MEDICAL UNIVERSITY OF LODZ
FACULTY OF PHARMACY
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, DRUG ANALYSES AND RADIOPHARMACY

NOVEL MULTIFUNCTIONAL ACRIDINE HYBRIDS AS ACETYLCHOLINESTERASE INHIBITORS FOR ALZHEIMER'S DISEASE TREATMENT

PhD THESIS

Nina Chufarova, MSc

Supervisor: Pawel Szymanski PhD DSc
Associate Professor
Vice-Dean for Didactics
Faculty of Pharmacy
Medical University
Department of Pharmaceutical Chemistry, Drug Analyses and Radiopharmacy

Lodz, Poland, 2018

Abstract

Alzheimer's disease (AD) is one of the forms of the dementia. One of the biggest risks for the development of the AD is age – the biggest risk is for the patients in age group above 65 years old.

Pathophysiology of the disease still remains unclear. One of the hypothesis is amyloid-β (Aβ) theory, which refers to the accumulation of the amyloid in neuronal cells. The amyloid plaques are formed by pathologic amyloidogenic polymeric peptides that are the result of enzymatic cleavage of the Aβ precursor protein. The other hypothesis is connected to the formation of neurofibrillary tangles that consist of tau protein in the brain cells. Tau protein is responsible for normal functioning of the neuronal cell cytoskeleton. Hyperphosphorylated tau protein forms tangles, which lead to destruction of neuronal cytoskeleton and thus neuronal degeneration. Nevertheless, the most popular hypothesis is the cholinergic one. It says that the progression of AD may be one of the results of decreased activity of acetylcholine transferase in cholinergic neurons. Simultaneously, the synthesis of the acetylocholine and serotonin also decreases. Drugs developed using this theory as the basic one are currently used in AD therapy; nevertheless, all of them provide only symptomatic treatment.

The current work represents synthesis, biological tests and computer modeling of the series of novel promising tacrine hybrids for therapy of the Alzheimer's disease (AD). Firstly, 16 new tacrine-acridine hybrids with different carbon linker length were synthesized. After synthesis, 8 of the synthesized compounds (as hydrochlorides) were tested *in vitro* for the ability to inhibit acetylocholinesterase (AChE) and butyrylcholinesterase (BuChE) activity. The most promising compound **3d** (IC_{50} = 7.6 pM for AChE and 1.7 pM for BuChE) appeared to have higher activity comparing to tacrine (IC_{50} = 89914.1 pM for AChE and 14982.4 pM for BuChE); kinetic studies revealed mixed type inhibition. After that, the most promising compound **3d** was tested for possible additional biological activity – ability to inhibit Aβ aggregation. The activity of compound **3d** ranged from 31.66% for the lowest concentration of 10 μM to 57.77% for 100 μM concentration. To evaluate possible toxic effects cytotoxicity tests were conducted. At the highest concentration (100 μM), compound **3d** exhibited 43% cell viability, that is increasing with the decrease in the concentration of the active compound. The compound showed no significant cytotoxic effect in the tested concentrations. At the end, docking studies using methods of computer modeling were performed to visualize binding mode of the inhibitor **3d**. It showed dual-binding mode for AChE, by binding to catalytic anionic site and peripheral anionic site simultaneously. Thus, compound **3d** is a promising multifunctional hybrid for further *in vitro* and *in vivo* studies for AD treatment.