered and complementary *in vitro* and *in vivo* investigations need to be performed to evaluate whether chemotherapic plus guaraná administration is safe to breast cancer patients. **Financial Support:** National Council for Scientific and Technological Development.

10.003

Preliminary investigation of cytotoxic potential of semi-synthetic chalcone against human cancer cells. Maranhão SS, Soares BM, Meira AS, Moraes MO, Cavalcanti BC, Pessoa CO UFC – Fisiologia e Farmacologia

Introduction: Chalcones are phenolic compounds derived from secondary plant metabolism. These compounds are precursors in the biosynthesis of flavonoids and isoflavones and exhibit bactericidal, fungicidal, antiviral, antiprotozoal, insecticidal, anthelmintic, anti-inflammatory and anticancer activities. The present study evaluated the cytotoxicity of semi-synthetic chalcone 3-(3,5-dimethoxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-ona (AFC4) on three human tumor (lung, prostate and ovarian) cell lines and peripheral blood mononuclear cells (PBMC). **Methodology:** Cytotoxicity of AFC4 (0.001 to 17 μM) was assessed by MTT assay after 72 h exposure. Cell viability was evaluated by trypan blue exclusion test, and morphological cell changes after treatment were analyzed through differential staining with acridine orange/ethidium bromide as well as panoptic histological staining procedure after 24 h exposure. **Results and discussion:** Over a period of 72 h exposure, AFC4 exerted cytotoxic effects against all tumor cells with IC_{50} values equal to 5.5 μM for NCI-H358M (lung carcinoma) cells, 7.5 μM for OVCAR-8 (ovarian adenocarcinoma) cells, and 5.7 μM for PC-3M (metastatic prostate carcinoma). Also, the cytotoxic effect of AFC4 was assessed in PBMC, which showed a moderate cytotoxicity (IC_{50} 18.2 μM). Preliminary studies on mechanisms of action were performed using ovarian OVCAR-8 cancer cell line after AFC4 treatment (4, 8 and 16 μM) for 24 h. Morphological analysis of OVCAR-8 cells AFC4-treated showed apoptotic markers such as nuclear condensation, DNA fragmentation, and plasma membrane blebs formation at cultures treated with 4 and 8 μM. Also, necrosis was observed only at cultures exposed to highest concentration (16 μM). In summary, the studied chalcone AFC4 elicited significant cytotoxicity against tumor cells when compared to non-tumor cells (PBMC). Regarding ovarian OVCAR-8 cancer cells, our preliminary data highlights apoptosis as a main pathway of cell death mechanisms induced by AFC4. **Financial agencies and acknowledgements:** CNPq, FUNCAP, Capes, BioTechcell.

10.004

Antitumor effects of the mesoionic compound sydnone-1 in walker-256 carcinosarcoma model. Galuppo LF[1], Martins GG[1], Lívero F[1], Cadena SMSCC[2], Beltrame OC[3], Dittrich RL[3], Echevarria A[4], Acco A[1] [1]UFPR – Pharmacology, [2]UFPR – Biochemistry and Molecular Biology, [3]UFPR – Veterinary Medicine, [4]UFRJ – Chemistry

Introduction: The number of people diagnosed with cancer is increasing worldwide. One of the possible treatments is the chemotherapy, however it is known that chemotherapeutic agents are highly cytotoxic, have several side effects, and poor selectivity for tumor cells (Chen, 2009). Because of these characteristics the studies of new agents capable of combating the cancer with lower side effects, providing welfare to the patient, are necessary. The objective of this work was to study the antitumoral activity of the mesoionic compound Sydnone 1 (Syd-1) in the Walker-256 (W256) tumor model in rats, by assessing *in vivo* antineoplastic, metabolic and toxicological parameters.

- All the procedures in male Wistar rats were approved by the institutional committee for the animal care (CEUA nº 765). The W256 tumor cells were maintained by intraperitoneal inoculation (ip) of 10^7 cells/ animal. After 4-5 ip passages, and after verifying their feasibility by the method of Trypan blue in a Neubauer chamber, carcinosarcoma cells were inoculated subcutaneously (10^7 cells per rats) into the right hind limb of animals. In the next day after the tumor cells inoculation the oral (gavage) treatment started for the groups as follows: 1) Basal group, non-tumor bearing animals that received vehicle (80% Tween Saline); 2) Control group, rats with tumor treated with vehicle (80% Tween Saline); and 3) Syd-1 group, rats with tumor that received Syd-1 (75 mg/kg). After 12 days of treatment the animals were anesthetized with ketamine and xylazine for collecting material. Liver, tumor and plasma were collected and frozen for later analysis. Also the tumor volume and weigh were evaluated. The oxidative stress in tumor and liver tissue was checked by the measurements of the enzymes GST, Cat and SOD, and by the LPO and GSH levels. Biochemical and hematological parameters, as well as the weight of the organs (liver, kidney, spleen and lung) were assessed as toxicological biomarkers.
- It was observed a decrease in tumor volume and weight of animals treated with Syd-1. The tumor suppression caused by the treatment was about 34%. No significant differences were observed in parameters of oxidative stress in the treated group compared with baseline and control groups. A significant decrease in plasma alkaline phosphatase was observed in both treated and control group. The hematology of the Syd-1-treated group showed an increase in the size of red blood cells, mean corpuscular volume, and in granulocytes and leukocytes number, as well as an expressive splenomegaly.
- The results confirmed the antitumor action of Syd-1. This effect may not be related with the oxidative stress induction. More studies are needed to elucidate the mechanism by which the tumor suppressor occurs and the effects upon spleen and red blood cells. **Financial support**: Capes and Fundação Araucária. **Reference:** CHEN Y. Design of anticancer prodrugs for reductive activation. *Med. Res. Rev.* v. 29, p. 29, 2009.

10.005

In vitro **evaluation of a novel series of quinoxaline-derived chalcones in oral squamous cell carcinoma: relevance of a-ring methoxylation.** Mielcke TR[1], Erig TC[2], Mascarello A[3], Chiaradia LD[3], Nunes RJ[3], Campos MM[4] [1]PUCRS – Medicine and Health Sciences, [2]PUCRS – Pharmacy, [3]UFSC – Chemistry, [4]INTOX-PUCRS – Medicine and Health Sciences

Introduction: Molecules based on natural products continue to play a relevant role in oncology drug discovery. Extensive research identified numerous natural compounds with anti-cancer effects that might present beneficial effects when used in combination with known chemotherapeutic drugs (Newman *et al.*, *J Nat Prod.* 75(3): 311, 2012). Several studies have shown that chalcones, which are natural precursors of flavonoid and isoflavonoids (Lin *et al.*, *J Nutr Biochem.* 23(4): 368, 2012), display a wide variety of proprieties, including anti-cancer activities (Hseu *et al.*, *J Agric Food Chem.* 60(9): 2385, 2012). We have previously demonstrated (Mielcke *et al.*, *Eur J Med Chem.* 48: 255, 2012) that quinoxaline-derived chalcones, based on the selective PI3Kγ inhibitor AS605240, show marked antiproliferative effects against different glioma cell lines. These results prompted us to test these chalcones and additional related compounds in the oral squamous cell carcinoma (OSCC), due to its high prevalence in Brazil and the poor outcomes. **Methods**: After reaching confluence, the rat and human OSCC cell lines (SCC158 and HN30, respectively) were seeded at $5x10^3$ cells/well in DMEM/10% FBS, in 96-well plates. They were exposed to increasing concentrations (0.1 to 10 μg/mL) of 20 different quinoxaline-based chalcones, for incubation periods of 24, 48 and 72h. Parallel control experiments were carried out with the addition of 10% FBS (cell viability control) or DMSO (vehicle control, 0.01%). Cell viability was evaluated by MTT assay, which provides a quantitative measure of the number cells with metabolically active mitochondria. In separate experiments, SCC158 and HN30 cells were seeded at $1x10^4$ cells/well, in 24-well plates, and allowed to grow for 24 h. The medium was changed prior to treatment with four different chalcones (5 μg/mL), selected on the basis of MTT assay. After 48 h of incubation, the number of cells was determined in a haemocytometer. **Results:** From the 20 chalcones screened in the MTT assay, six of them, namely N15, N16, N17, N18, N20, N23 displayed concentration- and time-related inhibitory effects on the viability of both tested cell lines. From the six active compounds, we selected four quinoxaline-derived chalcones (N15, N17, N18 and N23) which displayed inhibitions superior to 50%, at the 5 μg/mL concentration, to be tested in the cell counting assay. These chalcones were able to reduce cell proliferation by 88 ± 3%, 87 ± 3%, 84 ± 7%, and 90 ± 1%, for SCC158 cells, and 67 ± 12%, 82 ± 12%, 68 ± 12%, and 98 ± 1%, for the HN30 lineage. **Discussion:** The structure-activity screening of 20 quinoxaline-derived chalcones in rat and human OSCC cell lineages revealed remarkable effects for four compounds, which present a similar chemical structure, having methoxy groups at the ring A. Further studies are in progress in order to gain further insights into the mechanisms of action of the selected compounds. It is tempting to point out these compounds as potential useful alternatives for treating OSCC, even when used alone or in combination with currently available chemotherapy agents. **Acknowledgements:** CNPq, Capes, PUCRS.

