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Evaluation of nicotine and cotinine analogs as potential neuroprotective agents for Alzheimer's disease

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Abstract

The currently available therapies for Alzheimer's disease (AD) and related forms of dementia are limited by modest efficacy, adverse side effects, and the fact that they do not prevent the relentless progression of the illness. The purpose of the studies described here was to investigate the neuroprotective effects of the nicotine metabolite cotinine as well as a small series of cotinine and nicotine analogs (including stereoisomers) and to compare their effects to the four clinically prescribed AD therapies.

Keywords

Neuroprotection; nicotinic; multi-target-directed ligands; multifunctional compounds; amyloid; glutamate; excitotoxicity; neurodegeneration; dementia; disease modifying

Alzheimer's disease (AD) is the most common neurodegenerative disease in the elderly and its prevalence is expected to rise sharply in the next several decades.[1] Unfortunately, the currently available therapies (acetylcholinesterase inhibitors and the glutamate, NMDA antagonist, memantine) are limited by modest efficacy, adverse side effects, and the fact that they do not prevent or even significantly delay the relentless progression of the illness. The varied symptoms of AD which include cognitive deficits, non-cognitive behavioral symptoms (e.g., agitation, hallucinations), and the complex pathophysiology (amyloid-β neurotoxicity, tau hyperphosphorylation, glutamate excitotoxicity etc.) support the argument that novel compounds that affect multiple drug targets (i.e., multi-target-directed ligands" or MTDLs) or that have multifunctional properties (e.g., pro-cognitive and neuroprotective, pro-cognitive and antipsychotic actions) are needed for more optimal therapeutic interventions.[2–5]

Interestingly, the tobacco alkaloid nicotine has been shown to possess multifunctional properties including pro-cognitive effects in humans, rodents, and non-human primates[6–7]

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and neuroprotective activities in a variety of model systems.[8] The use of nicotine as a therapeutic agent, however, is clearly limited by its short half-life, abuse potential, and cardiovascular side effects.[9] An increasing body of evidence suggests that the most predominant metabolite of nicotine in mammalian species, cotinine, might retain the positive features of nicotine while exhibiting fewer limitations. In vitro, cotinine protects against toxic insults in PC12 cells with potency similar to that of nicotine[10], suppresses the release of oxygen free radicals from neutrophils[11], augments PI3K-dependent anti-inflammatory pathways in human monocytes[12], protects against 6-OHDA-toxicity in SH-SY5Y cells[13], and reduces death induced by Aβ neurotoxicity in primary cortical neurons.[14] In vivo, cotinine has been observed to prevent memory loss in transgenic (Tg) 6799 Alzheimer's disease mice as well as to stimulate the Akt/GSK3β pathway and reduce Aβ aggregation in their brains.[15] Cotinine has also been evaluated across a variety of additional behavioral assays in rodents and non-human primates for potential effects on information processing and cognition. In monkeys cotinine elicited dose-dependent improvements of a delayed match to sample (DMTS) task as well as a modified version of the task (DMTS-D) where randomly-presented (task-relevant) distractors were presented.[16] Cotinine also attenuated deficits of DMTS in monkeys produced by the glutamate NMDA receptor antagonist ketamine[17] and it attenuated the deficits of sustained attention in rats induced by the NMDA receptor antagonist MK-801.[18] Cotinine also improved prepulse inhibition (PPI) of the acoustic startle response in pharmacological impairment models[19], a property that may predict the efficacy of compounds as antipsychotic agents as well as cognitive enhancers.

Collectively, the results described above indicate that cotinine has neuroprotective properties and that it improves information processing, attention, and memory-related task performance in model systems that have relevance to both AD and other neuropsychiatric disorders such as schizophrenia. Given the much longer half-life of cotinine compared to nicotine, its considerably lower toxicity[20], and apparent lack of abuse potential[9], it may serve as a superior prototypical therapeutic agent for neuropsychiatric disorders.

The purpose of the studies described here was to further investigate the neuroprotective potential of cotinine (and nicotine) as well as a small series of their analogs (including stereoisomers) which are commercially available (see Fig 1) and to compare their effects to the four clinically prescribed AD therapies. The purpose of evaluating the analogs was to establish a preliminary structure–activity relationship (SAR) and define the features of the molecules that might be optimal for neuroprotective activity. We focused on neuroprotection against amyloid β (Aβ) and glutamate-mediated toxicity which are well established as major contributing factors to the neurodegeneration of AD.[21,22] The neuroprotection assays are based on methods described previously[23,24] with modifications.[25–26]

Concentration-effect relationships for $A\beta_{1–42}$ and glutamate treatment on the viability of rat primary cortical neurons are illustrated in Fig 2A and 2B, respectively. As illustrated, after exposure to either the $A\beta_{1–42}$ peptide or glutamate for 24 hours, there was a concentration-dependent decrease in cell viability as indicated by the MTT assay. From these concentration-response curves, $A\beta_{1–42}$ [200 nM] and glutamate [20μM] were selected for subsequent neuroprotection evaluations with each compound reducing cell viability to approximately 60% of control (specifically, 60.8 ± 2.4% for $A\beta_{1–42}$ exposure and 58.6±

3.2% for glutamate exposure when compared with the vehicle-treated sample). In a second set of (confirmatory) experiments, these selected concentrations of $A\beta_{1–42}$ and glutamate produced a similar decrease in cell viability as indicated by the Trypan blue exclusion method. Note the increase in nonviable cells in the representative photomicrographs in the neurotoxin treated cultures (compared to vehicle-treated controls) which are membrane-porous and stain blue, whereas the viable cells exclude trypan blue stain due to their intact cell membranes.

