Communications to the Editor

Novel Isoxazoles which Interact with Brain Cholinergic Channel Receptors Have Intrinsic Cognitive Enhancing and Anxiolytic Activities

David S. Garvey,*,† James T. Wasicak, Michael W. Decker, Jorge D. Brioni, Michael J. Buckley, James P. Sullivan, George M. Carrera, Mark W. Holladay, Stephen P. Arneric, and Michael Williams

Neuroscience Research Division, D-47W, Pharmaceutical Discovery, AP-10, Abbott Laboratories, Abbott Park, Illinois 60064

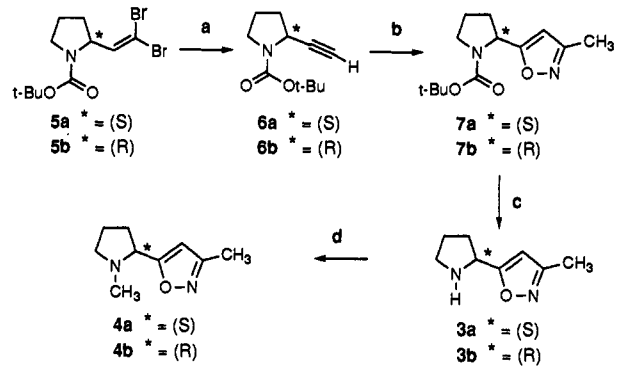
Received December 9, 1993

Alzheimer's disease (AD) is a central nervous system (CNS) neurodegenerative disorder which leads to dramatic personality changes as well as a profound dementia in individuals who are afflicted. Studies performed on autopsied brain tissue have shown that AD affects a number of brain neurochemical systems with the most pronounced and consistent change being a deficiency in markers of cholinergic tone in the neocortex and hippocampus. Decreases in markers of cholinergic activity such as choline acetyltransferase, high-affinity choline uptake, and acetylcholine esterase activity[1, 2] as well as regional reductions in the population of cholinergic receptors which recognize either muscarinic or nicotinic ligands have been found to occur in AD brains.[3–7] The decreases in cholinergic markers have been correlated with the severity of dementia, and thus, these findings have led to the cholinergic hypothesis of AD-related memory loss.[8–10]

Experimentally, it has been demonstrated that (*S*)-nicotine (**2a**) can improve the cognitive performance of rodents[11–13] and primates[14] in a variety of behavioral paradigms. Compound **2a** is also known to produce anxiolytic effects in humans.[15] More recently, pilot clinical studies have suggested that **2a** may be useful in the palliative treatment of deficits in attention and information processing associated with AD.[16,17] Patients with AD show a significant reduction in the number of neuronal cholinergic channel receptors (CCR's) in regions of the brain associated with cognitive function;[10,18,19] however, clinical studies have shown an inverse relationship between smoking and the disease.[20] Furthermore, chronic exposure to **2a** has been shown to upregulate neuronal CCR's in both rodents[21, 22] and in humans.[23] Chronic **2a** treatment additionally appears to elicit a beneficial neuroregenerative/neuroprotective effect in animal models utilized to evaluate neuronal survival.[24, 25]

The prototypical agonist for CCR's, **2a**, is also known to mediate a number of adverse side effects including irritations of the gastrointestinal tract[26,27] and negative effects on the cardiovascular system.[28–32] Thus, **2a** itself represents a poor therapeutic choice in the search for a safe and effective treatment for the elderly population with Alzheimer's dementia. Recent advances in the field of molecular biology have, however, led to the discovery of the existence of a number of potential subtypes of CCR's.[33,34] These new findings have raised the possibility that one may be able to design CCR ligands that may be differentiated from **2a** by selectively interacting with subtypes of CNS CCR's. Such cholinergic channel activators (ChCA's) represent a potentially novel class of psychoactive drugs for the safe and effective treatment of AD.[35] To this end, medicinal chemistry efforts have focused upon the preparation of ligands which will possess the beneficial behavioral effects associated with **2a** yet have significantly reduced side effects. Herein is described a novel series of compounds in which a substituted isoxazole has been incorporated as a bioisosteric replacement of the pyridine ring found in the enantiomers of **2**.

Scheme 1



[a] Reagents: (a) *n*-BuLi,THF, −78 °C, then $NaHCO_{3(aq)}$; (b) PhNCO, Et_3N, $C_2H_5NO_2$, benzene; (c) TFA–CH_2Cl_2; (d) 37% $(CH_2O)_{aq}$, 88% HCO_2H, reflux.

