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

Jie Gao, Bao-Ling Adam, Alvin V. Terry Jr. *

*Department of Pharmacology and Toxicology, CB-3545, Georgia Regents University, 1120 Fifteenth Street, Augusta, GA 30912-2450, United States*

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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.



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] 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]

*Abbreviations:* Aβ, amyloid β; AChEI, acetylcholinesterase inhibitor; AD, Alzheimer's disease; nAChR, nicotinic acetylcholine receptor.

* Corresponding author. Tel.: +1 706 721 9462; fax: +1 706 721 2347.
*E-mail address:* aterry@gru.edu (A.V. Terry).

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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 Figure 2A and B, respectively. As illustrated, after exposure to either the $A\beta_{1-42}$ peptide or glutamate for 24 h, 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 (20 μM) 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 Figure 3 and Table 1. In Figure 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 h 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

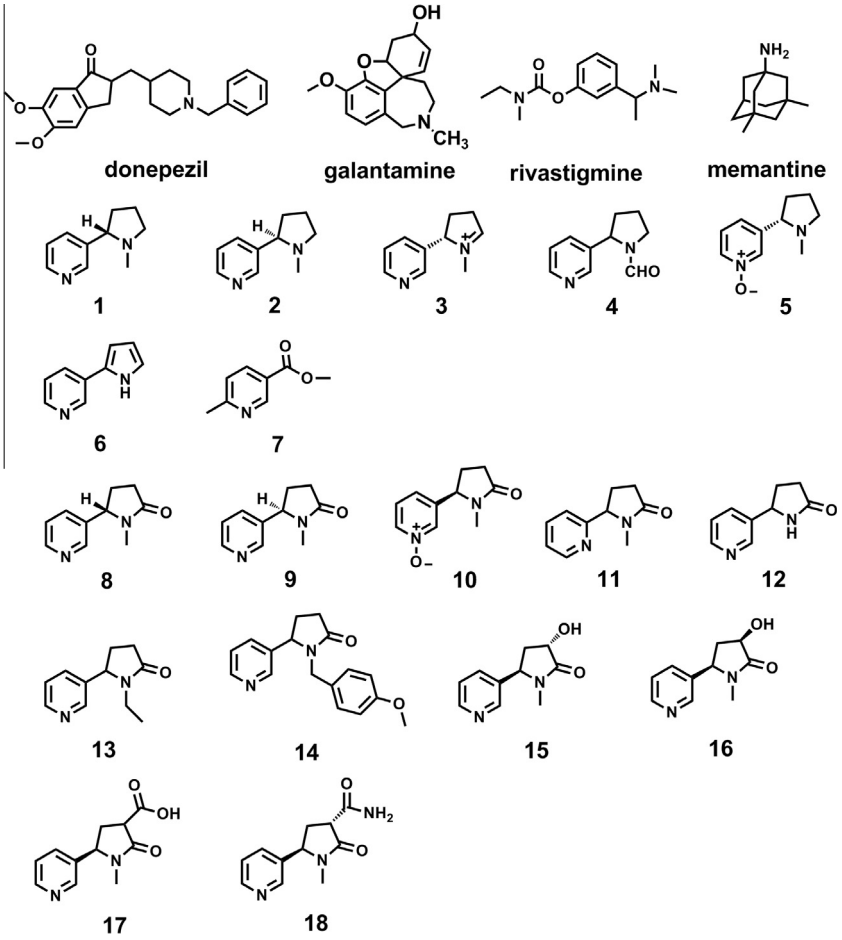


**Figure 1.** Chemical structures of the currently prescribed AD therapeutic agents, commercially available nicotine analogs (compounds **1**–**7**), and commercially available cotinine analogs (compounds **8**–**18**).