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Cinnamic acid derivatives as the potential modulators of prion aggregation

Sofia A. Tishina,[a] Victor S. Stroylov,[b] Ivan A. Zanyatkin,[a] Aleksandra K. Melnikova,[a] Vladimir I. Muronetz[a,c] and Yulia Yu. Stroylova*[c,d]

[a] *Department of Bioengineering and Bioinformatics, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*

[b] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation*

[c] *A. N. Belozersky Institute of Physico-Chemical Biology, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax +7 495 939 3181; e-mail: stroylova@belozersky.msu.ru*

[d] *Institute of Molecular Medicine, I. M. Sechenov First Moscow State Medical University, 119991 Moscow, Russian Federation*

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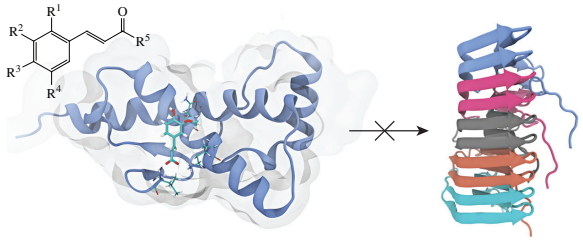
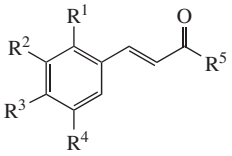
Eight cinnamic acid derivatives were studied as prion inhibitors *in vitro* and their cytotoxicity was evaluated. Ferulic (3-methoxy-4-hydroxycinnamic) acid, 3,4,5-trimethoxycinnamic acid and methyl 3-ethoxy-4-acetamidoxycinnamate were found to inhibit amyloid fibril formation, seeding, and spontaneous aggregation of recombinant ovine prion protein. None of the compounds demonstrated cytotoxicity on human neuroblastoma SH-SY5Y cells.



Transmissible spongiform encephalophaties (TSEs), also known as prion diseases, are neurodegenerative disorders deadly to both humans and animals.[1,2] A viable approach to the prophylaxis and treatment of prion diseases (and other conformational pathologies)[3] is based on the compounds capable of binding to the prion protein (or its aggregates), thus inhibiting its conversion into the pathologic form and ultimately slowing down further aggregation. The naturally ocurring polyphenolic compound curcumin is known to bind to normal, oligomer, and fibril forms of prion protein, inhibiting pathological aggregation;[4] however, it suffers from extremely low bioavailability.

Using a molecular docking approach, we have recently suggested a binding site in native prion protein for cinnamic acid derivatives[5] based on the fact that the above compounds represent a curcumin molecule virtually cut in halves. We studied the activity of eight cinnamic acid derivatives (Figure 1), synthesized according to known procedures, in amyloid fibril formation, 'seeding' and spontaneous aggregation assays. Note that ferulic acid (L8) is a naturally occuring component of foods and herbs including coffee beans, red wine and *Scutellaria baicalensis*.[6–8]



	R^1	R^2	R^3	R^4	R^5
L1	H	OMe	OEt	H	OH
L2	OMe	OMe	OMe	H	OH
L3	H	OMe	OMe	OMe	OH
L4	H	OEt	OPr^i	H	OH
L5	H	OCH_2O		H	OH
L6	H	OEt	$OCH_2C(=O)NH_2$	H	OMe
L7	H	OMe	$OCH_2C(=O)NH_2$	H	OH
L8	H	OMe	OH	H	OH

Figure 1 Structural formula of the test compounds.

Prion protein (PrP)† amyloid fibril‡ formation assay perfomed with the test compounds resulted in more than 40% reduction in Thioflavin T (ThT) fluorescence§ in case of ligands L6, L7, and L8. Other tested compounds have shown lesser influence on fibril formation, while compounds L1 and L4 were inactive in a 200 μM concentration (Figure 2).

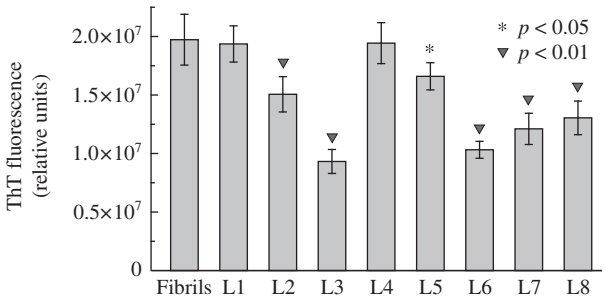


Figure 2 Inhibition of fibril formation by 200 μM of compounds L1–L8, mean ± SD, $n = 4$.

