Abstract

Alzheimer's disease (AD) is a chronic neurodegenerative disorder in which the aggregation of amyloid β (Aβ) and tau protein plays a key role. Despite advances in immunochemical and imaging methods, early, accessible, and cost-effective AD diagnostics remain challenging. One promising option is small fluorescent molecular probes that could detect AD biomarkers in body fluids by fluorescence measurements. This thesis focused on designing, synthesizing, and evaluating small organic D–π–A fluorophores for the detection of AD-associated aggregates.

We prepared four generations of rod-like fluorophores, differing in electron donor (D) and electron acceptor (A) groups, as well as in the length and structure of the π-conjugated system (π). The first generation comprises ketones, β-diketones, β-enaminones, and malononitriles with phenyl, naphthyl, naphthyl–phenyl, and stilbene π-skeletons. The second generation is based on variously substituted 2*H*-pyran-2-ones, the third on 3-acetyl and BF_2-β-diketonate 2*H*-pyran-2-ones, and the fourth on conjugated alkenes with a nitro group. The fluorophores were synthesized using conventional and contemporary organic synthetic methods, including the Suzuki–Miyaura and Heck reactions, the Horner–Wadsworth–Emmons reaction, the Bucherer reaction, Buchwald–Hartwig amination, mixed Claisen condensation, Knoevenagel condensation, cyclization to the 2*H*-pyran-2-one ring, and the Henry reaction. In this way, four structurally diverse generations of compounds were prepared by systematically varying the electron-donating groups (dimethylamino, azetidinyl, pyrrolidinyl, piperidinyl, and *N*-methyl-2-hydroxyethylamino), the π-conjugated systems (phenyl, naphthyl, phenyl–naphthyl, and stilbene systems, as well as extended conjugated alkenes containing phenyl, naphthyl, or stilbene aromatic scaffolds), and the electron-accepting fragments (ketones, β-diketones, β-enaminones, malononitriles, variously substituted 2*H*-pyran-2-ones, their BF_2-β-diketonate analogues, and the nitro group). This enabled us to systematically investigate the influence of the donor, π-conjugated system, and acceptor on the optical and binding properties of the compounds.

The optical properties of the prepared fluorophores were studied in solvents of different polarity, namely hexane, dichloromethane, acetonitrile, methanol, and HEPES buffer. We showed that absorption and emission maxima, Stokes shifts, and quantum yields are significantly influenced by the nature of the electron acceptor A and the length and structure of the π-conjugated system. Extension of the π-system and stronger electron acceptor motifs generally led to a bathochromic shift of absorption and emission, in some cases even into the near-infrared region.

An important part of the thesis also involved *in vitro* evaluation of the optical and binding properties of the prepared fluorophores in the presence of $A\beta_{1-42}$ fibrils. The results showed that these properties are not determined solely by the length of the π-conjugated system, but also by the nature of the aromatic skeleton and by the electron acceptor and electron donor groups. Across several generations of compounds, an appropriate combination of these structural features provided a favorable balance between affinity for $A\beta_{1-42}$ fibrils and the position and intensity of the emission maxima upon binding.

Keywords: Alzheimer's disease, amyloid β, D–π–A, fluorophore, 2*H*-pyran-2-one, nitroalkene, optical properties, binding properties.