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Brief Article

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Multifunctional Mercapto-tacrine Derivatives for Treatment of Age-Related Neurodegenerative Diseases

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Supporting Information

ABSTRACT: Cooperating mercapto groups with tacrine in a single molecular, novel multifunctional compounds have been designed and synthesized. These mercapto-tacrine derivatives displayed a synergistic pharmacological profile of long-term potentiation enhancement, cholinesterase inhibition, neuroprotection, and less hepatotoxicity, emerging as promising molecules for the therapy of age-related neurodegenerative diseases.

INTRODUCTION

Age-related neurodegenerative diseases, especially Alzheimer's disease (AD), is a multifaceted disease characterized by the progressive loss of memory and cognitive functions, which depends on a combination of aging, genetic, and environmental factors. AD is initiated by a cascade of molecular events creating dysfunctions in different neurotransmitter systems.[1] Currently, the approved therapy for AD was based on enhancing cholinergic transmission through inhibition of cholinesterase (ChE). These anticholinesterase drugs, such as galantamine, donepezil, and rivastigmine, have been shown to only offer a modest improvement in memory and cognitive function.[2,3] Because of the complexity of AD, it is unlikely that a unitary mechanism of action will provide a comprehensive therapeutic approach to such multifaceted neurodegenerative disease.[4] Efficient therapy is more likely to be achieved by drugs that incorporate several pharmacological effects into a single chemical entity.

Synaptic plasticity, especially the long-term potentiation (LTP) in hippocampus, is widely considered as a key cellular mechanism underlying learning and memory.[5] Deficits in learning and memory accompanied by age-related neurodegenerative diseases are closely related to the impairment of synaptic plasticity. Generally, many studies have revealed that an impairment of hippocampal LTP usually occurred in aged animals.[6] Therefore, modulation of hippocampal LTP has been proposed as a potential therapeutic strategy for improving cognitive function of AD patients. More recently, it has been reported that some natural compounds can improve cognitive impairment via enhancing hippocampal LTP.[7,8] It has been well documented in our laboratory and other groups that chemical entities containing mercapto group, such as dithiothreitol (DTT), glutathione (GSH), and *N*-acetyl cysteine (NAC) (Figure 1), can facilitate the induction of LTP in the normal rats and even reverse the LTP impairment in aged rats.[9−11]

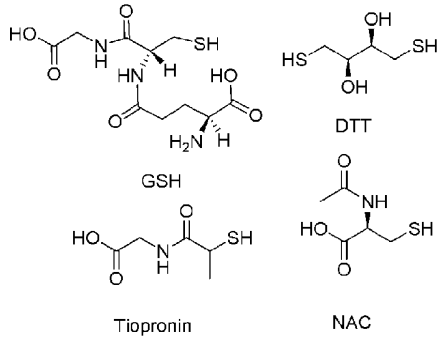

Figure 1. Chemical structure of GSH, DTT, Tiopronin, and NAC.

In addition, chemical entities containing mercapto group have attracted our interest because of their multiple pharmacological actions. Given that some exogenous sulfhydryl compounds could maintain the level of intracellular glutathione, the major antioxidant in nerve cells when exposed to the reactive oxygen species (ROS) insults, they may also lead to neuroprotection in the central nervous system (CNS).[12] The serious hepatotoxicity of tacrine was the main limitation for its clinical use.[13] Tacrine can cause ROS production stimulation and glutathione depletion in the human liver cell, suggesting that oxidative insults might be involved in the hepatotoxicity of tacrine. It has been reported that tacrine-induced oxidative stress can be prevented by glutathione or vitamin E.[14,15] Additionally, some chemical entities containing mercapto

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group, such as Tiopronin (Figure 1), were clinically applied to treat the liver injury caused by chemical insults.[16] Therefore, tacrine derivates cooperated with additional antioxidant fragment might be favorable to conquer the toxicity of tacrine. Indeed, some tacrine–antioxidant hybrids have been reported such as lipocrine that protects cells against oxidative stress and NO-donor-tacrine hybrids that showed hepatoprotective properties.[3,13,17]

In this context, we proposed that tacrine derivates cooperating with metcapto group in a single molecular entity may work in a synergistic manner. These compounds can serve as a safer and more effective therapeutic method for treatment of age-related neurodegenerative diseases. Thus, we designed and synthesized a series of mercapto-tacrine derivatives (Figure 2) and then investigated their effects on ChE activity, synaptic plasticity, neuroprotection, and liver function.