Chemistry. The nornicotine enantiomers **1a** and **1b** were resolved via HPLC on a chiral column.[36] The 3-methyl-5-(2-pyrrolidinyl)isoxazoles were prepared as outlined in Scheme 1. Briefly, the dibromovinyl derivative **5**[37] prepared from *N*-(*tert*-butyloxycarbonyl)-protected (*S*)- or (*R*)-prolinal was converted to acetylide **6** according to Corey's procedure.[38] Reacting compound **6** with the nitrile oxide generated *in situ* from nitroethane afforded the protected isoxazole derivatives **7**. Nitrogen deprotection and salt formation gave the product amines **3a** (A-79814) and **3b** (A-82695). Both enantiomers were converted to the corresponding N-methylated tertiary amines **4a** (ABT-418) and **4b** (A-81754) via Eschweiler–Clarke conditions. The enantiomeric purities of **4a** and **4b** were determined to be >99% ee by HPLC assay on a chiral column.[39]

Biology. The binding-affinity profile of each compound at neuronal CCR's was determined by measuring the displacement of [^{3}H]cytisine from a preparation of whole rat brain[40] according to the procedure of Pabreza et al.[41] A further CCR binding selectivity profile was completed by measuring displacement of [^{125}I]-α-bungarotoxin (α-BgT) from rat brain using a modification of the procedure of Marks et al.[42] and from the membranes of *Torpedo californica* electroplax according to the method of Charurvedi et al.[43] Brain muscarinic receptor binding was determined in rat brain by the displacement of [^{3}H]-oxotremorine M using a modification of the procedure of Birdsall et al.[44] The binding results of the test compounds

† Anti-infective Research Division, D-47N.

0022-2623/94/1837-1055$04.50/0

Table 1. Cholinergic Binding Profile

compd	binding affinity[a] (K_i, nM) [^{3}H]cytisine (rat brain)		[^{125}I]α-BgT (rat brain)	[^{125}I]α-BgT (*Torpedo* electroplax)	[^{3}H]Oxo-M (rat brain)	formula	anal.
1a	22.0 ± 3.0	(56)[b]	>20000	11000	>20000	$C_9H_{12}N_2 \cdot 2HCl \cdot 0.2H_2O$	C,H,N
1b	17.0 ± 2.0	(41)[b]	>20000	300 ± 98	>20000	$C_9H_{12}N_2 \cdot 2HCl$	C,H,N
2a	1.1 ± 0.4	(1.4)[b]	4000	>20000	>20000		
2b	16.0 ± 3.4	(32)[b]	>20000	1500	>20000		
3a	333 ± 50		>20000	>20000	>20000	$C_8H_{12}N_2O \cdot C_2H_2O_4$	C,H,N
3b	7.4 ± 0.7		>20000	3400	8000	$C_8H_{12}N_2O \cdot C_2H_2O_4$	C,H,N
4a	4.2 ± 0.6		>20000	>20000	>20000	$C_9H_{14}N_2O \cdot 1.1C_2H_2O_4$	C,H,N
4b	44 ± 12		>20000	>20000	>20000	$C_9H_{14}N_2O \cdot HCl$	C,H,N

[a] Values are the means ±SEM. [b] Literature value for displacement of [^{3}H]nicotine from ref 51.

have been normalized to their equilibrium dissociation constants (K_i).[45]

Behavioral studies were performed utilizing CD1 mice. Drug enhancement of cognitive performance was evaluated utilizing an inhibitory avoidance (IA) paradigm,[46] while anxiolytic-like activities were assayed utilizing the elevated plus-maze (EPM) paradigm.[47,48]

For the IA task, animals are initially trained 15 min after an intraperitoneal (ip) administration of a solution of either the drug or saline. During the training session animals are placed into the illuminated half of an apparatus of which also contains a darkened area. Rodents, being nocturnal animals, have a natural tendency to move into the dark area. Upon stepping into the darkened space, the animal receives a mild foot shock, after which it is returned to its home cage. Twenty-four hours later each animal is again placed into the same illuminated area and the latency to step through to the darkened compartment is a measure of how well the animal has remembered the test (i.e., the aversive experience which was learned during the training session). Longer latencies above those of the saline-treated control group of animals are attributed to the cognitive-enhancing properties of the drug.

The EPM test is a pharmacologically validated animal model of anxiety. The maze consists of four arms elevated above the floor with two of the arms having side walls and the other two being open. Naive animals are administered drug or saline ip and 15 min later placed in the center of the maze. Each animal is then given 5 min to explore the apparatus. The amount of time that drug-treated animals spend in the open arms of the maze over and above that of the saline-treated control group is a measure of the anxiolytic-like properties of the drug.

