CNS Drug Reviews
Vol. 10, No. 2, pp. 167–182


# ABT-089: Pharmacological Properties of a Neuronal Nicotinic Acetylcholine Receptor Agonist for the Potential Treatment of Cognitive Disorders

Lynne E. Rueter, David J. Anderson, Clark A. Briggs, Diana L. Donnelly-Roberts, Gary A. Gintant, Murali Gopalakrishnan, Nan-Horng Lin, Mark A. Osinski, Glenn A. Reinhart, Michael J. Buckley, Ruth L. Martin, Jeffrey S. McDermott, Lee C. Preusser, Terese R. Seifert, Zhi Su, Bryan F. Cox, Michael W. Decker, and James P. Sullivan

*Abbott Laboratories, Global Pharmaceutical Research and Development, Abbott Park, IL, USA*

**Keywords:** ABT-089 — Alzheimer's disease — Attention deficit disorder — Cognitive disorders — Neuronal nicotinic acetylcholine receptor.

## ABSTRACT

ABT-089 [2-methyl-3-(2-(S)-pyrrolidinylmethoxy)pyridine dihydrochloride salt] is a selective neuronal nicotinic receptor (NNR) modulator with cognitive enhancing properties in animal models of cognitive functioning. Amongst NNR subtypes, ABT-089 shows selectivity for the cytisine binding site on the $\alpha_4\beta_2$ receptor subtype as compared to the α-bungarotoxin (α-BgT) binding sites on the $\alpha_7$ and $\alpha_1\beta_1\delta\gamma$ receptor subtypes. In functional *in vitro* electrophysiological and cation flux assays, ABT-089 displays differential activity including agonism, partial agonism and antagonism depending upon the NNR subtype and assay. ABT-089 is as potent and efficacious as (–)-nicotine at evoking acetylcholine (ACh) release from hippocampal synaptosomes. Furthermore, ABT-089 is neuroprotective against excitotoxic glutamate insults, with even greater potency seen after chronic treatment. Similarly, ABT-089 is effective in models of cognitive functioning, including enhancement of baseline functioning as well as improvement of impaired cognitive functioning seen following septal lesioning and natural aging. In neuroprotective assays the compound is most potent by chronic administration. In stark contrast to the positive effects in the cognitive models, ABT-089 shows little propensity to induce adverse

Address correspondence and reprint requests to Lynne Rueter, Abbott Laboratories, Neuroscience Research, R4N5, AP9A, 100 Abbott Park Rd., Abbott Park, IL 60064-6115, USA.
Fax: +1 (847) 938-0072; E-mail: lynne.e.rueter@abbott.com

effects such as ataxia, hypothermia, seizures, cardiovascular or gastrointestinal side effects. Together these data suggest that ABT-089 is a NNR modulator with the potential for treating cognitive disorders with markedly limited adverse cardiovascular and gastrointestinal side effects.

## INTRODUCTION

Despite years of effort, there are few pharmacological therapies for cognitive disorders such as attention deficit hyperactivity disorder (ADHD) and Alzheimer's disease (AD) available in the clinic. Until very recently, the only approved medications for ADHD were stimulants such as methylphenidate. However, this medication class of controlled substances has had limitations due to fear of abuse, mood swings and/or motor side effects (39). In addition to stimulants, antidepressants, particularly those with noradrenergic activity, have been prescribed off-label (39). In keeping with this, the norepinephrine reuptake inhibitor atomoxetine (Strattera®) has recently received FDA approval for the treatment of ADHD. Initial indications suggest atomoxetine has similar efficacy to stimulants with reduced side effects, however, some of the side effects present are presumably target-mediated, e.g., changes in cardiovascular function (42). Similar to ADHD, there are very limited pharmacological therapies available for AD. To date, the only approved treatments are the cholinesterase inhibitors such as donepezil and NMDA receptor antagonists such as memantine, which have been shown to temporarily improve cognitive function and/or slow deterioration (14, 43).

One intriguing possibility for a novel pharmaceutical agent for the treatment of cognitive dysfunction in ADHD and AD is a modulator of the NNR. The promise of this approach lies in the effects of nicotine itself. In adults with ADHD, nicotine patches have been shown to significantly improve attentional performance (32). Similarly, administration of nicotine to AD patients enhances performance on memory tasks, particularly the attentional aspects of those tasks. (32). These results mirror preclinical work demonstrating enhanced cognitive function and/or reversal of cognitive dysfunction with nicotine or nicotinic agonists (22,32).

NNRs are pentameric ligand-gated ion channels made up of differing combinations of subunits. For the present review, four of these subunit combinations are important. First is the most common subunit combination in the central nervous system (CNS), the $\alpha_4\beta_2$ receptor. Knockout studies suggest these subunits play a role in some of the therapeutic actions of nicotinic agonists such as antinociception, cognitive enhancement and neuroprotection (9). Second, the $\alpha_7$ receptor, a homomeric receptor, is believed to be involved in neuroprotection and cognitive functioning (9,22). Third, expression of the $\alpha_3\beta_4$ subunit combination is strong in both the CNS and peripheral nervous system (PNS), and this receptor subtype is believed to play an important role in the autonomic nervous system, including cardiovascular function (9,38). Finally, the $\alpha_1\beta_1\delta\gamma$ is a subunit combination existing only in the PNS that plays an important role in the control of skeletal muscle (20).

The following article is a summary of the preclinical pharmacology of ABT-089, a NNR modulator with differential affinity and activity at multiple NNR subunit combinations. As a partial agonist at the $\alpha_4\beta_2$ and $\alpha_7$ subtype receptors and an antagonist at the $\alpha_3\beta_4$ subtype receptor, ABT-089 is a NNR modulator with a substantially reduced side effect profile compared to nicotine and other NNR modulators.