10.006

Inhibition of proliferation due to cell cycle arrest caused by sublethal concentrations of extract and fraction from *Casearia sylvestris* (Salicaceae). Felipe KB[1], Kviecinski MR[1], Ourique F[1], Bucker NF[1], Farias MS[1], Grinvícius VMAS[1], Gatti FM[2], Rossi MH[2], Castro LSEPW[1], Pedrosa RC[1], Mota NS[1] [1]UFSC – Bioquímica, [2]CPDSA-Instituto Biológico

Introduction: *Casearia sylvestris* is a tree found in tropical America. In Brazil it is known mainly as Guaçatonga. Studies have confirmed the presence of some cytotoxic constituents in this plant. But some useful antitumor drugs have more a cytostatic effect than a cytotoxic one. The aim of this work is to investigate the antiproliferative potential of the crude extract and chloroform fraction of *C. sylvestris* at sublethal concentrations. **Material and Methods:** The human breast cancer cell line MCF-7 was used. Non cytotoxic concentrations were selected by the MTT assay. The antiproliferative effect was assessed by the colony forming unit assay. Effects on the cell cycle were observed through flow cytometry and a propidium iodide kit. The effect on some key proteins of DNA damage, the phosphorylation on the histone H2Ax and cell cycle control p53, p16, CDK2 was evaluated through immunoblot. **Results and discussion:** The EC_{50} values found at 24 h on MCF-7 cells were 141 µg/mL for hydroethanol crude extract (CHE) and 66 µg/mL for chloroformic fraction (f-$CHCl_3$). Inhibition on proliferation was recorded at concentrations as low as 4 µg/mL in the case of the f-$CHCl_3$ (up to 40%) and up to 50 % when CHE was added at 9 µg/mL. The cell cycle arrest was demonstrated by the reduction in terms of number of cells in phases G2/M and S, up to 15.4 and 7.4 % when cells were treated with CHE and 11.6 and 5.2 %, respectively when cells were treated with f-$CHCl_3$. The number of cells in phases G2/M and S presented by the control group (CTLR) was 25.2 and 15.4 %, respectively. The number of cells in G1 was increased when the cells were treated with CHE (5.1%) or f-$CHCl_3$ (4.6 %), when compared with CTRL (2.7%). Key proteins of cell cycle control were affected. The treatments caused activation of p53, p16 and DNA damage found by the appearance of bands corresponding to γ-H2Ax. On the other hand, the treatments caused reduction of CDK2. **Conclusion:** Whether the extracts from *C. sylvestris* are cytotoxic at high concentrations, the low concentrations have antiproliferative effect and can be useful to complement the conventional cytotoxic cancer chemotherapy. **Financial Support:** Capes

10.007

Comparative analysis of toxicological parameters in mice with melanoma treated and untreated with cisplatin. Ferreira NH, Furtado RA, Oliveira PF, Acésio NO, Souza LDR, Magalhães GM, Nassar EJ, Tavares DC Unifran

Introduction: Currently, the therapeutic modality based on the use of cytotoxic drugs is still the primary form of approach for cancer treatment. Unfortunately, this approach results are quite disappointing in most cases, despite widespread use. Among the most commonly used drug is cisplatin. It is estimated that 50-70% of cancer patients worldwide are treated with cisplatin[1]. This compound is a potent antineoplasic drug widely used for the treatment of various types of cancer. Despite its success, cisplatin has several disadvantages that include severe toxicity such as nephrotoxicity and neurotoxicity, limiting the treatment[2]. In the present study, we performed a comparative analysis of the toxic effects in tumor-bearing animals treated with cisplatin and those untreated. **Methods:** The syngeneic murine B16/F10 tumor model was employed. At the time the C57BL/6 mice showed a tumor with a size of 100 mm3, these were treated subcutaneously with cisplatin at dose of 10 mg/kg or DMSO (Dimethylsulfoxide, 1%) for five days. The parameters analyzed were: DNA damage in hepatocytes by comet assay, levels of creatinine and urea in serum, macroscopic and histopathologic evaluation of spleen, kidney and liver. **Results and discussion:** The treatment with cisplatin resulted in reduction of the tumor size and weight and also reduction of body weight. The animals were visibly weakened. This treatment group showed frequencies of DNA damage significantly higher compared with negative control group. The animals with tumors no submitted to treatment with cisplatin showed no statistically significant increase in the frequency of DNA damage. The group treated with cisplatin showed levels of creatinine and urea significantly higher when compared to those of the negative control group and those with tumors untreated with cisplatin. Regarding the analysis of the tissues, it was found that the animals with tumor showed significantly higher numbers of mitoses compared to the group treated with cisplatin, indicating cell proliferation. The group treated with cisplatin showed larger quantities of intratumoral necrosis and hemorrhagic areas. The animals of the group treated with cisplatin showed a significantly decrease in liver weight, necrosis in the spleen and signs of toxicity were observed macroscopically as change in color and size in these tissues. Thus, this study contributes to a better understanding of antitumoral and toxic effects of the cisplatin chemotherapy. [1]Vaccaro, M. Chem Commun, v. 21, p. 1404, 2009. [2]Wong, E. Chem Rev, v. 99, p. 2451, 1999. Protocol number of the Ethics Committee on Animal Use at the University of Franca: 007/13. **Financial support**: São Paulo Research Foundation (Fapesp, Brazil, grant # 2012/24427-0).

10.008

Cytotoxic action of secretions from Amazon amphibians. Machado KC[1], Lima DJB[2], Debiasi BW[3], Oliveira SFC[1], Machado KC[1], Noronha JC[3], Rodrigues DJ[3], Sinhorin AP[3], Pessoa C[2], Vieira-Júnior GM[3], Ferreira PMP[1] [1]UFPI, [2]UFC, [3]UFMT

Introduction: Skin secretions of amphibians are a fascinating source of active substances. Herein, it was carried out a bioprospecting in nine extracts of *Rhinella marina* (RM) and *Rhaebo guttatus* (RG) toads occurring in the Southern Amazon of Mato Grosso, Brazil in search of venoms with cytotoxic activity against tumor and normal cells. **Methods**: Firstly, the cytotoxic properties were assessed by MTT [leukemia (HL-60), colon (HCT-116), glioblastoma (SF-295) and ovarian (OVCAR-8) tumor cells] and Alamar Blue™ assays [peripheral blood mononuclear cells (PBMC)] after 72 h exposure and maintaining in supplemented RPMI-1640 at 37°C in a 5% CO_2 atmosphere. Control groups (negative and positive) received the same amount of dimethylsulfoxide solvent (0.1% DMSO) as test groups. Doxorubicin (Dox, 0.005-5.0 µg/mL) was used as positive control. For hemolytic activity, extracts (1.56-200 µg/mL) were incubated in 96- well plates for 60 min at 25°C in a suspension of mice erythrocytes (2%) in 0.85% NaCl containing 10 mM $CaCl_2$. Hemoglobin levels in the supernatants were spectrophotometrically determined at 540 nm. Directly quantify cell proliferation was determined by BrdU labeling in treated HL-60 cells with *R. marina* extracts (RM-1, RM-2, RM-3, RM-4 and RM-5: 0.1 and 1 µg/mL) after 24 h. These protocols were approved and registered (IBAMA, SISBIO: number 30034-1; Ethical Committee on Animal Research 0102/2011). **Results and discussion:** The discovery and development of medicines has established a respectable arsenal of useful chemotherapeutic agents, as well as a number of important successes in the treatment and management of human cancer (HARRIS, A.R., *Nat. Rev. Cancer*, v. 2, p.38, 2002). All extracts of *R. marina* venoms revealed higher cytotoxic activity, with IC_{50} values ranging from 0.01 µg/mL (SF-295) to 0.23 µg/mL (OVCAR-8). Meanwhile, RG venom extracts exhibited IC_{50} values being around 2.9-6.6 µg/mL. Interestingly, higher IC_{50} values were found for proliferating leukocytes (0.8-13.9 µg/mL). Statistically, there were no differences in the cytotoxicity outcomes between samples obtained from female and male animals belonging to the same species ($p > 0.05$). Only, *R. guttatus* venom extracts led to hemolysis, with EC_{50} values ranging from 20.8 (RG-8) to 33.7 µg/mL (RG-6). All extracts (RM-1, RM-2, RM-3, RM-4 and RM-5) decreased BrdU incorporation, showing labeling of 35.4 ± 3.4, 30.7 ± 1.0, 25.1 ± 1.8, 28.0 ± 1.7 and 38.3 ± 2.6 % at 0.1 µg/mL and 19.7 ± 1.3, 19.6 ± 1.2, 15.8 ± 1.8, 16.5 ± 0.8 and 29.5 ± 1.6 % at 1 µg/mL, respectively, when compared to untreated cells (73.0 ± 3.2 %) ($p<0.05$). Dox (0.1 and 1 µg/mL) treatment resulted in 22.6 ± 1.9 and 12.7 ± 0.9% BrdU incorporation ($p<0.05$). Extracts from *R. marina* and *R. guttatus* venoms showed pronounced lethal and discriminating effects in tumor lines, especially those from *R. marina*, highlighting toad parotoid gland secretions as a promising source of novel lead anticancer compounds. **Financial Suport**. CNPq, FAPEMAT, FUNCAP e FAPEPI.

10.009

Cytotoxic activity of lapachol analogues on HL60 with or without antioxidant co-treatment. Costa AM[1], Gomes SLS[2], Costa PRR[2], Silva AJM[3], Costa-Lotufo LV[1], Pessoa CO[1], Moraes MO[1], Militão GCG[4] [1]UFC – Fisiologia e Farmacologia, [2]CCS-UFRJ – Química Bioorgânica, [3]CCS-UFRJ – Catálise Orgânica, [4]UFPE – Fisiologia e Farmacologia

Introduction: Lapachol, a naphthoquinone first isolated from *Tabebuia sp.*, present cytotoxic activity on tumor cells by, in part, enhancing the oxygen reactive species production. Two synthetic analogues to lapachol, 1,4-dioxyl-3-phenil-1,4-dihidronaphthalen-2-yl dietilcarbamate (**1**) and 3-(3,4-dimetoxiyenil)-1,4- dioxyl -1,4-dihidronaphthalen-2-yl dietilcarbamate (**2**) was synthesized in order to improve the cytoxic potential of this natural compound. Thus, the objective this work was to evaluate the cytotoxic activity on cells HL-60 of two naphthoquinones analogous of lapachol (compounds 1 and 2) with and without co-treatment with the antioxidant n-acetylcysteine (NAC). **Methods**: Firstly, the antiproliferative effect of the compounds was evaluated by MTT on leukemic cells (HL-60) and on peripheral blood mononucleated cells (PBMC) after 72 and hours of treatment. Secondly, a 24 h treatment with and without co-treatment with the antioxidant n-acetylcysteine (NAC) was evaluated by MTT assay and May-Grunwald-Giemsa staining. **Results and discussion:** The IC_{50} values for Hl60 after 72 h of treatment was of 12 µM, 2.3 µM and 4.3 µM for lapachol, compound **1** and compound **2**, respectively; while IC_{50} for PBMC was 13.7 µM and 34.0 µM for compound **1** and **2**, respectively. The IC50 values in cells HL-60 after 24 hours incubation without NAC was 42.9 µM, 2.7 µM and 4.3 µM for lapachol, compound **1** and compound **2** respectively; IC_{50} with NAC (5 µM) co-treatment after 24 hours of exposure to lapachol, compound **1** and compound **2** was 180.0 µM, 46.0 µM and 18.0 µM, respectively Analysis of HL60 morphological changes induced by compounds 1 an 2 after 24h treatment reveled cell shrinkage, DNA fragmentation and pyknotic nuclei. All morphological alterations induced by compounds was abolished by co-treatment with NAC. The cytotoxicity of two lapachol analogues is related to the increase of oxygen reactive species, since co-treatment with an antioxidant drastic reduced its pharmacological effects. **Financial support**: CNPq and Capes.