The results of experiments designed to assess the potential neuroprotective effects of nicotine, cotinine and structural analogs against the compromised neuronal viability induced by the $A\beta_{1–42}$ peptide are illustrated in Fig 3 and Table 1. In Fig 3, concentration-effect relationships for the most effective compounds (in the MTT assay) are illustrated in the bar plots and the effects of optimal concentrations (confirmed by the trypan blue exclusion method) are illustrated in the representative photomicrographs. As shown, 24 hr incubation with the $A\beta_{1–42}$ peptide [200 nM] decreased cell survival by about 40% in each series of experiments. (−)-Nicotine (compound 1), (−)-cotinine (compound 8) and compounds 3 and 12 significantly protected against Aβ-induced neurotoxicity. In fact, all of the concentrations of nicotine, cotinine and compounds 12 evaluated (10.0 nM to 100 μM) offered some degree of protection ($p<0.05$) while the highest 3 concentrations of compound 3 afforded significant protection. Compound 12 appeared to offer the greatest degree of protection with the 100 μM concentration producing cell viability greater than 90% of control values. As indicated in Table 1, nine of the experimental compounds evaluated ((−)-nicotine, (−)-cotinine and their analogs) offered some degree of neuroprotection, while none of the currently prescribed AD therapies (donepezil, galantamine, rivastigmine, or memantine) were effective. The highest dose of donepezil (100 μM) was, in fact, associated with an increase in neurotoxicity compared to $A\beta_{1–42}$ peptide exposure alone. It is also important to note that while the (+) isomers of nicotine (compound 2) and cotinine (compound 9) offered some degree of neuroprotection in these experiments, they were considerably less effective that the (−) isomers.

The results of experiments designed to assess the potential neuroprotective effects of nicotine, cotinine and structural analogs against glutamate neurotoxicity are illustrated in Fig 4 and Table 2. Similar to the toxicity associated with the $A\beta_{1–42}$ peptide, 24 hr incubation with glutamate (20 μM) decreased cell survival by about 40% in each series of experiments. Based on the number of concentrations that afforded significant protection against glutamate neurotoxicity, (−)-nicotine, memantine, and compounds 3 and 12 were most effective. In Fig 4, concentration-effect relationships for these compounds (using the MTT assay) are illustrated in the bar plots and the effects of optimal concentrations (confirmed by the trypan blue exclusion method) are illustrated in the representative photomicrographs. In these experiments, (−)-nicotine (compound 1) and memantine were clearly the most effective compounds with their highest concentrations (100 μM) improving cell viability to over 85% of control. The (+) isomer of nicotine (compound 2) did not retain the neuroprotective activity of the (−) isomer. There were a few other instances where some level of neuroprotection was afforded against glutamate neurotoxicity depending on the compound and drug concentration evaluated. For example, two concentrations of donepezil (1.0 and 10.0 μM) improved cell viability; while (similar to the case of $A\beta_{1–42}$ toxicity) the highest

concentration (100 μM) appeared to increase glutamate toxicity. One concentration of galantamine (10 μM) and one concentration of compound 14 (100 μM) also improved cell viability.

The data obtained in the experiments described in this manuscript provide: 1) confirmatory evidence that (−)-nicotine and its most predominant metabolite (−)-cotinine have neuroprotective properties in vitro, 2) that the protective effect of the (−) isomers of nicotine and cotinine is significantly reduced or lost in the (+) isomers, 3) that some of commercially available analogs of nicotine and cotinine also possess neuroprotective activity in vitro: 4) and that (−)-nicotine and at least two of the nicotine/cotinine analogs (by exhibiting efficacy in two neurotoxicity models) appear to be superior as neuroprotective agents when compared to the currently prescribed AD therapeutic agents.

In the $A\beta_{1–42}$ neurotoxicity model, the (−)-nicotine and (−)-cotinine analogs could be categorized into two main groups: those affording protection similar to or better than their parent compounds (e.g. Compounds 4 and 12) and those that showed complete loss of activity (e.g. compound 11 and 15). These results allowed for an initial prediction of the molecular features that might underlie nicotine/cotinine's protective activity. First, oxidation of the nitrogen in the pyridine ring with a positive charged cation (compound 5 and 10) preserved neuroprotective activity of the parent compounds. However, compound 11, where the substituted position on the pyridine ring was switched from *meta* to *ortho*, lost the protective activity. Second, when the pyrrolidine ring is reduced to an aromatic pyrrole ring (compound 6) or is replaced by a chain ester substituent (compound 7), the protective activities were also reduced. However, compound 3, where the pyrrolidine ring is replaced with a 3,4-dihydro-2H-pyrrol-1-ium, retained neuroprotective activity. These data suggest that the flexibility of this ring system might be essential for optimum neuroprotective activity, given that the aromatization of the pyrrolidine introduced conformational changes in the structure and restricted the carbon positions in the ring. Third, a small substituent on the nitrogen of the pyrrolidine appears to be important for neuroprotective activity (in the $A\beta_{1–42}$ neurotoxicity model) since the effect was lost by the addition of a *para*-methoxylmethylbenzyl group as observed in compound 14, while compound 12 and 13 without any substituent or with a small ethyl group, exhibited comparable activities to the parent compounds. Fourth, the substituted groups on the pyrrolidine ring (except for the nitrogen) might also be critical based on the mild decrease in activity in the compounds with the hydroxyl substituent (compounds 15 and 16) and complete loss of activity in the compound with an amide substituent (compound 18). However, compound 17 with the carboxylic group retained activity which suggested that a strong electronegative group might be favorable for neuroprotective activity.

