de tumores, e a sua inibição está associada a segregação cromossómica aberrante com consequente morte celular. Também, o aumento da expressão proteica da família anti-apoptótica BCL-2, é encontrado em diferentes tipos de cancro e sua inibição é capaz de potencializar a ação de fármacos combinados, aumentando a atividade citotóxica tumoral [2,3]. **Objetivos:** Avaliar o potencial antitumoral da combinação do inibidor da MPS-1 (BAY1217384) e do inibidor da família de proteínas anti-apoptóticas BCL-2 (Navitoclax), em culturas 2D e 3D de células de cancro do pulmão. **Material e Métodos:** Nas culturas 2D, a atividade citotóxica dos compostos BAY1217384 e Navitoclax, foi avaliada através da determinação do IC50 pelo método de MTT na linha tumoral A549 (adenocarcinoma alveolar humano). Os efeitos da combinação (efeitos antagónicos/aditivos/sinérgicos) foram determinados com recurso ao software Combenefit®. A indução da morte celular foi avaliada pelo método do TUNEL e por citometria de fluxo através da marcação com anexina V/Iodeto de Propídeo. Para avaliação da atividade antiproliferativa, foi realizado o ensaio de formação de colónias. Nas culturas celulares 3D, a viabilidade celular dos esferoides foi determinada pelo CellTiter-Glo®. **Resultados:** Nas culturas 2D, o IC50 dos inibidores correspondeu a 5,03±1,09 µM para o BAY1217384 e 13,22±0,87 µM para o Navitoclax. Verificou-se a presença de diversos pontos com efeitos sinérgicos, sendo que a combinação com as menores concentrações farmacológicas foi utilizada para as demais experiências (0,5 µM de BAY1217384 e 2 µM de Navitoclax). A combinação induziu a morte celular por apoptose (> 50%) e inibiu a formação de colónias (< 20% de sobrevivência) nas culturas 2D. Além disso, nos modelos esferoides 3D, a combinação terapêutica também foi capaz de reduzir a viabilidade celular. **Conclusões:** A combinação BAY1217384 + Navitoclax potenciou a atividade citotóxica nas culturas celulares 2D e nos esferoides 3D, destacando o potencial antitumoral da combinação em estudo.

Palavras-chave: MPS-1; BAY1217384; apoptose; navitoclax; combinação terapêutica; atividade antitumoral.

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POSTER 102

AMB-Fubinaca, AB-Chminaca and HU-308's effects on in vitro differentiation and proliferation of NG108-15 cells

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Doi: https://doi.org/10.51126/revsalus.v4iSup.369

Resumo

Introduction: Synthetic Cannabinoids (SCs) are the largest group of new psychoactive substances monitored by the EMCDDA through the EU Early Warning System. The abuse of these substances embodies major public health and social risks as they have been responsible for numerous intoxications and deaths. In particular, their abuse by adolescents and young adults (including pregnant women and women of childbearing age), is concerning as the exposure of the developing brain to these substances may lead to the onset of neurodevelopmental disorders. **Objectives:** Thus, this work hypothesizes that the abuse of SCs may also exert profound negative effects during neurogenesis. **Material and Methods:** To test this hypothesis, a neuroblastoma x glioma hybrid cell line NG108-15 was exposed to 3 SCs, AMB-FUBINACA, AB-CHMINACA and HU-308 (a selective agonist of CB2), at concentrations considered biologically relevant (1pM, 1nM and 1µM). Cell differentiation was

assessed by measuring the differentiation ratios (i.e., percentage of primary neurites per total cell number) and the total length of neurites, after 72-h incubations in differentiation medium. Cell proliferation was evaluated by the sulforhodamine B assay (SRB) up to 72 h. **Results:** AMB-FUBINACA ($p<0.01$, at 1pM and 1µM) increased the differentiation ratios and the total length of primary neurites ($p<0.05$, at 1nM). On the other hand, neither AB-CHMINACA nor HU-308 significantly affected neuronal differentiation. Since NG108-15 cells do not express the CB2 receptor, data obtained with HU-308 suggest that SC-induced neurodifferentiation in these cells does not depend on CB2 activation, although this hypothesis still needs to be clarified. None of the drugs affected cell proliferation in this cell line at the concentrations tested. **Conclusion:** These results show that one of SCs most consumed worldwide (AMB-FUBINACA) impacts in vitro neuronal differentiation, suggesting that significant post-exposure effects, that depend on the abused SC, may also occur during human neurodevelopment.

Keywords: synthetic cannabinoids (SCs); neurodevelopmental disorders; cell differentiation; cell proliferation; sulforhodamine B assay (SRB).

Acknowledgments: This work was supported by FCT—Fundação para a Ciência e a Tecnologia in the scope of the project NeuroSCANN (POCI-01-0145-FEDER-029584) and the grants UIDP/04378/2021 and UIDB/04378/2021 of the Applied Molecular Biosciences—UCIBIO) and the project LA/P/0140/2021 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB).

POSTER 103

Enantiomeric estimation of drugs consumption by gas chromatography – the role of suspended particulate matter in wastewater epidemiology

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Doi: https://doi.org/10.51126/revsalus.v4iSup.370

Resumo

Introduction: The abusive consumption of licit and illicit psychoactive drugs (PADs) is ubiquitous all over the world and is a serious public health problem [1]. Wastewater based epidemiology (WBE) is a relatively recent approach that nowadays is used worldwide as a complementary tool to the traditional drug monitoring methods to estimate drug consumption at a community level. In this context, the suspended particulate matter (SPM) plays an important role concerning the determination of PADs by WBE approach, because PADs may be adsorbed to SPM, depending on their physico-chemical properties [2]. Moreover, the evaluation of enantiomeric fractions (EF) of chiral PADs, beyond the importance for environmental risk assessment, is unexpendable to discriminate between consumption, direct disposal and synthesis pathways for identification of manufacturing locations [3,4]. **Objectives:** The aim of this study is to develop and validate an indirect method by gas chromatography coupled to mass spectrometry (GC–MS) based on chiral derivatization using (R)-(–)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride, for enantiomeric quantification and estimation of community consumption of PADs including amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxymethamphetamine (MDMA), buphedrone (BPD), butylone, 3,4-dimethylmethcathinone (3,4-DMMC), 3-methylmethcathinone (3-MMC), as well as for a better understanding on the behaviour and distribution of PADs in SPM. **Material and Methods:** Raw sewage samples collected from the inlet of a wastewater treatment plant were filtered and the SPM was extracted