Results and Discussion. [^{3}H]-(−)-Cytisine has been shown to bind with high affinity to the $\alpha4\beta2$ subtype of CCR's, the major subtype in rodent brain accounting for greater than 90% of (−)-nicotine binding sites.[49,50] Consistent with previously published results,[51] initial examination of the data shows that there is a significant difference in the binding potencies of the enantiomers of nicotine at the site labeled by [^{3}H]cytisine (Table 1). The (*S*)-enantiomer, **2a** ($K_i \cong 1.2$ nM), is 1 order of magnitude more potent than the corresponding (*R*)-enantiomer ($K_i \cong 16$ nM). This is in contrast to the nornicotine enantiomers which have nearly identical binding potencies ($K_i \cong 20$ nM). Comparing the trends in binding potencies of the nornicotine and nicotine enantiomers, it was noted that N-methylation enhanced the potency in the (*S*)-enantiomeric series while it had virtually no effect on the affinity of the (*R*)-enantiomers.

From the results with **3b** ($K_i \cong 7.4$ nM) and **4a** ($K_i \cong 4.2$ nM), it is apparent that potent nanomolar binding affinity at brain CCR's can be achieved with either enantiomer of the isoxazoles. Of particular note is that the trend in potency for each enantiomeric isoxazole series is different. That is, for the (*S*)-enantiomeric compounds, the N-methylated pyrrolidine **4a** is approximately 80-fold more potent than the desmethyl analog **3a**. Although the potency enhancement is not as dramatic, this is the same trend observed upon comparison of the pyridine derivatives **1a** and **2a**. The opposite trend is observed with the two isoxazoles in the (*R*)-enantiomeric series. Analog **3b**, the compound with the unsubstituted pyrrolidine nitrogen, is 7-fold more potent than the *N*-methyl derivative **4b**. This enhanced binding potency is not observed upon N-demethylation of **2b** to afford **1b**.

In contrast to the activity at the $\alpha4\beta2$ nicotinic acetylcholine receptor (nAChR) subtype labeled by [^{3}H]-cytisine, all of the compounds were nearly 3 orders of magnitude less potent in displacing [^{125}I]-α-BgT binding from the α-BgT-sensitive CCR subtype present in rat brain membranes (Table 1). However, a trend in the structure–activity binding profile emerged when the binding of [^{125}I]-α-BgT to the subtype of nAChR present in *Torpedo* electroplax, which is similar to the neuromuscular CCRs, was examined. At the [^{125}I]-α-BgT site defined in *Torpedo*, the general trend observed was that (*R*)-enantiomers of the isoxazole and pyridine derivatives were slightly more potent than the corresponding derivatives in the (*S*)-enantiomeric series. With the exception of **4a**, N-demethylation in both enantiomeric series enhanced binding affinity. None of the compounds had appreciable activity at muscarinic cholinergic receptors labels by [^{3}H]-oxotremorine-M (Table 1), highlighting the predominant selectivity of these compounds for the ligand-gated ion channel cholinergic receptors found in brain.

On the basis of the potent binding affinities of **4a** and **3b** at the $\alpha4\beta2$ brain CCR subtype, it was decided to evaluate these two analogs in comparison to **2a** in the rodent IA and EPM behavioral paradigms. The results of these compounds in the IA task is shown in Figure 1. Although each of the compounds was able to produce a significant enhancement in cognitive performance, of great interest was the fact that both **4a** and **3b** were significantly more potent than **2a**. Cognitive enhancing effects were observed with **4a** at a dose of 0.036 μmol/kg and with **3b** at 0.062 μmol/kg, while the effects with **2a** required a much higher dose of 0.620 μmol/kg. Although the $\alpha4\beta2$ brain binding affinities were slightly less potent, the behavioral results with the two isoxazole compounds showed them to be at least 10-fold more potent than **2a** in their ability to produce a cognitive enhancing effect.