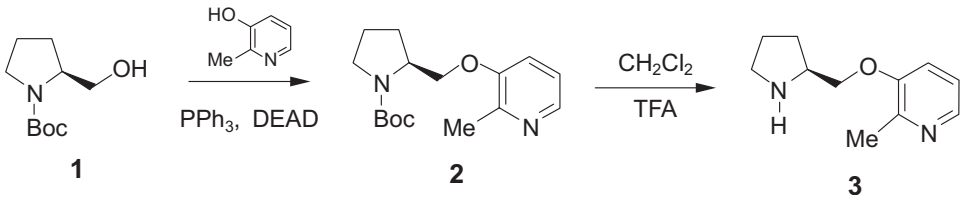


**Fig. 1.** Original synthesis of ABT-089.

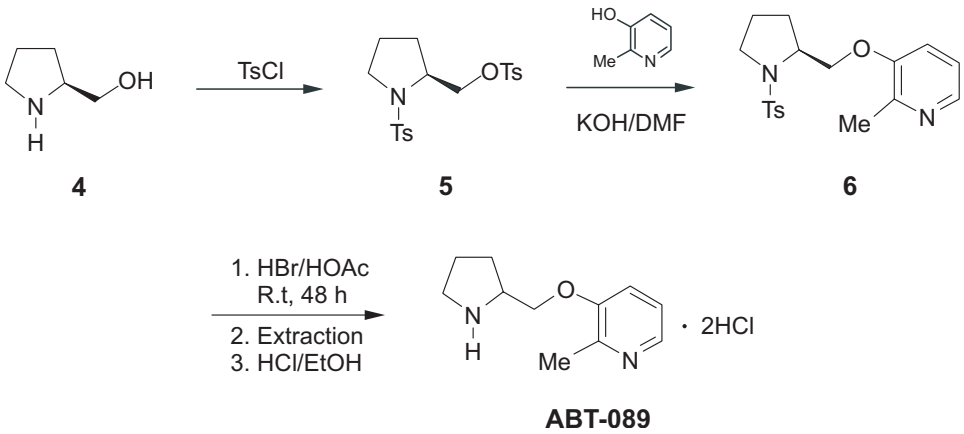


**Fig. 2.** Modified synthesis of ABT-089.

## CHEMISTRY

ABT-089 [2-methyl-3-(2-(S)-pyrrolidinylmethoxy)pyridine dihydrochloride salt] is a nonhygroscopic white crystalline powder with a melting point of 253°C. The aqueous solubility of the hydrochloride salt exceeds 500 mg/mL over a pH range of 2–9. The hydrochloride salt is stable at 25°C (i.e., less than 10% degradation was observed over a range of pH (2–9) during 62 days). It exhibited a $t_{90}$ of 20.3 days under extreme ultraviolet light at pH 7.3 and 40 °C.

The original synthesis of ABT-089 has been previously published and is summarized in Fig. 1 (25). The *tert*-butoxycarbonyl (Boc)-prolinol building block was prepared from commercially available Boc-L-proline in high enantiomeric purity.

A modified route (Fig. 2) has been developed to facilitate the large-scale synthesis of ABT-089. Reaction of L-prolinol (Fig 2, molecule 4) with toluenesulfonyl chloride resulted in both the protection of the nitrogen atom and activation of the primary alcohol for nucleophilic displacement. The coupled product (Fig. 2, molecule 6) is deprotected, extracted from aqueous solution and converted to the corresponding hydrochloride salt in a three-step sequence.

# BIOCHEMICAL PHARMACOLOGY

## Radioligand Binding

ABT-089 interacts with high affinity at the $\alpha_4\beta_2$ subtype of both rat and human NNRs. Affinities were measured by displacement of specific [$^3$H](–)-cytisine binding from rat brain membranes ($K_i$ = 17 nM) and from human $\alpha_4\beta_2$ NNRs stably expressed in a HEK cell line. In contrast, (–)-nicotine displaces [$^3$H](–)-cytisine binding to rat brain and human NNRs with $K_i$s of 1 nM. ABT-089 has insignificant affinity ($K_i$ > 10,000 nM) for displacement of [$^{125}$I]α-bungarotoxin (α-BgT) from both rat brain membranes and from HEK cells that are stably expressing the human $\alpha_7$ NNR subtype. (–)-Nicotine displaces [$^{125}$I]α-BgT binding from rat brain and human $\alpha_7$ with $K_i$ values of 6000 nM and 2000 nM, respectively. ABT-089 and (–)-nicotine are also weak inhibitors ($K_i$ > 1000 μM) of [$^{125}$I]α-BgT binding to the $\alpha_1\beta_1\delta\gamma$ NNR subtype found on Torpedo electroplax membranes (40).

In 45 other receptor binding and enzyme activity assays, ABT-089 shows negligible affinity ($K_i$ > 10,000 nM). Among those assayed for were muscarinic, GABA, 5-HT$_3$ and the benzodiazepine receptors, members of the ligand-gated ion channel superfamily, channel proteins, members of G-protein coupled receptor superfamily, neurotransmitter uptake sites, as well as several enzymes (40).

## *In Vitro* Functional Studies

### *Ion flux*

Unlike (–)-nicotine, ABT-418 [(S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole] and ABT-594 [(R)-5-(2-azetedinylmethoxy)-2-chloropyridine mono-tosylate salt], all three cholinergic channel activators, ABT-089 displays a complex pattern of pharmacological activity in various functional assays. At the major subtype in the CNS, the $\alpha_4\beta_2$ receptor, ABT-089 is significantly less potent and efficacious relative to (–)-nicotine in the $Rb^+$ efflux assay exhibiting an $EC_{50}$ > 300 mM and efficacy <10% (human receptor; Table 1). In contrast, in the mouse thalamic synaptosomal $Rb^+$ efflux assay, ABT-089 displays partial agonist activity with a potency of 5 μM and 34% efficacy, an effect attenuated by the noncompetitive NNR antagonist mecamylamine (Table 1). As previously reported, the NNR-induced cation efflux from mouse thalamic synaptosomes is indicative of the activation of an $\alpha_4\beta_2$ subtype (31). The difference in activity at the $\alpha_4\beta_2$ subtypes in these two preparations could be due to an NNR species difference, to a difference between recombinant and native NNR pharmacology, or to effects on native NNR subtypes other than the recombinant subtypes evaluated to date.

*TABLE 1.* In vitro *functional properties of ABT-089 in $Rb^+$ efflux assays*

| $EC_{50}$ (μM) (efficacy) | ABT-0891[1] | (–)-Nicotine[1] |
|---|---|---|
| Mouse thalamus | 5 (34%) | 1.5 (100%) |
| Human IMR-32 | 150 (<20%) | 20 (100%) |
| Human TE 671 | 30 (60%) | 180 (100%) |

[1] Data summarized from ref. 40.

The effects of ABT-089 were also investigated in a NNR-mediated $Rb^+$ efflux assay using the IMR-32 cells, which express a "ganglionic-like" NNR, reflective of an $\alpha_3\beta_4$ subtype (28). In the IMR-32 cells, ABT-089 exhibits weak partial agonist activity with an $EC_{50} > 300$ μM and 15% efficacy (Table 1). ABT-089 also exhibits weak activity ($IC_{50} \approx 100$ μM) as an antagonist to block nicotine-induced cation efflux. The weak activity observed in this cell line parallels the diminished ability of this compound to elicit adverse cardiovascular side effects in anesthetized dogs, and this functional IMR-32 data supports this observed correlation (see below).

In the TE 671 cell line, ABT-089 exhibits partial agonist activity with efficacy of 60% and potency greater than (–)-nicotine with an $EC_{50}$ value of 30 μM (Table 1). This effect is blocked by pretreatment with mecamylamine, indicating that it is NNR-mediated. The TE 671 cells express muscle-type NNRs (27) as well as some subunits of the neuronal subtypes (40). However, as previously noted, ABT-089 displays little affinity for [$^{125}$I]-α-BgT binding sites on TE 671 cells. Therefore, this raises the possibility that the TE 671 cell line contains neuronal subunits that form a unique pharmacology explaining the enhanced pharmacological activity of ABT-089 (40).

