Proceedings of the 35th European Peptide Symposium
Patrick B. Timmons, Chandralal M. Hewage, Michal Lebl (Editors)
European Peptide Society & PSP, 2018

New 4-Aminopyridine derivatives containing peptide fragment designed for the treatment of Alzheimer disease and multiple sclerosis

Lyubomir Vezenkov[1], Borislav Anchev[1], Ivanka Kostadinova[2], Kristian Fidanov[1], Daniela S. Tsekova[1], Nikolay G. Vasilev[3], Nikolai D. Danchev[1]

[1]University of Chemical Technology and Metallurgy, Bulgaria
[2]Medical University, Sofia, Bulgaria
[3]Bulgarian Academy of Sciences, Bulgaria

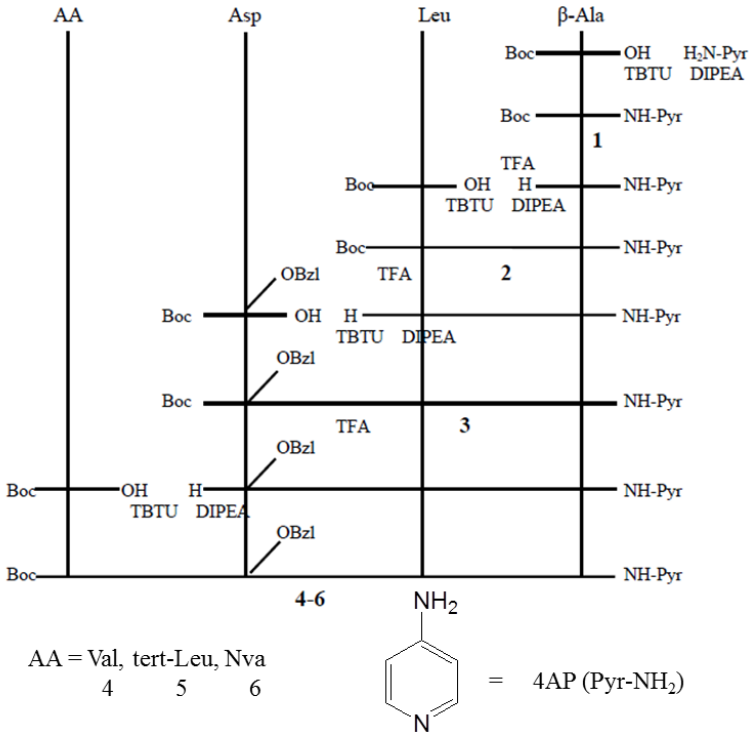
https://doi.org/10.17952/35EPS.2018.241

Alzheimer's disease (AD) and Multiple sclerosis (MS) are neurodegenerative diseases. The AD process is associated with plaques and tangles in the brain. MS is a disease that causes inability of the CNS cells to communicate each to other. Neurodegeneration leads to problems with cognitive function (dementias) and / or movement. According to the literature data, no cure for AD and MS, but the treatments can help for the changing of the diseases progression. Among the most effective medicals used for both diseases treatment are 4-aminopyridine (4-AP) and galanthamine. 4-AP has very high toxicity, limiting its use in the treatment of neurodegenerative diseases, Based on the literature [1-3], we propose an approach to produce new hybrid molecules between 4-aminopyridine and different peptide fragments possessing β–secretase inhibitoty activity, thus we expect to combine important pharmacological effects to influence AD and MS: Inhibition of β-secretase and a significant reduction in toxicity due to peptide fragments; Blocking of potassium channels leads to an increase in the level of acetylcholine in the brain, an anti-inflammatory action as well as easier passage through BBB, due to 4-aminopyridine. Here we report the synthesis of three new hybrid compounds containing 4-AP and a peptide fragment AA- Asp(OBzl)-Leu-Ala, where AA is Val, Nva or tert-Leu (see Scheme 1).

Results and Discussion

Synthesis. The obtaining of the new compounds were performed according to Scheme 1. The synthesis of the final compounds 5-7 were carried out in solution by sequentially attachment of the protected amino acids Boc-Ala-OH, Boc-Leu-OH, Boc-Asp(OBzl)-OH and Boc-Val-OH/Boc-Nva-OH/Boc-tert-Leu-OH to 4-AP. The condensation was carried out by TBTU method, with the reagents being dissolved in a minimal amount of DMF. The resulting crude products were recrystallized to chromatographically pure products. The compounds were characterized by m.p. thin layer chromatography and NMR.

Toxicological tests. The new derivatives of 4-AP, containing peptide fragments were investigated for acute toxicity by OECD-425-FDA-USA method by intraperitoneal (IP) application on male mice line H, weight 20-25 g. [4] The data for LD50 are presented as mg/kg body weight in the Table 1. The newly compounds are considerably less toxic (70-80 times) than 4-AP that has toxicity LD50 = 19 mg/kg (highly toxic compound according to Hodge and Sterner classification [4]).



