COMPARATIVE IMAGING OF THE HAEE-CY5 AND EEAH-CY5 TETRAPEPTIDE BINDING TO THE Aβ AGGREGATES ON THE SH-SY5Y CELLS

Ivanova AV[1,2] ✉, Chmelyuk NS[1,2], Kuzmichev IA[1], Shilyaeva MI[1], Abakumov MA[1,2]

[1] Research Institute of Translational Medicine, Pirogov Russian National Research Medical University, Moscow, Russia

[2] Institute of Biomedical Engineering, MISIS University of Science and Technology, Moscow, Russia

Due to high diagnostic value of β-amyloid aggregates, the target ligands capable of specific binding to abnormal Aβ aggregates are of special interest. The study aimed to perform comparative characterization of the HAEE-Cy5 (Ac-His-Ala-Glu-Glu-Gly-Gly-Gly-Gly-Lys(ε-Cy5)-NH2) and EEAH-Cy5 (Ac-Glu-Glu-Ala-His-Gly-Gly-Gly-Gly-Lys(ε-Cy5)-NH2) tetrapeptide capability of binding to the Aβ aggregates in the SH-SY5Y human neuroblastoma cell line by confocal microscopy. It has been shown that the HAEE-Cy5 tetrapeptide demonstrates specific binding yielding typical cytoplasmic clusters and clear co-localization with the amyloid aggregates, while the EEAH-Cy5 peptide with the inverted sequence totally loses the binding capability. Quantification has confirmed high specificity of the HAEE-Cy5 binding to the Aβ aggregates (Manders' colocalization coefficient 0.58 ± 0.03). It has been found that the histidine N-terminal position is a critical determinant of the interaction specificity. The findings offer the prospects of using the HAEE peptide as a platform for the development of targeted disgnostic systems for amyloid disorder imaging.

Keywords: HAEE peptide, EEAH peptide, Aβ aggregates, SH-SY5Y, Alzheimer's disease

Funding: the work was carried out under the State Assignment "Development of a radiopharmaceutical for the diagnosis of Alzheimer's disease using the HAEE tetrapeptide as a vector molecule", EGISU R&D registration number 1024110600012-8-3.2.25;3.2.26;3.2.12.

Author contribution: Ivanova AV — literature review, manuscript writing; Chmelyuk NS — fixation, cell sample preparation for microscopic imaging, image acquisition and analysis using a confocal microscope; Kuzmichev IA — synthesis of Ac-HAEEGGGGK(ε-Cy5)-NH2 and Ac-EEAHGGGGK(ε-Cy5)-NH2 fluorescent peptides; Shilyaeva MI — cell sample preparation; Abakumov MA — goal setting, developing the study design, manuscript writing; all the authors contributed to preparation of the paper equally, they confirmed compliance of their authorship with the international ICMJE criteria.

✉ **Correspondence should be addressed:** Anna V. Ivanova
Ostrovityanova, 1, str.1, Moscow, 117513, Russia; super.fosforit@yandex.ru

Received: 15.10.2025 **Accepted:** 13.11.2025 **Published online:** 24.11.2025

DOI: 10.24075/brsmu.2025.061

СРАВНИТЕЛЬНАЯ ВИЗУАЛИЗАЦИЯ СВЯЗЫВАНИЯ ТЕТРАПЕПТИДОВ HAEE-CY5 И EEAH-CY5 С АГРЕГАТАМИ Aβ НА КЛЕТКАХ SH-SY5Y

А. В. Иванова[1,2] ✉, Н. С. Чмелюк[1,2], И. А. Кузьмичев[1], М. И. Шиляева[1], М. А. Абакумов[1,2]

[1] Научно-исследовательский институт трансляционной медицины, Российский национальный исследовательский медицинский университет имени Н. И. Пирогова, Москва, Россия

[2] Институт биомедицинской инженерии, Университет науки и технологий МИСИС, Москва, Россия

В связи с высокой диагностической значимостью β-амилоидных агрегатов при болезни Альцгеймера особый интерес представляют целевые лиганды, способные к специфическому связыванию с патологическими агрегатами Aβ. Целью исследования было провести методом конфокальной микроскопии сравнительную характеристику связывающей способности тетрапептидов HAEE-Cy5 (Ac-His-Ala-Glu-Glu-Gly-Gly-Gly-Gly-Lys(ε-Cy5)-NH2) и EEAH-Cy5 (Ac-Glu-Glu-Ala-His-Gly-Gly-Gly-Gly-Lys(ε-Cy5)-NH2) с агрегатами Aβ на клеточной линии нейробластомы человека SH-SY5Y. Показано, что тетрапептид HAEE-Cy5 демонстрирует специфическое связывание с образованием характерных цитоплазматических скоплений и четкой колокализации с амилоидными агрегатами, в то время как пептид EEAH-Cy5 с обращенной последовательностью полностью утрачивает способность к связыванию. Количественный анализ подтвердил высокую специфичность связывания HAEE-Cy5 с агрегатами Aβ (коэффициент колокализации Мандерса 0.58 ± 0.03). Установлено, что N-концевое положение гистидина является критическим детерминантом специфичности взаимодействия. Результаты работы открывают перспективы применения пептида HAEE в качестве платформы для разработки целевых диагностических систем визуализации амилоидной патологии.

Ключевые слова: пептид HAEE, пептид EEAH, агрегаты Aβ, SH-SY5Y, болезнь Альцгеймера

Финансирование: работы выполнены в рамках Государственного задания «Создание радиофармацевтического лекарственного препарата для диагностики болезни Альцгеймера с использованием тетрапептида HAEE в качестве векторной молекулы», регистрационный номер ЕГИСУ НИОКТР 1024110600012-8-3.2.25;3.2.26;3.2.12.