## *NNR channel currents*

The $\alpha_7$ NNR subtype is known to be highly $Ca^{2+}$ permeant (5,16,37,36) and has been found to enhance neurotransmitter release in several neuronal systems (2,3,15,17,19,21, 23,24,29,41). At human $\alpha_7$ NNR expressed in *Xenopus* oocytes, ABT-089 is a weak partial agonist. The amplitude of the response to a high concentration of ABT-089 (1 μM) is only 1.5% as large as the response to nicotine (6,40). However, effects dependent upon a small but relatively persistent influx of $Ca^{2+}$ may be more sensitive to ABT-089 than is apparent from consideration of the amplitude alone (Fig. 3; see also ref. 33). Among such effects may be modulation of neurotransmitter release and neuroprotection.

Like other agonists, ABT-089 also inhibits $\alpha_7$ NNR through desensitization (6,7) Thus, systemic application of ABT-089 could elicit a small but persistent increase in $\alpha_7$ channel openings, rather like an increase in spontaneous activity, while inhibiting synaptic $\alpha_7$ activation by acetylcholine (ACh) released rapidly at high concentration.

To further dissect the actions of ABT-089 at $\alpha_7$ NNR, its effects at human α7V274T mutagenized NNR were determined (7). In this NNR, one amino acid in the channel-lining TM2 segment is changed from valine to threonine, resulting in a receptor that appears to maintain an open channel in the desensitized state (4). Like other full and partial agonists, ABT-089 is much more potent and efficacious at α7V274T than at the wild-type receptor. However, ABT-089 remains a partial agonist at α7V274T (40%), unlike other partial agonists such as (–)-cotinine and GTS-21 [(E)-3-(2,4-dimethoxybenzylidene)-3,4,5,6-tetrahydro-2,3′-bipyridine dihydrochloride] which are full agonists at α7V274T. The actions of ABT-089 at $\alpha_7$ NNR are complex and appear to include direct desensitization as well as channel activation.

## *Neurotransmitter release*

ABT-089 is nearly as potent as (–)-nicotine in its ability to evoke [$^3$H]ACh release from rat hippocampal synaptosomes. The $EC_{50}$ of [$^3$H]ACh release for ABT-089 is 3 μM while that for (–)-nicotine is 1 μM (40). In addition, ABT-089 has full efficacy as compared to (–)-nicotine. The ABT-089-evoked release of ACh may partially account for its ability to enhance cognition.

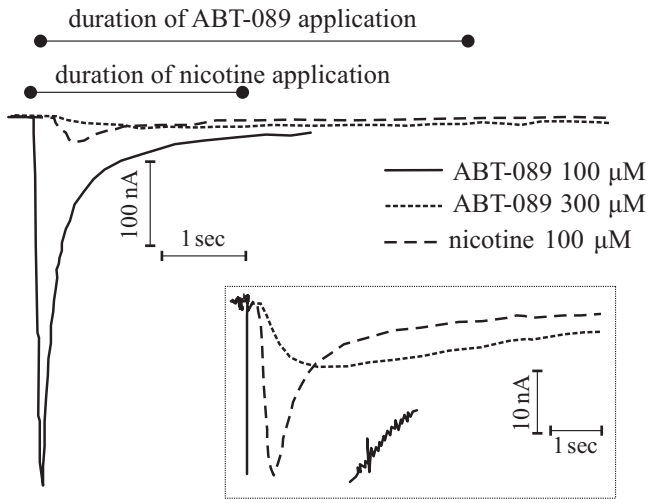


**Fig. 3.** Activation and decay rate of a human $\alpha_7$ receptor mediated ion current in *Xenopus* oocytes. Traces compare responses to 100 μM (–)-nicotine and two concentrations of ABT-089, 100 and 300 μM applied to the bathing solution as indicated by the straight lines above the traces. The inset shows the same responses on an expanded scale in order to illustrate the small but extended effect of ABT-089 on ion current. Modified figure reproduced with permission from ref. 40.

Another neurotransmitter that is released in response to ABT-089 is dopamine. In stimulating the release of [$^3$H]dopamine from rat striatal slices, ABT-089 is a partial agonist with 70% efficacy. The selective antagonist, dihydro-β-erythroidine (DHβE), blocks the response. ABT-089 is also 25-fold less potent than (–)-nicotine with an $EC_{50}$ of 1.1 μM for ABT-089 compared with 0.04 μM for (–)-nicotine (40).

Thus, the potency and efficacy of ABT-089 to elicit neurotransmitter release is not well explained by its activity at the human recombinant receptors evaluated to date. The reason for this discrepancy is not known. It may relate to species differences between rodent and human NNR pharmacology, or it may signal a discord between recombinant and native NNR structure and pharmacology. Alternatively, it is possible that ABT-089 is selective for an NNR subunit combination, at which it has not yet been tested, such as $\alpha_6\beta_2$ or $\alpha_4\alpha_5\beta_2$.

## In vitro *cytoprotection*

Pretreatment of primary cortical cultures or IMR-32 cells with ABT-089 protects cells against several cytotoxic insults as measured by a lactate dehydrogenase release assay (13). ABT-089 exhibits a dose-dependent protection against glutamate insult with $EC_{50}$ values of 3 μM and 10 μM for IMR-32 and primary cortical cells, respectively (Fig. 4). The ABT-089-induced neuroprotection is time-dependent with maximal effects observed at 2 h after pretreatment with ABT-089. The protective effect of ABT-089 is mediated via an interaction with the NNRs since pretreatment with the NNR antagonists, mecamylamine and α-BgT, attenuate the observed protective effects (40). These results indicate the neuroprotective effects of ABT-089 might be via the $\alpha_7$ NNR subtype.

