New BODIPYs for PET Diagnosis on the A549 Lung Cancer Cell Line

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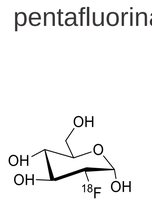
Introduction

Lung cancer is one of the most prevalent diseases in the world. Positron Emission Tomography (PET), as an accurate and metabolic associated methodology, is one of the most effective medical diagnostic methods for this pathology. Most PET radiopharmaceuticals are based on radioactive ^{18}F, which has a half-life of 109.7 minutes and can be obtained by proton bombardment of $H_2{}^{18}O$. As an important tool for medical imaging, new drugs containing ^{18}F have been investigated as a complement of the glucose derivative ^{18}F-FDG (scheme 1, structure **(1)**) or as new more efficient drugs that can correlate with new biological carriers or address new cancer targets.

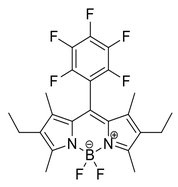
The BODIPYs (boron dipyrromethenes) are an interesting class of boron and fluor containing organic molecules that have great structural versatility and intense absorption and fluorescence optical properties. These molecules can withstand large media variations in polarity or pH, and present low self-aggregation in solution[2]. But most interestingly, BODIPYs can be radiolabeled by isotopic exchange (^{19}F to ^{18}F) on the boron center and this property is now starting to be exploited in PET. BODIPYs **(2)**, **(3)**, and **(4)** were synthetized with a boron pattern substitution that can lead to a faster radiolabeling procedure.

In this communication we show our results of synthesis and biocompatibility of BODIPY-meso-pentafluorinated **(2)**, BODIPY-phenol **(3)** and BODIPY-catechol **(4)**.



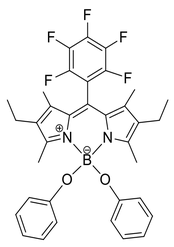
2-Deoxy-2-[18F]fluoroglucose

(1)



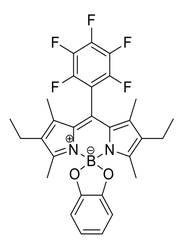
2,8-diethyl-5,5-difluoro-1,3,7,9-tetramethyl-10-(perfluorophenyl)-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide

(2)



2,8-diethyl-1,3,7,9-tetramethyl-10-(perfluorophenyl)-5,5-diphenoxy-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide

(3)



2',8'-diethyl-1',3',7',9'-tetramethyl-10'-(perfluorophenyl)spiro[benzo[*d*][1,3,2]dioxaborole-2,5'-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin]-4'-ium-12-uide

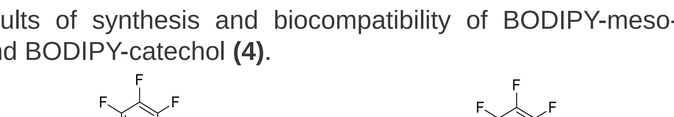
(4)

Objectives

The objectives of this work were to synthetize and characterize BODIPYs **(2-4)** and study their biocompatibility in A549 cancer cell line with the MTT test.

Materials and Methods

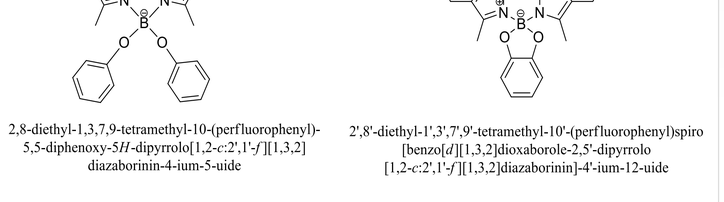
The BODIPY-meso-pentafluorinated **(2)** is formed by coupling the 3-ethyl-2,4-dimethyl-1H-pyrrole with the pentafluor-benzaldehyde in the presence of catalytic quantities of trifluoroacetic acid (TFA), resulting in the dipyrromethane that is oxidized by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), followed by the addition of boron trifluoride (BF_3Et_2O).



(2)

2,8-diethyl-5,5-difluoro-1,3,7,9-tetramethyl-10-(perfluorophenyl)-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide

The synthesis of BODIPY-phenol **(3)** and BODIPY-catechol **(4)** is based on the replacement of the fluorine atoms at the boron centre of the compound **(2)** by the phenol or catechol molecule, in the presence of a Lewis acid catalyst ($AlCl_3$).



