Abstract

The development of facile and reliable methods to image and detect important biomolecules has drawn considerable attention owing to their potential applications in clinical, bioanalytical and forensic analysis. One-photon microscopy (OPM) has traditionally been used in cell biology research. However, probes based on OPM are associated with shortcomings including photobleaching, cell damage, and intracellular autofluorescence interference. Many researchers are seeking better tools to overcome these obstacles. Two-photon microscopy (TPM) is a convenient and powerful tool to explore the intracellular environment and provides the opportunity to overcome the abovementioned obstacles. Probes based on TPM have become important for bioimaging and sensing because of their low photodamage, reduced fluorescence interference, and better tissue penetration depth. With the development of fluorescence molecules in recent decades, a wide range of organic fluorescence probes based on TPM has been rapidly developed and used in biomedicine and bioimaging. Cyanine dye, one of the classic synthetic dyes, continues to be used in many fields, especially in bio-related applications, owing to its ability to interact with biomolecules through non-covalent and electrostatic bonds. Based on cyanine models, we designed a series of structural modifications of cyanine fluorophores used as two-photon (TP) probes to detect and image the intracellular environment in which new cyanine compounds, namely SLSO₃, SLCOOH-Pr, F-SLOH, SLOH, Me-SLM, SLE, SAM, SAOH, SLG, F-SPG, SLOH-Pr, SLAD, F-SLAD, Me-SLG, SLNA, SLAD-Pr, SLCOOH, SLAce, SLM, SPC, SIOH, PSIOH, DMA-SLOH, DBA-SLOH, DPA-SLM, GBPM, HBBM, HBLM, SBM, SIBM, SIM, PLOH, and PTM, was successfully synthesized. All of these newly designed compounds were characterized with ¹H
NMR, $^{13}$C NMR, and HRMS and found to show good agreement with the desired structures. To our surprise, some of the novel cyanine molecules were also able to detect and image amyloid-β (Aβ) peptide species and showed excellent biological properties including neuroprotective effects against the cytotoxicity induced by different forms of Aβ species, blood–brain barrier permeability, and high in vivo stability.

The photophysical and biological properties of these newly synthesized compounds included optical properties such as UV-vis absorption, emission, fluorescence quantum yield in different solvents, dissociation constant determined by fluorescence titration, and circular dichroism spectroscopy, cytotoxicity assay, neuroprotection, and inhibition of Aβ aggregation were investigated.
SBM

SIBM

SIM

SIOH

PSIOH

PLOH

DPA-SLM

R = (CH₂)₂O(CH₂)₂OCH₃
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