Вклад авторов: А. В. Иванова — обзор литературы, подготовка рукописи; Н. С. Чмелюк — фиксация, подготовка клеточных образцов для съемки на микроскопе, получение и анализ изображений на конфокальном микроскопе; И. А. Кузьмичев — синтез флуоресцентных пептидов Ac-HAEEGGGGK(ε-Cy5)-NH2 и Ac-EEAHGGGGK(ε-Cy5)-NH2; М. И. Шиляева — подготовка образцов клеток; М. А. Абакумов — постановка цели, разработка дизайна исследования, подготовка рукописи; все авторы внесли равнозначный вклад в подготовку публикации, подтверждают соответствие своего авторства международным критериям ICMJE.

✉ **Для корреспонденции:** Анна Валерьевна Иванова
ул. Островитянова, д. 1, стр.1, г. Москва, 117513, Россия; super.fosforit@yandex.ru

Статья получена: 15.10.2025 **Статья принята к печати:** 13.11.2025 **Опубликована онлайн:** 24.11.2025

DOI: 10.24075/vrgmu.2025.061

Alzheimer's disease (AD) is the most globally prevalent neurodegenerative disorder that usually results in the neuronal death and brain atrophy. It is accompanied by accumulation of abnormal deposits: senile plaques consisting of the aggregated β-amyloid (Aβ) and neurofibrillary tangles formed by the hyperphosphorylated tau protein [1, 2]. Despite decades of research, the amyloid hypothesis remains one of the central to explain the AD pathogenesis. Accumulation of the oligomeric Aβ forms and their subsequent aggregation into mature, stable fibrils is considered to be the key event triggering the neurotoxicity and neuroinflammation cascade [3–5]. Since Aβ aggregates represent the main structural component of senile plaques, these are a priority target for the development of the diagnosis and treatment methods. One promising approach is the search for compounds capable of inhibiting the Aβ aggregation. Currently, various classes of such compounds are being developed, including small molecules [6, 7], monoclonal antibodies (aducanumab, lecanemab) [8, 9], peptides [10], natural ligands [11], multifunctional hybrid molecules [12], however, many of these face the problems of low bioavailability and limited efficacy in late-stage AD, or serious adverse effects, such as ARIA (amyloid-related imaging abnormalities), in cases of antibody therapy [13, 14]. In this context, of special interest are the short peptides conbining targeted effects and the fundamentally better penetration capacity. The HAEE tetrapeptide is a candidate compound. According to some data [15], HAEE acts as a specific molecular tool selectively binding to the metal-binding domain of the Aβ peptide (11EVHH14) to form a stable complex in the presence of Zn^{2+}. Such interaction confirmed by the surface plasmon resonance, nuclear magnetic resonance, and molecular modeling methods significantly disturbes the Zn^{2+}-dependent Aβ monomer dimerization, thereby preventing production of toxic oligomers. The *in vivo* experiments on the blood-brain barrier (BBB) provided important evidence of the HAEE efficacy: the peptide not only inhibited the Zn^{2+}-induced amyloid accumulation, but completely prevented the associated abnormal phenotypes, including paralysis and shortened lifespan of transgenic nematodes [15]. No signs of the peptide toxicity were reported in these experiments, which suggested the peptide beneficial safety profile in this model system. Furthermore, one more key property was reported for HAEE: pharmacokinetic studies and molecular modeling suggest that it was capable of crossing the BBB [15]. It should be noted that HAEE is a peptide derived from the sequence of the nicotinic acetylcholine receptor α4-subunit (nAChRα4) [16]. Thus, HAEE has properties of the unique candidate for AD therapy: it has a determined mechanism of action targeting the key pathogenesis link and demonstrates its efficacy at the whole-body level. The direct detection of the HAEE binding to the Aβ aggregates under the cell culture conditions can be a simple and clear method to further confirm this mechanism. Fluorescence microscopy, specifically when combined with immunocytochemistry analysis, is among the most popular and accessible methods to complete the task.

Thus, the study aimed to perform direct imaging and confirm binding of the HAEE-Cy5 fluorescent conjugate to the Aβ aggregates in the SH-SY5Y human neuroblastoma cell line by fluorescence microscopy in order to assess its specificity and the prospects of using the HAEE peptide as a target ligand for the AD diagnosis and treatment.

METHODS

All the experiments were conducted at the laboratory of the Deparment of Medical Nanobiotechnology of the Research Institute of Translational Medicine, Pirogov Russian National Research Medical University. Confocal microscopy was performed at the Biomedical Nanomaterials laboratory of the Bioengineering Institute, MISIS University of Science and Technology.

The following compounds were tested in the experiment: HAEE-Cy5 (Ac-His-Ala-Glu-Glu-Gly-Gly-Gly-Gly-Lys(ε-Cy5)-NH_2, EEAH-Cy5 (Ac-Glu-Glu-Ala-His-Gly-Gly-Gly-Gly-Lys(ε-Cy5)-NH_2, Cy5-NH2. The test peptides HAEE-Cy5 and EEAH-Cy5 (purity > 95% based on the HPLC data) were synthesized and provided by the laboratory of the Department of Medical Nanobiotechnology, Research Institute of Translational Medicine, Pirogov Russian National Research Medical University. The peptides cosist of the tetrapeptide “head” (HAEE or EEAH) connected to the Cy5 fluorescent dye via a linker of four glycine residues (GGGG).

The L-lysine residue (K) in the linker provides a conjugation site for the dye. The chemical structure of the test compounds is provided in Fig. 1.

