6-Methyluracil derivatives as acetylcholinesterase inhibitors for treatment of Alzheimer’s disease


**BACKGROUND:** Alzheimer’s disease (AD) is the major age-related progressive neurodegenerative disorder. The brain of AD patients suffers from loss of cholinergic neurons and decreased number of synapses [1]. AD is caused by an imbalance between Aβ production and clearance, resulting in increased amount of Aβ in various forms [2]. Reduction of Aβ production and increasing clearance of Aβ pathogenic forms are key targets in the development of potential therapeutic agents for AD treatment. Unfortunately, only nosotropic approaches for treatment of AD are currently effective in humans. These approaches mainly focus on the inhibition of brain acetyl-cholinesterase (AChE) to increase lifetime of cerebral acetylcholine [3]. It is important to emphasize that AChE itself promotes the formation of Aβ fibrils in vitro and Aβ plaques in the cerebral cortex of transgenic mouse models of AD [4]. This property of AChE results from interaction between Aβ and the peripheral anionic site of the enzyme (PAS) [5]. Dual binding site inhibitors of both catalytic active site (CAS) and PAS can simultaneously improve cognition and slow down the rate of Aβ-induced neural degeneration. Unfortunately, the assortment of AChE PAS ligands is still extremely limited.

**OBJECTIVE:** To study putative advantages of AChE non-charged PAS inhibitors based on 6-methyluracil derivatives for the treatment of Alzheimer’s disease.

**METHODS:**

*In vitro studies.* Concentration of drug producing 50% of AChE/BuChE activity inhibition (IC50) was measured using the method of Ellman et al. [6]. Toxicological experiments were performed using IP injection of the different compounds in mice. LD50, dose (in mg/kg) causing lethal effects in 50% of animals was taken as a criterion of toxicity [7]. The ability of compound to block in vitro AChE-induced Aβ1−40 aggregation was studied using a thioflavin T (ThT) fluorescent probe [8].

*In vivo biological assays.* For in vivo blood–brain barrier permeation assay brains were removed 30 min after IP injection of LD50 dose of tested compound injection. The inhibitory potency was measured using the method of Ellman.
Scopolamine and transgenic models of AD were used to evaluate the influence of compound 35 on spatial memory performance. Water solution of scopolamine was injected to mice (ip) 20 minutes before starting memory test during 14 days [9]. Mice were assigned to 7 groups, including 4 groups receiving injection (ip) of compound in different dosages, donepezil-treated mice (donepezil is conventionally used to treat Alzheimer’s disease), positive and negative control groups. Double transgenic (APP/PS1) mice expressing a chimeric mouse/human amyloid precursor protein and a mutant of human presenilin-1 [10] were assigned to 4 groups, including transgenic animals injected (ip) with compound 35 or donepezil solution, positive (transgenes injected with water) and negative (wild-type mice) controls.

To evaluate spatial memory performance, mice were trained on a reward alternation task using a conventional T-maze [11]. The criterion for a mouse having learned the rewarded alternation task was 3 consecutive days of at least 5 correct responses out of the 6 free trials.

For β-amyloid peptide load was evaluated quantitatively as a number and summary area of Thioflavine S fluorescent spots in cerebral cortex and hippocampal images using Image J program. Statistical analyses were performed using the Mann-Whitney test.

**RESULTS:** We evaluated the acute toxicity of the most active compounds. The most potent AChE inhibitor compound 35 (IC50 (AChE) = 5 ± 0.5 nM) exhibited the lowest LD50 values (51 mg/kg) and inhibited brain AChE by more than 71 ± 1%. Compound 35 at 10 nM, exhibited a significant (35 ± 9%) inhibitory activity toward human AChE-induced Aβ aggregation.

Scopolamine injection induced significant decrease in correct choice percentage in T-maze, as well as decrease in percentage of mice reaching criterion for learning the task by day 14. This memory deficit was relieved to some extent either by compound 35 (5 mg/kg) or donepezil (reference compound) treatment (0.75 mg/kg). Interestingly, higher doses of compound 35 (10 and 15 mg/kg) produced less therapeutic effect on spatial memory deficit.

Group of APP/PS1 mice showed 3 times lower percentage of reaching behavioral criterion and lower percentage of correct choice in T-maze alternation task comparing to WT mice, whereas compound 35 (5 mg/kg) or Donepezil treatment effectively improved these parameters in APP/PS1 mice.

Compound 35 treatment (5 mg/kg) during 14 days significantly reduced percentage of summary area and number of β-amyloid peptide (βAP) deposits visualized in sections of cerebral cortex, dentate gyrus, and hippocampal CA3 area in APP/PS1 mice. The most prominent reduction of βAP load by compound 35 treatment was found in CA3 area and cerebral cortex. Meanwhile, Donepezil treatment (1 mg/kg) during 14 days significantly reduced βAP load in cerebral cortex but not in dentate gyrus and CA3 area.

**CONCLUSIONS:** Experiments showed that the most potent AChE inhibitor compound 35 (6-methyluracil derivative) permeated the blood-brain barrier, improved working memory in the APP/PS1 transgenic mice and significantly reduced the number and area of Aβ plaques in the brain. Thus, compound 35 is a promising candidate as a bi-functional inhibitor of AChE for treatment of AD.

Keywords: Alzheimer’s disease, acetyl-cholinesterase inhibitors, methyluracil derivatives

**Conflict of interest statement:** Authors declare no conflict of interests.

**References**