Figure 2 graphically depicts the results obtained with these three compounds in the EPM behavioral test. With

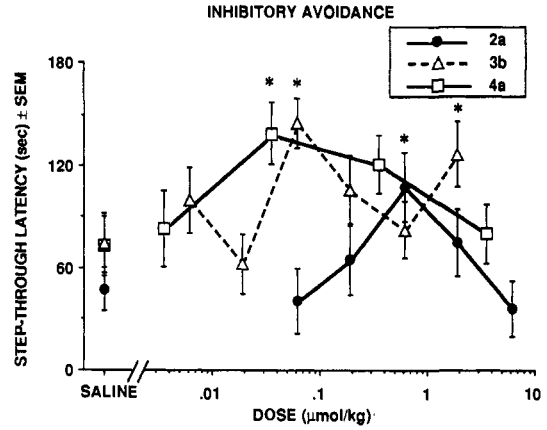


Figure 1. The effects of **3b** and **4a** on retention of inhibitory avoidance training in mice. Shown are the mean test latencies in seconds (± SEM). Significant improvement in retention test performance was observed for **4a** [$F(4,52) = 2.59$; $p < 0.05$] and for **3b** [$F(6,92) = 2.62$; $p < 0.05$] **2a** data redrawn from ref 46 for comparative purposes. *Significantly different from appropriate saline group, $p < 0.05$.

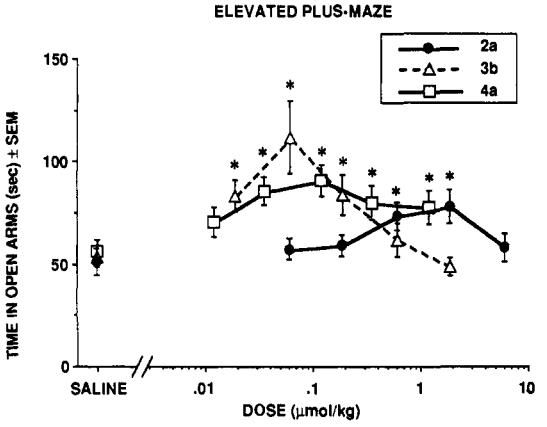


Figure 2. The effects of **3b** and **4a** on elevated plus-maze exploration in mice. Shown are the mean times spent in the open arms in seconds (± SEM). Significant improvement in retention test performance was observed for **4a** [$F(5,69) = 3.21$; $p < 0.05$] and for **3b** [$F(5,63) = 5.06$; $p < 0.001$]. **2a** data redrawn from ref 46 for comparative purposes. *Significantly different from appropriate saline group, $p < 0.05$

regard to the anxiolytic effects of the compounds, a similar trend in potency to that which was observed with the compounds in the IA paradigm was apparent. The minimal effective doses for **4a** and **3b** to produce significant anxiolytic effects were again at concentrations much lower than the lowest dose at which **2a** was active. The lowest dose at which **4a** produced anxiolytic effects was at 0.036 μmol/kg while **3b** produced beneficial effects at a dose as low as 0.019 μmol/kg. Thus, the anxiolytic activities of the two isoxazoles in the EPM paradigm is manifested at levels which are 3–30-fold lower than the dose of 0.62 μmol/kg minimally required for **2a** to show activity.

To assess potential side-effect liabilities of the new isoxazole compounds, **2a**, **3b**, and **4a** were evaluated for their abilities to modulate the general locomotor activity of the rodents as well as to determine their effects on the animals' body temperature. As shown in Figure 3, **2a** significantly reduced body temperature at a dose which was at least three-fold lower than that observed with either of the isoxazoles tested. As summarized in Figure 4, a significant reduction in locomoter activity was observed with **2a** at a dose which was minimally 6-fold lower than the dose required to produce a comparable hypolocomotor

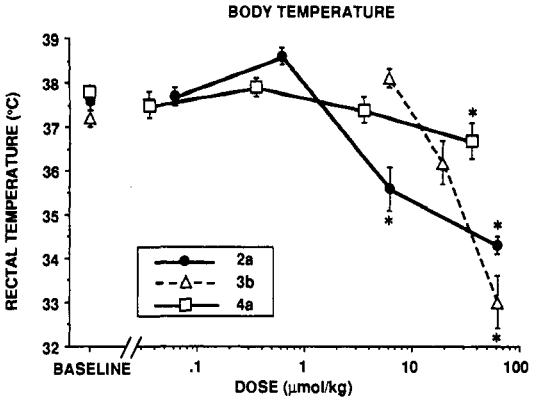


Figure 3. The effects of **2a**, **3b**, and **4a** on body temperature taken 15 min after injection. Shown are mean rectal temperatures in °C ± SEM. Differences from baseline performance were evaluated by paired t tests. *Significantly different from pre-injection baseline, $p < 0.05$.