ABT-089 is also neuroprotective in primary cortical cultures against cytotoxic insults by aggregated $A\beta_{1-42}$, thought to be one of the first peptides formed in AD plaques (35)

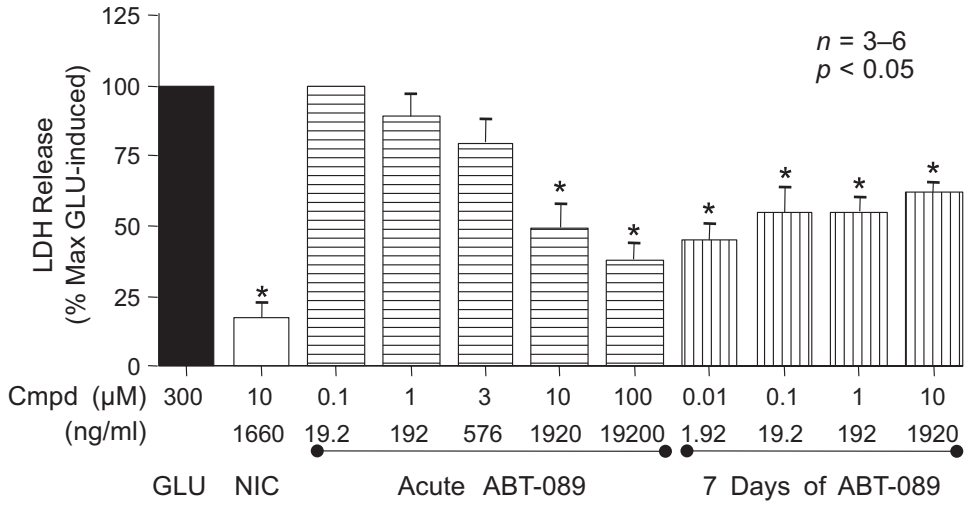


**Fig. 4.** Effects of acute and subacute (7 day) exposure to ABT-089 and acute exposure to (–)-nicotine on the protection of cortical neurons against a glutamate neurotoxic insult. Compounds were applied to the cells 2 h prior to exposure to glutamate and the amount of lactate dehydrogenase (LDH) released was assessed 24 h later. Values are normalized to the maximal glutamate induced LDH release (means ± S.E.M.). Modified figure reproduced with permission from ref. 40.

and gp120, the neurotoxic soluble HIV-1 coat protein (26). This neuroprotection by ABT-089 (10–100 μM) is via an interaction with NNRs because it can be attenuated by mecamylamine (12). In addition, it has been shown to be $Ca^{2+}$ dependent since removal of $Ca^{2+}$ from the extracellular medium prevents nicotinic agonist-induced neuroprotection (13).

Curiously, the potency and efficacy of ABT-089 to elicit neuroprotection correlates better with $\alpha_7$ desensitization than with $\alpha_7$ channel activation. However, if neuroprotection depends upon a small-amplitude but persistent $Ca^{2+}$ influx, then the potency and efficacy of a weak partial agonist like ABT-089 would be enhanced relative to that measured in the standard electrophysiological technique involving synchronous channel activation by bolus drug application.

As discussed in the behavorial section, the potency and efficacy of ABT-089 is enhanced after subacute treatment (10). Therefore, studies examining subacute exposure of primary cortical cells to ABT-089 (0.01 to 10 μM for 7 days), prior to glutamate insult, were performed. As shown in Fig. 4, subacute exposure of ABT-089, at concentrations of 0.01–10 μM, induces significant neuroprotection against glutamate (300 μM) insult. Similar doses of ABT-089, given acutely (2 h), would not elicit neuroprotection against a glutamate insult. It should be noted that the lower doses (0.01–0.1 μM) used in the subacute exposure are approximately equal to the cognitive-enhancing plasma and brain levels of ABT-089 after subacute exposure in rodents (10). It is unclear whether the effects of ABT-089 in an *in vitro* model of neurotoxicity include an upregulation of the NNR subtypes.

## *IN VIVO* PHARMACOLOGY

### Cognitive Assessment

ABT-089 has been examined in a series of preclinical assays in normal and aging animals designed to assess its potential for the treatment of cognitive dysfunction. In the 24-h inhibitory avoidance paradigm in the mouse, ABT-089 was injected prior to the training phase of the experiment. Upon testing for retention of learning 24 h later, it was found that ABT-089 enhances inhibitory avoidance at a minimum dose of 0.62 μmol/kg, i.p. (25). In this regard, the compound performs as well as (–)-nicotine (25). A similar assay was performed in young and aged rats. ABT-089 was administered chronically via minipump for two weeks, and then animals were exposed to the training phase for inhibitory avoidance and tested for retention 72 h later with pump on board. Under these conditions, ABT-089, at doses as low as 1.3 μmol/kg/d, (plasma concentration of 5.3 ng/mL) improves performance in the aged (non-significant trend), but not in the young rats (10).

ABT-089, following acute administration of 1.9, 6.2, or 19 μmol/kg, i.p., does not alter performance of young rats in the Morris Water maze nor does it reverse the performance deficits seen in septal lesioned young rats. In contrast, following continuous infusion for 2 weeks, ABT-089 significantly attenuates lesion-induced deficits in water maze performance (10). The effect is seen at 1.3 and 4.0 μmol/kg/d (plasma concentrations of 5.3 and 13.7 ng/mL, respectively), i.e., doses lower than those required for (–)-nicotine to attenuate septal lesion-induced deficits in the Morris Water maze (19 and 62 μmol/kg/d) (10). The effects of chronically administered ABT-089 are specific to the cognitive deficits induced by the lesions as shown by the lack of effect of the compound on lesion-induced changes in locomotor activity or startle reactivity (10). Finally, in a pharmacologically induced model of cognitive deficits, repeated administration of ABT-089 attenuates scopolamine-induced deficits in the Morris water maze (Fig. 5).

ABT-089 enhances cognitive performance in monkeys, similarly to aged and lesioned rats. In a standard delayed-match-to-sample (DMTS) paradigm, ABT-089 improves the accuracy of performance in mature monkeys when the test is performed with a medium length delay between stimulus exposure and response (trend seen 10 to 32.4 nmol/kg, significance seen at 64.8 nmol/kg; 10). Similarly, there is a trend toward improving performance after a long delay with ABT-089, at doses of 32.4 and 64.8 nmol/kg (corresponding to peak plasma concentrations of approximately 13 and 26 ng/mL, respectively). Performance of aged monkeys in the standard DMTS paradigm with a long delay is markedly improved by ABT-089, at 4 and 8 nmol/kg (peak plasma concentrations of approximately 1.5 and 3 ng/mL, respectively) (10). As a comparator, (–)-nicotine improves the performance of aged monkeys at doses of 31 and 62 nmol/kg (8). Finally, in a modified DMTS paradigm with a distractor, ABT-089, at doses of 4.1 to 32.8 nmol/kg, significantly enhances performance of adult monkeys. In comparison, (–)-nicotine significantly improves performance in this task only at the dose of 43.4 nmol/kg (34).