10.010

Genetic profile analysis and *in vitro* drug sensibility from primary culture obtained from glioblastoma. Kipper FC[1], Becker R[1], Mendonça LC[1], Vanacôr CN[2], Confortin G[2], Marc A[2], Paglioli-Neto E[2], Morrone FB[3], Lenz G[1] [1]UFRGS – Biofísica e Centro de Biotecnologia, [2]HSL-PUCRS – Neurocirugia, [3]PUCRS – Farmácia

Gliomas are the most common and aggressive tumors that appear in central nervous system. These tumors present high mortality rates with a mean survival of 12 months after diagnosis, despite radio and chemotherapy, but recent chemotherapeutic regimes have increased the survival by only two months. *The Cancer Genome Atlas* effort subtyped gliomas in four molecular groups based on gene expression, mutation and deletion and retrospectively linked these subtypes to chemotherapy response. The goal of this project is to analyze gene expression and drug sensitivity to prospectively contribute to therapeutic decisions. For this, we prepared 18 cell cultures from 18 biopsis of brain tumors and treated them with a range of chemotherapy agents (temozolomide, carmustine, lomustine, cisplatin, etoposide, paclitaxel, irinotecan, procarbazin, mebendazol, vincristin and vinblastin) at plasmatic concentration. Cells were counted for at least 10 days after treatment or tested for viability after 7 or 14 days. None of the cultures analyzed responded to temozolomide (the first line chemotherapy for this tumor) in plasmatic concentration after a single administration. From eleven tested cultures nine were sensitive to at least one of the drugs, with less than 40% of cells remaining after 14 days; the non-sensitive cultures were derived from one Glioblastoma grade IV and one meduloblastoma. More pronounced effects were observed using drugs against microtubules. The cell cultures from three of five patients display EGFR over-expression and four of five present p53 and p21 expression. From these data we conclude that brain tumors can be routinely grown and tested for drug sensitivity in culture for future correlation with therapeutic outcome. **Financial support**: FAPERGS, CNPq e Capes. Ethical protocol number UFRGS: 420.856; PUCRS: 429.849

10.011

Role of GSK3-β signaling on telocinobufagin-induced cell death. Amaral LS, Cunha-Filho GA, Noël F, Quintas LEM ICB-UFRJ

Introduction: Bufadienolides are a class of cardiotonic steroids widely found in nature and have been identified in mammals. They are considered specific Na+ /K+ -ATPase inhibitors but also promote the activation of multiple signal transduction pathways via this enzyme. We postulate that such diverse endogenous bufadienolides may evoke unique cellular effects. We have shown that the structurally similar endogenous bufadienolides telocinobufagin (TCB) and marinobufagin (MBG) have opposite effects in renal cells; while MBG induced cell proliferation, TCB had no effect or even had an antiproliferative. MBG effect is mediated by ERK1/2 pathway, but TCB's is still unclear. Inhibition of GSK3-β has been recently associated to osteosarcoma cell apoptosis by cinobufagin (Yin *et al.*, *Toxicol* Lett., 218:129, 2013). Here we evaluated the involvement of GSK3-β in TCB antiproliferative effect. **Methods:** LLC-PK1 cells (porcine proximal renal tubule) were treated with 10 or 100 nM TCB or 500 nM BIO (GSK3-β inhibitor) for 15 min, lysed with RIPA buffer, centrifuged at 13,000g for 15 min and the supernatants were used in Western blot for the evaluation of inhibition (phosphorylation) of GSK3-β. Inhibition curves of GSK3-β with BIO were also assessed after treatment for 72 h. Also, the cells were treated for 72 h with 10, 25, 50 and 100 nM TCB, with or without the presence of 500 nM BIO and cell viability/quantity was measured by counting the number of Trypan blue-viable cells. **Results:** TCB 10 and 100 nM as well as 500 nM BIO significantly increased phosphoGSK3-β fraction after 15 min (28,9% and 29,6% of control, $p<0.05$, $n=3$). However, no inhibition was achieved when the cells were treated for 72 h with concentrations of BIO from 25 to 1000 nM as well as changes in cell number. In accordance with previous results, increasing concentrations of TCB significantly decreased LLC-PK1 cell number after 72 h in a concentration-dependent fashion. More importantly, this TCB effect was largely potentiated when combined to a fixed concentration of BIO that did not affect cell quantity. **Discussion:** TCB-induced inhibition of GSK3-β seems to be involved in its cell death effect. Considering that the structurally related bufadienolides TCB and MBG mediate divergent effects by different signaling pathways following the interaction with one and only receptor, Na+ /K+ -ATPase, these results strongly indicate that mammalian endogenous cardiotonic steroids present functional selectivity **Financial support:** Capes, CNPq, Faperj.

10.012

C-terminus of protein S100A9 inhibits adhesion, proliferation and migration of endothelial cell on extracellular matrix components. Moraes NF[1], Melo RL[2], Sampaio SC[1], Giorgi R[1] [1]IBu – Fisiopatologia, [2]IBu – Proteômica Funcional

Introduction: Our group has showed that both S100A9 and a synthetic peptide identical to the terminal region of this protein (mS100A9p) induce antinociceptive effect. In addition, the mS100A9p inhibits mice adherent peritoneal cells functions, cells critical for interactions with both malignant and stromal cells in the local microenvironment. Literature data shows that the complex S100A8/A9 is being associated to malignancy. In cancers, the proliferative state is accompanied by intense angiogenesis, a process that evolves new vase formation and cell migration. However, studies focusing the effect of the mS100A9p on tumor progression and angiogenesis have not been carried out yet. So, the aim of the present study was to evaluate the *in vitro* effect of the mS100A9p on events involved in angiogenesis. **Methods: Proliferation assay:** $5x10^4$ thymic endothelial cells (tEnd.1) were incubated in a 6 well plate for 1h with culture medium (control) or culture medium containing different peptide concentrations (0.585; 1.17; 2.35; 4.7 or 9.4µM/well). The plate was washed and after 24h incubation with RPMI, the cells were stained with Trypan blue and cells number determined in Neubauer Chamber. **Cell migration assay** (***wound healing***)**:** $1x10^6$ tEnd.1 cells were adhered on type I collagen coated coverslips in 24 well plates and after became confluent a wound was made with a sterile tip. Cells were incubated with only RPMI 1640 medium at 10% bovine fetal serum or mS100A9p (0.585; 1.17; 2.35; 4.7 or 9.4µM/well) for 1h. It was washed with PBS and left for 24h with RPMI 1640 1%. After this period were counted the cells which migrated to the wound. **Adhesion assay:** 96 well plate were sensitized with different extracellular matrix components (fibronectin-3µg/well, collagen type I-10µg/well and laminin-10µg/well) for 16h and after was incubated for 1h with 1% BSA. $5x10^5$ tEnd.1 cells/well treated or not with mS100A9p (0.585; 1.17; 2.35; 4.7µM/well for fibronectin and collagen or 2.35µM/well for laminin) for 1 hour were added to the plate for another hour. Adhered cells were incubated with MTT for 3h and then with SDS for 18h and read in ELISA (Multiskan EX, Labsystem). **Results and discussion:** It was found that all the mS100A9p concentrations, except the 9.4µM/well, inhibited cell proliferation. For migration, all the concentrations inhibited it, but 2.35µM was the most effective (56%, in relation to control group). On the adhesion assay to collagen type I the peptide induced an inhibitory effect with the 2.35 (36%) and 4.7µM/well (54%) concentrations. The most effective inhibition of the peptide on the adhesion to fibronectin was observed with 2.35µM/well (74%). Based in these results, the concentration of the mS100A9p utilized on the adhesion to laminin assay was 2.35µM/well and the inhibition observed was of 22%. Altogether, it shows that mS100A9 peptide is capable of modulating *in vitro* events involving angiogenesis. Considering that tumor growth needs a considerable blood supply to guarantee enough oxygen on tumoral microenvironment and that the inhibition of events involved in angiogenesis means a possible inhibition on tumor development, the mS100A9p may be used as a therapeutic tool for the control of tumor growth. Supported by Fapesp 2013/01295-4 e CNPq 830619/1999-2

10.013

Bioprospecting anticancer compounds from the octocoral *Stragulum bicolor* (Anthozoa, Subclass: Octocorallia) on the Ceará coast. Santos EA[1], Jimenez PC[2], Torres MCM[1], Gomes BA[1], Sousa TS[1], Pessoa ODL[1], Cutignano A[3], Nuzzo G[3], Fontana A[3], Costa-Lotufo LV[1] [1]UFC, [2]Unifesp, [3]CNR-Italia