The lyophilized peptides were dissolved in the sterile deionized water to the concentration of 5–10 mM, and aliquots of solutions were stored at –20 °C for no longer than 3 months. Before applying the peptides to the cells the solutions were

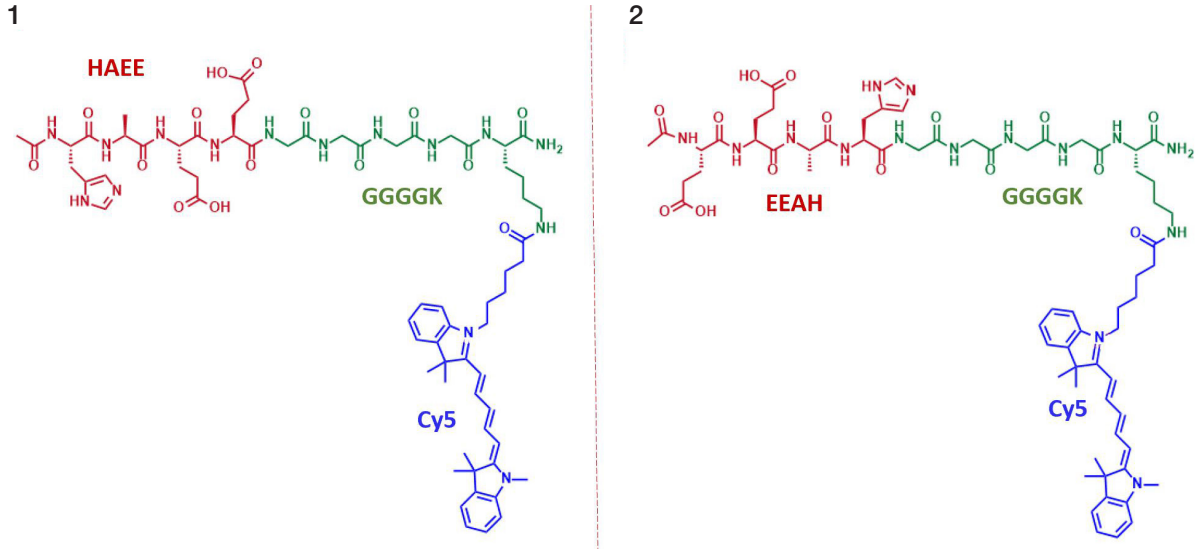

Fig. 1. Chemical structure of the test conjugated peptides

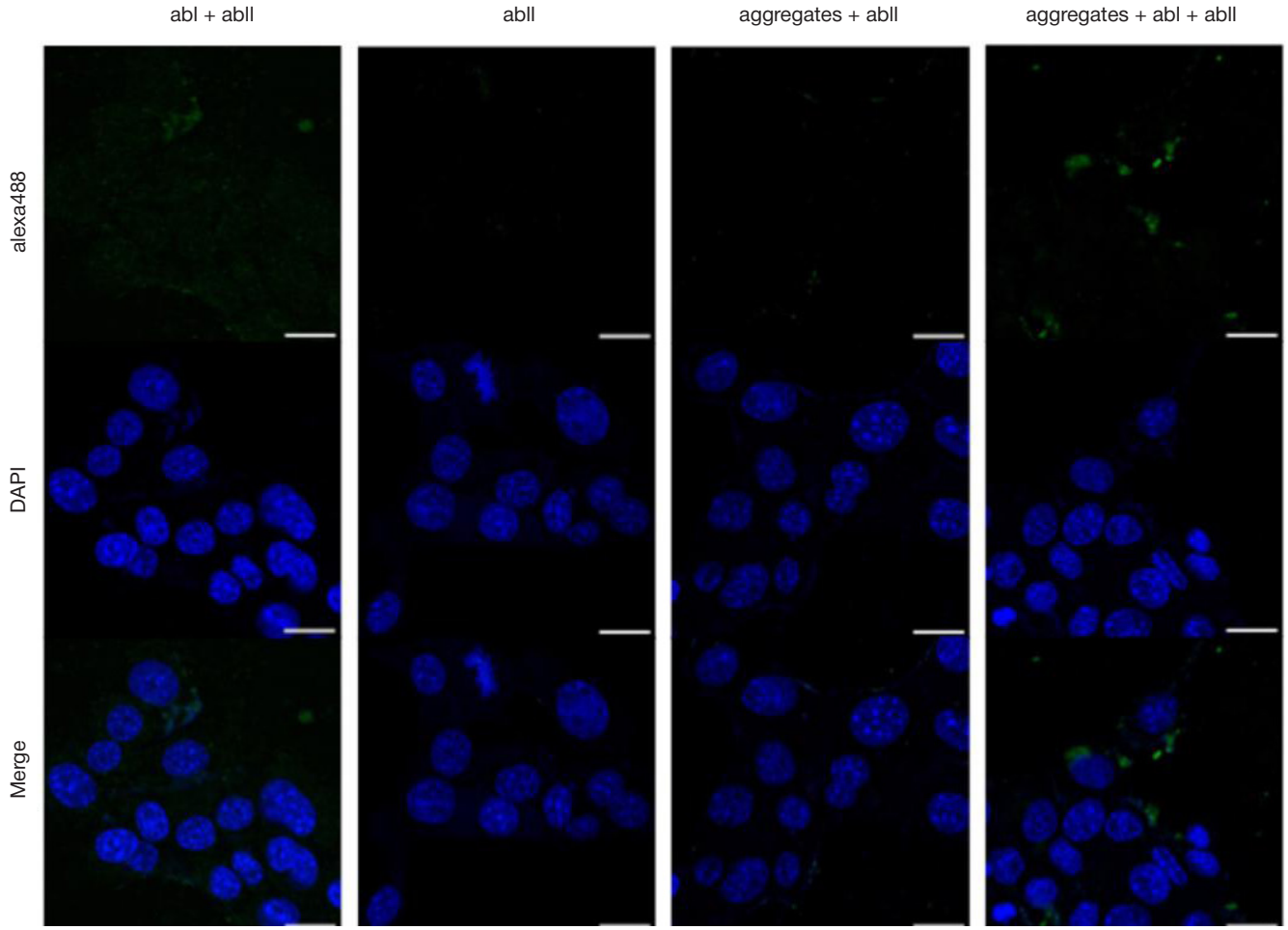

Fig. 2. Control of immunofluorescence staining specificity in the SH-SY5Y cells. Alignment of channels demonstrates localization of the alexa 488-stained target antigens (*green*) on the Aβ aggregates. The nuclei are DAPI stained (*blue*). Scale bar 20 μm

diluted in the DMEM/F12 cell culture medium containing no fetal bovine serum (FBS) to the concentration of 5 μM.