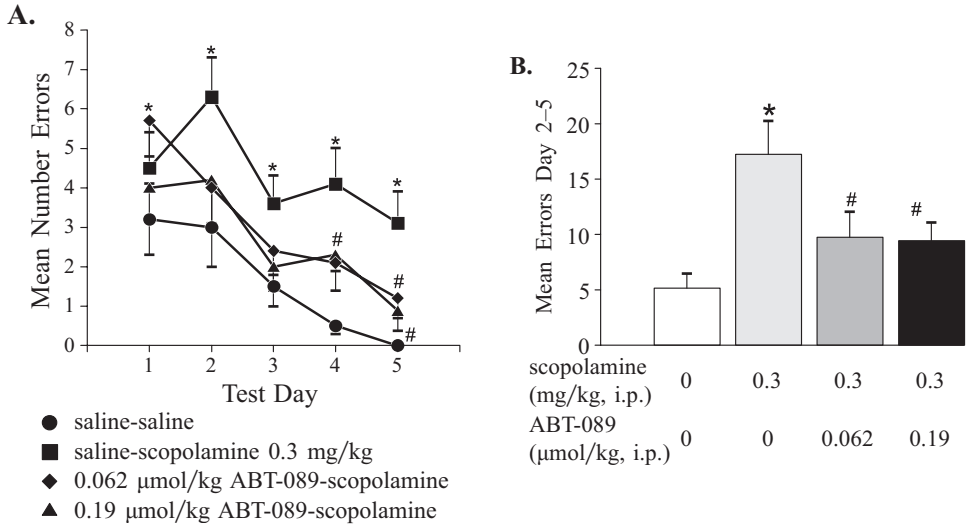


**Fig. 5.** Effect of ABT-089 on scopolamine-induced deficits in the rat Morris water maze. Each day, ABT-089 was injected 15 min prior to the injection of scopolamine. Testing occurred 15 min after the injection of scopolamine. The measure was the number of errors (means ± S.E.M.) made in attempting to find the escape platform. **A.** Effect of ABT-089 across the five days of testing. Note the impairment due to scopolamine is attenuated at days 4 and 5 by ABT-089. **B.** Data collapsed across days 2 to 5. * indicates significantly different ($p < 0.05$) compared to respective saline-saline control. # indicates significantly different compared to respective saline-scopolamine control.

## Other Behavioral Assessment

NNR modulators commonly induce unwanted behavioral effects such as decreased locomotor activity, hypothermia, ataxia and seizures. In contrast to its equal or greater potency compared to nicotine in cognitive behavioral models, ABT-089 is markedly less potent than nicotine in inducing unwanted behavioral effects. Therefore, whereas (–)-nicotine decreases locomotor activity in mice ($ED_{50} = 0.62$ μmol/kg) and rats ($ED_{50} = 1.9$ μmol/kg), ABT-089 does not decrease locomotor activity in doses up to 190 μmol/kg in either mice or rats (11,25). As stated above, this lack of effect on locomotor activity is also apparent after chronic treatment via minipump (10). Similarly, ABT-089 does not induce ataxia or decreased motor coordination as measured by the rotarod assay until supra-efficacious doses of 300 μmol/kg. In this model, (–)-nicotine disrupts performance at doses as low as 12.4 μmol/kg (30). In mouse hypothermia studies, ABT-089 does not induce hypothermia at doses up to 62 μmol/kg as compared to the marked induction of hypothermia seen with (–)-nicotine at 6.2 μmol/kg (25). Furthermore, in the mouse, ABT-089 is almost 20× less potent at inducing seizures than is (–)-nicotine ($ED_{50} = 774$ μmol/kg vs. $ED_{50} = 41$ μmol/kg, respectively; 25). Similarly, EEG studies indicate that ABT-089, in doses up to 19 μmol/kg, does not induce cortical activation, whereas (–)-nicotine elicits these effects at doses as low as 0.62 μmol/kg (1). Finally, in the rat, ABT-089, at doses up to 5000 μmol/kg, has no effect on respiration.

## PHARMACOKINETICS AND METABOLISM

The pharmacokinetics of ABT-089 has been evaluated in Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys (Table 2) (1,25). Following intravenous administration to rats, ABT-089 has a very short plasma elimination half-life (~1 h) and rapid plasma clearance (3.4 L · h/kg). In either dogs or monkeys, the plasma half-life is approximately 2-fold longer (~2 h), with a corresponding decrease in plasma clearance (2.0 L · h/kg). In rats and dogs ABT-089 is rapidly absorbed upon oral administration, with peak plasma concentrations observed within the first 40–50 min. Absorption is slightly slower in the monkey, with peak plasma concentrations achieved 1.4 h after oral administration. Oral bioavailability of ABT-089, in aqueous solution, averages 33, 26, and 62% in the rat, monkey, and dog, respectively (1).

In addition, brain concentrations of ABT-089 have been assessed in rats. Peak concentrations and area under the curve (AUC) values for ABT-089, by i.p. administration, are approximately 10-fold higher in the brain than in the plasma. However, it is interesting to note that there is an approximately 1:1 brain: plasma ratio shortly after administration (<15 min). Following oral administration, maximal plasma and brain levels are reached after approximately one hour. Similar to i.p. administration, a marked preferential distribution to brain relative to plasma is observed at 2 h after oral administration of ABT-089.

## METABOLISM AND DISPOSITION

Preliminary *in vitro* metabolism studies using 9000 g supernatant fraction (S9) from mouse, rat, dog, monkey, and human livers indicate that ABT-089 undergoes only minimal metabolism (<10%). At least four metabolites have been detected, two of which appear to be the lactam (Fig. 6, molecule 7) and pyrrolidine N′-oxide (Fig. 6, molecule 8).

*TABLE 2. Pharmacokinetic properties of ABT-089 across multiple species*

| | Dose | | $t_{1/2}$[1] | $V_\beta$ | $Cl_p$ | $C_{max}$ | $T_{max}$ | $F$ |
|---|---|---|---|---|---|---|---|---|
| | μmol/kg | mg/kg | (min) | (L/kg) | [L/(h · kg)] | (ng/mL) | (h) | (%) |
| **Rats** | | | | | | | | |
| i.v. | 2 | 0.53 | 66 | 5.5 | 3.4 | | | |
| Oral | 2 | 0.53 | 213 | | | 9.3 | 0.6 | 33.4 |
| **Dogs** | | | | | | | | |
| i.v. | 5 | 1.32 | 108 | 5.0 | 2.0 | | | |
| Oral | 5 | 1.32 | 102 | | | 9.4 | 0.8 | 61.5 |
| **Monkeys** | | | | | | | | |
| i.v. | 5 | 1.32 | 112 | 5.1 | 1.9 | | | |
| Oral | 5 | 1.32 | 119 | | | 3.1 | 1.4 | 26.3 |

[1] The terms in the table are defined as follows: $t_{1/2}$, elimination half life; $V_\beta$, volume of distribution; $Cl_p$, plasma clearance; $C_{max}$, maximum plasma concentration; $T_{max}$, time of maximum plasma concentration; $F$, oral bioavailability.