In the glutamate neurotoxicity model, the low number of effective nicotine and cotinine analogs prevented any clear predictions as to the optimal structural features for neuroprotection. The fact that compound 3 (a nicotine analog) and 12 (a cotinine analog) each afforded significant neuroprotection in both the $A\beta_{1–42}$ and the glutamate neurotoxicity model suggests that the extra carbonyl group in the cotinine structure may (alone) have little influence on neuroprotective activity. The observation that compound 14 with a bulky substituent on the pyrrolidine ring did not exhibit protective activity in the $A\beta_{1–42}$

neurotoxicity model, whereas it exhibited a strong neuroprotective effect (83.9 ± 2.7% of control cell viability) in the glutamate neurotoxicity model (albeit at a single concentration), further suggests that the substituent size of the nitrogen in the pyrrolidine ring might be an important target for structural modifications. The fact that memantine (a glutamate NMDA antagonist) was effective in the glutamate neurotoxicity model was not surprising and it effectively served as a positive control for the later series of experiments described in this manuscript. There may be features of this molecule that could be combined with the structure of nicotine or cotinine to enhance activity against glutamate neurotoxicity.

The mechanisms of the neuroprotective effects of the various compounds observed in this study are unclear. It has been reported that the neuroprotective effects of nicotine and acetylcholinesterase inhibitors (AChEIs) observed previously in $A\beta_{1-42}$ and glutamate neurotoxicity models is related to direct (nicotine) and indirect (AChEIs) effects at $\alpha_4\beta_2$ and α_7 nicotinic acetylcholine receptors (nAChRs) as well as effects on the PI3K-Akt pathway, activation of calcineurin, and L-type calcium channels.[27–30] In older nAChR binding assays, cotinine was found to be approximately 100–1000 fold less potent than nicotine at displacing radiolabeled nAChR ligands[31–34], therefore, it appears unlikely that the neuroprotective effects of cotinine observed in the $A\beta_{1-42}$ neurotoxicity assay (i.e., at similar concentrations to nicotine) could be fully explained by direct effects at nAChRs. Interestingly, effectiveness of nicotine and cotinine and some other compounds (e.g., choline analogs) in memory-related behavioral tasks has been correlated with their effectiveness in producing nAChR desensitization.[35] It would, therefore, be interesting to determine if such a relationship could be made between nAChR desensitization and neuroprotective activity. To our knowledge the nicotine and cotinine analogs evaluated in the current studies have not been assessed in nAChR binding or functional assays. The neuroprotective effects of some of the compounds evaluated in this study might also be related to effects on growth factors (i.e., neurotrophins) and/or their receptors. Interestingly, nicotine has been shown in culture systems (SH-SY5Y cells) to increase the release of Brain-Derived Neurotrophic Factor (BDNF) and to increase the cell surface expression of TrkB receptors.[36] Likewise, nicotine, in primary cultures of rat basal forebrain neurons, was found to increase the release of nerve growth factor (NGF) and to increase TrkA receptors.[37] Such effects on neurotrophin-related proteins might be especially relevant to the observations in the current study given that the test compounds (i.e., including nicotine) were administered first then washed out of the culture medium prior to toxin exposure (i.e., indicative of a prolonged neuroprotective effect). It is important to note that (to date) the effects described above have only be shown with nicotine, therefore, future experiments will be required to determine if such effects occur after exposure to the analogs of nicotine, cotinine, and cotinine analogs.

In conclusion, the results of this study indicated that S-(−)-nicotine, S-(−)-cotinine, and nine of their analogs (especially compounds 3 and 12) exhibited neuroprotective activities against amyloid-β neurotoxicity while only four of the compounds evaluated, nicotine, compounds 3 and 12, and the clinically prescribed NMDA antagonist, memantine exhibited significant protective effects against glutamate-mediated toxicity. The results with the analogs also indicated that the substituent size of the nitrogen in the pyrrolidine portion of these compounds is critical for neuroprotective activity and that the extra carbonyl group in the cotinine structure has little influence on this activity. The efficacy of (−)-nicotine and

compounds 3 and 12 in both neuroprotection models used in these experiments suggest superior potential as disease-modifying agents when compared to the available prescription therapies (acetylcholinesterase inhibitors and the glutamate, NMDA antagonist, memantine). Given the limitations of nicotine as a potential therapeutic agent (e.g., cardiovascular side effects, abuse potential), compounds 3 and 12 may serve as superior prototypical compounds for the treatment of neurodegenerative conditions such as AD. Further, their structural features may aid in future rational drug design approaches.

Acknowledgments

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List of abbreviations

Aβ	amyloid β
AChEI	acetylcholinesterase inhibitor
AD	Alzheimer's disease
nAChR	nicotinic acetylcholine receptor

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25. Chemicals and suppliers-The source of drugs and materials used are as follows: Cell culture materials (Gibco, Grand Island, NY, USA); Invitrogen Vybrant® MTT Cell Proliferation Assay Kit (Molecular probes, Eugene, OR, USA), Aβ1–42 (American Peptide, Sunnyvale, CA, USA), R-(+)-Nicotine, S-(−)-Cotinine, R-(+)-Cotinine, (+/−)-ortho-Cotinine Perchlorate, trans-3'-Hydroxy Cotinine (10mg), cis-3'-Hydroxy Cotinine, (S)-Cotinine N-Oxide, (2'S)-Nicotine 1-Oxide, N-(4-Methoxybenzyl)cotinine, trans-Cotinine Amide, N-Formylnornicotine, S-(−)-Nicotine-Δ1'(5')-iminium Diperchlorate Salt, (R,S)-norcotinine, β-Nornicotyrine, (R,S)-N-Ethylnorcotinine (Toronto Research Chemicals, Toronto, ON, Canada), Methyl 6-methylnicotinate (Alfa Aesar, Ward Hill, MA, USA), trans-1-Methyl-4-carboxy-5-(3-pyridyl)-2-pyrrolidinone (TCI America, Portland, OR, USA), (−)-Nicotine, Memantine hydrochloride, MK-801, (±)-Nornicotine, L-glutamic acid monosodium salt hydrate (Sigma, St. Louis, MO, USA), Donepezil, Galantamine (A&A Pharmachem, Shenzhen, China).