† The ovine recombinant prion protein (variant PrP VRQ – V136, R154, Q171, full-length amino acid sequence 23–234, without N-terminal signal peptide and C-terminal peptide, with one additional serine residue on the N-terminus) was expressed and purified according to a previously described procedure.[9]

‡ Prion fibrils were formed using 87 μM PrP in a 100 mM acetate buffer (pH 4.0), 0.03% sodium azide with 1 M guanidine hydrochloride at 37 °C during two days with shaking and stirring.[10]

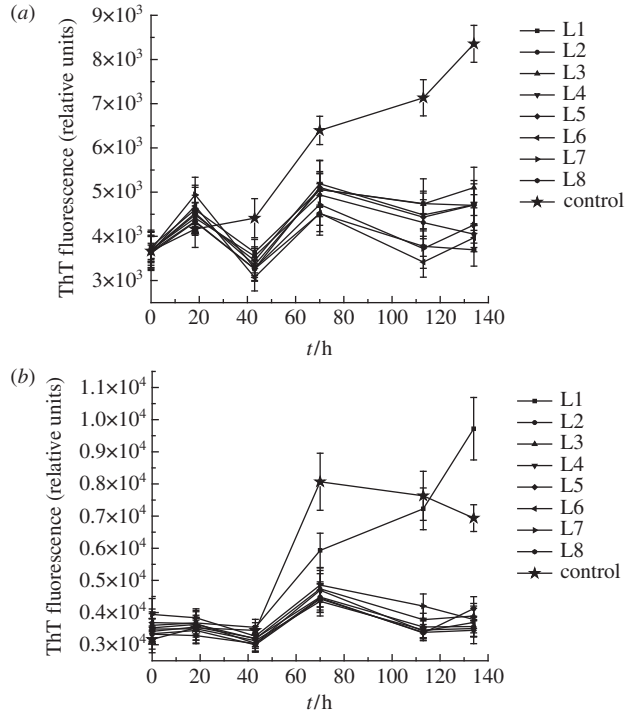


Figure 3 (*a*) Seeding and (*b*) spontaneous aggregation inhibition by 100 μM of compounds L1–L8. Mean ± SD, $n = 3$. For all ligands in seeding experiments at 70 h, $p < 0.01$; from 113 h, $p < 0.001$. For L2–L8 in spontaneous aggregation experiment starting from 70 h, $p < 0.001$.

The pre-incubation of PrP with inhibitors led to a significant reduction in ThT fluorescence in a seeding assay,[¶] and the most pronounced effect was observed with compounds L6, L7 and L8 (Figure 3).

In a spontaneous aggregation assay performed by incubating a 0.5 mg ml^{-1} solution of prion protein in a MOPS buffer (pH 7.5) at 37 °C with intense stirring for 138 h, all test ligands, except for L1, were able to prevent PrP aggregation.

Based on the obtained results, a brief structure–activity relationship (SAR) analysis was performed with cinnamic acid derivatives as PrP aggregation inhibitors. Substituents in the *meta*- and *para*-positions exerted the most pronounced effect on the anti-aggregation activity of the test compounds. Compounds L6 and L7 bearing an acetamidoxy group in the *para*-position exhibited the most promising effect in PrP aggregation assays; therefore, we suppose that a polar substituent in this position is crucial for ligands with increased potency. We also suppose that substituents larger than a methoxy group lead to a significantly lower activity, which is corroborated by the fact that ferulic acid is the most potent inhibitor, while compounds L1, L4 and L5 are the least active. A comparison between compounds L2 and L3 showed that a substituent in the 2-position leads to a decreased activity.

The cytotoxicity of the most potent compounds (L2, L4, L6, L7 and L8) was tested using an MTT assay on SH-SY5Y neuroblastoma cell line.[††] Upon incubation for three days with cells, the test compounds in 25, 50, 100 and 200 μM concentrations exerted no toxic effect. Moreover, 100 and 200 μM concentrations of test items were shown to increase cell viability compared to the vehicle-treated control (Figure 4).