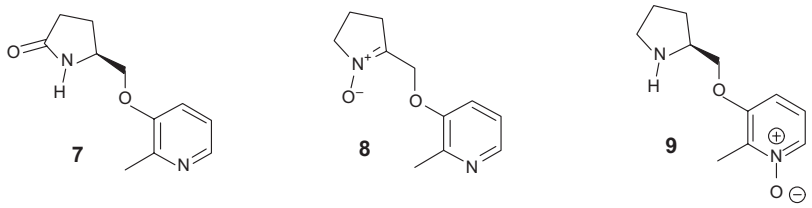


**Fig. 6.** Metabolism of ABT-089.

The pyridine N-oxide (Fig. 6, molecule 9) and hydroxylamine are thought to be two additional metabolites.

Preliminary metabolism and excretion studies have been conducted in a single rat and a single dog after bolus i.v. administration of radiolabelled ABT-089 (3 and 1 μmol/kg, respectively). In both species, the majority (65%) of the dose (as measured by total radioactivity) was excreted in urine within the first 24 h after i.v. administration. Only a small fraction (<10%) was present in feces (internal data).

In the rat, HPLC analysis of urine excreted in the first 24 h indicates the presence of 45% of the parent drug and more than 50% of one major metabolite that was identified (Fig. 6, molecule 9). The parent drug and the pyridine N-oxide (Fig. 6, molecule 9) were also detected in plasma at 15 and 60 min after dosing. Only trace amount of metabolites (Fig. 6, molecules 7 and 8) were detected in urine and plasma.

In the dog, the parent drug accounts for only 20% of total radioactivity in the urine during the first 24 h after i.v. administration. At least 6 metabolites have been detected in urine, accounting for 71% of radioactivity. As in the rat, the pyridine N-oxide (Fig. 6, molecule 9) represents one of the major metabolites.

## CARDIOVASCULAR PROFILE

In order to assess the cardiovascular safety profile of ABT-089, three primary assays were performed. The first two, the Purkinje fiber repolarization assay and the hERG ionic current assay were performed *in vitro*. The final assay was the complete cardiovascular profiling of the compound *in vivo* in the anesthetized dog (1).

### *In Vitro* Studies

Changes in action potential duration of canine cardiac Purkinje fibers *in vitro* have been assessed to evaluate the effects of ABT-089 on ventricular repolarization. In this assay, electrical activity of isolated Purkinje fibers from adult beagle dogs of either gender are monitored using standard microelectrode techniques under physiologic conditions (superfusion with bicarbonate-buffered Tyrode's solution, $[K^+]_o = 4$ μM, 37°C); slow stimulation rates (0.5 Hz) are applied to exaggerate potential drug effects on repolarization. There is no effect of ABT-089 on the action potential duration at plasma concentrations ranging from 20.7 to 2070 ng/mL (Fig. 7A) (1).

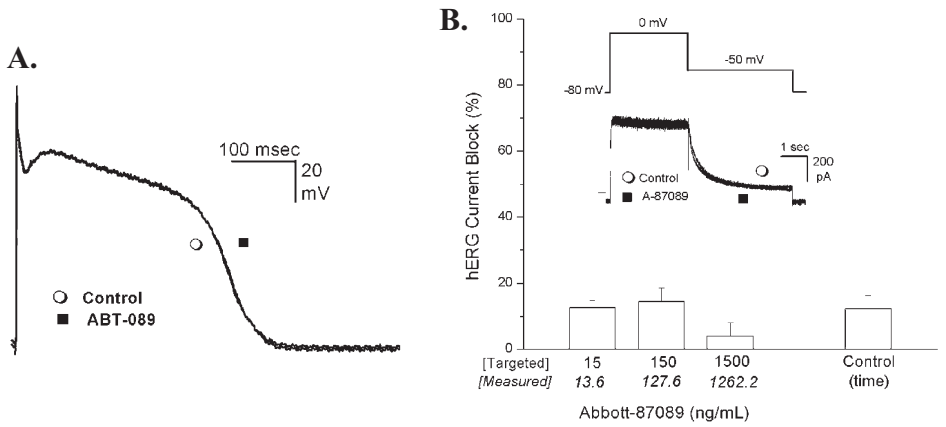


**Fig. 7.** Effect of ABT-089 on *in vitro* cellular cardiac electrophysiology. **A.** Lack of effect of ABT-089 on canine Purkinje fiber repolarization. Illustrated are representative action potentials obtained in the absence and in the presence of the highest concentration of ABT-089 tested (2070 ng/mL) during slow stimulation (2 sec basic cycle length [0.5 Hz]); the action potentials are superimposed. Comparable results were observed in each of six fibers studies. **B.** Effect of ABT-089 on hERG tail current amplitude. ABT-089 had no effect on hERG tail current at all concentrations tested (ANOVA, $n = 6$ per group). Targeted concentrations refer to expected bath concentrations, while measured concentrations represent analytical assay results from bath superfusate (mean ± S.E.M. values measured from 5–6 experiments). Inset: voltage clamp protocol employed.

In addition, the effect of ABT-089 on hERG current has been evaluated using HEK 293 cells stably expressing hERG. In this assay, drug effects are evaluated based on changes of tail currents measured during 4-sec repolarizing test pulses to –50 mV preceded by a 3-sec depolarizing activating pulse to 0 mV (holding potential of –80 mV, pulses applied once every 15 sec). Experiments are conducted at 36.5–37°C with a 5 μM external $K^+$ HEPES-buffered Tyrode's solution.

ABT-089 was evaluated at target concentrations of 15, 150, and 1500 ng/mL (measured bath concentrations of 13.6 ± 1.0, 127.6 ± 6.0, and 1,262.2 ± 59.4 ng/mL; mean ± S.E.M., $n = 5$–6 per concentration). Under these wide-ranging concentrations, ABT-089 had no effect on hERG tail currents (relative to water-vehicle controls). Furthermore, ABT-089 did not affect the amplitude of hERG activating current during depolarizing activating pulses to 0 mV (Fig. 7B) (1).

## *In Vivo* Studies

The *in vivo* cardiovascular profile of ABT-089 has been evaluated in anesthetized dogs. In this assay, male beagle dogs, weighing approximately 8–11 kg are anesthetized with pentobarbital (35 mg/kg, i.v.), immediately placed on a constant intravenous infusion of pentobarbital (6.0 mg/kg/h) and instrumented for measurement of cardiovascular and hemodynamic function parameters. ABT-089, administered by intravenous infusion (0.02 mL/kg/min) at doses of 24.0, 240, and 2400 mg/kg, produced peak plasma concentrations of 5.2 ± 0.4, 51.9 ± 2.1, and 499.5 ± 51.2 ng/mL, respectively. At these plasma concentrations, ABT-089 produced no physiologically significant changes in mean ar-

terial, systolic or diastolic arterial pressures relative to control animals. In addition, ABT-089 exerted no physiologically significant effects on heart rate, central venous pressure, left ventricular-end diastolic pressure, cardiac output, systemic vascular resistance, pulmonary vascular resistance or the hematocrit when compared to vehicle controls. Changes in indices of cardiac contractility ($dP/dt_{max}$ and $dP/dt$ at 50 mm Hg) were not significantly different from those in control animals. ABT-089 had no effect on cardiac electrophysiologic function. When infused at the same doses, ABT-089 had no significant effects on either the QTc (Bazett's formula) or PR intervals during the treatment or post-treatment periods. To further investigate the cardiovascular potential of ABT-089 was infused to dogs at higher doses (6.5, 19.5, and 65 mg/kg). Again, at plasma levels as high as 2.91 ± 0.5 μg/mL, ABT-089 had no physiologically significant effects on any hemodynamic, myocardial or cardiac electrophysiological parameters. (1).