Sessile marine organisms, such as sponges and corals are known to produce chemically diverse metabolites with a wide spectrum of biological activities (McClintock, J. B., Introduction to the Chemical Ecology of Marine Natural Products. In Marine Chemical Ecology, CRC Press: Boca Raton, Fl, p. 3, 2001; *Coll, J. C., Chem. Rev.* v. 92, p. 613, 1992). The octocorals (Anthozoa: subclass: Octocorallia) represent a group of cnidarians with acknowledged relevance in discovery of new natural products. The presence of diterpenoids, steroids and other chemical compounds with pharmacological activities such as antitumor, antiviral and anti-inflammatory has been reported for this class of soft corals (Berrue, F. Nat. Prod. Rep., v. 26, p. 681, 2009; Chao, C.H., Mar. Drugs, v.10, p. 439, 2012; Correa, H. *J Inflamm*, v. 6, p. 5, 2009; Huang, K. J., Mol., v. 18, p. 2914, 2013; Rodríguez, I.I., v. 67, p. 1672, J. Nat. Prod. 2004). However, little information is available regarding the biomedical potential of octocorals from the coast of Ceará. Therefore, the aim of the present work was to evaluate the *in vitro* cytotoxic activity of metabolites from octocoral *Stragulum bicolor* (Octocorallia: Clavulariidae) in tumor cells through bioassay guided fractionation. Fragments of the colony of this species were collected at Caponga Beach, on the east coast of Ceará state, and superficially decontaminated with ethanol 70%. The material was extracted with methanol (100%) and filtered and concentrated by rotary evaporation to yield a crude extract. Then, it was performed a solid phase extraction (SPE) of the crude extract followed by chromatography (silica gel) eluted with a combination of solvents of increasing polarity. The isolated compounds were confirmed by nuclear magnetic resonance and high-resolution mass spectrometry. The extracts and fractions were screened for cytotoxicity using the MTT assay in tumor cell lines: HCT-116 (humam colon carcinoma), PC-3 (prostate carcinoma), PC-3M (metastatic prostate carcinoma), OVCAR-8 (ovarian carcinoma) and MALME-3M (metastatic melanoma). The crude extract showed significant cytotoxicity in three tumor cell lines (HCT-116, OVCAR-8, MALME-3M), displaying IC_{50} values ranging 0.5 to 2.87 µg/mL. Among the extracts obtained from SPE, only the fraction with intermediate polarity showed an inhibitory effect on cell growth greater than 80%. This fraction was subjected to silica gel chromatography, yielding 12 fractions. Of these, 2 fractions were identified as macrolides members of amphidinolides. One macrolide was identified as amphidinolide P, already reported in the literature for its antitumor proprieties (Kobayashi, J., Nat. Prod. Rep. v. 21, p. 77, 2004) and the other is still an unpublished molecule. This study reports for the first time the biomedical potential of compounds isolated for this recently described octocoral and the isolation of a novel amphidinolide. However, further studies will be conducted in order to evaluate its mechanism of action. Nevertheless, the results herein confirm that the soft corals have a great value as a source of compounds with pharmaceutical applications. Keywords: cytotoxicity, octocoral, amphidinolides. **Financial support**: CNPq/CNR, Capes and FUNCAP.

10.014

Evaluation of the toxicity of the association of cerium oxide and zinc oxide nanoparticles after nine days of treatment. Batista TM[1], Xavier A[1], Brito MT[1], Sousa TKG[1], Mangueira VM[1], Beltrão DM[1], Moura APG[1], Santos CCL[2], Keyson D[2], Souza AG[2], Farias IAP[3], Sampaio FC[3], Albuquerque AJR[3], Sobral MV[1] [1]UFPB – Ciências Farmacêuticas, [2]UFPB – Química, [3]UFPB – Biotecnologia

Introduction: The association of cerium oxide and zinc oxide nanoparticles (NCZ) exhibit significant *in vivo* antitumor activity against Ehrlich Ascites Carcinoma (EAC). However, the evaluation of the toxicity of these nanoparticles is crucial for the applicability and pharmacological optimization of these effects. **Methods**: This assay was approved by the Animal Studies Committee of the Federal University of Paraíba (no. 0812110). EAC cells were implanted ($2x10^6$ cells/animal) into female mice peritoneum (n = 6). 24 hours after and for 9 days, NCZ was administered (i.p.) at doses 10, 20 and 40 mg/kg. 5-Fluorouracil (5-FU), 25 mg/kg, was the standard drug. After the treatment, biochemical and hematological parameters, and the indexes of organs (mg organ)/g animal), liver, heart, kidneys, spleen and thymus were evaluated. Data were presented as mean ± standard error compared with the control group by ANOVA followed by Tukey, and significant when $p < 0.05$. **Results**: No significant changes in the organs index of the treated animals were observed. Administration of NCZ in the experimental animals induced significant increase in the AST activity in all of the groups treated compared to the control group (Control: 54.4 ± 5.5; 10 mg/kg: 159.9 ± 16.6*; 20 mg/kg: 163.5 ± 7.5*; 40 mg/kg: 230.1 ± 27.7*). It was also observed a significant increase in ALT activity in the group treated with 20 mg/kg of NCZ compared to the control group (Control: 37.1 ± 5.2; 20 mg/kg: 64,86 ± 6,28*). Regarding renal function, a significant decrease was observed in serum urea in the animals treated with 10 mg/kg of NCZ compared to the control group (Control: 60.1 ± 6.4; 10mg/kg: 40.6 ± 3.2). We also observed a significant increase in serum creatinine concentration in the animals treated with doses of 20 mg/kg and 40 mg/kg of NCZ when compared to the control group (Control: 0.23 ± 0.03; 20 mg/kg: 0.49 ± 0.03*; 40mg/kg: 0.59 ± 0.07*). Concerning the hematological parameters, it was observed a significant increase in MCH (Mean Corpuscular Hemoglobin) parameter of the animals treated with NCZ at doses of 20 and 40 mg/kg (Control: 18.30 ± 0.47; 20 mg/kg: 20.78 ± 0.64*; 40 mg/kg: 21.38 ± 0.64*). **Discussion**: AST is found in high concentrations in the cytoplasm and mitochondria of hepatic cells, it is also found in cardiac and skeletal muscle, kidney, pancreas and erythrocytes. When any of these tissues is damaged, AST is released into the blood. ALT is only found in high concentrations in the cytoplasm of hepatocytes, which makes it useful for diagnosis of liver injury, with relative specificity. High levels of serum creatinine is associated with a reduction in glomerular filtration rate. The urea is synthesized in the liver and advanced liver disease is often associated with reduced plasma levels of urea. Increased levels of mean corpuscular hemoglobin, may indicate the presence of macrocytosis. From these results we can conclude that NCZ showed moderate toxicity in the parameters analyzed. **Financial support**: CNPq

10.015

Screening of antitumor activity of the synthetic piperinic derivatives in Ehrlich ascites carcinoma model. Sousa TKG[1], Mangueira VM[1], Batista TM[1], Fernandes HMB[1], Meireles DRP[1], Guimarães ARBV[1], Souza HDS[2], Lira BF[2], Filho PFA[2], Sobral MV[1] [1]UFPB – Ciências Farmacêuticas, [2]UFPB – Química

Introduction: Natural products are available in abundance and offer the best possibilities for the isolation of substances or synthesis prototypes of therapeutic interest. Piperine is one of the first and most important alkaloid amides isolated from *Piper nigrum* (Epstein, W. W., Journal of Chemical Education, 70: 598, 1993). Piperine has cytotoxic, anti-inflammatory, antipyretic, analgesic, antioxidant, antitumor, antifungal and bactericidal activity (Bong, C.F.J., Molecules, 15: 2398, 2010). HE-01, HE-02 and HE-03 are new synthetic compounds derived from piperine. The present investigation was carried out to evaluate the antitumor activity *in vivo* of the three synthetic piperinic derivatives in experimental model of Ehrlich Ascites Carcinoma (EAC). **Methods:** EAC cells were implanted (2×10^6 cells/animal) into female mice peritoneum (n = 4). 24 hours after and for 9 days, HE-01, HE-02 and HE-03 were administered (ip) at a dose of 50 mg/kg. 5-Fluorouracil (5-FU) (25 mg/kg) was the standard drug. On the 11th day the animals were euthanized and the ascites fluid collected for the assessment of tumor weight (g) and volume (mL), and total number of tumor cell ($\times 10^7$). Data presented as mean ± standard error compared with the control group by ANOVA followed by Tukey, and significant when $p<0.001$. This assay was approved by the Animal Studies Committee of the UFPB (no. 0901/14). **Results:** The treatment with 50 mg/kg of HE-01 induced reduction of the tumor weight and volume, and total number of tumor cell (0.0 ± 0.0 g, 0.01 ± 0.0 mL, 0.001 ± 0.0005 $\times 10^7$ cells, respectively). The values for HE-02 were 0.02 ± 0.03 g for tumor weight, 0.05 ± 0.04 mL for tumor volume and 0.0003 ± 0,0001 $\times 10^7$ cells for total number of tumor cell. And, for the HE-03 the tumor weight and volume, and total number of tumor cell was 0.0 ± 0.0 g, 0.01 ± 0.0 mL and 0.0002 ± 0.00005 $\times 10^7$ cells, respectively. All of the molecules showed significant activity on this screening in comparison with the control group (7.93 ± 0.41 g, 7.05 ± 0.36 mL, 145.8 ± 7.7 $\times 10^7$ cells for tumor weight and volume, and total number of tumor cell, respectively). The values for 5-FU were 0.07 ± 0.03 g for tumor weight, 0.01 ± 0.001 mL for tumor volume and 0.0001 ± 0.00004 $\times 10^7$ cells for total number of tumor cell. **Discussion:** The treatment with 50 mg/kg of synthetic piperinic derivatives and 25 mg/kg of 5-FU showed significant decrease in tumor weight and volume in comparison to the control group. There was no significant difference between the HE-01, HE-02, HE-03 and 5-FU. Total number of tumor cell ($\times 10^7$ cells) also reduced in the groups treated with the synthetic piperinic derivatives and 5-FU compared with the control group. HE-01, HE-02 and HE-03 showed potent antitumor activity in EAC model. Thus, additional studies are being conducted to evaluate the antitumor activity of the other parameters, as well as to elucidate the mechanism of action of these substances. **Financial support:** CNPq

10.016

Role of Purinergic P2 receptors on proliferation and viability of esophageal cancer cells lines. Santos AAJr[1], Paccez JD[2], Pinto LF[3], Zerbini LF[2], Morrone FB[1] – [1]PUCRS – Biologia Celular e Molecular, [2]ICGEB – Cancer Genomics, [3]INCa

Introduction: Oesophageal cancer is one of the most aggressive cancers and is the sixth leading cause of cancer death worldwide (Kamangar *et al*, J Clin Oncol. 10:2137, 2006). ATP is known to inhibit the growth of cancer cells in a variety of models and through several mechanisms, including the activation of P2X7 purinergic receptors (Chueh *et al*, *J Neurochem* 61:1782, 1993). Aim: To investigate the role of ATP on the proliferation and viability of oesophageal cancer cells lines and to investigate the expression of P2X7 receptor in cancer cells lines. **Methods**: The viability of the cell lines OE21 and KYSE450 (oesophageal squamous cell carcinoma), OE19 and OE33 (oesophageal adenocarcinoma), was evaluated through MTT assay, the proliferation was analyzed by cell counting, the expression of P2X7 protein was analyzed by Western Blot in EPC2, KYSE30, KYSE450, KYSE520 and WHCO1. **Results:** The treatment with ATP (3 and 5mM) along 24h was able to reduce the viability of OE19 (17 ± 4% and 27 ± 5 %, respectively), OE21 cells (38 ± 4% and 47 ± 4%, respectively) and OE-33 (19 ± 6% e 23 ± 3%, respectively), while on KYSE450 just the treatment with ATP 5 mM along 24h was able to reduce the viability (20 ± 2 %), ATP treatment (3 and 5 mM), after 48h, reduced cell viability on OE19 (18 ± 1% and 22 ± 1%, respectively), OE21 (62 ± 3% and 72 ± 5%) and OE33 cells (32 ± 5% and 31 ± 4%). The treatment with ATP 1mM for 48h also reduced OE21 cells viability (31 ± 3%). ADP treatment (50, 100 and 200μM) was not able to alter viability of the OE19, OE21 and OE22 cells. The treatment with ATP (1, 3 and 5mM) reduced the number of OE21 cells after 24h (36 ± 5%, 69 ± 3% and 79 ± 6% respectively). We also found a higher immunoreactivity of P2X7 protein in KYSE450, KYSE520 and WHCO1 cells (Figure 1 K) when compared to normal cells (EPC2). **Discussion**: The preliminary findings allow us to infer that ATP, through P2X receptors, has a role in the control of proliferation and viability of OE cells, and that this effect is time and concentration-dependent. Furthermore, the presence of P2X7 protein in KYSE450, KYSE520 and WHCO1 cells, can lead us to further investigate the effect of this receptor in this type of cancer. **Acknowledgements**: Capes, FINEP (PUCRSINFRA) # 01.11.0014-00, PUCRS.