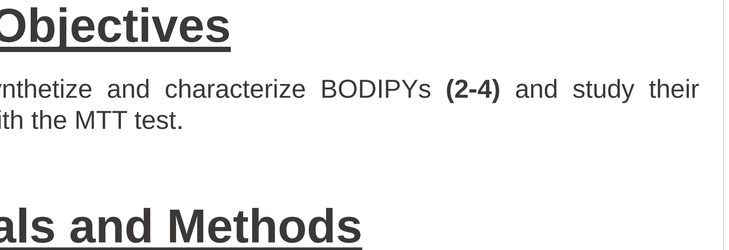
(2)

(3)

2,8-diethyl-1,3,7,9-tetramethyl-10-(perfluorophenyl)-5,5-diphenoxy-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide

For the instrumental analysis, absorption spectrums and Protonic Nuclear Magnetic Resonance (^1H NMR) were obtained.

The cells were cultured on a humidified atmosphere with 95% air and 5% CO_2, at a temperature of 37° C. Cells were seeded at a density of 200.0000 cells/mL, and then concentrations of BODIPYs between 1 and 100 µM were added to each well, and cells were incubated again.

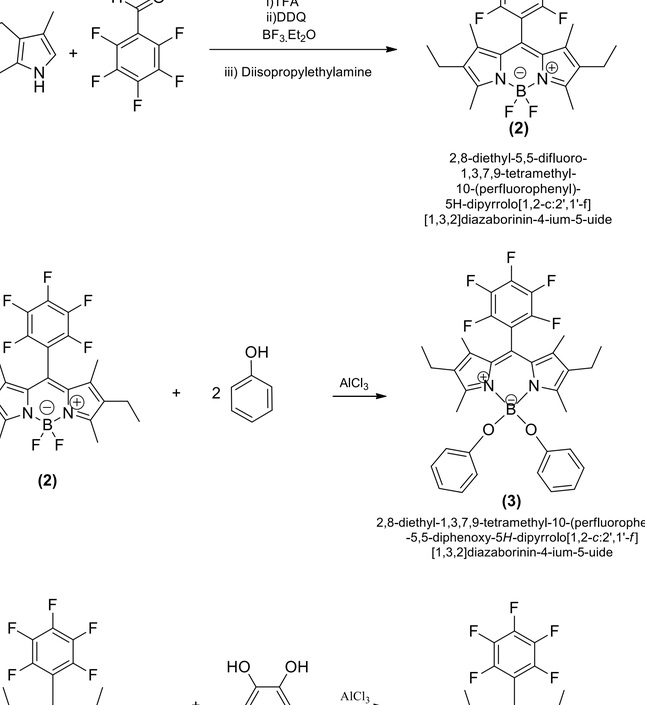


(2)

(4)

2',8'-diethyl-1',3',7',9'-tetramethyl-10'-(perfluorophenyl)spiro[benzo[d][1,3,2]dioxaborole-2,5'-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin]-4'-ium-12-uide

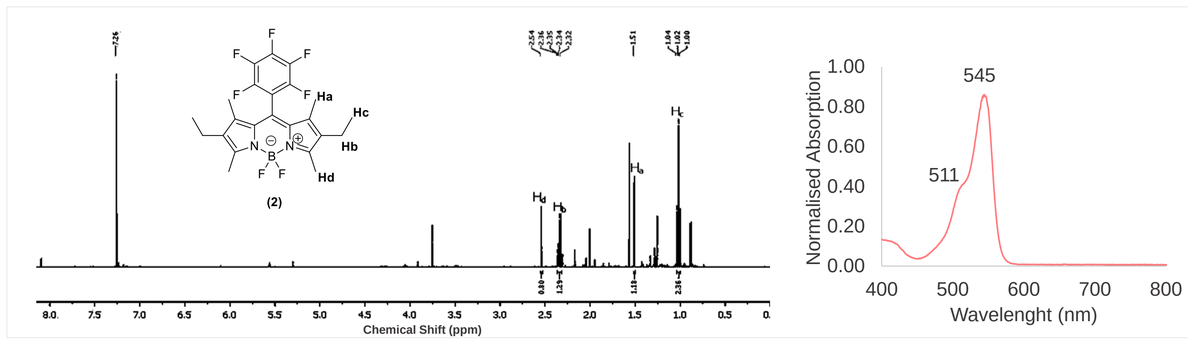
To perform the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, it was necessary to remove the culture medium from the cells (A) and wash the cells with PBS (B). 100µL of MTT were added to each well (C) and the cells were incubated (D). Then, the insoluble formazan crystals were dissolved with 100µL of a solution of 0.04M HCl in isopropanol (E). The absorbance was measured at a wavelength of 570nm, using 620nm as a reference wavelength (F), in a multiwell plate reader.