26. Neuroprotection Assays-Cortical neuronal cultures were derived from the cerebral cortex of Sprague–Dawley rat embryos (E17–18) as described previously [32]. Briefly, cells dissociated from the cerebral cortex of embryos were seeded at a density of 5×105 cells/mL onto poly-D-lysine pre-coated 96-well plates for neuronal cytotoxicity assay. For image analysis, cells were

seeded at a density of 2×105 cells/mL onto poly-L-lysine pre-coated 12mm glass coverslips in 24-well plates (Corning, Corning, NY, USA). Cultures were incubated in neurobasal medium supplemented with 2% B27, 0.5M L-glutamax, 100 U/mL penicillin and streptomycin and maintained at 37°C in a 5% CO2 humidified atmosphere. Experiments were performed at 37°C on the culture day 7–8. In order to determine concentration-effect relationships for the neurotoxic effects of the Aβ1–42 peptide or glutamic acid, cultured neurons were exposed to Aβ1–42 (100, 200, 400, 800 and 1000nM) or glutamate (10, 20, 40, 80 and 100uM) for 24 h. To determine the neuroprotective effects of the test compounds against Aβ- and glutamate-induced neurotoxicity, neurons were pre-treated with test compounds alone (10nM, 100nM, 1uM, 10uM and 100uM) for 24 hours. The cells were washed and then challenged with 200nM Aβ or 20uM glutamate for another 24 hours. For each condition described above, a total of 2–4 independent experiments were performed with 7 replicates per drug concentration evaluated. Cell viability was determined using a commercially available MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay (Invitrogen Vybrant® MTT Cell Proliferation Assay Kit). Briefly, after exposures to neurotoxins and/or test compounds, primary cortical neurons were treated with 0.5 mg/ml MTT for 4 h at 37°C. 100 μl of the SDS-HCl solution were added to each well and mixed thoroughly and incubated for another 14 hours. The absorbance was measured at 570 nm. As a secondary method of confirming the results obtained with the MTT assay, cell viability was confirmed via the Trypan blue exclusion method. Briefly, cultured cells were incubated in 1.5% trypan blue PBS solution for 10 minutes at room temperature and fixed in 4% paraformaldehyde (pH 7.2, 2–4°C), and then rinsed with PBS. Culture fields were photographed with a Zeiss Axioplan 2 Microscope with AxioCam camera. Results are expressed as percentage of control values obtained from cultures not exposed to glutamate or the Aβ1–42 peptide. Differences were analyzed for statistical significance using one-way ANOVA, followed by Holm-Sidak post hoc comparison method. Significance was set at $p < 0.05$.

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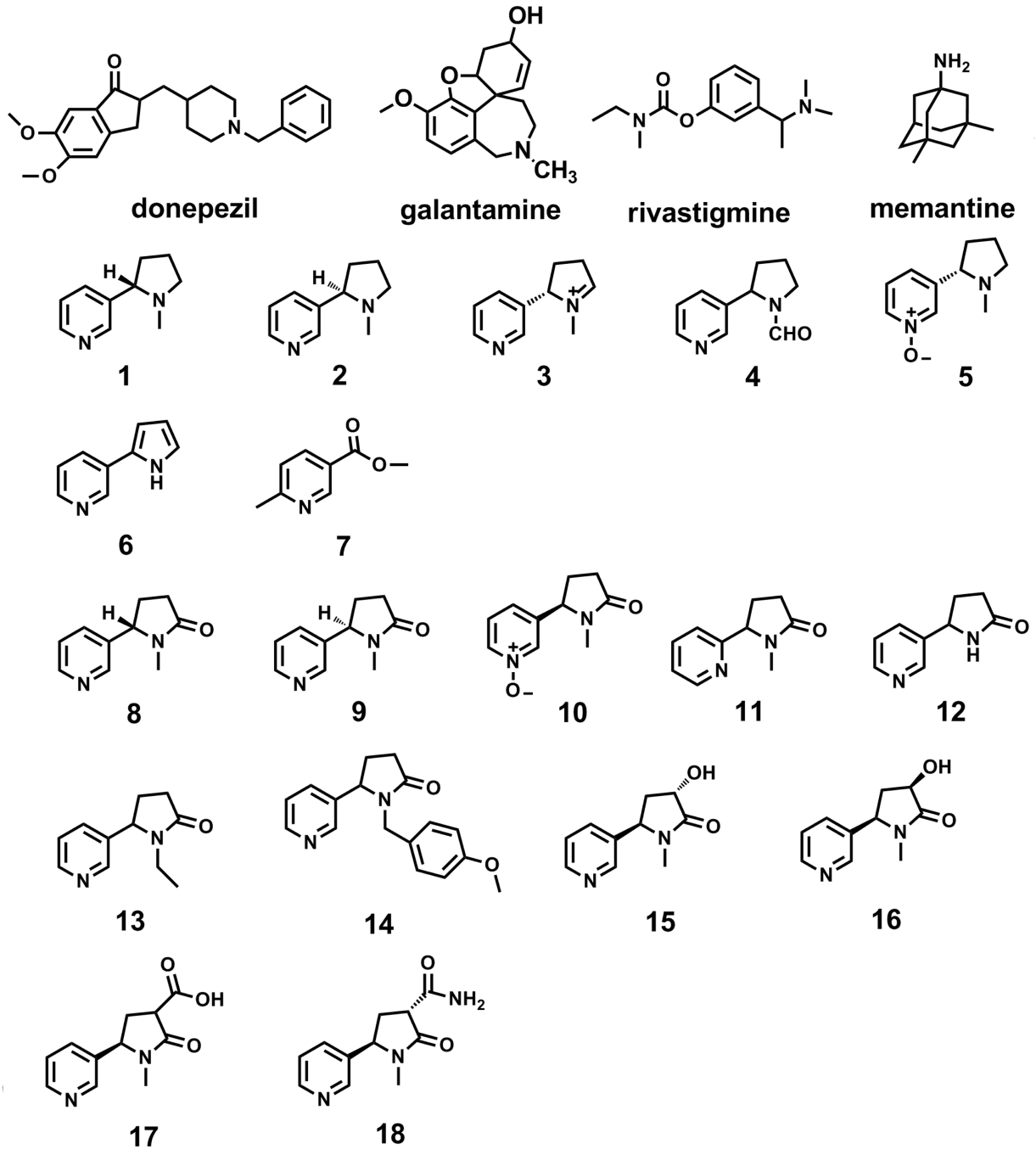

Fig 1.