Scheme 1: Synthesis of 4-Aminopyridine derivatives containing a peptide fragment: compounds 4, 5 and 6

Table 1: Acute toxicity of final compounds

code	compound	LD50 (mg/kg)
4 (LK-6)	Boc-Val-Asp(OBzl)-Leu-β-Ala-4-AP	>1500
5 (LK-7)	Boc-tert-Leu-Asp(OBzl)-Leu-β-Ala-4-AP	>1500
6 (LK-4)	Boc-Nva-Asp(OBzl)-Leu-β-Ala-4-AP	>1500

Cytotoxic tests. Cytotoxicity tests were applied also on the the newly compounds. Cell toxicity screening was performed towards the following two types cell cultures: 1. Mice neuroblastoma cell Neuro 2a; 2. Human chronic myeloid leukemia - BV-173. The data are presented in the Fig. 1, Fig. 2 and in the Table 2.

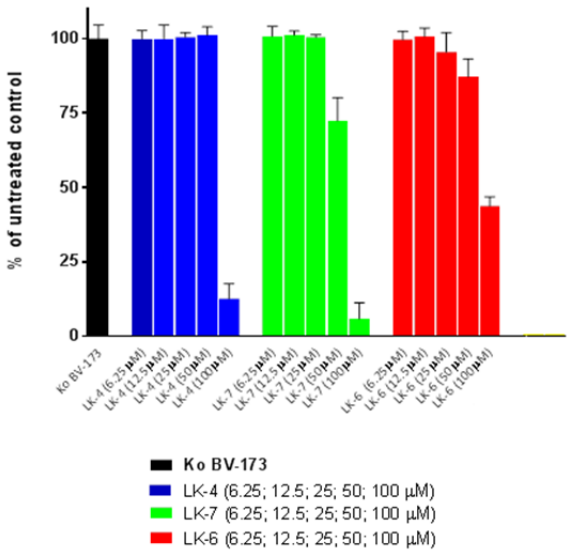


Figure 1: Viability of BV-173 cells following 72h exposure to LK-4, LK-7 and LK-6.

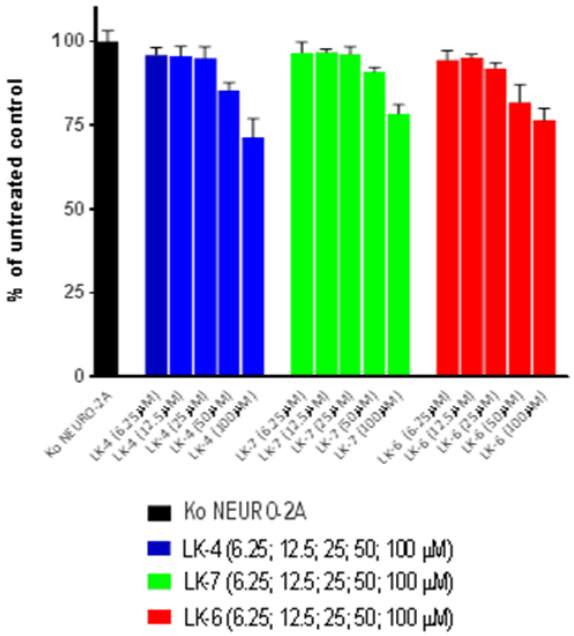


Figure 2: Viability of NEURO-2A cells following 72h exposure to LK-4, LK-7 and LK-6

Table 2: IC50 values of test compounds on the corresponding cell lines (µM).

code	compound	Cell line	
		BV-173	NEURO-2A
LK-4	Boc-Nva-Asp(OBzl)-Leu-β-Ala-4-AP	88.7 ± 23.1	> 100
LK-7	Boc-tert-Leu-Asp(OBzl)-Leu-β-Ala-4-AP	59.8 ± 4.1	> 100
LK-6	Boc-Val-Asp(OBzl)-Leu-β-Ala-4-AP	92.2 ± 6.3	> 100

Conclusions. A series of new 4-AP derivatives comprising peptide fragment were synthesized. Our study on acute toxicity shows that the investigated compounds are less toxic than 4-AP (about 80 times). Results from cytotoxicity studies are in a good correlation with results from acute toxicity test. Obviously, the used peptide fragments decrease significantly *in vivo* toxicity on mice and cytotoxicity on the studied cell cultures.

Acknowledgements

This study is supported by the Scientific Research Fund of Bulgaria through the project DH 03/8, "Galanthamine's and 4-aminopyridine's derivatives containing peptide motif with expected effect on the Alzheimer's disease and multiple sclerosis".

References

1.Niacin, an Old Drug, has New Effects on Central Nervous System Disease Jieli Chen and Michael Chopp The Open Drug Discovery Journal, 2010, 2: 181-186.

2. LT. Vezenkov, D I. Zaharieva, ND. Danchev, NG. Vasilev, AT. Bakalova, TchB. Ivanov. Proceedings of the 33rd EPS 31 Aug-5 Sep 2014; Sofia, Bulgaria, pp 200-201.

3. I. Kostadinova, N. Danchev, I. Nikolova, L. Vezenkov, Tch. Ivanov, Pharmacia, 2017,64(3): 22-39

4. Hodge, A. and Sterner, B. (2005) .Toxicity classes. In:Canadian center for occupational Health and safety. Retrieved from https://www.ccohs.ca/oshanswers/chemicals/ld50.html

5. OECD Guidelins for the testing of chemicals, Health effects. 16 oct. 2008