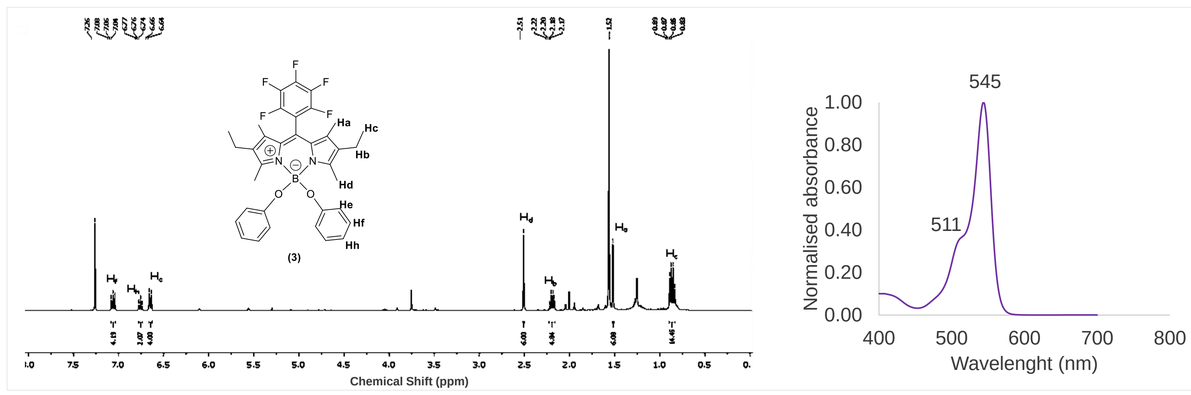


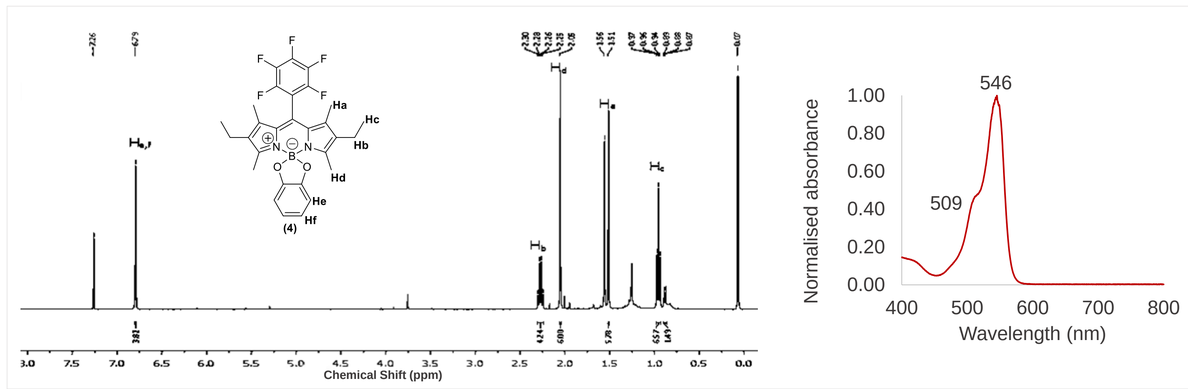
The statistical analysis of the experimental values was performed using the software GraphPad Prism version 9.0.1 for Windows, GraphPad Software, USA. The comparison of the metabolic activity of the cells for each concentration was done with the One-Sample t test (assuming a normal distribution of values). Significances were corrected with the Bonferroni-Dunn test, with a significance level of 0.05. When $p< 0.05$, significances are represented by *, $p< 0.01$ significances are represented by **, and with $p<0.01$ by ***.