10.017

Cytotoxic potential of compounds isolated FROM *Hyptis carvalhoi.* Moura AF[1], Araújo AJ[1], Lima KSB[2], Silveira ER[2], Moraes MO[1], Costa-Lotufo LV[1] – [1]UFC – Fisiologia e Farmacologia, [2]UFC – Química Orgânica e Inorgânica

Introduction: *Hyptis* is one of the largest genus of the Lamiaceae family, with about 580 species, and several of them are found in North and Northeast of Brazil, especially in the Brazilian "*cerrado*" (JUDD, *et al.*; 1999). Previous studies on the constituents of species of *Hyptis* have revealed mainly triterpenes and diterpenoids (ARAÚJO, LIMA e SILVEIRA, 2004). The aim of this work was to evaluate the cytotoxic effect of compounds isolated from the hexane extract of the roots of *Hyptis carvalhoi* Harley in cancer cell lines. **Methods:** The hexane extract from the roots of *H. carvalhoi* Harley was fractionated over silica gel column using *n*-hexane/CH_2Cl_2 (1:1), CH_2Cl_2, CH_2Cl_2/EtOAc (1:1), EtOAc e MeOH as eluents. The resultant fractions were submitted to successive column chromatography, followed by HPLC to yield seven different compounds. These compounds were tested against four cancer cell lines: HL-60 (leukemia), HCT-116 (colon), SF-295 (brain) and OVCAR-8 (ovarian), 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 and discussion:** The fractionation resulted in four abietane diterpenes: 7*β*-hydroxy-11,14-dioxoabieta-8,12-diene (**1**), 7*α*-hydroxy-11,14-dioxoabieta-8,13-diene (**2**), 12-hydroxyabieta-8,11,13-trien-20-ol (pisiferol) (**3**), 12-methoxy-*trans*-carnosic acid (**4**), an unknown nor-diterpene: 8(7),10(7)-diepoxy-12-hydroxy-20-*nor*-8,11,13-abietatriene (**5**), one labdane diterpene: 11-ketosandaracopimar-15-en-8*β*-ol (**6**) and a compound mixed biosynthesis: 3*β*-[4'-acetoxyangeloyloxy]-tremetone (**7**). The compounds **6** and **7** showed no cytotoxic effect with IC_{50} values greater than 70 µM. The abietane diterpene 7*β*-hidroxi-11,14-dioxoabieta-8,13-dieno (**1**) was the most active against HL-60 cells (IC_{50} 0.38 µM), and in addition showed moderate activity against other cell lines with IC_{50} values ranging from 40.3 µM in OVCAR-8 and HCT-116 cells up to 69.2 µM in SF-295, indicating selectivity for leukemic cells. The abietane diterpenes have attracted particular attention, because many of them present interesting biological activities. Among the abietane diterpenes that exhibit those activities, were observed several highly oxygenated compounds, that can present either the C-ring saturated or unsaturated, aromatic, and also as ortho or para-naphtoquinones. The high activity of the compound (**1**) probably was due to the presence of the *para*-naphtoquinone C-ring and of the *β*-positionated hydroxyl. In conclusion, the constituents isolated from the roots of *H. carvalhoi* could explain, at least in part, the ethnopharmacological use of this plant in the treatment of cancer. **Supported by:** CNPq, PRONEX, Capes and FUNCAP. **References:** JUDD, W. S. *et al.*, **Plant Systematics: a phylogenetic approach**, (1999); ARAÚJO, E. C. C. *et al.*, *MRC*, 42, 1049, (2004).

10.018

Nor-β-Lapachone PLGA microparticules: development, characterization and cytotoxicity activity.Feitosa ACS[1], Costa MP[1], Oliveira FCE[1], Silva Junior EN[2], Dias GG[2], Sales FAM[3], Freire VN[3], Pessoa CO[1], Caetano EWS[4] [1]UFC – Fisiologia e Farmacologia, [2]UFMG – Química, [3]UFC – Física, [4]IFCE

Introduction: In recent years, efforts are being made to develop new lapachone analogues, more potent and less toxic. Nor-β-Lapachone (NβL), a semisynthetic derivative from nor-lapachone, is a cytotoxic agent against several cancer cell lines. To overcome its liposolubility and non-specific toxicity, this drug was encapsulated. This work investigated the characteristics of Nor-β-lapachone-loaded PLGA microcapsules fabricated using the emulsion-solvent evaporation technique. **Methods:** The encapsulation efficiency, size, morphology, zeta potential, drug release kinetics and *in vitro* cytotoxicity of the drug-loaded microcapsules were determined. Classical molecular dynamics and classical annealing allowed us to estimate the binding energy range and typical adsorption geometries for NβL on the surface of PLGA microparticles. **Results and discussion:** Spherical microcapsules with a size range of 1.03 ± 0.46 μm were obtained. The encapsulation efficiency of the microcapsules was approximately 19 %. The NβL-loaded PLGA microcapsules exhibited a pronounced initial burst release with 90.05 ± 6.76 % of the drug released within 24 h. Classical molecular dynamics simulations indicated that NβL can be adsorbed on the surface of PLGA microparticles with binding energies as large as –32 kcal.mol^{-1}, contributing to the initial phase of the drug release process. After the *in vitro* treatment with NβL-loaded PLGA microcapsules, a clearly phagocytosis of the spheres was observed in a few minutes. NβL-loaded microcapsules were more sensitive in prostate cancer cells. **Conclusions:** Based on these results, we can conclude that PLGA microcapsules containing NβL could be a promising drug delivery system for anticancer applications. **Financial support:** Capes, CNPq and FUNCAP. **Acknowledgments:** Capes, CNPq and FUNCAP.

10.019

Evaluation of the effects of thalidomide-loaded biodegradable devices in solid ehrlich tumor. Pereira BG[1], Fialho SL[1], DE FREITAS MAA[2], De Souza CM[3], Cassali GD[3], Silva-Cunha A[2] [1]FUNED – Pharmaceutical and Biotechnological Development, [2]UFMG – Pharmacy, [3]UFMG – General Pathology

Introduction: Thalidomide has been considered a potent inhibitor of angiogenesis and extensively evaluated in recent years as a potential drug for treatment of cancer, including solid tumors[1,2]. Despite its promising biological activities, thalidomide has unfavorable physicochemical characteristics[3], problematic pharmacokinetic after oral administration[4] and severe side effects that limit its use[5]. For this reason, we proposed a new biodegradable polymeric implant to this drug. **Methods:** For preparation of 5 mg implants, thalidomide and polylactide-co-glycolide (PLGA) were dissolved in a water and acetonitrile mixture (1:4 ratio), lyophilized and obtained by hot molding. The anti-angiogenic effect of the implants was evaluated on chicken chorioallantoic membrane (CAM)[6]. Five days after fertilization and exposure of chorioallantoic membrane, biodegradable thalidomide and blank implants were applied over the CAM surface. PBS (pH 7.4) was applied over the CAM surface as negative control and Avastin® (bevacizumab) as positive control. After 48h the CAMs were removed from the eggs and analyzed. The effects of thalidomide implant were also evaluated in Ehrlich tumor growth model[7]. Five days after inoculation of female Swiss mice dorse with $2.5x10^6$ Ehrlich tumor cells, thalidomide and blank implants were placed into a subcutaneous pouch (through 1 cm long dorsal incision). The volume of the tumor was calculated, body weights of animals were determined periodically and tumor size was measured at the end of the experiment. 60 days after implantation, necropsy was performed with tumor removal of each animal for histopathological and morphometric analysis. The study was approved by Committee in Animal Experimentation of the Ezequiel Dias Foundation (Protocol 027/2011, Brazil). **Results:** The average percentage of blood vessels remaining in the CAM after application of thalidomide-loaded PLGA implants (75.20 ± 5.04) were significantly lower than the negative control (set as 100%) but significantly higher than that of the positive control (70.07 ± 5.41). There was no significantly difference between blank implant (98.95 ± 4.98) and the negative control ($p<0.05$). In the Ehrlich tumor growth model, thalidomide implant was able to reduce the tumor volume in 47% with no alteration in body weight of the animals. We also observed decrease in blood vessel formation, less inflammation areas and increased necrosis in the thalidomide implant group compared with the blank implant group. No difference was observed in the percentage of neoplastic areas between the groups. **Discussion:** In this study we evaluated the antiangiogenic activity and antitumor effect of thalidomide when incorporated in PLGA implants, a dosage form that permits prolonged drug release. We estimate that a major inhibition of tumor growing can be achieved by adjustment of dose and release rate. Thus, the polymeric implants of thalidomide may be an option to improve the treatment of cancer. **References:** 1 EICHHOLZ, A. OncoTargets and Ther v.3, p.69, 2010. 2 ISRAYELYAN, A. Cancer Chemother Pharmacol v. 64, p. 1201, 2009. 4 KALE, R. Drug Dev Ind Pharm v. 34, p.149, 2008. 5 YAKOUB-AGHA, I. Eur J Haematol v. 88, p. 249, 2012. 6 NOWAK-SLIWINSKA, P. Microvasc Res v. 79, p.21, 2010. 7 ZAHRAN, M.A., Bioorg Med Chem v. 16, p. 9708, 2008. **Financial Agencies:** CNPq/MCT and Fapemig

