1. Raziskovalna organizacija:

Univerza v Ljubljani, *Fakulteta za farmacijo*

2. Ime in priimek mentorja:

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3. Področje znanosti iz šifranta ARRS:

1.09 Farmacija

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5. Kratek opis programa usposabljanja:

Alzheimerjeva bolezen (AB) je najpogostejša oblika ireverzibilne demence, ki se konča z dokončnim propadom kognitivnih funkcij. Bolniki so v zadnjih stadijih bolezni povsem odvisni od nege svojcev ali medicinskega osebja, smrt pa nastopi približno devet let po diagnosticiranju. Kljub temu, da AB predstavlja veliko breme tako za svojce bolnika kot za celotno družbo, zdravila, ki bi odpravilo vzroke za razvoj AB, še niso odkrili. Trenutno zdravijo bolnike z AB z zaviralci acetilholin esteraze (AChE) in butirilholin esteraze (BChE), kot sta donepezil in rivastigmin. Ta zdravila povišajo koncentracijo acetilholina v sinapsi in za nekaj mesecev ublažijo kognitivni upad.

V zadnjem času intenzivno preučujejo strategije, s katerimi bi lahko spremenili potek AB. V možganih bolnikov z AB so našli senilne plake z ekstracelularnimi depoziti amiloida β (Aβ). Z nadaljnjimi poskusi so ugotovili, da ima Aβ, ki je sicer proteolitski derivat velikega transmembranskega proteina APP, zgodnjo in pomembno vlogo pri vseh oblikah AB. Še posebej je pomemben najbolj netopen, 42 aminokislin velik peptid Aβ42. Odkrili so, da tako monomerne oblike Aβ42, kot njegovi agregati v obliki amiloidnih plakov, delujejo nevrotoksično. Agregacijo Aβ42 povzroča več dejavnikov, med njimi tudi kovinski ioni in periferno mesto na encimu AChE. Nedavno so odkrili, da lahko tudi katepsin B cepi APP do Aβ, zato ta encim predstavlja novo atraktivno tarčo za razvoj nevroprotektivnih spojin. Z vplivom na nastanek in agregacijo Aβ42 bi lahko odpravili enega izmed vzrokov AB in tako spremenili sam potek bolezni.

Mladi raziskovalec se bo posvetil načrtovanju, sintezi in biološkemu vrednotenju novih spojin s potencialnim delovanjem na razvoj in simptome AB. Spojine bodo načrtovane tako, da bo v eno molekulo združeno delovanje na več tarč, povezanih z AB. Spojine bodo zavirale AcChE, BChE in katepsin B, zavirale agregacijo Aβ in kompleksirale nekatere kovinske ione. Nove zaviralce tarčnih encimov bomo iskali s pomočjo virtualnega rešetanja na osnovi objavljenih kristalnih struktur kompleksov z ligandi. Iz začetne knjižnice "ZINC drug like" bomo s filtriranjem najprej odstranili neželene spojine. Filtrirano in obogateno knjižnico spojin bomo sidrali v aktivno mesto encimov s pomočjo različnih programov in na koncu naročili najbolje ocenjene spojine. Le-te bomo biokemijsko ovrednotili v encimskih testih. V kontekstu izboljšanja aktivnosti bomo najprej naročili 2D in 3D analoge najbolj aktivne spojine, nato pa bomo sintetizirali tudi ciljano knjižnico sorodnih spojin. V nadaljevanju bomo s sintezo ciljanih knjižnic spojine strukturno optimizirali. Pričakujemo, da bomo s pomočjo računalniških metod odkrili mikro- do nanomolarne zaviralce tarčnih encimov, ki jim bomo dokazali delovanje v in-vitro modelih AB.

Raziskave, ki jih bo izvajal mladi raziskovalec, predstavljajo izviren, inovativen in do sedaj neizkoriščen pristop k predkliničnemu razvoju novih zdravil za zdravljenje Alzheimerjeve bolezni.

Alzheimer's disease (AD) is the most common form of irreversible dementia, which ends with the complete collapse of the cognitive functions. Patients in the last stages of the disease are completely dependent on the care of relatives or medical personnel. Death occurs approximately nine years after the diagnosis. Despite the fact that AD is a heavy burden for both patients's relatives and the society as a whole, drugs that eliminate the causes for the development of AB have not yet been discovered. Acetylcholine esterase (AChE) inhibitors, such as donepezil and rivastigmine, are prescribed for patients with early to mid-stage AD. These drugs increase the levels of acetylcholine in the synapse and postpone the cognitive decline for a few months. The enzyme butyrylcholinesterase (BChE), which is structurally and functionally related to AChE, also hydrolyses and inactivates acetylcholine. It has been shown that the activity of AChE in patients with late stage AB is reduced, while the expression and concentration of BChE are compensatory increased, suggesting that in the late stages of AD the enzyme BChE modulates the hydrolysis of acetylcholine. Furthermore, the BChE knockout mice showed no physiological disadvantages and silent mutants in humans had a slower rate of cognitive decline, which inspired the hypothesis that BChE may be an attractive target enzyme for developing anti-AD drugs.

Currently, the strategies that could alter the course of AB are being intensively studied. In the brains of patients with AD, senile plaques were found with extracellular deposits of amyloid β (Aβ). Further experiments revealed that Aβ, which is a proteolytic derivative of the large APP transmembrane protein, plays an important early role in all forms of AD. Recently, cathepsin B (CatB) was demonstarted to participate in the cleavage of APP to Aβ. The knock-out animal models and irreversible inhibitors further validated CatB as a new target for the development of cure for AD. Since the neurotoxicity is associated with the formation and accumulation of Aβ species (mainly $A\beta_{1-42}$), targeting the Aβ aggregation represents an emerging approach for the discovery of novel neuroprotective agents. Aβ42 aggregation is caused by several factors, including metal ions. The impact on the formation and aggregation of Aβ42 could eliminate one of the causes of AD and could also change the course of the disease itself.

Young researcher will focus his attention to design, synthesis and evaluation of new compounds as potential agents for the treatment of AD. All compounds will be carefully designed so that they will incorporate the activities against different targets (AcChE, BChE cathepsin B, inhibition of Aβ aggregation, and complexation of ions) in a single molecule. First, the »ZINC drug like library« will be filtered in order to remove the compounds with unwanted properties. Filtered and enriched library of compounds will then be docked to the active sites of target enzymes through a variety of programs and finally the hits will be ranked with the scoring functions. The best ranked compounds will be evaluated in biochemical enzymatic assays. The analogues of the most potent compounds will be purchased after performing 2D and 3D computational similarity searches, or focused libraries of related compounds will be synthesised. We expect that we will discover micro- or nanomolar inhibitors of target enzymes, which will also be active in-vitro AD models.

This research performed by young researcher will constitute an original, innovative and so far unexploited approach to preclinical development of new drugs for the treatment of Alzheimer's disease.