As noted previously, the plasma concentration for behavioral efficacy of ABT-089 is approximately 2–25 ng/mL. The results of the *in vivo* cardiovascular studies demonstrate that at plasma concentrations approximately 60–1500 fold higher than the estimated preclinical efficacious concentrations, ABT-089 exerts no physiologically significant effects on hemodynamics, cardiovascular function or myocardial electrophysiological function in the anesthetized beagle dog, and, therefore, exhibits no nicotine-like cardiovascular effects.

## GASTROINTESTINAL PROFILE

One of the dose-limiting effects of oral (–)-nicotine is significant activity at the level of the enteric nervous system of the gastrointestinal tract. In particular, development of an oral formulation of an NNR agonist for the clinic requires substantially diminished gastrointestinal activity compared to nicotine. To assess the gastrointestinal profile, ABT-089 has been evaluated *in vitro* and *in vivo*. *In vitro*, ABT-089, 100 μM, does not evoke contractility of the guinea-pig ileal smooth muscle, unlike (–)-nicotine or the muscarinic agonist, methylcholine (1). In addition, ABT-089 does not inhibit the activity of other contractile agents such as ACh, histamine, serotonin or $K^+$ (1).

In addition, *in vivo* studies have been carried out to examine the effects of ABT-089 on gastrointestinal transit and emesis. In a conscious rat model, ABT-089 was administered at doses of 3, 10, 30, and 100 mg/kg, and a charcoal meal suspension was administered by gavage 30 min after the drug. At doses up to 100 mg/kg ABT-089 produces no significant effects on gastrointestinal transit. Additionally, ABT-089 has been evaluated for emetic liability in conscious ferrets, a model for the evaluation of clinical emetic potential. ABT-089 was administered by oral gavage at 3, 10, 30, and 50 mg/kg and animals were observed for emesis over the next 90-min period. There was no notable emesis at any dose of ABT-089 tested. Similarly, as previously reported (1), ABT-089 does not induce emesis in either dogs or monkeys. Taken together, these data suggest that, unlike nicotine, ABT-089, at behaviorally relevant concentrations, does not interfere with gastrointestinal motility.

## CONCLUSIONS

ABT-089 is an orally active compound with good brain penetration that shows activity in multiple models of cognitive function, including models of impaired function such as septal lesions and aged animals. Furthermore, these effects are seen in at least three species. The cognitive enhancement seen in the preclinical models is similar to that seen with (–)-nicotine, a compound that has been shown to improve cognitive functioning in humans with ADHD and Alzheimer's disease (32). ABT-089 has a markedly improved safety profile compared to (–)-nicotine; most importantly, it shows little or no evidence of cardiovascular or gastrointestinal liabilities. This selective profile is likely based on the binding and *in vitro* functional profile of the compound. The binding and activity profile of ABT-089 is unique in that, while it shows selective binding at the $\alpha_4\beta_2$ receptor subtype, it has only weak agonist activity at the receptor. At other recombinant receptor subtypes, it shows weak partial agonism and/or antagonism. Nevertheless, ABT-089 is effective in stimulating neurotransmitter release from rat brain *in vitro*. To date, it is unclear which of these properties underlie the efficacy seen in preclinical models. Regardless, the preclinical results suggest that ABT-089 may be efficacious in the treatment of cognitive disorders such as ADHD and AD with a limited potential for adverse cardiovascular and gastrointestinal side effects.

We have recently completed clinical Phase I studies. As suggested by preclinical studies, ABT-089 demonstrates good cardiovascular and gastrointestinal tolerability at plasma concentrations expected to be therapeutic. In addition, the compound has an excellent pharmacokinetic profile in humans.

## REFERENCES

1. Arneric SP, Bannon AW, Brioni JD, et al. ABT-089: an orally effective cholinergic channel modulator (ChCM) with cognitive enhancement and neuroprotective action. In: Becker R and Giacobini E, Eds. *Alzheimer Disease: From Molecular Biology to Therapy.* Boston: Birkhäuser, 1996;287–291.
2. Ashworth-Preece M, Jarrott B, Lawrence AJ. Nicotinic acetylcholine receptors in the rat and primate nucleus tractus solitarius and on rat and human inferior vagal (nodose) ganglia: Evidence from *in vivo* microdialysis and [$^{125}$I] α-bungarotoxin autoradiography. *Neuroscience* 1998;83:1113–1122.
3. Beani L, Antonelli T, Tomasini MC, Marani L, Bianchi C. The nicotinic modulation of [H-3]D-aspartate outflow in primary cultures of rat neocortical neurons: Effect of acute and long term nicotine treatment. *Neuropharmacology* 2000;39:2646–2653.
4. Bertrand D., Devillers-Thiéry A, Revah F, et al. Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. *Proc Natl Acad Sci USA* 1992;89:1261–1265.
5. Bertrand D, Galzi J-L, Devillers-Thiéry A, Bertrand S, Changeux J-P. Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal $\alpha_7$ nicotinic receptor. *Proc Natl Acad Sci USA* 1993;90:6971–6975.
6. Briggs CA, McKenna DG. Activation and inhibition of the human $\alpha_7$ nicotinic acetylcholine receptor by agonists. *Neuropharmacology* 1998;37:1095–1102.
7. Briggs CA, McKenna DG, Monteggia LM, et al. Gain of function mutation of the $\alpha_7$ nicotinic acetylcholine receptor: Distinct pharmacology of the human α7V274T variant. *Eur J Pharmacol* 1999;366:301–308.
8. Buccafusco JJ, Jackson WJ. Beneficial effects of nicotine administered prior to a delayed matching-to-sample task in young and aged monkeys. *Neurobiol Aging* 1991;12:233–238.
9. Cordero-Erausquin M, Marubio LM, Klink R, Changeux J-P. Nicotinic receptor function: New perspectives from knockout mice. *Trends Pharmacol Sci* 2000;21:211–217.