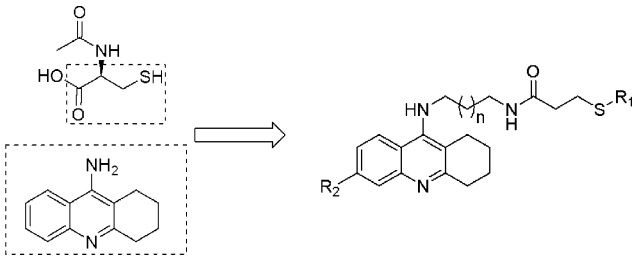
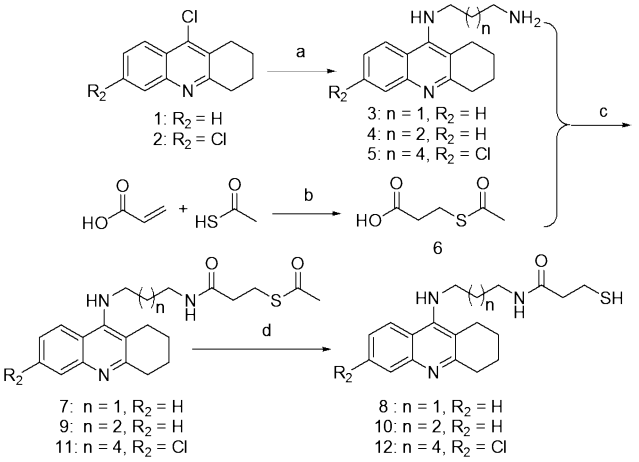


Figure 2. Design strategy for compounds **7**–**12**.

RESULTS AND DISCUSSION

Compounds **7**–**12** were synthesized following the convergent synthetic approach depicted in Scheme 1. Intermediates **1** or **2** were reacted with alkylenediamines to produce the important intermediates 9-aminoalkylamino-1,2,3,4-tetrahydroacridines (**3**, **4**, **5**). 3-(Acetylthio) propanoic acid (**6**) was produced by mixing propenoic acid with thioacetic acid in tetrahydrofuran. Compounds **3**, **4**, or **5** were condensed with **6** in the presence of 1-ethyl-3-(3-diethylaminopropyl) carbodiimide (EDC·HCl) and 1-hydroxy-7-azabenzo triazole (HOBt) in dichloromethane to yield **7**, **9**, and **11**. Compounds **8**, **10**, and **12** were prepared by deacetylation of **7**, **9**, and **11** in the presence of methanol/hydrochloric acid mixture.

Scheme 1. Synthesis of 7–12[a]



[a]Reaction conditions: (a) diaminealkane, pentanol, 180 °C,18 h; (b) THF, 50 °C, 18 h; (c) EDC·HCl, HOBt, CH_2Cl_2, 25 °C, 12 h; (d) MeOH/HCl, 40 °C, 10–12 h.

The ChE inhibitory activity of **7**–**12** was measured in vitro by Ellman's assay (Table 1).[18] Acetylcholinesterase (AchE) from electric eel and butyrylcholinesterase (BuchE) from equine serum were used. The results showed that **7**–**12** generally retained the ChE inhibitory effect (Table 1). Compound **11** displayed the most potent inhibitory activity against AchE among all the tested compounds. However, compared with tacrine, **7**–**10** and **12** showed a decrease in the inhibitory activity against AchE and BuchE. The mercapto-tacrine derivatives were more potent inhibitors of BuchE than AchE similar to that of tacrine, except for **11**, which had higher selectivity for AchE. The reduced potency of **11** against BuchE may cause by steric hindrance of the chloride atom with specific residues of the BuchE binding site.[19,20] The IC_{50} for AchE inhibition by **11** were not significantly different among 2, 10, and 40 min incubation, suggesting that **11** inhibited AchE activity in a nontime-dependent manner.[3] An analysis of the Lineweaver–Burk reciprocal plots of **11** against AchE in Figure 3 revealed that there were an increasing slope and an increasing intercept with higher inhibitor concentration. This result indicated that **11** caused a mixed-type inhibition, which was similar to that of tacrine and other reported tacrine derivates.[3]