Chemical Structures of the currently prescribed AD therapeutic agents, commercially available nicotine analogs (compounds 1–7), and commercially available cotinine analogs (compounds 8–18).

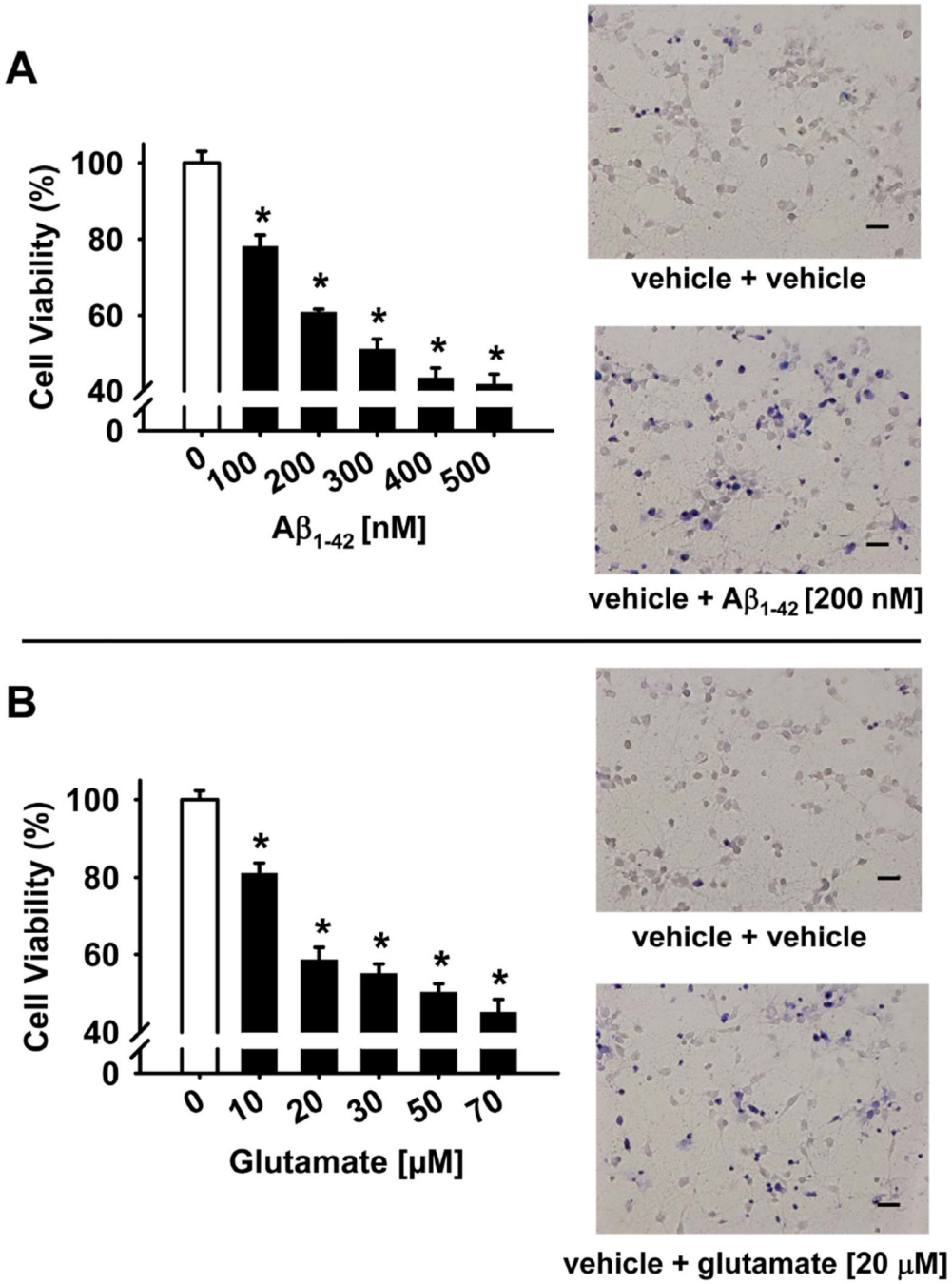

Fig 2.

Concentration-effect relationships for $A\beta_{1–42}$ and glutamate treatment on cell viability in primary cultures of rat cortical neurons. Cultures were exposed to various concentrations of the $A\beta_{1–42}$ peptide (A) or glutamate (B) for 24 hours and cell viability was determined in an MTT assay (see Materials and Methods), calculated as percentage survival rate, and compared to a negative control (i.e., cultures without the $A\beta_{1–42}$ peptide). Each bar represents the mean ± S.E.M (derived from 2–4 independent experiments with 7 replicates per drug concentration). *$p < 0.05$ compared to wells with no $A\beta_{1–42}$ peptide. The effects of

the selected concentrations of the $A\beta_{1-42}$ peptide and glutamate to be used in subsequent neuroprotection experiments were confirmed via a Trypan Blue exclusion assay (see Materials and Methods) and are illustrated in representative photomicrographs. Scale bar = 100 μm.

