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Novel BODIPY Photosensitizers and corresponding nano-conjugates in an *in vitro* antimicrobial and anticancer study

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ABSTRACT

Introduction

The photon-based initiated therapeutic modalities particularly photodynamic therapy (PDT), have shown significant clinical potential in surface-based and deep-seated tumor treatment as well as antimicrobial and antiviral treatment. Photodynamic therapy involves the use of photosensitizers, a directed light of specific wavelength, and molecular oxygen to generate phototoxic oxygen species of which cell death is realized. Boron dipyrromethene (BODIPY) photosensitizers (PSs) have in recent years gained much attention due to their interesting photophysical and photochemical properties and their potential application as anti-microbial and anti-cancer agents. Despite the progress made in this field, the design, synthesis, and modification of BODIPY PSs as anti-cancer and anti-microbial agents has been slow.

Methods

This study focuses on the design, synthesis, functionalization of BODIPY PSs, and Platinum-based nanoconjugates and their characterization. An acid condensation of an aldehyde and a pyrrole method was used for the synthesis of novel BODIPY PSs. The functionalization of some BODIPY PSs was carried out using the amide bond linker to afford sequential extended conjugation of iodine functional groups. The influence of functional groups on the photoactivity of the novel PSs was investigated with the attention on BODIPY PSs containing carboxylic acid (-COOH), amine (NH_2), sulfamoyl- (SO_2NH_2), Sulfonic acid (SO_3H), and iodine (I) groups. Synthesized PSs were screened for their anti-microbial activity using the disc diffusion technique.

Stock solutions of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis* were obtained from the Department of Oral Biological Science

microbiology laboratory, University of the Witwatersrand. The minimum inhibitory concentration assays were carried out in 96 well plates, bacterial cultures were cultured in Mueller Hinton broth (MHB) and *Candida* was cultured in sabouraud dextrose broth (SAB). For preliminary cytotoxicity studies, the normal HaCat cells and human UCT Mel-1 cancer cells were obtained from the University of Cape Town, gifted by Dr. E.L. Wilson, Department of Haematology, at the Groote Schuur Hospital, Cape Town. All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin incubated at 37 ºC, 5% CO_2 until 90% confluence was reached. Phototoxicity of microorganisms with BODIPY PSs was carried out using the 32 (65 mW/cm^2) LED lights mounted on a stand, placed at a height of 5 cm from cells, and irradiated for 45 minutes. Comparative phototoxicity study of HaCat and UCT Mel-1 cell lines with BODIPY PSs and hypericin was carried out with a ~1.5 J/cm^2 lightbox with two F15T8 15W/UVA PUVA lamps (wavelength 320-410 nm/peak 351 nm).

Results

BODIPY PSs were successfully synthesized their photochemical properties were studied with UV-Vis and fluorescence. The results showed that upon oxidation the fluorescence is quenched by 40% for BODIPY 1 and 50% for BODIPY 3. Moreover, following functionalization with bulk units the fluorescence intensity was quenched by 45 % for BODIPY 8 to 10. Furthermore, functionalization with iodine groups resulted in negligible to no fluorescence of BODIPY PSs. Their structural elucidation was confirmed with mass spectrometry, FTIR, XRD, and NMR and the results confirmed the synthesis of the desired BODIPY PSs. BODIPY 1 to 5, 7 to 9, and 6.1 to 6.5 showed negligible phototoxicity in anti-microbial studies with MIC >10-30 mg/mL. However, BODIPY 10 showed activity with a MIC 2.08 mg/mL for *S. pyogenes* and 4.17 mg/mL for *C. Albicans*. Furthermore, BODIPY 6 showed anti-microbial activity at MIC of 0.26 mg/mL against bacteria and >0.25 pM against fungal species. After further purification with silica, biobeads and Sephadex BODIPY 6 PSs showed a MIC of 65-130 µg/mL for *Candida*. BODIPY 6.6 with extended iodine moieties showed MIC of 130 µg/mL upon irradiation for 45 minutes. The Pt-nanoconjugates showed an improvement of 10% for bacterial and 18% for fungal induced cell killing capacity of the BODIPY PSs. For cytotoxicity studies, both BODIPY 6 and BODIPY 6.6 showed phototoxicity efficacy at a concentration <3 µM. BODIPY 6 showed a cell viability of 56% compared to its counterpart hypericin with 46% while the cell viability for BODIPY 6.6 was

60%. Pt-nanoconjugates also demonstrated an improved cell killing efficacy of BODIPY PSs which is desired for targeted and enhanced PDT activity.

Conclusion

BODIPY PSs have demonstrated potential for use as agents for photodynamic inactivation of microbial species as well as anti-cancer PDT agents for melanoma cell lines. BODIPY PSs showed that the activity for antimicrobial application follows the order $H<COOH<NH_2<SO_3H<SO_2NH_2$ respectively. Furthermore, iodine substituted BODIPY induced both anti-microbial (65 µM concentration) and anticancer (<3 µM concentration) properties even at lower concentrations. No statistically significant differences between hypericin and BODIPY 6 p-value 0.40 were observed, but in comparison with BODIPY 6.6, there was a significant statistical difference p-value 0.0074 after irradiation with 1 J/cm^2 light. These results demonstrated that BODIPY 6 showed more activity for both microorganisms compared to its counterpart BODIPY 6.6. Pt-nanoconjugates improved the solubility and photoactivity of the novel BODIPY PSs. Phototoxicity analysis studies demonstrate that BODIPY 6 and BODIPY 6.6 exhibit potential for their application in photodynamic inactivation (PDI) and PDT of microorganisms and melanoma skin cancer cells respectively. Therefore, these BODIPY PSs have the potential to serve as anti-cancer and antimicrobial agents.