Table 1. Inhibition of AchE and BuchE (pIC_{50} Values) by Mercapto-tacrine Derivatives

				pIC_{50} ± SEM[a]	
compd	*n*	R_1	R_2	AchE[b]	BuchE[b]
7	1	acetyl	H	5.76 ± 0.05	6.64 ± 0.07
8	1	H	H	5.22 ± 0.04	6.74 ± 0.03
9	2	acetyl	H	5.14 ± 0.04	6.81 ± 0.03
10	2	H	H	5.08 ± 0.02	6.89 ± 0.15
11	4	acetyl	Cl	7.37 ± 0.02	7.04 ± 0.03
12	4	H	Cl	6.75 ± 0.07	6.75 ± 0.04
Tacrine				7.19 ± 0.03	8.42 ± 0.11

[a]Data are the mean values of at least three determinations. pIC_{50} = $-\log IC_{50}$. [b]AchE from electric eel and BuchE from equine serum were used.

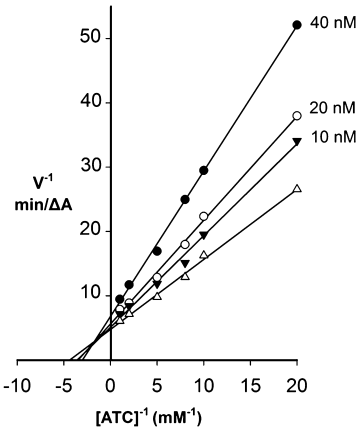


Figure 3. Kinetic study on the mechanism of AchE inhibition by **11**. Lineweaver–Burk reciprocal plots of AchE initial velocity at increasing substrate concentration (0.05–1 mM) in the absence of inhibitor and in the presence of **11** (10–40 nM) are shown.

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To evaluate the regulation of mercapto-tacrine derivatives on synaptic plasticity, **7**, **8**, and **12** with diverse substructure were chosen to test their effects on high-frequency stimulation (HFS)-induced LTP in the CA1 region of Sprague–Dawley (SD) rat hippocampal slices. It was found that HFS induced a dramatic increase in the magnitude of LTP when incubation of hippocampal slices with 50 μM **8** for 10 min (control, 133.7 ± 3.6% of baseline; **8**, 160.8 ± 4.4% of baseline; n = 9 for each group, P < 0.01 vs control, Figure 4B). Likewise, application of 50 μM **12** also enhanced the magnitude of LTP (control, 134.3 ± 3.9% of baseline; **12**, 157.1 ± 5.7% of baseline; n = 8 for each group, P < 0.01 vs control, Figure 4C). However, when the mercapto group was acetylated, **7** failed to enhance LTP (control, 136.3 ± 5.3% of baseline; **7**, 136.2 ± 3.1% of baseline; n = 8 for each group, Figure 4A). Treatment of hippocampal slices with the equimolar dose of tacrine also did not significantly alter the induction of LTP (control, 132.6 ± 4.6% of baseline; tacrine, 136.2 ± 3.6% of baseline; n = 6 for each group, Figure 4D). Additionally, we further investigated the regulation of **12** on synaptic plasticity under the condition of in vivo drug administration. Hippocampal slices were recorded 3 h after intracerebroventricular (icv) injection of **12** (final concentration 50 μM). It was found that in vivo injection of **12** also enhanced the hippocampal LTP (control, 126.0 ± 3.6% of baseline; **12**, 149% ± 4.5% of baseline; P < 0.01 vs control, n = 8 for each group, Figure 5). Above results indicated that the mercapto-tacrine derivatives could strengthen the induction of LTP via its mercapto group.