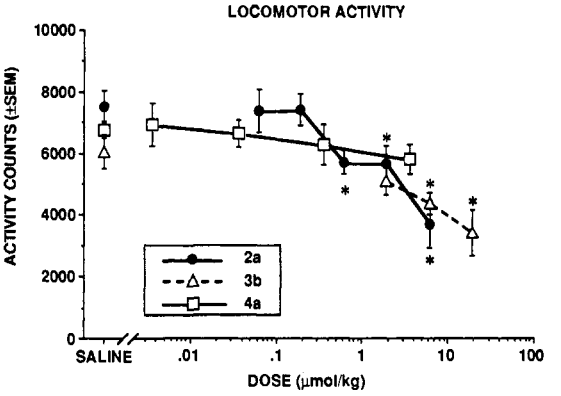


Figure 4. The effects of **2a**, **3b**, and **4a** on locomotor activity measured for 20 min in an open field beginning approximately 2 min after injection. Shown are mean activity counts (infrared beam breaks) ± SEM. Significant effects were noted for **2a** [$F(5,42) = 6.65$; $p < 0.0001$] and **3b** [$F(3,28) = 4.30$; $p < 0.05$], but not for **4a** [$F(4,32) = 0.74$; $p = 0.57$]. *Significantly different from appropriate saline group, $p < 0.05$.

effect with either **3b** or **4a**. With **2a**, locomoter activity was reduced at the same dose which facilitated memory performance and produced anxiolytic effects. Thus, on the basis of the results of these in vivo experiments, the potential therapeutic utility of the isoxazoles **3b** and **4a** is significantly higher than that of **2a**. With **2a**, side effects are produced at the same dose level as that required for the production of the beneficial behavioral activities while for **3b** and **4a** the cognitive enhancing and anxiolytic activities occur at dose levels which are at least 10-fold lower than those which produce hypolocomotive or hypothermic effects.

In summary, the discovery a novel series of neuronal CCR ligands in which a substituted isoxazole ring has been incorporated as a bioisosteric replacement for the pyridine ring of nicotine is reported. Receptor binding studies show that this modification is favorable in both the (*S*)- and (*R*)-enantiomeric series to interact with the $\alpha4\beta2$ subtype of neuronal CCR, the major receptor subtype with the (−)-nicotine binding site in brain.[49,50] Remarkably beneficial cognitive enhancing and anxiolytic effects have also been produced in mice with **4a** and **3b** at dosage levels averaging 1 order of magnitude lower than those observed with **2a**. Further in vitro[52] characterization of **4a** has indicated that it is a potent and selective agonist of some putative subtypes of neuronal CCR's. Moreover, **4a**·HCl

had less emetic liability in dogs compared to **2a** and was less potent in eliciting seizures and death in mice.[53] Ligands for neuronal CCR's possessing such a behavioral profile and with a more favorable therapeutic window with respect to nicotinically mediated adverse side effects may be useful in the treatment of AD related personality changes and memory loss. This novel series of isoxazole compounds represents an exciting new area of investigation where safe and effective ChCAs to treat CNS disorders involving loss of cholinergic function will continue to be explored.

Acknowledgment. We thank Pamala Adams, David J. Anderson, Diana Donnelly-Roberts, and Joanna L. Raszkiewicz for performing the receptor binding experiments.