10. Decker MW, Bannon AW, Curzon P, et al. ABT-089 [2-methyl-3-(2-(S)-pyrrolidinylmethoxy)pyridine dihydrochloride]: II. A novel cholinergic channel modulator with effects on cognitive performance in rats and monkeys. *J Pharmacol Exp Ther* 1997;283:247–258.
11. Decker MW, Majchrzak MJ, Arneric SP. Effects of lobeline, a nicotinic receptor agonist, on learning and memory. *Pharmacol Biochem Behav* 1993;45:571–576.
12. Donnelly-Roberts DL, Brioni JD. Preclinical evidence on the neuroprotective effects of nicotinic ligands. In: Arneric, SP, Brioni, JD, Eds. *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities*. Indianapolis: Wiley-Liss, Inc., 1999:337–348.
13. Donnelly-Roberts DL, Xue IC, Arneric SP, Sullivan JP. *In vitro* neuroprotective properties of the novel cholinergic channel activator (ChCA), ABT-418. *Brain Res* 1996;719:36–44.
14. Ferris SH. Evaluation of memantine for the treatment of Alzheimer's disease. *Expert Opin Pharmacother* 2003;4:2305–2313.
15. Fu Y, Matta SG, Sharp BM. Local α-bungarotoxin-sensitive nicotinic receptors modulate hippocampal norepinephrine release by systemic nicotine. *J Pharmacol Exp Ther* 1999;289:133–139.
16. Fucile S, Renzi M, Lax P, Eusebi F. Fractional $Ca^{2+}$ current through human neuronal alpha 7 nicotinic acetylcholine receptors. *Cell Calcium* 2003;34:205–209.
17. Girod R, Barazangi N, McGehee DS, Role LW. Facilitation of glutamatergic neurotransmission by presynaptic nicotinic acetylcholine receptors. *Neuropharmacology* 2000;39:2715–2725.
18. Haass M, Kubler W. Nicotine and sympathetic neurotransmission. *Cardiovasc Drugs Ther* 1997 10:657–65.
19. Kaiser S, Wonnacott S. alpha-Bungarotoxin-sensitive nicotinic receptors indirectly modulate [H–3]dopamine release in rat striatal slices via glutamate release. *Mol Pharmacol* 2000;58:312–318.
20. Karlin A, Akabas MH. Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. *Neuron* 1995;15:1231–1244.
21. Kofalvi A, Sperlagh B, Zelles T, Vizi ES. Long-lasting facilitation of 4-amino-n-[2,3-H-3]butyric acid ([H–3]GABA) release from rat hippocampal slices by nicotinic receptor activation. *J Pharmacol Exp Ther* 2000;295:453–462.
22. Levin ED. Nicotinic receptor subtypes and cognitive function. *J Neurobiol* 2002;53:633–640.
23. Li X, Rainnie DG, McCarley RW, Greene RW. Presynaptic nicotinic receptors facilitate monoaminergic transmission. *J Neurosci* 1998;18:1904–1912.
24. Liang SD, Vizi ES. Positive feedback modulation of acetylcholine release from isolated rat superior cervical ganglion. *J Pharmacol Exp Ther* 1997;280:650–655.
25. Lin N-H, Gunn DE, Ryther KB, et al. Structure-activity studies on 2-methyl-3-(2(S)-pyrrolidinylmethoxy)pyridine (ABT-089): An orally bioavailable 3-pyridyl ether nicotinc acetylcholine receptor ligand with cognition-enhancing properties. *J Med Chem* 1997;40:385–390.
26. Lipton SA, Rosenberg, PA. Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* 1994;330:934–940.
27. Lukas RJ. Characterization of curaremimetic neurotoxin binding sites on membrane fractions derived from the human neuroblastoma clonal line TE 671. *J Neurochem* 1986;45:1936–1941.
28. Lukas RJ. Expression of ganglia-type nicotinic acetylcholine receptors and nicotinic ligand binding sites by cells of the IMR-32 human neuroblastoma clonal line. *J Pharmacol Exp Ther* 1993;265:294–302.
29. Marchi M, Risso F, Viola C, Cavazzani P, Raiteri M. Direct evidence that release-stimulating alpha 7* nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. *J Neurochem* 2002;80:1071–1078.
30. Marks MJ, Burch JB, Collins AC. Genetics of nicotine response in four inbred strains of mice. *J Pharmacol Exp Ther* 1983;226:291–302.
31. Marks MJ, Farnham DA, Grady SR, Collins AC. Nicotinic receptor function determined by stimulation of rubidium efflux from mouse brain synaptosomes. *J Pharmacol Exp Ther* 1993;264:542–552.
32. Newhouse PA, Kelton M. Nicotinic systems in central nervous systems disease: Degenerative disorders and beyond. *Pharm Acta Helv* 2000;74:91–101.
33. Papke RL, Porter Papke JK. Comparative pharmacology of rat and human alpha 7 nAChR conducted with net charge analysis. *Br J Pharmacol* 2002;137:49–61.
34. Prendergast MA, Jackson WJ, Terry AV Jr, Decker MW, Arneric SP, Buccafusco JJ. Central nicotinic receptor agonists ABT-418, ABT-089, and (–) nicotine reduce distractability in adult monkeys. *Psychopharmacology* 1998;136:50–58.
35. Roch J-M, Puttfarcken PS. Biological actions of the β-amyloid protein and its precursor. *Curr Drugs* 1996;1:9–16.

36. Sands SB, Costa ACS, Patrick JW. Barium permeability of neuronal nicotinic receptor $\alpha_7$ expressed in *Xenopus* oocytes. *Biophys J* 1993;65:2614–2621.
37. Séguéla P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. Molecular cloning, functional properties, and distribution of rat brain $\alpha_7$: A nicotinic cation channel highly permeable to calcium. *J Neurosci* 1993;13: 596–604.
38. Skok VI. Nicotinic acetylcholine receptors in autonomic ganglia. *Auton Neurosci* 2002;18:1–11.
39. Spencer TJ, Biederman J, Wilens TE, Faraone SV. Novel treatments for attention-deficit/hyperactivity disorders in children. *J Clin Psychiatry* 2002;63(Suppl 12):16–22.
40. Sullivan JP, Donnelly-Roberts D, Briggs CA, et al. ABT-089 [2-methyl-3-(2-(S)-pyrrolidinylmethoxy)pyridine]: I. A potent and selective cholinergic channel modulator with neuroprotective properties. *J Pharmacol Exp Ther* 1997;283:235–246.
41. Tucci SA, Genn RF, File SE. Methyllycaconitine (MLA) blocks the nicotine evoked anxiogenic effect and 5-HT release in the dorsal hippocampus: Possible role of alpha 7 receptors. *Neuropharmacology* 2003;44:367–373.
42. Wernicke JF, Kratochvil CJ. Safety profile of atomoxetine in the treatment of children and adolescents with ADHD. *J Clin Psychiatry* 2002;63(Suppl 12):50–55.
43. Yamada K, Toshitaka N. Therapeutic approaches to the treatment of Alzheimer's disease. *Drugs Today* 2002;38:631–637.