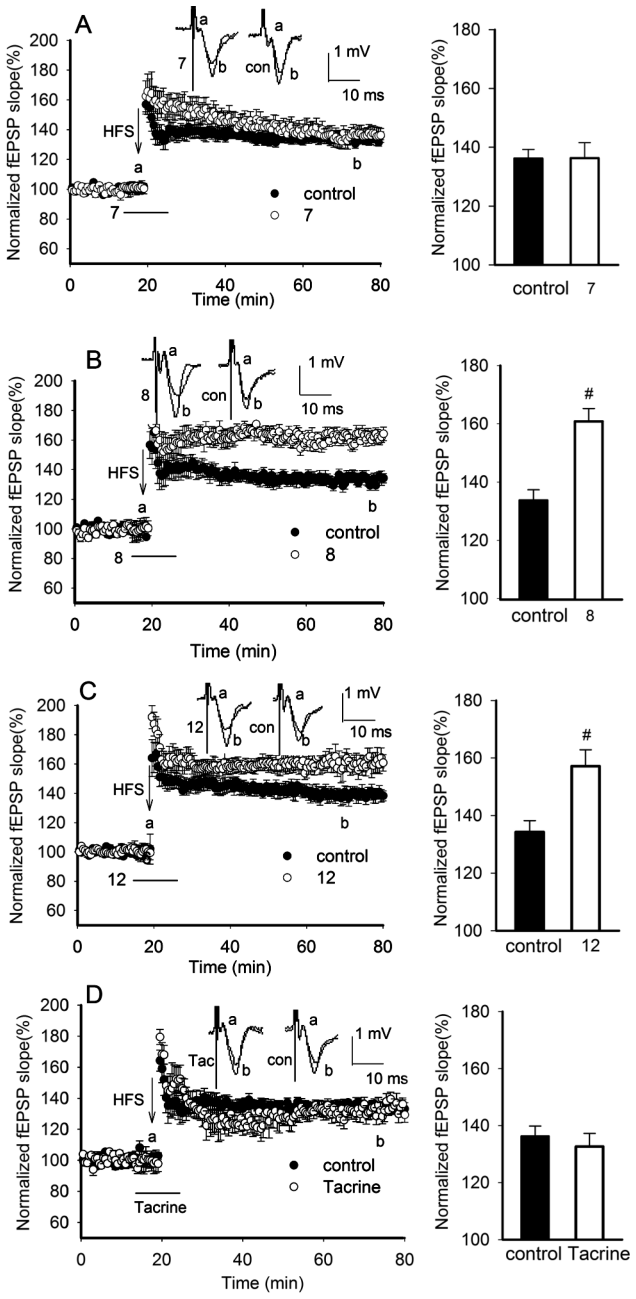

Figure 4. Effects of mercapto-tacrine derivatives on the magnitude of LTP in the hippocampal slices of rat. The bar graph illustrating the fEPSPs slope averaged from the last 15 min recordings of control and treated slices. (A) **7** (n = 8); (B) **8** (# P < 0.01 vs control, n = 9); (C) **12** (# P < 0.01 vs control, n = 8); (D) tacrine (n = 6).

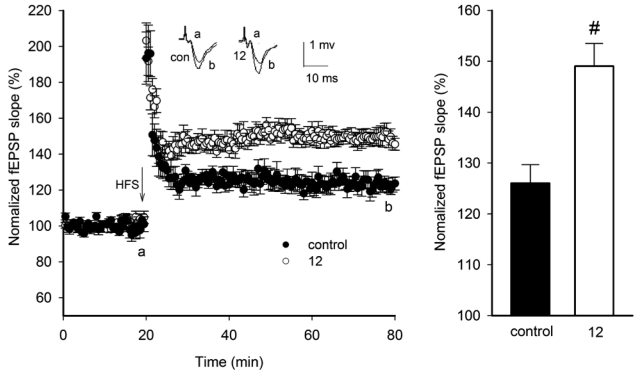

Figure 5. Effects of **12** on the magnitude of hippocampal LTP under the condition of in vivo administration. Hippocampal slices were recorded 3 h after intracerebroventricular injection of **12** (final concentration 50 μM). The bar graph illustrating the fEPSPs slope averaged from the last 15 min recordings of control and treated slices. # P < 0.01 vs control, n = 8.