References

(1) Sims, N. A.; Bowen, D. M.; Allen, S. J.; Smith, C. C. T.; Neary, D.; Thomas, D. J.; Davison, A. N. Presynaptic Dysfunction in Patients with Dementia. *J. Neurochem.* **1983**, *40*, 503–509.
(2) Bowen, D. M.; Allen, S. J.; Benton, J. S.; Goodhardt, M. J.; Haan, E. A.; Palmer, A.M.; Sims, N. R.; Smith, C. C. T.; Spillane, J. A.; Esiri, M. M.; Neary, D.; Snowden, J. S.; Wilcoch, G. K.; Davison, A. N. Biochemical Assessment of Serotonergic and Cholinergic Dysfunction and Cerebral Atrophy in Alzheimer's Disease. *J. Neurochem.* **1983**, *41*, 266–272.
(3) Norberg, A.; Adem, A.; Hardy, J.; Winblad, B. Change in Nicotinic Receptor Subtypes in Temporal Cortex of Alzheimer Brains. *Neurosci. Lett.* **1986**, *86*, 317–321.
(4) Shimohama, S.; Taniguchi, T.; Fujiwara, M.; Kameyama, M. Changes in Nicotinic and Muscarinic Cholinergic Receptors in Alzheimer-Type Dementia. *J. Neurochem.* **1986**, *46*, 288–293.
(5) Flynn, D. D.; Mash, D. C. Characterization of L-[^{3}H] Nicotine Binding in Human Cerebral Cortex: Comparison Between Alzheimer's Disease and the Normal. *J. Neurochem.* **1986**, *46*, 1948–1954.
(6) Whitehouse, P. J.; Martino, A. M.; Antuono, P. G.; Lowenstein, P. R.; Coyle, J. T.; Price, D. L.; Kellar, K. J. Nicotinic Acetylcholine Binding Sites in Alzheimer's Disease. *Brain Res.* **1986**, *371*, 146–151.
(7) Whitehouse, P. J.; Martino, A. M.; Wagster, M. V.; Price, D. L.; Mayeux, R.; Atach, J. R.; Kellar, K. J. Reductions in [^{3}H] Nicotinic Acetylcholine Binding in Alzheimer's Disease and Parkinson's Disease: An Autoradiographic Study. *Neurology* **1988**, *38*, 720–723.
(8) Perry, E. K. The Cholinergic Hypothesis – Ten Years On. *Br. Med. Bull.* **1986**, *42*, 63–69.
(9) Bartus, R. T.; Dean, R. L.; Fisher, S. K. Cholinergic Treatment for Age-Related Memory Disturbances: Dead or Barely Coming of Age? In *Treatment Development Strategies for Alzheimer's Disease*; Crook, T., Bartus, R. T., Ferris, S., Gershon, S., Eds.; Mark Powley Assoc.: Madison, CT, 1986; pp 421–450.
(10) Coyle, J. T.; Price, D. L.; DeLong, M. R. Alzheimer's Disease: A Disorder of Cortical Cholinergic Inervation. *Science* **1983**, *219*, 1184–1190.
(11) Bovet, D.; Bovet-Nitti, F.; Olivero, A. Effects of Nicotine on Avoidance Conditioning of Inbred Strains of Mice. *Psychopharmacology* **1966**, *10*, 1–5.
(12) Evangelista, A. M.; Gattoni, R. C.; Izquierdo, I. Effect of Amphetamine, Nicotine, and Hexamethone on Performance of a Conditioned Response During Acquisition and Retention Trials. *Pharmacology* **1970**, *3*, 91–96.
(13) Haroutunian, V.; Barnes, E.; Davies, K. L. Cholinergic Modulation of Memory in Rats. *Psychopharmacology* **1985**, *87*, 266–271.
(14) Buccafusco, J.; Jackson, W. Beneficial Effects of Nicotine Administered Prior to a Delayed Matching-To-Sample Task in Young and Aged Monkeys. *Neurobiol. Aging* **1991**, *12*, 233–238.
(15) Pomerleau, O. F.; Turk, D. C.; Fertig, J. B. The Effects of Cigarette Smoking on Pain and Anxiety. *Addict. Behav.* **1984**, *9*, 265–271.
(16) Newhouse, P. A.; Sunderland, Y.; Tariot, P. N.; Blumhardt, C. L.; Weingartner, H.; Mellow, A. Intravenous Nicotine in Alzheimer's Disease: A Pilot Study. *Psychopharmacology* **1988**, *95*, 171–175.
(17) Sahakian, B.; Jones, G.; Levy, R.; Gray, J.; Warburton, D. The Effects of Nicotine on Attention, Information Processing, and Short-Term Memory in Patients with Dementia of the Alzheimer Type. *Br. J. Psych.* **1989**, *154*, 797–800.
(18) Nordberg, A.; Winblad, B. Reduced Number of [^{3}H] Nicotine and [^{3}H] Acetylcholine Binding Sites in the Frontal Cortex of Alzheimer's Brains. *Neurosci. Lett.* **1986**, *72*, 115–119.