10.020

Much more than cell cycle arrest: the multitude of mechanisms induced by vincristine in glioma cells. Thome MP, Filippi-Chiela EC, Bueno e Silva MM, Lenz G UFRGS

Introduction: Vincristine (VCR) is a vinca alkaloid that binds to tubulin dimers, leading to cell cycle arrest at the metaphase due to the inhibition of microtubule assembly. Despite its wide use in cancer treatment, the mechanism of cell death triggered by VCR after cell cycle arrest in cancer cells is poorly understood. **Methods** We treated U87 ($p53^{wt}$, $PTEN^{mut}$) and U251 ($p53^{mut}$,$PTEN^{mut}$) human glioma cell lines with VCR at 1, 5, 10, 50 and 100 nM for 24h or 48h, followed by the assessment of cell growth arrest and cell death mechanisms. In addition, treated cells were replated in Drug-Free Medium (DFM) and grown to assess long term effects on cell number and senescence as well as clonogenic capacity. **Results** Acutely (24h and 48h), VCR reduced cell number in a dose-dependent way in both cell lines. U251 cells were more sensitive to the drug, while U87 cells treated with VCR 1 nM even increased cell number in relation to control. In agreement with cell number data, VCR triggered high levels of apoptosis in U251, while in U87 only 50 and 100nM of VCR induced apoptosis, at low levels. U251 cells were also more sensitive to VCR-induced G2/M cell cycle arrest, while an increase of polyploidy cells was found in U87 cells. Concerning nuclear morphometry, both cell lines presented nuclear alterations that resemble mitotic catastrophe. At a long term, VCR decreased clonogenic capacity of U251 cells after 15 days but did not induce senescence. On the other hand, the drug induced senescence in U87 cells in a dose-dependent manner. Interestingly, U87 cells treated with 1nM of VCR increased the clonogenic capacity in comparison to control cells. **Discussion** VCR exerted its toxicity through a multitude of mechanisms in glioma cells, including cell cycle arrest, apoptosis, mitotic catastrophe and senescence. In U251 cells, which are mutated for p53, VCR triggered higher sensitivity, apoptosis and G2/M arrest than U87 $p53^{wt}$ cells. On the other hand, in this latter cell line, VCR increased the number of polyploidy cells and, at a long term, induced senescence. These results suggest that the presence of p53 confers resistance to microtubules inhibitors such as VCR, allowing cells to reach a polyploidy and/or a senescent state. **Financial agencies**: CNPq, Capes and FAPERGS

10.021

Antileukaemic and antioxidant properties of eugenol-derived isozaxolines. Mendonça DS[1], Putarov NB[2], Carvalho EM[3], Aguiar AP[2], Sampaio ALF[1] [1]Farmanguinhos-Fiocruz – Farmacologia Molecular, [2]IME – Síntese Orgânica, [3]Farmanguinhos-Fiocruz – Síntese

Introduction: Rational Discovery and development of new drugs for leukaemia is still a challenge for medicinal chemistry. Bioactive isozaxoline derivatives, planned by molecular hybridization strategy, are compounds with some interesting antitumor activities such as inhibition of angiogenesis, decrease of proliferation rate, viability and induction of apoptosis in tumor cells (Kaur,K, Eur.J.Med.Chem.77.p121.2014). In the current study, we have chosen eugenol as starting material for synthesis of isozaxoline derivatives. **Methods:** The cycloaddition of four different nitrile oxides to eugenol in low pH conditions affored four hybrid prototypes: FAR2013057 – R-H; FAR2013055 – R-Br; FAR2013058 – R-OCH3; FAR2013059; R-NO2. The compounds were tested for antioxidant activity, using the DPPH assay and tumour cell proliferation (SKMEL28 and K562 cell lines) using MTT reduction assay. **Results:** Eugenol (18.7 to 300 μM) showed a low antioxidant activity, even at high concentration, 11.87% at 300 μM. The first prototype, FAR2013057 – R-H, have considerably increased the antioxidant activity to 33.82% at the same concentration ($p<0.05$). The use of different substituents at aromatic ring of nitrile oxide leads to a further increase in the antioxidant activity. The prototypes with Br (FAR2013055) and OCH3 (FAR2013058) have shown greater antioxidant activity at 300 μM (49.12% and 49.19%, respectively) when compared to the compound FAR2013057 ($p<0.05$). On the other hand, the compound FAR2013059, with a NO_2 group, have had the antioxidant activity reduced (18.63), when compared to the FAR2013057 (33.82%). All compounds when tested on the melanoma cell line SKMEL28, showed little or no significant antiproliferative effect. In the K562 assay (Chronic Myeloid Leukaemia) using eugenol (0.01-100μM; IC50 2.29 μM) was observed a little antiproliferative effect in the intermediary concentration of 1 μM (14.6%) with little improvement ($p>0.05$). The hybrid compound FAR2013055 (R-Br) have shown an important inhibitory activity at 100 μM (77.2%) that have dropped to 25.5% at the intermediary concentration of 1 μM. On the other hand, compound FAR2013058 (R-OCH_3; IC50 0.08 μM) have shown an important inhibitory activity (77.9%) in the higher concentration that have been maintained elevated in the lower concentrations 54.7% at 1 μM and 25.2% at 0.01 μM. The compounds FAR2013055 (R-Br) FAR2013058 (R-OCH3) have shown higher ($p<0.05$) inhibitory effect at intermediary concentration of 1 μM when compared to FAR2013057 (R-H). Compounds FAR2013058 and FAR2013055 have shown the best results in the DPPH and K562 proliferation assays. **Discussion:** With the data obtained, we cannot directly connect the antiproliferative effect of these compounds with their antioxidant activity. The mechanism of action of FAR2013058 and FAR2013055, for their inhibitory effect on K562, is an ongoing study and our results suggest that these compounds have a good potential for the development of new drugs for Leukaemia treatment. **Support:** Faperj E – 26 /110.032/2011.

10.022

Cytotoxicity evaluation of amazon plant extracts in cancer cell lines. Ramos INF[1], Barreto LH[1], Pinto LC[1], Soares BM[1], Uchoa AV[1], Pinheiro ACV[2], Silva MN[2], Burbano RMR[1], Montenegro RC[1] [1]UFPA – Citogenética Humana, [2]UFPA – Cromatografia Líquida

Introduction: The use of natural products to obtain antitumor drugs, reveal a huge diversity of chemical structures often unpublished and with big biological activity potential. The Amazon biome is then presented as an excellent alternative, since they have a lot of inputs that may be used on the discovery of new drugs, as well as prototypes for semi-synthesis and synthesis of new compounds. **Objective:** The aim of this study was to evaluate the cytotoxic effect of 34 extracts from Leguminosae and Connaraceae families from the amazon region in three types of human cell cancer lines: colon (HCT-116), gastric (AGP-01) and melanoma (SKMEL-19) and compared to normal cell line MRC-5 (Fibroblasts). **Methods:** To test cell survival, we performed the MTT assay. The extracts were screened for cytotoxicity against the four line cells. Cells were treated with concentrations 1,56-100 µg/mL to determine IC50 of extracts that showed better activity.The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl 2Htetrazolium bromide (MTT) and IC50's were calculated according to their standard errors and their averages using Graphpad Prism program. **Results:** Out of 34 extracts, four of them displayed cytotoxicity against all tested cell line. The most active extract was BEHGGM with a range of 3.4 and 5.4 µg/mL in SKMEL-19 and HCT-116, respectively. BEHCM presented an IC_{50} of 18.57, 26.74, 28.33 and 28.5 µg/mL; BEAGGM presented an IC_{50} of 6.8, 11.3, 11.8 and 14 µg/mL; BEEGGM presented an IC_{50} of 23.6, 28.3, 25.6 and 31 µg/mL to AGP-01, HCT-116, SKMEL-19 and MRC-5, respectively. **Discussion:** Several studies have been demonstrated the potential cytotoxic of plant extracts against cell cancer [1]desmonstrated the extracts obtained from the aerial parts of *Picrolemma sprucei* (Simaroubaceae), from the leaves and stems of *Laetia suaveolens* (Salicaceae), from the aerial parts of *Abarema auriculata* (Fabaceae-Mimosoideae) and from the stem of *A. auriculata* were cytotoxic against Squamous cell carcinoma. Another work, demonstrated that extracts prepared from Ononis hirta (aerial parts) and Inula viscosa (flowers) were the most active fractions against MCF-7 cells with IC_{50} of 27.96 and 15.78 µg/mL respectively and they were less toxic against other cell lines[2]. Based upon the initial screening work reported here, further studies aimed at the identification of active components of these four extracts and the elucidation of their mechanisms as cancer therapeutics should be performed. **Financial Agencies:** CNPq, Capes, UFPA, Fapespa **References:** 1. OZI *et al.*, Braz Oral Res. v.25, p.519. 2011. 2. TALIB *et al.*, Sci Pharm. v.78, p.33. 2010. 3. NEWMAN *et al.*, J Nat Prod. v. 23, p.311. 2012. 4. SUFFREDINI *et al.*, Fitoterapia. v.78, p.223. 2007. 5. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bodesch, H., Kenney, S., Boyd, M.R. New colorimetric cytotoxicity assay for anticancer–drug screening. *J. Natl. Câncer Insti.*, v. 82, n.13, p.1107-1112, 1990.

10.023

Lepidotrichilins A and B, New protolimonoids with cytotoxic activity from *Trichilia lepidota* (Meliaceae). Freitas WR[1], Terra WS[2], Vieira IJC[3], Filho RB[3], Kanashiro MM[1], Torres MCM[4] [1]UENF – Biologia do Reconhecer, [2]IFF-Cabo Frio, [3]UENF – Ciências Químicas, [4]UFC – Química

Species of the genus *Trichilia* (Meliaceae) are known to contain varied limonoid structures and other terpenic metabolites which are responsible for various biological properties. Limonoids of various *Trichilia* with a wide spectrum of biological effects such as potential antiviral, analgesic, insecticidal, and insect growth inhibition activity have been documented[1,2]. Additionally, it has been proved that limonoids from *Citrus* fruits demonstrated *in vitro* the capacity to induce apoptosis and to inhibit the proliferation of neuroblastoma cells[3].

In the present work, we describe the isolation and characterization of the mixture of two new protolimonoids named lepidotrichilin A and B, along with the known deoxyflindissone.