The neuroprotective action of mercapto-tacrine derivatives against oxidative stress was measured in human neuroblastoma cell line SH-SY5Y. Cells were incubated with **7**–**12** at four concentrations (1, 3, 10, and 30 μM) 1 h before the addition of 200 μM hydrogen peroxide. Cell viability was measured by using the MTT assay after treatment of 200 μM hydrogen peroxide for 12 h. Trolox, the vitamin E antioxidant moiety, was selected as a positive control. All tested compounds (Table 2) displayed neuroprotection against hydrogen peroxide-induced damage at 1, 3, 10, and 30 μM. Compounds **7**, **9**, and **11**, the mercapto group of which was covered with the acetyl group,

Table 2. Neuroprotection (%) in the Human Neuroblastoma Cell Line SH-SY5Y against H_2O_2 (200 μM) at the Indicated Concentrations

compd	1 μM	3 μM	10 μM	30 μM
7	32.4[a]	35.9[a]	38.2[b]	50.6[b]
8	45.9[c]	22.0[a]	16.7	34.0[a]
9	21.7	30.1[a]	43.6[c]	56.5[b]
10	23.8[a]	5.8	21.5[a]	38.2[a]
11	8.1	17.1	28.5	34.0[a]
12	52.3[c]	27.2	24.5[b]	37.8[a]
Trolox			44.2[c]	59.1[c]

[a] P < 0.05. [b] P < 0.01. [c] P < 0.001 vs H_2O_2 group

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exhibited the neuroprotective effect mostly in a concentration-dependent manner, with the maximal effect at 30 μM (**7**, 50.6%; **9**, 56.5%; **11**, 34.0%). Interestingly, **8**, **10**, and **12** presented a U-shaped dose−effect curve. Compounds **8** and **12** showed significant protection at 1, 3, and 30 μM, and the maximal efficacy was observed at 1 μM (**8**, 45.9%; **12**, 52.3%). It was worth mentioning that the neuroprotective effect was decreased at higher concentrations, which was probably due to additional interactions with other biological targets. These phenomena were similar to those of some marketed compounds (e.g., galantamine or donepezil), which also showed a U-shaped concentration-dependent neuroprotective properties.[21]

To determine whether mercapto-tacrine hybrids were more safe than tacrine, **7** and **8** were chosen to test their hepatotoxicity. Rats were treated at the highest tolerated dose of tacrine (6 μmol/100 g body weight) or equimolar doses of **7** and **8**. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in serum samples were measured before administration (0 h) or 24, 36, 72, and 120 h after administration. As shown in Figure 6, administration of

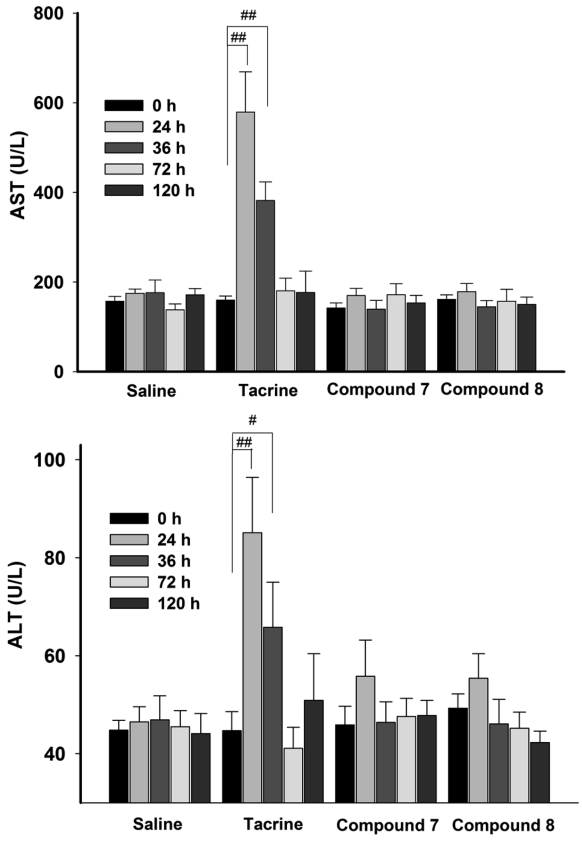

Figure 6. Influence of equimolar doses (6 μmol/100 g bwt) of tacrine and **7** and **8** on the activity of ALT (U/L), and AST (U/L). Results are compared to control values before administration. Statistical significant difference to control values before administration (Student's *t* test, n = 8−12). # $P < 0.05$. ## $P < 0.01$ vs 0 h.

the highest tolerated dose of tacrine caused a sharp rise in AST and ALT activities at 24 h. Although decreased at 36 h, they were still significantly higher than the level of before administration. The increase in activities of AST and ALT by single dose of tacrine returned to the normal levels at 3 days after administration. However, administration of saline or **7** and **8** did not dramatically increase the activities of AST and ALT from 0 to 120 h. These results indicated that **7** and **8** had little hepatotoxicity.