(19) Rinne, J.; Myllykyla, T.; Lonnberg, P.; Marjamaki, P. A Post-Mortem Study of Brain Nicotinic Receptors in Parkinson and Alzheimer's Disease. *Brain Res.* **1991**, *547*, 167–170.
(20) Duijn, C. V.; Hofman, A. Relation Between Nicotine Intake and Alzheimer's Disease. *Br. Med. J.* **1991**, *302*, 1491–1494.
(21) Schwartz, R. D.; Kellar, K. J. Nicotinic Cholinergic Receptor Binding Sites in the Brain: Regulation In Vivo. *Science* **1983**, *220*, 214–216.
(22) Ksir, C., Hakan, R.; Hall, D. P., Jr.; Kellar, K. J. Exposure to Nicotine Enhances the Behavioral Stimulant Effect of Nicotine and Increases Binding of [^{3}H] Acetylcholine to Nicotinic Receptors. *Neuropharmacology* **1985**, *24*, 527–531.
(23) Benwall, M. E. M.; Balfour, D. J. K.; Anderson, J. M. Evidence that Tobacco Smoking Increases the Density of (–) [^{3}H] Nicotine Binding Sites in Human Brain. *J. Neurochem.* **1988**, *50*, 1243–1247.
(24) Jason, A. M.; Fuxe, K.; Agnati, L. F.; Jasson, A.; Bjeklke, B.; Sundstrom, E.; Anderson, K.; Harfstrand, A.; Goldstein, M.; Owman, C. Protective Effects of Chronic Nicotine Treatment on Lesioned Nigrostriatal Dopamine Neurons in the Male Rat. *Prog. Brain Res.* **1989**, *79*, 257–265.
(25) Owman, C.; Fuxe, K.; Jason, A. M.; Kahrstrom, J. Studies of Protective Actions of Nicotine on Neuronal and Vascular Functions in the Brain of Rats: Comparison Between Sympathetic Noradrenergic and Mesostriatal Dopaminergic Fiber System, and the Effect of a Dopamine Agonist. *Prog. Brain Res.* **1989**, *79*, 267–276.
(26) Wormsley, K. G. Smoking and Duodenal Ulcer. *Gastroenterology* **1978**, *75*, 139–152.
(27) Eastwood, G. L. The Role of Smoking in Peptic Ulcer Disease. *J. Clin. Gastroenterol.* **1988**, *10*, 519–523.
(28) Gebber, G. L. Neurogenic Basis for the Rise in Blood Pressure Evoked by Nicotine in the Cat. *J. Pharmacol. Exp. Ther.* **1969**, *166*, 255–263.
(29) Larson, P. S.; Silvette, H. *Tobacco: Experimental and Clinical Studies*; Williams & Wilkins: Baltimore, 1968; Suppl. I.
(30) Larson, P. S., Silvette, H. *Tobacco: Experimental and Clinical Studies*; Williams & Wilkins: Baltimore, 1975; Suppl. III.
(31) Quick, C. R. G.; Cotton, L. T. The Measured Effect of Stopping Smoking on Intermittent Claudication. *Br. J. Surg.* **1982**, *69*, 524–526.
(32) Lusby, R. J.; Bauminger, B.; Woodcock, J. P.; Skidmore, R.; Baird, R. N. Cigarette Smoking: Acute Main and Small Vessel Hemodynamic Responses in Patients with Arterial Disease. *Am. J. Surg.* **1981**, *142*, 169–173.
(33) Deneris, E. S.; Connolly, J.; Rogers, S. W.; DuVoisin, R. Pharmacological and Functional Diversity of Neuronal Nicotinic Acetylcholine Receptors. *Trends Pharmacol. Sci.* **1991**, *12*, 34–40.
(34) Sargent, P. B. The Diversity of Neuronal Nicotinic Acetylcholine Receptors. *Annu. Rev. Neurosci.* **1993**, *16*, 403–443.
(35) Arneric, S. P.; Williams, M. Nicotinic Agonists in Alzheimer's Disease: Does The Molecular Diversity of Nicotine Receptors Offer the Opportunity for Developing CNS Selective Cholinergic Channel Activators? In *The Treatment of Neurodegenerative Disorders and Cognitive Function: Disease*; Capri: Italy, 1993; in press.
(36) Racemic *N*-(*tert*-butyloxycarbonyl)nornicotine was separated on a Chiralcel-OD (Daicel) column utilizing hexane/iPrOH (9:1) as the mobile phase. Deprotection with HCl–dioxane afforded the desired products as the dihydrochloride salts. Absolute stereochemical assignments were made by Eschweiler–Clarke N-methylation of the enantiomers and comparison with authentic (*S*)-nicotine and (*R*)-nicotine standards.