SH-SY5Y cell culturing

The SH-SY5Y human neuroblastoma cells (ATCC, USA) were grown in the cell culture flasks with the growth medium comprising DMEM/F12 (ServiceBio, China) supplemented with the 10% FBS (Cytiva (GE Healthcare Life Sciences HyClone), USA), antibiotic mixture (penicillin — 100 μg/mL, streptomycin — 100 μg/mL) (ServiceBio, China), and L-glutamine (100 mM) (ServiceBio, China) at 37 °C in the 5% CO_2 atmosphere.

Beta-amyloid preparations

The lyophilized Aβ (Amyloid β-Protein (1–42) (E-PP-0428), Elabscience, China) was dissolved in the 1% NH_4OH to the concentration of 1 mg/mL in the ultrasonic bath without heating for 10 min. Then the solution was aliquoted (10 μL) and stored at –80 °C. Before applying to the cells, the solution was ultrasound-treated again for 30 min at 37 °C in the final concentration (20 μg/mL).

Immunofluorescence analysis

To assess localization of the compounds, the SH-SY5Y cells were sown in the wells of the 24-well plate, 200 × 10^3 cells per well. After 24 h the cells were treated with the Aβ preparations (20 μg/mL) in the medium containing no FBS and incubated for 4 h. Then the cells were twice washed with the Hanks' solution, added the test compounds (HAEE-Cy5, EEAH-Cy5, NH2-Cy5) in a concentration of 5 μM, and incubated for 2 h. Then the cells were fixed for 15 min in the 4% paraformaldehyde at +4 °C. Permeabilization was performed in the blocking buffer of 0.2% Twin-20, 0.2% Triton X-100, and 2% goat serum for 30 min at room temperature. Then incubation with the primary antibody (mouse IgG1 antibody against himan beta-amyloid protein, abl, clone 6E10 BioLegend) diluted 1:100 000 (0.01 μg/mL) in the buffer (0.2% Twin-20, 0.2% Triton X-100, 0.2% goat serum, FBS) was performed for 60 min. After that the cells were triple washed with the solutions of 0.2% Twin-20, 0.2% Triton X-100 for 5 min. Then the cells were incubated with the secondary antibody (abll, goat antibody against IgG(H+L) conjugated with alexa 488 (E-AB-1056, Elabscience, China). Incubation with the secondary antibody was also conducted for 60 min; then the cells were triple washed with the solutions of 0.2% Twin-20, 0.2% Triton X-100 for 5 min and stained with the DAPI nuclear dye.

Confocal microscopy

Cell imaging was accomplished with the Nikon Eclipse Ti2 microscope (Nikon, Tokyo, Japan) equipped with the laser scanning system (ThorLabs, Newton, New Jersey, USA) and the Apo 60×/0.5–1.25 oil immersion lens. Scanning was performed using the ThorImageLS software (version 2.4) (Thorlabs, Newton, New Jersey, USA); images were processed using the Fiji 2.9.0 software tool.