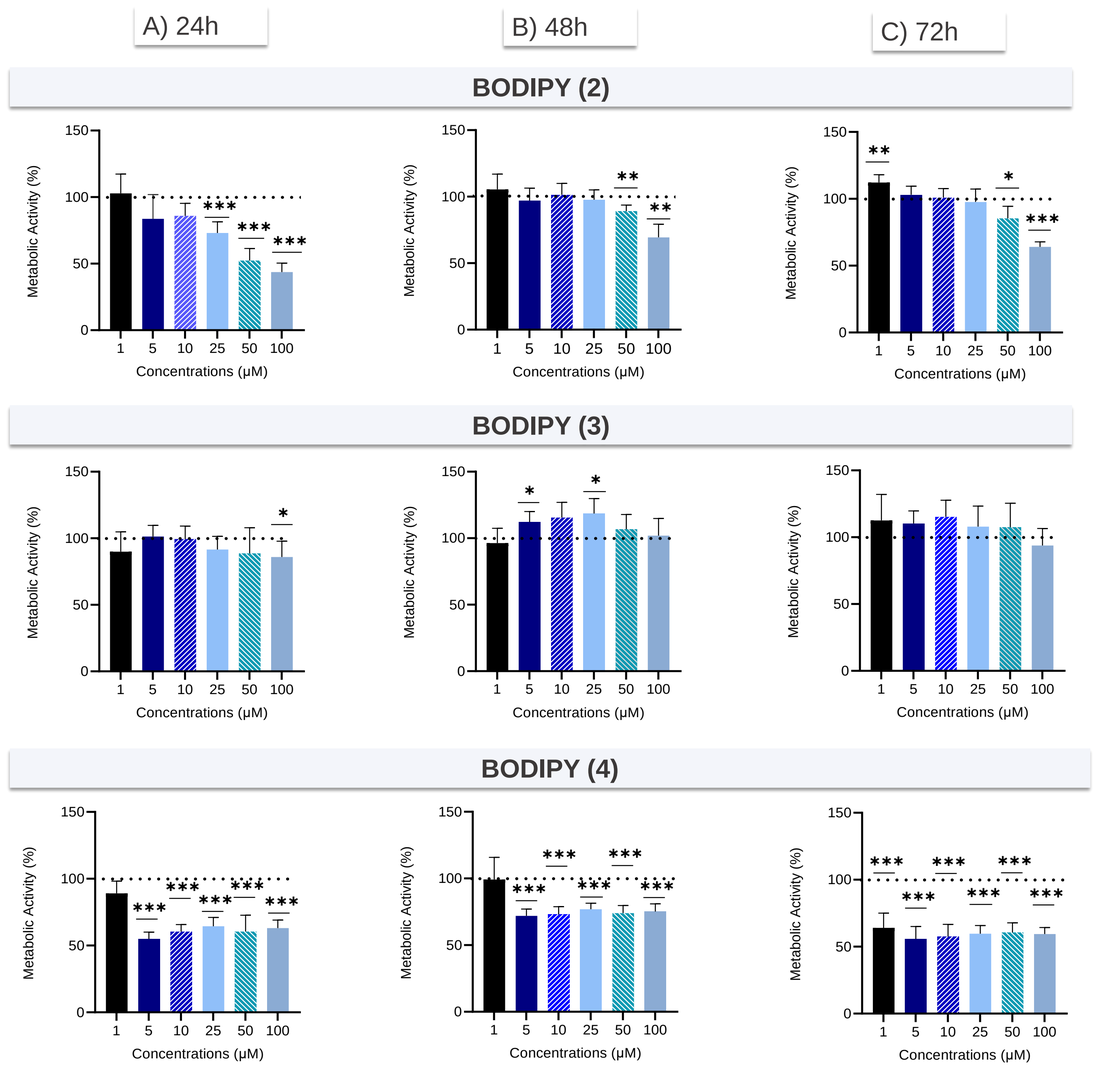
Results

Absorption Spectrums were obtained with an Ocean Optics UV-VIS-NIR Light Source, with CH_2Cl_2 as solvent; and ^1H RMN were obtained using a Bruker AVANCE III NMR (at 400 MHz for the proton) with $CDCl_3$ as solvent.









BODIDY **(2)** leads to a concentration-dependent decrease in metabolic activity. Despite the initial decrease in metabolic activity, cell cultures appear to recover at least at concentrations up to 25µM. The promising BODIDY **(3)** does not influence the cellular metabolic activity for the tested concentrations. BODIDY **(4)** leads to some decrease in metabolic activity, apparently in a time-dependent manner.

Conclusions

The synthesis and characterization of the target compounds was successfully made, and our MTT results showed that the cells tend to maintain their metabolic activity above 80% for BODIPYs **(2)** and **(3)** at concentrations such as 1, 5, 10 and 25 µM. In conclusion, Compounds **(2)** and **(3)** seem to be the best candidates for the next steps of isotopic exchange (^{18}F-^{19}F). Other biocompatibility studies are being started such as SRB (Sulforhodamine B), Cellular Uptake and Haemolysis and Coagulation tests.

References

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[2] Vaquero, J. J.; Kinahan, P. Annu. Rev. Biomed. Eng., 2015, 17 (1), 385–414.
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