The structures of the known and new compounds were established on the basis of spectral data, mainly ^{1}H- and ^{13}C-NMR (1D and 2D), HRESIMS spectra. This work also describes the viability of human leukemic lineages cells U937 and MOLT-4 treated with the protolimonoids lepidotrichilins A and B and the protolimonoid desoxyflindissone.

Cell viability was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MTT (5.0 $mg.mL^{-1}$, 20 $\mu L.well^{-1}$) was added to the cell culture after 48 h of treatments with different samples and incubated for 4 h at 37 ˚C. After incubation, 150 µL of medium from each one well was discarded and the purple crystals of MTT were dissolved by adding 100 µL of isopropanol solution with 0.0014% of HCl. After vigorous agitation the optical density of the samples was measured in multichannel spectrophotometer with wavelength adjusted for 570 nm.

The lower IC50 value 9.3 μgmL^{-1} was observed for protolimonoid deoxyflindissone for both cells line U937 and MOLT-4. Analysis of the IC50 values shows that the isolated protolimonoid deoxyflindissone was more efficient at inducing cellular cytotoxicity than the new protolimonoids lepidotrichilin A and B. Comparing the values of IC50 for LDH release and cellular cytotoxicity we can observe that the sample concentration to induce LDH release was much higher than the sample concentration to induce cytotoxicity. Those data suggested that cell death occurred by apoptosis.

The hexane extract from the fruits of *T. lepidota* provided three protolimonoids, which were isolated in a previous phytochemical investigation. The protolimonoids named lepidotrichilin A and B are described for the first time in the literature. The protolimonoids lepidotrichilins A and B (IC50 42.7 $\mu g.mL^{-1}$) and the protolimonoid deoxyflindissone (IC50 9.3 $\mu g.mL^{-1}$) exhibited significant cytotoxic activity against the MOLT-4 and U937 leukemic cell lines. **Acknowledgments:** Faperj, CNPq and Capes. **References**: 1. Simmonds, M.S.J. *et al* . Insect antifeedant activity of three tetranortriterpenoids from *Trichilia pallida J. Nat. Prod.* **2001**, *64*, 1117–1120. 2. Pupo, M.T. *et al.* Androstane and pregnane 2β,19-hemiketal steroids from *Trichilia claussenii. Phytochemistry* **1997**, *45*, 1495–1500. 3. Poulose, S.M. *et al. Citrus* limonoids induce apoptosis in human neuroblastoma cells and have radical scavenging activity. *J. Nutr.* **2005**, *135*, 870–877.

10.024

Characterization of cytotoxic activity of compounds derived from major constituents of the cashew nut shell liquid in human oral squamous cell carcinoma. Araujo LAN[1], Alves WG[1], Matos NS[1], Romeiro LAS[1], Silveira D[1], Santos ML[2], Motoyama AB[1] [1]University of Brasilia – Health Sciences, [2]UnB – Chemistry

Background: Cancer is the second leading cause of death worldwide, and oral cancer ranks tenth among all types [1]. Chemotherapy, radiotherapy and surgery are current therapeutic options; however these are not fully efficient. Permanent functional impairment and aesthetic scars are frequent [2]. In this scenario, it is crucial to find therapeutic alternatives, including those derived from the flora, which currently provides about 1/3 of all new medicines. The cashew nut shell liquid (herein referred to as "LCC") is mostly (> 90%) made of anacardic acids, cardanols and cardols, and is currently an industrial by-product with low economic value. These constituents are made of a phenolic ring connected to a long side chain (usually C15H31-n) that can bear several to none insaturations. Additionally, a methyl group can be found in the phenolic ring [3]. Apart from current industrial uses, it has been demonstrated that some of these compounds may exert microbicide and anti-oxidative activities. Anacardic acid has been shown to be cytotoxic to lung, liver and gastric tumor cells through epigenetic mechanisms by inhibiting histone acetyl-transferases (HATs) [4] and in a capase-independent manner [5]. However, given the possible molecular diversity obtained from LCC constituents, not all distinct LCC derivatives have yet been fully analyzed or characterized. **Aim:** The aim of the present study was to screen for compounds with cytotoxic activity in oral cancer cells and characterize the observed effect. **Methods:** Constituents of LCC were extracted, purified and subjected to chemical reactions to generate new, derivative compounds. Oral squamous carcinoma cells (OSCC-3) were treated with the parental and derived compounds (total of 8) at 25ng/uL for 24h, 48h and 72h, as well as with staurosporine (300nM) and ethanol (diluent of compounds); as positive and negative controls, respectively. Citotoxicity and cell viability were measured by spectrophotometry and crystal violet assays. To investigate dose-dependency, treatment with compounds with previous promising results was additionally carried out, at concentrations of 0, 5, 10, and 25ng/uL, and cell viability was measured. In order to identify the type of cell death, DNA fragmentation studies and Western Blot for caspases were performed. **Results:** Of the eight compounds tested, four showed initial cytotoxic activity at 25ng/mL, at all incubation times analyzed. When tested for dose-dependency, two compounds induced, at concentrations between 5 and 25ng/uL, a marked decrease in cell viability, which dropped from approximately 70% (5ng/uL) to less than 20% (25ng/uL). DNA fragmentation assay showed that these compounds also induced apoptosis. These results were further expanded by western blot analyses. **Conclusion:** Compounds derived from LCC have considerable cytotoxic activity towards oral cancer cells. Studies to characterize molecular motifs that mediate these effects are underway. In this light, new therapeutic agents may be developed from the compounds tested. **Financial Support:** Capes, CNPq and Decanato de Pesquisa e Pós-Graduação/UnB. **References:** 1. American Cancer Society Cancer Facts and Figures. 2012, 2. Scully & Bagan. Oral Oncol 43: 107, 2007, 3. Santos, ML *et al. J Braz Chem Soc*, Vol. 10, No. 1, 13, 1999. 4. Sung *et al* Blood. 15; 111(10):4880, 2008. 5. Sukumar-Ramesh, *et al.* J. Neurosurg. 114: 1681, 2011.

10.025

Anti-angiogenic activity of Bothropstoxin I from *Bothrops jararacussu* (jararacuçu) snake venom: Inhibition by a KDR fragment. Sousa NC, Lorenzetti R, Hyslop S Unicamp – Farmacologia

Introduction: Snake venoms can stimulate angiogenesis via venom VEGF-like factors, or inhibit this phenomenon through enzymes such as L-amino acid oxidases, disintegrins and phopholipases A_2 (PLA_2). Basic PLA_2 from snake venoms can inhibit angiogenesis through interaction with the VEGF receptor. In this work, we used an egg-yolk model to examine the anti-angiogenic activity of bothropstoxin-I (BthTX-I), a basic Lys49 PLA_2 isolated from *Bothrops jararacussu* snake venom, as well as its inhibition by a KDR fragment that corresponds to immunoglobulin-like domain 1-7 of the VEGF receptor (Fujisawa *et al.*, *J. Biochem. Methods* **411**, 516, 2008). This fragment is frequently used to assess interaction with the VEGF receptor. **Methods and Results:** BthTX-I was purified from *B. jararacussu* venom by a combination of gel filtration (Superdex 75) and ion exchange (CM-Sepharose) chromatographies; purity was confirmed by SDS-PAGE, RP-HPLC and mass spectrometry. Anti-angiogenic activity was assayed in embryonated eggs (Kusaka M *et al.*, *Biochem. Biophys. Res. Commun.* **174**, 1070, 1991) and was assessed after 24 h by counting the number of new vessels in the absence and presence of VEGF (4 ng) and bFGF (100 ng). The ability of the KDR fragment to inhibit the anti-angiogenic activity of BthTX-I was assayed by simultaneously applying the fragment to the discs containing BthTX-I in the egg-yolk assay. In chick embryos, 0.125 μg BthTX-1 reduced basal vessel formation by 25% (from 80 ± 4 to 60 ± 5 vessels/microscopic field at 5x magnification, mean ± SD, n=8 $p<0.05$, Student's *t*-test) and 0.5 μg BthTX-1 reduced basal vessel formation by 81% (from 80 ± 4 to 15 ± 3 vessels/field; n=8; $p<0.05$, Student's *t*-test); higher amounts of BthTX-1 (1 and 2 μg) inhibited angiogenesis to the same extent as 0.5 μg. BthTX-1 (0.5-2 μg) also attenuated VEGF and bFGF-induced angiogenesis by ≥90% and ≥84%, respectively (from 178 ± 17 to 18 ± 4 vessels/field and from 140 ± 5 to 20 ± 3 vessels/field in the case of 0.5 μg; n=3; $p<0.05$, Student's *t*-test). KDR (30 μg or 3 μg) inhibited VEGF-induced vessel formation by 80% (from 80 ± 4 to 17 ± 2 or 16 ± 3 vessels/microscopic field, respectively; n=3; $p<0.05$, Student's *t*-test. At these same concentrations, KDR attenuated the anti-angiogenic activity of 0.5 μg BthTX-I by 21% (from 80 ± 4 to 63 ± 6 and 68 ± 10 vessels/microscopic field for 30 μg and 3 μg of KDR, respectively; n=3; $p<0.05$, Student's *t*-test). **Conclusions:** These results show that the anti-angiogenic activity of BthTX-1 is effectively inhibited by a KDR fragment, indicating that this biological activity of the toxin is mediated by interaction with the VEGF receptor. The license number of the Animal Ethics Committees is 4578955/67. **Financial support:** Capes, CNPq, Fapesp.