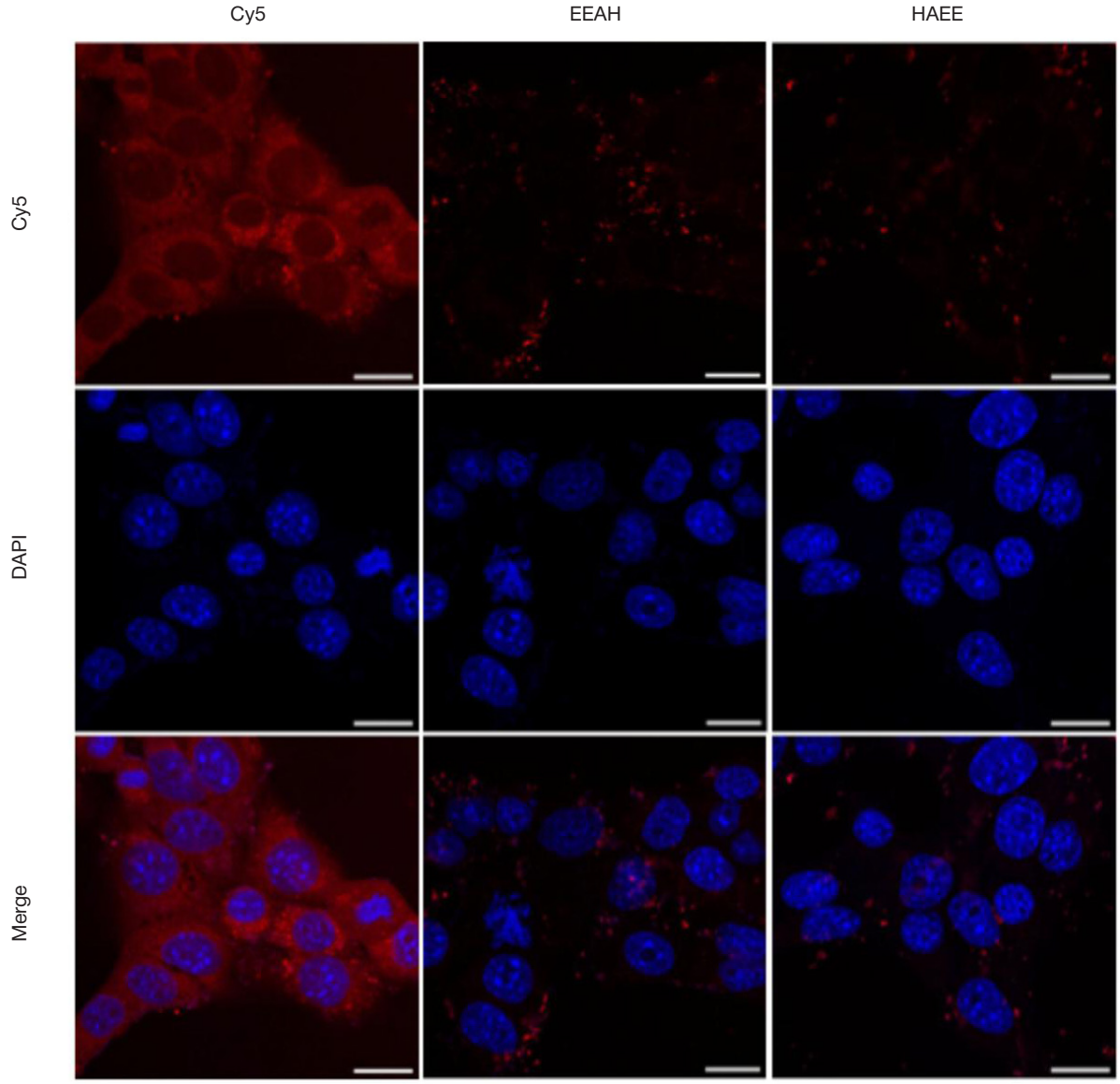

Fig. 3. Localization of the EEAH-Cy5 and HAEE-Cy5 compounds in the SH-SY5Y cells. Signal of the Cy5-labeled compounds (*red*). The nuclei are DAPI stained (*blue*). Scale bar 20 μm

Statistical analysis

Colocalization of images in the alexa488 and Cy5 channels was calculated using the Fiji software tool and Manders' coefficient; images (n = 6) were used for analysis.

RESULTS

To confirm immunofleorescence staining specificity in the SH-SY5Y cells, a number of control experiments was conducted (Fig. 2). When using only secondary antibodies conjugated with the alexa 488 (abII) fluorescent label, no significant fluorescent signal was detected. Likewise, incubation of cells with Aβ in the absence of primary antibody (abI) did not result in specific staining. Treatment of cells with abI or abII without Aβ yielded no significant fluorescent signal. Intense fluorescent staining was observed only when there were Aβ, abI, and abII at once; this corresponded to the expected localization of target antigens. The results obtained confirm that specific staining requires the presence of all system components and indicate specificity of the antibodies used.

Then the HAEE-Cy5 and EEAH-Cy5 localization of the cells was assessed. In the control experiment involving the use of the free Cy5-NH2 dye, the nonspecific internalization of the dye manifested by a diffuse signal on the cell membrane and in the cytoplasm was reported. The fact that such a hydrophobic compound is localized partially in the cytoplasm can be explained by permeabilization of the cells during the analysis. In contrast, the intense and structurally organized signal that was qualitatively different from the control was reported for the EEAH-Cy5 and HAEE-Cy5 compounds: this was characterized by the prominent membrane-bound component and formation of discrete clusters in the cytoplasm (Fig. 3). This suggests specific interaction of the test substances with the cell structures, which is not limited to non-specific accumulation of the dye.

Due to the detected specific EEAH-Cy5 and HAEE-Cy5 localization, direct verification of their capability of binding to the Aβ aggregates was performed. The analysis of confocal microscopy images showed the clear and intense colocalization of the HAEE-Cy5 signal (red) with the signal of Aβ deposits (alexa 488, green) visualized as large yellow regions in the combined image (Fig. 4), which unambiguously indicated the HAEE-Cy5 tetrapeptide high-affinity binding to amyloid aggregates. In contrast to HAEE-Cy5, no significant signal in