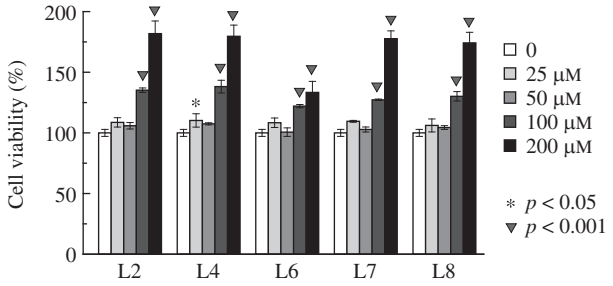


Figure 4 Cytotoxicity of compounds L2, L4, L6–L8 at various concentrations in neuroblastoma SH-SY5Y cells after incubation for three days. Mean ± SD, $n = 3$.

The test compounds were found active in amyloid fibril formation, 'seeding', and spontaneous aggregation assays, and the most pronounced effect was discovered for compounds L3, L6, L7 and L8 (ferulic acid). Ferulic acid prevents the amyloid aggregation of alpha-synuclein – a process implicated in the pathogenesis of Parkinson's disease.[12] The test compounds have no cytotoxic effect. Therefore, we conclude that cinnamic acid derivatives are promising candidates for the development of prion protein aggregation inhibitors, and ferulic acid, which is naturally abundant in coffee beans, is of special interest.

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References

1 S. B. Prusiner, *Science*, 1982, **216**, 136.
2 S. B. Prusiner, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 13363.
3 S. A. Kozin, V. A. Mitkevich and A. A. Makarov, *Mendeleev Commun.*, 2016, **26**, 269.
4 I. Hafner-Bratkovic, J. Gaspersic, L. M. Smid, M. Bresjanac and R. Jerala, *J. Neurochem.*, 2008, **104**, 1553.
5 I. Zanyatkin, Y. Stroylova, S. Tishina, V. Stroylov, A. Melnikova, T. Haertle and V. Muronetz, *Phytother Res.*, 2017, **31**, 1046.
6 M. Caruana, R. Cauchi and N. Vassallo, *Front Nutr.*, 2016, **3**, 31.
7 C. Monente, I. A. Ludwig, A. Irigoyen, M.-P. De Peña and C. Cid, *J. Agric. Food Chem.*, 2015, **63**, 4327.
8 Q. Zhao, Y. Zhang, G. Wang, L. Hill, J.-K. Weng, X.-Y. Chen, H. Xue and C. Martin, *Sci. Adv.*, 2016, **2**, e1501780.
9 H. Rezaei, D. Marc, Y. Choiset, M. Takahashi, G. Hui Bon Hoa, T. Haertlé, J. Grosclaude and P. Debey, *Eur. J. Biochem.*, 2000, **267**, 2833.
10 L. Breydo, N. Makarava and I. V. Baskakov, *Methods Mol. Biol.*, 2008, **459**, 105.
11 J. M. Wilham, C. D. Orrú, R. A. Bessen, R. Atarashi, K. Sano, B. Race, K. D. Meade-White, L. M. Taubner, A. Timmes and B. Caughey, *PLoS Pathog.*, 2010, **6**, e1001217.
12 R. Takahashi, K. Ono, Y. Takamura, M. Mizuguchi, T. Ikeda, H. Nishijo and M. Yamada, *J. Neurochem.*, 2015, **134**, 943.

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[§] A freshly prepared aqueous solution of Thioflavin T was added to 20 μM PrP at a molar ratio of 10 : 1. Thioflavin T was incubated for 15 min with protein samples before the measurements. ThT fluorescence was measured in 96-well plates; the fluorescence was excited at 430 nm and measured at 485 nm (PerkinElmer 2030 Multilabel Reader Victor X5).

[¶] Seeding was induced in the solution of 40 μM PrP in 20 mM MOPS buffer (pH 7.5) by the addition of fibrils suspension in a molar ratio of 1 : 50 with or without inhibitors and incubated for four to seven days with intense shaking as described elsewhere.[11]

[††] SH-SY5Y cells were plated in 96-well plates (3000 cells in 0.1 ml DMEM supplemented with 2% FBS) and treated with 25, 50 and 100 μM solutions of inhibitors. Each concentration was tested in quadruplicate. Cytotoxicity was measured using a standard MTT assay after drug exposure for 24 h. Results were quantified using a Universal Microplate Reader (Bio-Rad) at 570 nm, and a control absorbance in wells containing DMSO was designated as 100% of cell survival.