CONCLUSION

In the current study, we report a novel series of mercapto-tacrine derivatives endowed with cholinesterase inhibition, LTP enhancement, neuroprotective activity, and less hepatotoxicity. It is expected that these multifunctional compounds are more efficient to improve the memory and cognitive impairment with fewer side effects and diminish the oxidative damage caused by free radicals. Such interesting properties highlight them as good candidates for further studies directed to the development of novel drugs for age-related neurodegenerative diseases such as AD.

EXPERIMENTAL SECTION

^{1}H and ^{13}C NMR spectra were recorded using TMS as the internal standard in $CDCl_3$ or DMSO-d_6 with a Bruker spectrometer at 400 and 100 MHz, respectively. MS spectra were recorded on a Finnigan LCQ Deca XPTM instrument with an ESI mass selective detector. Reactions were monitored by thin layer chromatography and MS spectra. Flash column chromatography was performed with silica gel (200−300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purities of targeted compounds were confirmed by analytical HPLC on a HITACHI L-2000 instrument and a Diamonsil ODS-C18 HPLC column (250 mm × 4.6 mm, 5 μm) at 340 nm, eluted with a gradient of 70−80% solvent B (methanol) in solvent A (water) containing 0.2% phosphate buffer (pH = 7.23) at a speed of 1 mL/min. The purities of target compounds were determined to be ≥95% by HPLC unless otherwise indicated. Melting points were obtained on Taike X-5 microautomatic melting point detector and were uncorrected.

General Procedures for the Synthesis of 7, 9, and 11. To a stirred solution of 1.0 molar equiv of **6** in 30 mL of CH_2Cl_2 at 25 °C was added 2 molar equiv of 1-ethyl-3-(3-diethylaminopropyl) carbodiimide (EDC·HCl) and 2 molar equiv of 1-hydroxy-7-azabenzotriazole (HOBt). The resulting mixture was stirred at room temperature for 30 min before a solution of 1 molar equiv of compounds **3**, **4**, or **5** in 15 mL CH_2Cl_2 were added. The resulting mixture was stirred at 25 °C for 12 h before it was quenched with brine (5 mL) and diluted with CH_2Cl_2 (50 mL). The combined organic phase was washed with brine (3 × 50 mL), dried over Na_2SO_4, and filtered. After removal of the solvent under vacuum, the residue was purified by flash column chromatography with petroleum ether/ethyl acetate to yield the products.

General Procedures for the Synthesis of 8, 10, and 12. Compounds **7**, **9**, or **11** were dissolved in 10 mL of methanol containing 1 mL of 6 M HCl. The reaction mixture was stirred at 40 °C for 10−12 h under the protection of N_2 while being monitored by TLC and LC-MS until **7**, **9**, or **11** completely reacted. The reaction mixture was allowed to cool, diluted with 50 mL of CH_2Cl_2, and washed with $NaHCO_3$ aqueous solution and brine (3 × 50 mL). The combined organic phase was dried over Na_2SO_4 and filtered. The solvent evaporated to obtain the pure products.

ASSOCIATED CONTENT

Supporting Information

Spectroscopic data for **3**−**12**, AchE and BuchE inhibition assays, electrophysiological studies in hippocampal slices, neuroprotection assays, and hepatotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AD, Alzheimer's disease; ChE, cholinesterase; LTP, long-term potentiation; ROS, reactive oxygen species; DTT, dithiothreitol; GSH, glutathione; NAC, *N*-acetyl cysteine; AchE, acetylcholinesterase; BuchE, butyrylcholinesterase; HFS, high frequency stimulation; SD, Sprague−Dawley; fEPSPs, field excitatory postsynaptic potentials; AST, aspartate aminotransferase; ALT, alanine aminotransferase

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