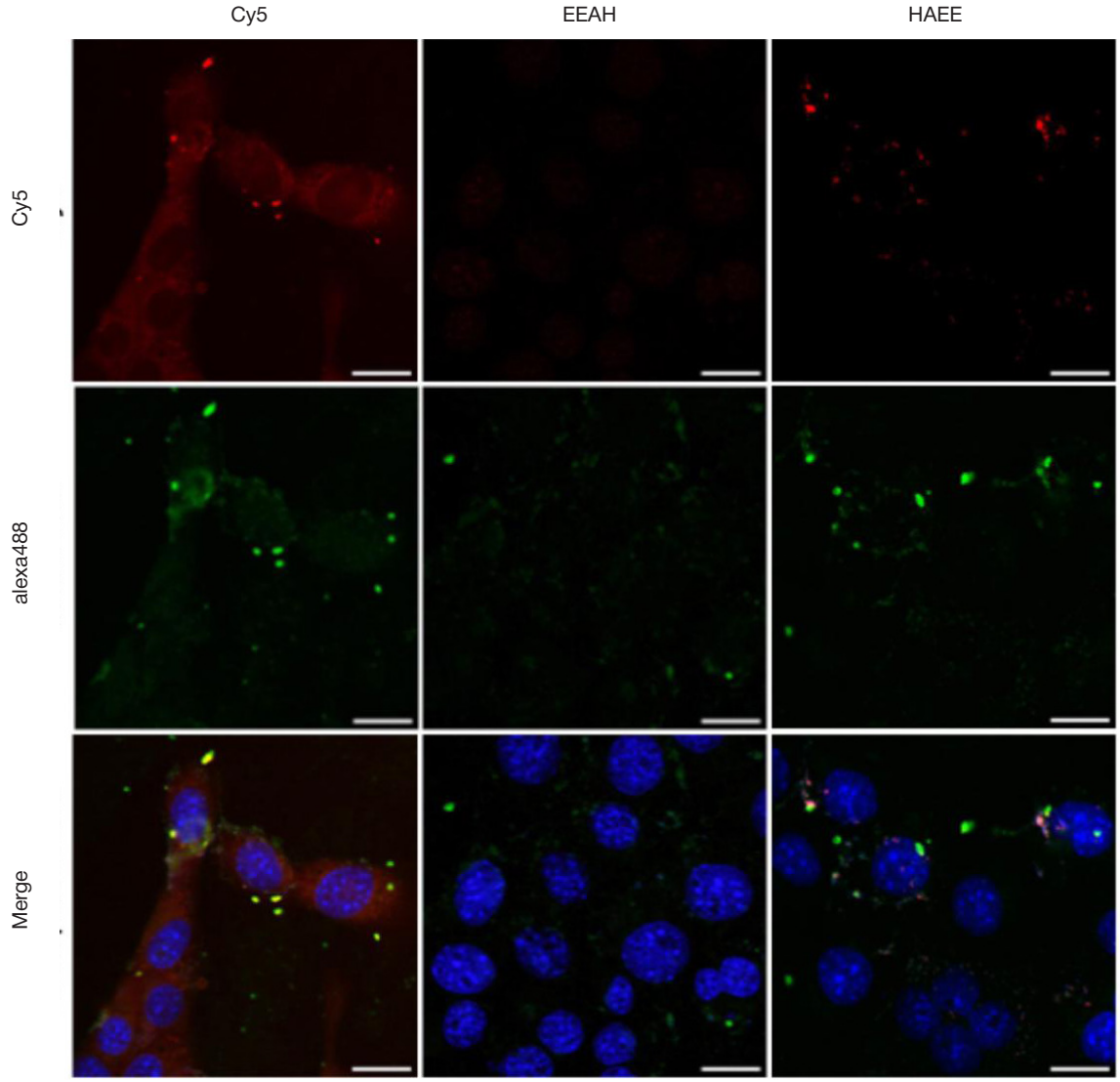

Fig. 4. Visualization of the HAEE-Cy5 and EEAH-Cy5 binding to the Aβ aggregates in the SH-SY5Y cells. Signal of the Cy5-labeled tetrapeptides (*red*). The alexa 488-labeled antibodies on Aβ (*green*). *Yellow* on the combined image indicates colocalization of peptides with Aβ aggregates. The nuclei are DAPI stained (*blue*). Scale bar 20 μm

the Cy5 channel was reported for the EEAH-Cy5 peptide, which suggested that it was unable to specifically interact with the studied target. Despite the fact that the source Cy5 dye showed some capability of binding to amyloid deposits, it was to the greater extent nonspecifically localized, which also confirmed high HAEE-Cy5 affinity for Aβ aggregates. To further confirm colocalization of the channels, the Manders' coefficient was calculated (between the channels of the Aβ aggregate images and test compounds for the images acquired): the highest coefficient value was reported for the HAEE-Cy5 compound and Aβ aggregates (0.58 ± 0.03), while the values between the Aβ and Cy5 channels and Aβ and EEAH-Cy5 channels were 0.22 ± 0.05 and 0.19 ± 0.02, respectively, which suggested no Cy5 and EEAH-Cy5 colocalization and higher extent of the HAEE-Cy5 binding to Aβ. The extremely low EEAH-Cy5 fluorescence signal can be associated with with its low capability of staying on the Aβ aggregates and in other cell compartments. At the same time, the Cy5 signal is significant, it is enhanced in the areas of Aβ aggregates, however, a large amount of fluorescence is distributed across the membrane and cytoplasm, which suggests low specificity of the Cy5 binding to Aβ.

DISCUSSION

The study conducted has made it possible to identify fundamental differences in the HAEE-Cy5 and EEAH-Cy5 capability of binding to the Aβ aggregates in the SH-SY5Y cells, which are likely to result from their structural features. Despite identical amino acid composition, the reversed EEAH-Cy5 sequence led to the complete functional activity loss, while HAEE-Cy5 showed high specificity for the studied target. The data obtained suggest that the histidine N-terminal position in the HAEE-Cy5 sequence is critically important for formation of specific interaction with Aβ. Our data clearly demonstrate that the histidine (His, H) shift from the first to fourth position in the tetrapeptide “head” of the ligand results in the rapidly decreased capability of binding to Aβ. It is well known that the Aβ peptide N-terminal domain comprises the main binding centers, specifically the His6, His13, His14 residues being the good σ-donors that are involved in coordination with metals [17], as well as the aromatic Phe4 and Tyr10 residues responsible for the π π interaction [17, 18]. It can be assumed that histidine being part of HAEE is involved in similar interaction with these sites. As for the inactive EEAH peptide, the presence of two negatively charged glutamic acid residues (Glu, E) in

the N-terminal position, as well as the presence of the Cy5 fluorescent dye in the ε-end of lysine (Lys, K), can cause the peptide chain folding or create steric hindrances disrupting spatial orientation and the key histidine residue availability. In such a configuration, histidine can be sterically unavailable for interaction with the Aβ binding sites.

The quantitative evidence to substantiate high specificity of the HAEE-Cy5 binding to amyloid aggregates is calculation of the Manders' colocalization coefficient. The coefficient value of 0.58 ± 0.03 for the HAEE-Cy5/Aβ pair is considerably higher compared to the values of control compounds (Cy5-NH2/Aβ and EEAH-Cy5/Aβ), which are close to zero. Quantification clearly demonstrates that the intense colocalization signal is not random, it confirms high HAEE peptide affinity specifically for the target Aβ aggregates, which is fully consistent with visual observation and demonstrates critical importance of the correct amino acid sequence for effective interaction.

The lack of the EEAH-Cy5 fluorescence signal upon clear detection of HAEE-Cy5 to extracellular Aβ aggregates suggests that it is not capable of specific interaction with the target. This difference resulting from suboptimal primary structure of the control peptide can be due to several factors: impaired binding to Aβ, increased susceptibility to proteolytic degradation, worse cellular permeability or accelerated removal from the cell. Thus, in contrast to HAEE-Cy5, the EEAH-Cy5 peptide fails to fulfill the target function, which confirms critical importance of certain amino acid sequence for effective binding. It is worth noting that in the absence of Aβ both peptides demonstrate similar intracellular localization, which suggests their stability and capability of entering the cell. However, the HAEE-Cy5 peptide intense and structurally organized signal manuifested by discrete cytoplasmic clusters suggests that it is capable of entering the cell and interacting with intracellular structures. Furthermore, according to the literature data [19], the reported colocalization with Aβ (Fig. 4) occurs in the extracellular space, which confirms the binding specificity. It is important to note that the HAEE-Cy5 distribution observed was qualitatively different from the diffuse signal of the free Cy5-NH2 dye, which precludes explanation by simple dye accumulation and emphasizes the role of peptide sequence in the targeted binding.