10.026

Caulibugulone A induces apoptosis by mitochondrial pathway with ROS production: a potential antineoplastic candidate. Silva MF[1], Freitas WR[1], Andreão A[2], Miranda PCML[3], Kanashiro MM[1] [1]UENF – Biology of Recognizing, [2]IFES-Aracruz, [3]Unicamp – Chemistry

Introduction: The A-D caulibugulones are four isolated isoquinoline quinones from marine Bryozoan *Caulibugula intermis*, an inhabitant of the Palau Indo-Pacific Ocean. A conducted study in the laboratory of chemical sciences of UENF showed a new route for the synthesis of caulibugulones A-D opening news perspectives for improving the knowledge on the cytotoxicity induced by these molecules. The objectives this work was to study the cytotoxic potential and mechanisms involved in death cell process induced by the synthetic caulibugulones A-D on human cancer cells. Methodology: The A-D caulibugulones were kindly provided Dr. Paulo Cesar Miranda (Unicamp) and tested against human neoplastic cell lines U937, MOLT4, COLO205, H460 and PBMC as normal cell control. The cytotoxicity was investigated by MTT assay. The apoptosis cell death was investigated by fluorescence microscopy with acridine orange and ethidium bromide staining, Annexin V-FITC and PI labeling followed by flow cytometry analysis and colorimetric assay for effector caspase 3. Apoptosis pathway was analyzed by kinetic profile of the initiator caspases (2, 4, 8 and 9), mitochondrial function with JC1 labeling and the induction of ROS production evaluated by DCFH-DA labeling. **Results and discussion:** The results obtained by MTT assay indicate that synthetic A-D caulibugulones were cytotoxic, acting selectively on tumor cells (IC50 among 0.3 – 3.8 µg/mL) compared to PBMC (IC50 greater than 2,7 µg/mL). To determine the apoptosis pathway induced by Caulibugulone A U937 cell was selected. Analysis by fluorescence microscopy showed typical apoptotic morphology in 40% of cells at 12 hours of treatment. Caspase 3 on U937 cells was activated after 24 hours of treatment with caulibugulone A. U937 cell incubated with caulibugulone A showed 96,9% of cells stained with annexin V-FITC. Analysis of initiator caspase on U937 cell treated with caulibugulone A showed at 3 hours of incubation the activity of caspases 2 and 9, suggestive of mitochondrial pathway activation. Mitochondrial activity evaluated by JC-1 labeling showed that 64.3% of U937 cell treated with caulibugulone A lost the membrane potential, confirming the intrinsic apoptosis pathway. The impairment of mitochondrial integrity promotes excessive generation of ROS, which promote the oxidation of cellular components, inducing cell death by apoptosis. U937 cell probed by DCFH -DA indicate that treatment with caulibugulone A induces strong production of ROS, with 71,65% of ROS-positive cells. NAC treatment, an inhibitor of ROS production, reduced the ROS-positive cells to 1,65% value and was also able to inhibit the cytotoxic effect of caulibugulone A on U937 cell treatment. Conclusions: The Caulibugulone A acts selectively on tumor cells and induces cell death by apoptosis through the mitochondrial pathway with ROS production. Our data point out a good perspective for further investigation of caulibugulone A on cancer treatment.

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Wipf P.; Beomjun J.; Nguyen T.; Lazo J.S. Synthesis and biological evaluation of caulibugulones AE. Org Biomol Chem 2004, 2, 2173-2174. **Funding Agencies**: CNPq, Faperj, Capes, UENF

10.027

Cytotoxic activity of (-)-chlorizidine A and derivatives. Guimarães LA[1], Jimenez PC[2], Rocha DD[1], Pinheiro DP[1], Hughes CC[3], Fenical W[3], La Clair JJ[4], Costa-Lotufo LV[1] [1]UFC, [2]Unifesp, [3]University of California, [4]Xenobe Research Institute

Marine *Streptomyces* has been extensively studied for its potential to yield unique chemical structures with anticancer activity. The marine alkaloid (-)-chlorizidine A (**1**) was recently isolated from an apparently new obligate marine *Streptomyces*, representing the first example of a natural product containing a 5H-pyrrolo[2,1-a]isoindol-5-one ring (Alvarez-Mico *et al.*, 2013. Org. Lett. 15: 988-991). In this study, the cellular activity of (-)-chlorizidine A and the semisynthetic acetate derivative **2** was explored using a combination of cellular and molecular **Methods:** (-)-Chorizidine A was also used to prepare an active immunoaffinity fluorescent (IAF) probe **3**. **1** and **2** were tested against HCT-116 and PC-3M tumor cell lines and the non-tumor cell line MRC-5 by the MTT assay. The viability of cells was investigated using flow cytometry analysis and confocal imaging at concentrations of 1 and 5 μM after 24h incubation. Compounds **1**, **2** and **3** presented cytotoxicity to both tested tumor cell lines, with IC_{50} values ranging from 2.2 to 10.6 μM, while they were inactive against the normal cell line. Analysis of cell viability showed a strong reduction in HCT-116 cells concentration after 24h incubation with compound **2** at 5μM, with an increasing number of non-viable cells, while compound **1** was not active at the tested concentration. Confocal analysis of **3** at 5 μM showed that the compound uptake occurs after 6h incubation with an increasing accumulation in the Golgi apparatus after 16h incubation, as observed by the counterstaining with norrisolide. Compound **3** was then used to identify the targeting of two cytosolic proteins GADPH and ENO1 that share common involvement in glycolysis. Studies are advancing towards the elucidation of the mode of action of these novel metabolites and the possible relation between molecular target and the cellular effect. Recently, glycolysis has been identified as a new avenue for therapeutic intervention in neurodegenerative disease and cancer. While in an early state of discovery, chlorizidine and related probes offer a new channel to further investigate the potential of GAPDH and related enzymatic pathways as viable chemotherapeutics. It is within this vein, that the discoveries within this presentation offer a refreshing look at natural product science. **Financial Support:** FUNCAP, CNPq, Capes and IFS.

10.028

Evaluation of the antitumoral activity and toxicity *in vivo* of the cerium oxide and zinc oxide nanoparticles association. Xavier AL[1], Brito MT[1], Pita JCLR[1], Santos CCL[2], Farias IAP[3], Albuquerque AJR[3], Keyson D[2], Sampaio FC[3], Antônio GS[2], Abrantes RA[4], Cruz RMD[4], Gonzaga JCO[4], Sobral MV[1] [1]UFPB – Natural Products and Bioactive Synthetics, [2]UFPB – Chemistry, [3]UFPB – Biotechnology, [4]UFPB – Pharmacy

Introduction: Nanoparticles of metal oxides have been widely used in chemical industry, semiconductor-related industries, cosmetics industry and biomedicine. However, there is little information about these particles both from a pharmacological point of view as toxicology. Thus, it has become interesting to study the ZnO + CeO nanoparticles association in order to evaluate a possible anti-tumor activity, as well as the acute toxicity induced by the intraperitoneal route. Objectives: To evaluate the antitumor activity *in vivo* model of Ehrlich Ascitic Carcinoma (EAC) of the nanometric ZnO + CeO nanoparticles association, as well as *in vivo* acute toxicity. **Methods:** Acute toxicological test in mice was performed according to OECD "Guidelines for Testing of Chemicals" n. 423/2001, and the classification was accordingly to Global Standard Harmonization (GSH). Swiss mice (*Mus musculus*), female, were submitted to single dose intraperitoneally (i.p.) of 300 and 50 mg/kg (n = 3). In order to map possible central and autonomic behavioral changes, so as death, observation to detect toxic signs was performed at the intervals: 0, 15, 30 and 60 minutes; after 4 hours and daily for 14 days (Almeida, R. N., Rev. Sci Farmacogn, 80: 72, 1999). In order to evaluate antitumor activity *in vivo*, EAC cells were implanted ($2x10^6$ cells/animal) into female mice peritoneum (n = 6). 24 hours after and for 9 days, ZnO + CeO nanoparticles association was administered (i.p.) at doses 10, 20 and 40 mg/kg. 5-Fluorouracil (5-FU) (25 mg/kg) was the positive control. The animals were euthanized and the ascites fluid collected for the assessment of total cellular count [($x10^7$ cells, which means cellular viability ($x10^6$ cells/mL) x volume (mL)], volume (mL) and tumor weight (g). Data presented as mean ± standard error of six animals compared with the control group by ANOVA followed by Dunns, and significant when $p<0.05$. All experiments were approved by the ethical committee for animal research of CBiotec / UFPB under CEUA No 0812/10. Results: Concerning *in vivo* toxicity, it was estimated the GSH 3 (> 50 – 300 mg/kg). There were no clinically relevant changes in the behavioral evaluated parameters. Concerning antitumor activity, treatments with 10, 20 and 40 mg/kg of ZnO + CeO nanoparticles association and 5-FU showed tumor weight of 5,98 ± 1,93; 3,93 ± 2,92; 0,0 ± 0,0 and 0,17 ± 0,17g; tumor volume of 5,80 ± 1,50; 2,68 ± 2,33; 0,09 ± 0,08 and, 0,01 ± 0,00mL, respectively, representing a significant decrease (control = 7,68 ± 0,32g; 7,05 ± 0,36mL). Total cellular count was reduced at 40 mg/kg (0,11 ± 0,10) and 5-FU ($8,53x10^{-4}$ ± $7,00x10^{-4}$; control = 118,60 ± 27,84). **Discussion:** ZnO + CeO nanoparticles association decreased volume, tumor mass and viability, showing potent antitumor activity *in vivo* in the EAC model. Regarding the *in vivo* assessment of toxicity, it was presented a moderate intraperitoneal toxicity of the sample when administered to mice. **Financial support**: CNPq, Capes.

10.029

Fractionation guided by cytotoxicity of extract from *Palythoa caribaeorum.* Costa AM[1], Pinto SCL[2], Pessoa ODL[2], Wilke DV[1], Costa-Lotufo LV[1], [1]UFC – Fisiologia e Farmacologia, [2]UFC – Química Orgânica e Inorgânica

Introduction: *Palythoa caribaeorum* widespread on the Brazilian coast. Previous studies have demonstrated that *Palythoa* genus is a source of steroids, nitrogenated compounds, prostaglandins, glycerol derivatives and fatty acids. From *P. caribaeorum*, we have already isolated two uncommon sulfonylated ceramides and several steroids. **Aim:** The aim of this study was to prospect antitumor compounds by bioassay-guided fractionation of the extract obtained from *P. caribeorum*. **Methods:** The extract and all fractions were assayed for their anti-proliferative activity agaisnt a human colon cancer cell line (HCT-116) through the MTT assay. The chemical investigation of this species, was conducted using methods such as Solid-Phase Extraction, High Performance Liquid Chromatography and Liquid Chromatography-Mass Spectrometry, as well the AntiMarin data base. **Results** The crude extract and its fractions (PCP-H, PCP-D, PCP-A and PCP-HD) showed potent cytotoxicity inhibiting HCT-116 cells in 5µg/mL range. Further investigation of these samples culminated on the isolation of ecdisteroids, carotenoids and fatty acids. However these compounds did not show citotoxicity. **Conclusion**: Bioguided fractionation of *Palythoa caribaeorum* extract yielded four fractions highly active, it was not possible identificate the citotoxic compounds yet. Further studies are ongoing to isolate the active substances of *P. caribaeorum* **Keywords**: Cytotocixity; *Palythoa caribaeorum*; ecdisteroids, carotenoids and fatty acids.