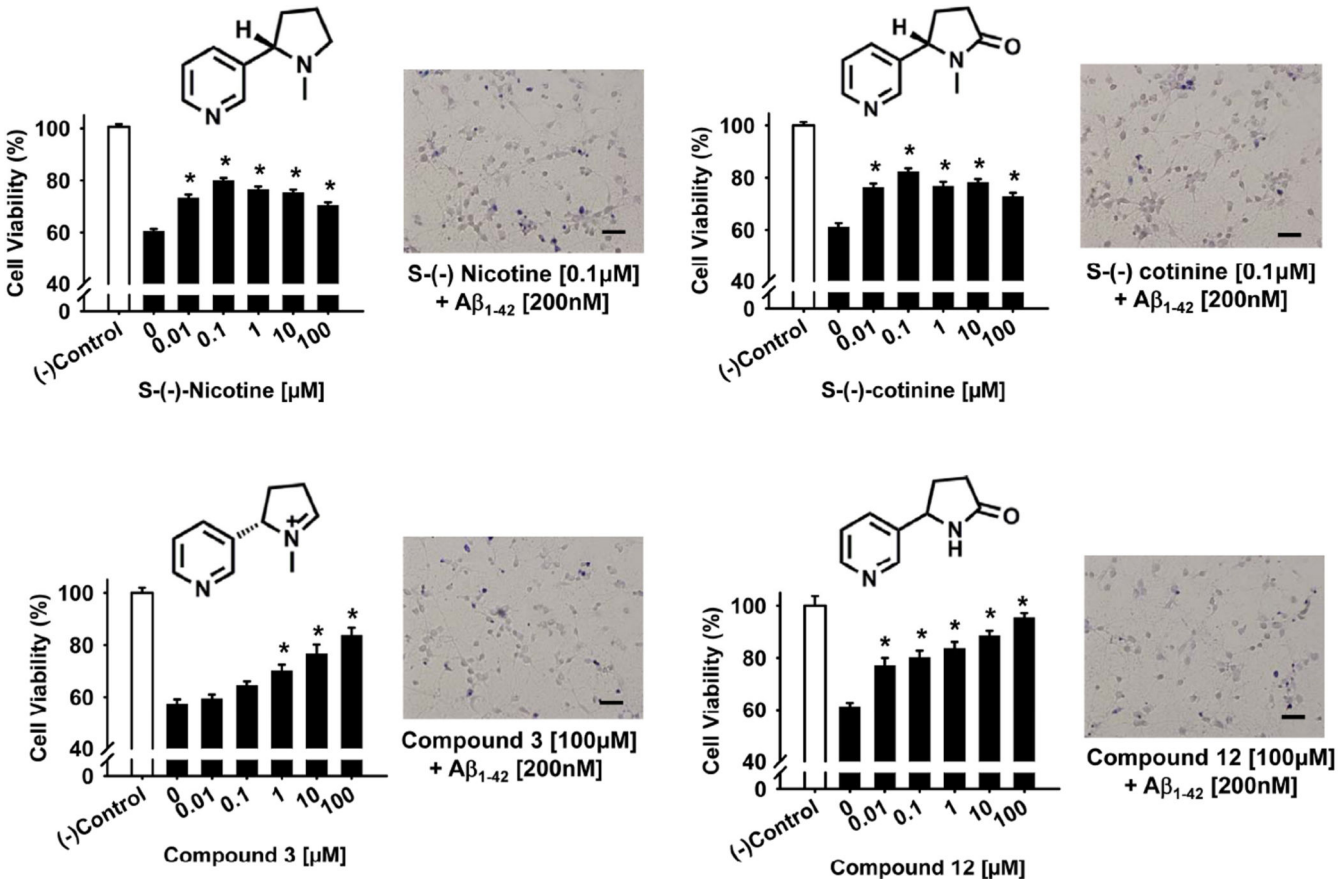

Fig 3.

Neuroprotective effects of nicotine, cotinine, and compounds 3 and 12 against the $A\beta_{1–42}$ peptide as determined in a cell viability assay in primary cultures of rat cortical neurons. Pretreatment of the cultures with various concentrations of nicotine, cotinine, and compounds 3 and 12 for 24 hours was followed by exposure to the $A\beta_{1–42}$ peptide (200 nM) for another 24 hours. Cell viability for each treatment was determined in an MTT assay (see Materials and Methods), calculated as percentage survival rate, and compared to a negative control (i.e., cultures without the $A\beta_{1–42}$ peptide or test compound). Each bar represents the mean ± S.E.M (derived from 2–4 independent experiments with 7 replicates per drug concentration). *$p<0.05$ compared to wells with the $A\beta_{1–42}$ peptide added, but no test compound. The effects of optimal concentrations of each compound were confirmed via a Trypan Blue exclusion assay (see Materials and Methods) and are illustrated in representative photomicrographs. Scale bar = 100 μm.