The findings are of high practical importance. The HAEE-Cy5 specificity for Aβ aggregates detected allows one to consider it is a promising ligand for the development of diagnostic means. In particular, it can be used as a foundation for the development of the following: MRI contrast agents for *in vivo* imaging of amyloid plaques, fluorescent probes for intraoperative detection of amyloid deposits, theranostic platforms for targeted delivery of drugs. In contrast to HAEE-Cy5, the EEAH-Cy5 peptide shows the total lack of binding activity. This clearly demonstrates that biological function is determined by not only amino acid composition, but also strict amino acid order. Further development of this research area requires the following: 1) thorough investigation of the HAEE interaction with Aβ by molecular docking and spectroscopy methods; 2) *in vitro* assessment of the HAEE capability of inhibiting the Aβ aggregation; 3) *in vivo* study of the HAEE distribution and bioavailability in transgenic AD models. Thus, the study results have not only identified the highly specific Aβ ligand, but also demonstrated that minimal changes in the peptide structure can have a dramatic effect on its functional properties, which is of fundamental importance for the design of peptide drugs.

CONCLUSIONS

In the study, we successfully validated the method to detect Aβ aggregates and visualized specific binding of the Cy5-conjugated HAEE tetrapeptide to amyloid aggregates in the SH-SY5Y cells, which was quantitatively confirmed by high Manders' colocalization coefficient (0.58 ± 0.03). The amino acid sequence critical importance for such interaction was determined based on the fact that the EEAH-Cy5 peptide with the reversed sequence showed the total lack of binding activity. Thus, it has been found that it is N-terminal position of histidine that is critical for formation of specific interaction with Aβ aggregates. The findings confirm the prospects of using HAEE as a target ligand for the development of diagnostic and theranostic agents for AD and emphasize the importance of stereochemical factors for construction of peptide drugs. To further develop the research area it is necessary to study molecular mechanisms of interaction and assess the peptide distribution *in vivo*.

References

1. Kolobova EA, Petrushanko IY, Mitkevich VA, Makarov AA, Grigorova IL. Novel method for detection of Aβ and Iso-D7-Aβ N-terminus-specific B cells and Iso-D7-Aβ-specific antibodies. Biol Methods Protoc. 2025; 10 (1).
2. Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, et al. Alzheimer disease. Nat Rev Dis Primers. 2021; 7 (1): 33.
3. Harris JR, Marles-Wright J, editors. Macromolecular Protein Complexes II: Structure and Function. Subcell Biochem. 2019; 93.
4. Walsh DM, Selkoe DJ. Amyloid β-protein and beyond: the path forward in Alzheimer's disease. Curr Opin Neurobiol. 2020; 61: 116–24.
5. Katzmarski N, Ziegler-Waldkirch S, Scheffler N, Witt C, Abou-Ajram C, Nuscher B, et al. Aβ oligomers trigger and accelerate Aβ seeding. Brain Pathol. 2020; 30 (1): 36–45.
6. Wilcock GK, Gauthier S, Frisoni GB, Jia J, Hardlund JH, Moebius HJ, et al. Potential of low dose leuco-methylthioninium bis(hydromethanesulphonate) monotherapy for treatment of mild Alzheimer's disease: cohort analysis as modified primary outcome in a phase III clinical trial. J Alzheimers Dis. 2018; 61 (1): 435–57.
7. Paul A, Viswanathan GKK, Huber A, Arad E, Engel H, Jelinek R, et al. Inhibition of tau amyloid formation and disruption of its preformed fibrils by naphthoquinone-dopamine hybrid. FEBS J. 2021; 288 (13): 4267–90.
8. Fedele E. Anti-amyloid therapies for Alzheimer's disease and the amyloid cascade hypothesis. Int J Mol Sci. 2023; 24 (19): 14499.
9. Kepp KP, Robakis NK, Høilund-Carlsen PF, Sensi SL, Vissel B. The amyloid cascade hypothesis: an updated critical review. Brain. 2023; 146 (9): 3969–90.
10. Tucker M, Liao GY, Park JY, Rosenfeld M, Wezeman J, Mangalindan R, et al. Behavioral and neuropathological features of Alzheimer's disease are attenuated in 5xFAD mice treated with intranasal GHK peptide. bioRxiv. 2023; 20: 2023.11.20.567908.
11. Kryscio RJ, Abner EL, Caban-Holt A, Lovell M, Goodman P, Darke AK, et al. Association of antioxidant supplement use and dementia in the Prevention of Alzheimer's Disease by Vitamin E and Selenium Trial (PREADViSE). JAMA Neurol. 2017; 74 (5): 567–73.
12. Ramesh M, Govindaraju T. Multipronged diagnostic and therapeutic strategies for Alzheimer's disease. Chem Sci. 2022; 13 (46): 13657–89.
13. Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. N Engl J Med. 2014; 370 (4): 322–33.