(37) Garvey, D. S.; Wasicak, J. T., Chung, J. Y.-L.; Shue, Y. K., Carrera, G. M.; May, P. D.; McKinney, M. M.; Anderson, D.; Cadman, E.; Vella-Rountree, L.; Nadzan, A. M.; Williams, M. Synthesis and In Vitro Characterization of Novel Amino Terminally Modified Oxotremorine Derivatives for Brain Muscarinic Receptors. *J. Med. Chem.* **1992**, *35*, 1550–1557.
(38) Corey, E. J.; Fuchs, P. L. A Synthetic Method for Formyl→Ethynyl Conversion. *Tetrahedron Lett.* **1972**, 3769–3772.
(39) The enantiomers were cleanly separated on a Chiralcel-OD (Daicel) column utilizing hexane/EtOH (97:3) as the mobile phase monitoring with UV detection at 215 nM.
(40) Enna, S. J.; Synder, S. H. Properties of γ-amino butyric acid (GABA) Receptor Binding in Rat Brain Synaptic Membrane Fractions. *Brain Res.* **1975**, *100*, 81–97.
(41) Pabreza, L. A; Dhawan, S.; Kellar, K. J. [^{3}H] Cytisine Binding to Nicotinic Cholinergic Receptors in Brain. *Mol. Pharmacol.* **1990**, *39*, 9–12.
(42) Marks, M. J.; Stitzel, A.; Romm, E.; Wehner, J. M.; Collins, A. C. Nicotinic Binding Sites in Rat and Mouse Brain: Comparison of Acetylcholine, Nicotine, and α-Bungarotoxin. *Mol. Pharmacol.* **1986**, *30*, 427–436.
(43) Chaturvedi, V; Donnelly-Roberts, D.; Lentz, T. L. Effects of Mutations of *Torpedo* Acetylcholine Receptor α1 Subunit Residues 184–200 on α-Bungarotoxin Binding in a Recombinant Fusion Protein. *Biochemistry* **1993**, *32*, 9570–9576.
(44) Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, E. C. The Binding of Agonists to Brain Muscarinic Receptors. *Mol. Pharmacol.* **1978**, *14*, 723–736.
(45) Cheng, Y.-C; Prusoff, W. H. Relationship Between the Inhibition Constant (Ki) and the Concentration of Inhibitor which Causes 50 Per Cent Inhibition (I_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(46) Brioni, J.; Arneric, S. P. Nicotinic Receptor Agonists Facilitate Retention of Avoidance Training: Participation of Dopaminergic Mechanisms. *Behavioral Neural Biol.* **1993**, *59*, 57–62.
(47) Lister, R. G. The Use of a Plus-Maze to Measure Anxiety in the Mouse. *Psychopharmacology* **1987**, *92*, 180–185.
(48) Brioni, J.; O'Neil, A. B.; Kin, D. J. B.; Decker, M. W. Nicotinic Receptor Agonists Exibit Anxiolytic-like Effects on the Elevated Plus Maze. *Eur. J. Pharmacol.* **1993**, *238*, 1–8.
(49) Flores, C. M.; Rogers, S. W.; Pabreza, L. A.; Wolfe,B.; Kellar, K. J. A Subtype of Nicotinic Cholinergic Receptor is Composed of $\alpha 4$ and $\beta 2$ Subunits and is Upregulated by Chronic Nicotine Treatment. *Mol. Pharmacol.* **1992**, *41*, 31–37.
(50) Whiting, P.; Schoepfer, R.; Lindstrom, J.; Priestly, T. Structural and Pharmacological Characterization of the Major Brain Nicotinic Acetylcholine Receptor Subtype Stably Expressed in Mouse Fibroblasts. *Mol. Pharmacol.* **1992**, *40*, 463–472.
(51) Zhang, X.; Nordberg, A. The Competition of (−)-[^{3}H] Nicotine Binding by the Enantiomers of Nicotine, Nornicotine and Anatoxin-a in Membranes and Solubilized Preparations of Different Brain Regions of Rat. *Arch. Pharm.* **1993**, *348*, 28–34.
(52) Arneric, S. P.; Sullivan, J. P.; Briggs, C. A.; Donnelly-Roberts, D.; Anderson, D. J.; Raszkiewicz, J. L.; Hughes, M.; Cadman, E. D.; Adams, P.; Garvey, D. S.; Wasicak, J.; Williams, M. ABT-418: A Novel Cholinergic Ligand with Cognition Enhancing and Anxiolytic Activity: I. In Vitro Characterization. *J. Pharmacol. Exp. Ther.*, in press.
(53) Decker, M. W.; Brioni, J. P.; Sullivan, J. P.; Buckley, M. J.; Sullivan, J. P.; Radek, R. J.; Raszkiewicz, J. L.; Kang, C. H.; Kim, D. J. B.; Giardina, W. J.; Wasicak, J.; Garvey, D. S.; Williams, M.; Arneric, S. P. ABT-418: A Novel Cholinergic Ligand with Cognition Enhancing and Anxiolytic Activity: II. In Vivo Characterization. *J. Pharmacol. Exp. Ther.*, in press.