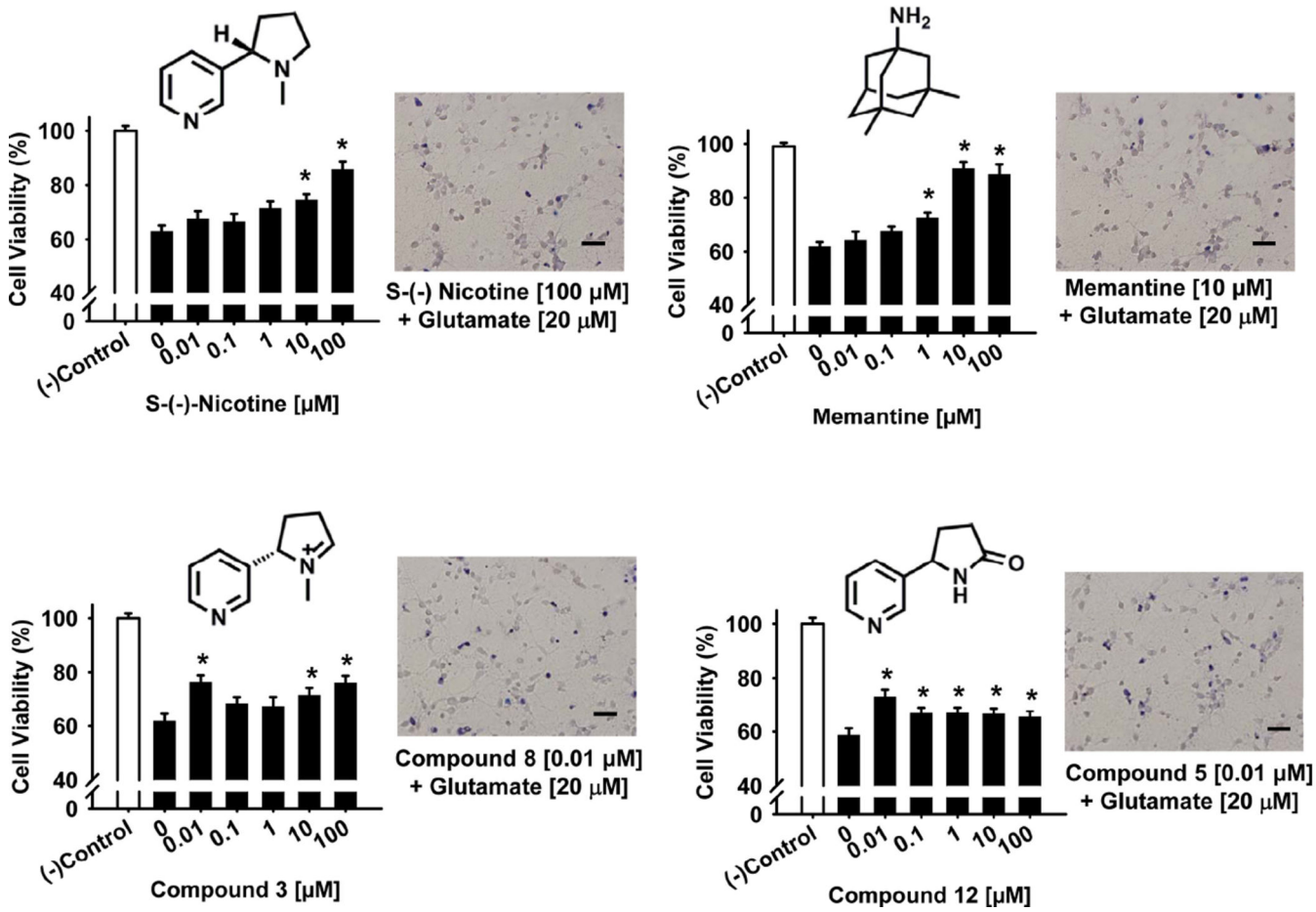

Fig 4.

Neuroprotective effects of nicotine, memantine, and compounds 3 and 12 against the glutamate toxicity as determined in a cell viability assay in primary cultures of rat cortical neurons. Pretreatment of the cultures with various concentrations of nicotine, memantine, and compounds 3 and 12 for 24 hours was followed by exposure to glutamate (20 μM) for another 24 hours. Cell viability for each treatment was determined in an MTT assay (see Materials and Methods), calculated as percentage survival rate, and compared to a negative control (i.e., cultures without glutamate or test compound). Each bar represents the mean ± S.E.M (derived from 2–4 independent experiments with 7 replicates per drug concentration). *$p < 0.05$ compared to wells with glutamate added, but no test compound. The effects of optimal concentrations of each compound were confirmed via a Trypan Blue exclusion assay (see Materials and Methods) and illustrated in representative photomicrographs. Scale bar = 100 μm.

Table 1

Protective effect of test compounds against the decreases in cell viability induced by $A\beta_{1-42}$ [200nM] in primary cortical neuronal cultures.

Compound	Cell Viability (% of Control)				
	10nM	100nM	1uM	10uM	100uM
Donepezil	67.0 ± 2.1%	64.2 ± 2.5%	63.2 ± 3.0%	58.3± 2.3%	48.7 ± 1.8%#
Galantamine	66.5 ± 2.0%	65.4 ± 1.7%	65.4 ± 1.9%	65.3 ± 2.0%	65.9 ± 2.5%
Rivastigmine	63.4 ± 1.3%	64.5 ± 1.8%	64.5 ± 1.1%	60.9 ± 1.2%	64.5 ± 1.2%
Memantine	63.3 ± 1.8%	63.9 ± 1.7%	62.2± 1.6%	63.2± 2.2%	60.2± 1.9%
1	73.3±1.4%*	80.0 ± 1.0%*	76.4 ± 1.3%*	75.3 ± 1.1%*	70.5 ± 1.1%*
2	63.8 ± 1.3%	64.6 ± 1.2%	68.1 ± 1.6%	65.8 ± 1.4%	66.5 ± 1.7%
3	62.0 ± 1.7%	67.4 ± 1.5%	73.3 ± 2.3%*	80.1 ± 3.5%*	87.5 ± 2.9%*
4	69.1 ± 1.9%*	71.0 ± 2.4%*	76.3 ± 3.7%*	80.8 ± 2.9%*	93.2 ± 2.8%*
5	66.6 ± 2.2%	71.9 ± 2.3%*	70.3 ± 2.2%*	64.6 ± 1.8%	64.4 ± 2.4%
6	60.0 ± 2.5%	60.5 ± 2.8%	62.1 ± 2.4%	63.3 ± 2.6%	65.7 ± 3.5%
7	61.4 ± 4.0%	61.7 ± 3.7%	62.2 ± 3.7%	64.5 ± 3.5%	64.2 ± 3.4%
8	76.3 ± 1.4%*	82.3 ± 1.2%*	76.8 ± 1.7%*	78.1 ± 1.4%*	72.8 ± 1.4%*
9	63.1 ± 1.6%	63.5 ± 1.7%	66.2 ± 1.6%	66.2 ± 1.5%	69.9 ± 1.5%
10	74.1 ± 2.9%*	74.9 ± 3.1%*	73.3 ± 3.8%*	76.9 ± 3.2%*	87.0 ± 2.9%*
11	64.7 ± 3.1%	66.6 ± 2.9%	62.7 ± 3.1%	67.1 ± 4.8%	58.5 ± 4.1%
12	75.5 ± 3.0%*	78.6 ± 2.6%*	82.0 ± 2.6%*	86.8 ± 1.8%*	93.6 ± 1.7%*
13	63.6 ± 2.0%	69.5 ± 1.8%*	72.6 ± 1.9%*	75.5 ± 2.8%*	82.4 ± 2.5%*
14	63.9 ± 3.6%	68.9 ± 2.8%	67.9 ± 2.3%	66.3 ± 1.3%	65.3 ± 2.5%
15	69.2 ± 2.8%	67.4 ± 6.0%	67.3 ± 3.5%	68.4 ± 3.4%	74.3 ± 4.6%
16	62.3 ± 3.1%	66.0 ± 3.7%	68.8 ± 1.9%	69.4 ± 2.7%	58.5 ± 2.3%
17	70.1 ± 1.6%*	73.1 ± 1.6%*	77.0 ± 1.3%*	78.9 ± 2.2%*	86.0 ± 1.8%*
18	57.4 ± 2.1%	58.3 ± 2.0%	57.0 ± 1.9%	59.1 ± 1.4%	58.9 ± 1.8%