14. Ivanova AV, Kutuzova AD, Kuzmichev IA, Abakumov MA. Alzheimer's disease: from molecular mechanisms to promising therapeutic strategies. Int J Mol Sci. 2025; 26 (19): 9444.
15. Mitkevich VA, Barykin EP, Eremina S, Pani B, Katkova-Zhukotskaya O, Polshakov VI, et al. Zn-dependent β-amyloid aggregation and its reversal by the tetrapeptide HAEE. Aging Dis. 2023; 14 (2): 309–14.
16. Barykin EP, Garifulina AI, Tolstova AP, Anashkina AA, Adzhubei AA, Mezentsev YV, et al. Tetrapeptide Ac-HAEE-NH2 protects α4β2 nAChR from inhibition by Aβ. Int J Mol Sci. 2020; 21 (24): 1–18.
17. Silva DES, Cali MP, Pazin WM, Carlos-Lima E, Salles Trevisan MT, Venâncio T, et al. Luminescent Ru(II) phenanthroline complexes as a probe for real-time imaging of Aβ self-aggregation and therapeutic applications in Alzheimer's disease. J Med Chem. 2016; 59 (19): 9215–27.
18. Yao S, Cherny RA, Bush AI, Masters CL, Barnham KJ. Characterizing bathocuproine self-association and subsequent binding to Alzheimer's disease amyloid β-peptide by NMR. J Pept Sci. 2004; 10 (4): 210–7.
19. Petrushanko IY, Mitkevich VA, Anashkina AA, Adzhubei AA, Burnysheva KM, Lakunina VA, et al. Direct interaction of beta-amyloid with Na, K-ATPase as a putative regulator of the enzyme function. Sci Rep. 2016; 6: 27738.

Литература

1. Kolobova EA, Petrushanko IY, Mitkevich VA, Makarov AA, Grigorova IL. Novel method for detection of Aβ and Iso-D7-Aβ N-terminus-specific B cells and Iso-D7-Aβ-specific antibodies. Biol Methods Protoc. 2025; 10 (1).
2. Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, et al. Alzheimer disease. Nat Rev Dis Primers. 2021; 7 (1): 33.
3. Harris JR, Marles-Wright J, editors. Macromolecular Protein Complexes II: Structure and Function. Subcell Biochem. 2019; 93.
4. Walsh DM, Selkoe DJ. Amyloid β-protein and beyond: the path forward in Alzheimer's disease. Curr Opin Neurobiol. 2020; 61: 116–24.
5. Katzmarski N, Ziegler-Waldkirch S, Scheffler N, Witt C, Abou-Ajram C, Nuscher B, et al. Aβ oligomers trigger and accelerate Aβ seeding. Brain Pathol. 2020; 30 (1): 36–45.
6. Wilcock GK, Gauthier S, Frisoni GB, Jia J, Hardlund JH, Moebius HJ, et al. Potential of low dose leuco-methylthioninium bis(hydromethanesulphonate) monotherapy for treatment of mild Alzheimer's disease: cohort analysis as modified primary outcome in a phase III clinical trial. J Alzheimers Dis. 2018; 61 (1): 435–57.
7. Paul A, Viswanathan GKK, Huber A, Arad E, Engel H, Jelinek R, et al. Inhibition of tau amyloid formation and disruption of its preformed fibrils by naphthoquinone-dopamine hybrid. FEBS J. 2021; 288 (13): 4267–90.
8. Fedele E. Anti-amyloid therapies for Alzheimer's disease and the amyloid cascade hypothesis. Int J Mol Sci. 2023; 24 (19): 14499.
9. Kepp KP, Robakis NK, Høilund-Carlsen PF, Sensi SL, Vissel B. The amyloid cascade hypothesis: an updated critical review. Brain. 2023; 146 (9): 3969–90.
10. Tucker M, Liao GY, Park JY, Rosenfeld M, Wezeman J, Mangalindan R, et al. Behavioral and neuropathological features of Alzheimer's disease are attenuated in 5xFAD mice treated with intranasal GHK peptide. bioRxiv. 2023; 20: 2023.11.20.567908.
11. Kryscio RJ, Abner EL, Caban-Holt A, Lovell M, Goodman P, Darke AK, et al. Association of antioxidant supplement use and dementia in the Prevention of Alzheimer's Disease by Vitamin E and Selenium Trial (PREADViSE). JAMA Neurol. 2017; 74 (5): 567–73.
12. Ramesh M, Govindaraju T. Multipronged diagnostic and therapeutic strategies for Alzheimer's disease. Chem Sci. 2022; 13 (46): 13657–89.
13. Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. N Engl J Med. 2014; 370 (4): 322–33.
14. Ivanova AV, Kutuzova AD, Kuzmichev IA, Abakumov MA. Alzheimer's disease: from molecular mechanisms to promising therapeutic strategies. Int J Mol Sci. 2025; 26 (19): 9444.
15. Mitkevich VA, Barykin EP, Eremina S, Pani B, Katkova-Zhukotskaya O, Polshakov VI, et al. Zn-dependent β-amyloid aggregation and its reversal by the tetrapeptide HAEE. Aging Dis. 2023; 14 (2): 309–14.
16. Barykin EP, Garifulina AI, Tolstova AP, Anashkina AA, Adzhubei AA, Mezentsev YV, et al. Tetrapeptide Ac-HAEE-NH2 protects α4β2 nAChR from inhibition by Aβ. Int J Mol Sci. 2020; 21 (24): 1–18.
17. Silva DES, Cali MP, Pazin WM, Carlos-Lima E, Salles Trevisan MT, Venâncio T, et al. Luminescent Ru(II) phenanthroline complexes as a probe for real-time imaging of Aβ self-aggregation and therapeutic applications in Alzheimer's disease. J Med Chem. 2016; 59 (19): 9215–27.
18. Yao S, Cherny RA, Bush AI, Masters CL, Barnham KJ. Characterizing bathocuproine self-association and subsequent binding to Alzheimer's disease amyloid β-peptide by NMR. J Pept Sci. 2004; 10 (4): 210–7.
19. Petrushanko IY, Mitkevich VA, Anashkina AA, Adzhubei AA, Burnysheva KM, Lakunina VA, et al. Direct interaction of beta-amyloid with Na, K-ATPase as a putative regulator of the enzyme function. Sci Rep. 2016; 6: 27738.