* $P < 0.05$, compared with $A\beta_{1-42}$ treatment only.

Data represent the mean ± S.E.M. derived from 2–4 independent experiments with 7 replicates per drug concentration.

Table 2

Protective effect of test compounds against the decreases in cell viability induced by glutamate [20μM] in primary cortical neuronal cultures

Compound	Cell Viability (% of control)				
	10nM	**100nM**	**1uM**	**10uM**	**100uM**
Donepezil	63.2 ± 1.3%	65.3 ± 1.5%	69.5 ± 1.4%*	70.0± 2.0%*	43.6 ± 1.8%#
Galantamine	54.4 ± 3.8%	57.1 ± 3.3%	64.1 ± 2.5%	71.7 ± 3.5%*	63.5 ± 2.4%
Rivastigmine	60.0 ± 1.8%	66.7 ± 1.1%	60.0 ± 2.5%	67.4 ± 1.5%	70.6 ± 2.6%
Memantine	62.1 ± 3.3%	65.4 ± 1.7%	70.3± 1.9%*	88.0± 2.4%*	85.8 ± 3.8%*
1	65.2± 2.8%	63.6 ± 2.8%	67.0 ± 3.1%	72.0 ± 2.2%*	85.8 ± 2.9%*
2	57.3 ± 2.2%	61.5 ± 1.8%	65.4 ± 1.4%	66.4 ± 2.2%	67.1 ± 1.3%
3	74.3 ± 2.6%*	66.6 ± 2.3%	65.5 ± 3.5%	69.7 ± 2.7%*	74.0 ± 2.6%*
4	59.4 ± 2.2%	59.5 ± 2.0%	56.9 ± 1.7%	55.7 ± 2.6%	54.8 ± 2.4%
5	63.2 ± 2.0%	58.5 ± 2.7%	56.7 ± 2.2%	58.9 ± 3.7%	66.3 ± 2.8%
6	69.2 ± 3.4%	64.1 ± 2.7%	67.6 ± 2.2%	67.3 ± 3.2%	68.9 ± 3.1%
7	55.2 ± 2.7%	58.7 ± 1.9%	59.1 ± 1.9%	60.4 ± 2.8%	60.4 ± 3.4%
8	60.5 ± 1.9%	61.7 ± 1.9%	63.8 ± 1.7%	61.6 ± 1.7%	66.5 ± 2.0%
9	60.5 ± 1.7%	60.7 ± 1.6%	62.6 ± 1.8%	61.9 ± 1.9%	64.3 ± 2.0%
10	62.6 ± 4.7%	65.7 ± 3.3%	68.6 ± 2.8%	65.6 ± 3.2%	67.2 ± 4.5%
11	60.5 ± 1.1%	62.9 ± 2.9%	66.2 ± 3.6%	65.6 ± 4.6%	64.9 ± 4.1%
12	74.5 ± 2.7%*	68.3 ± 2.2%*	68.3 ± 2.2%*	68.0 ± 2.2%*	66.9 ± 2.2%*
13	66.6 ± 2.6%	62.6 ± 2.5%	60.0 ± 3.3%	63.9 ± 2.8%	66.3 ± 3.0%
14	68.0 ± 4.5%	66.4 ± 2.8%	64.4 ± 2.8%	66.8 ± 2.6%	83.9 ± 2.7%*
15	61.8 ± 3.0%	60.3 ± 3.1%	57.9 ± 2.5%	57.3 ± 2.0%	59.2 ± 2.2%
16	66.8 ± 2.9%	62.9 ± 2.9%	59.6 ± 2.8%	58.3 ± 3.8%	56.8 ± 3.2%
17	54.9 ± 3.5%	61.5 ± 2.4%	61.0 ± 3.5%	63.4 ± 4.5%	63.7 ± 4.1%
18	63.6 ± 1.5%	63.8 ± 1.5%	61.6 ± 2.5%	59.4 ± 2.5%	59.2 ± 2.3%

* $P < 0.05$, compared with glutamate treatment only.

Data represent the mean ± S.E.M. derived from 2–4 independent experiments with 7 replicates per drug concentration.