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TC-1734: An Orally Active Neuronal Nicotinic Acetylcholine Receptor Modulator with Antidepressant, Neuroprotective and Long-Lasting Cognitive Effects

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ABSTRACT

The development of selective ligands targeting neuronal nicotinic acetylcholine receptors to alleviate symptoms associated with neurodegenerative diseases presents the advantage of affecting multiple deficits that are the hallmarks of these pathologies. TC-1734 is an orally active novel neuronal nicotinic agonist with high selectivity for neuronal nicotinic receptors. Microdialysis studies indicate that TC-1734 enhances the release of acetylcholine from the cortex. TC-1734, by either acute or repeated administration, exhibits memory enhancing properties in rats and mice and is neuroprotective following excitotoxic insult in fetal rat brain in cultures and against alterations of synaptic transmission induced by deprivation of glucose and oxygen in hippocampal slices. At submaximal doses, TC-1734 produced additive cognitive effects when used in combination with tacrine or donepezil. Unlike (–)-nicotine, behavioral sensitization does not develop following repeated administration of TC-1734. Its pharmacokinetic (PK) profile (half-life of 2 h) contrasts with the long lasting improvement in working memory (18 h) demonstrating that cognitive improvement extends beyond the lifetime of the compound. The very low acute toxicity of TC-1734 and its receptor activity profile provides additional mechanistic basis for its suggested potential as a clinical candidate. TC-1734 was very well tolerated in acute and chronic oral toxicity studies in mice, rats and dogs. Phase I clinical trials demon-

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strated TC-1734's favorable pharmacokinetic and safety profile by acute oral administration at doses ranging from 2 to 320 mg.

The bioavailability, pharmacological, pharmacokinetic, and safety profile of TC-1734 provides an example of a safe, potent and efficacious neuronal nicotinic modulator that holds promise for the management of the hallmark symptomatologies observed in dementia.

INTRODUCTION

Memory impairment and mental deterioration are common occurrences in the elderly population resulting either from senile dementia or from a normal aging process. Neurotoxicity associated with β-amyloid deposition, alteration in cerebral perfusion, and proinflammatory processes within the brain have also been reported to precipitate neuronal damage and contribute to the deterioration of brain function in health and disease. Within the last decade, there has been increasing experimental support for therapeutics targeting the acetylcholine neurotransmitter system, especially nicotinic acetylcholinergic receptors (nAChRs), in neurodegenerative pathologies (for review, see refs. 8,33).

To date, the only symptomatic treatments for Alzheimer's disease with proven efficacy have been acetylcholinesterase inhibitors that prevent the hydrolysis of acetylcholine in the synaptic cleft, thereby prolonging its activity. Although these agents have some benefit in alleviating cognitive impairment, they are palliative at best due to their low and limited clinical efficacy and tolerability.

Nicotinic agonists interact with nAChRs to improve cognition in animal models (2,12, 22–24), improve memory in Alzheimer's patients (18,29,31) and promote the release of acetylcholine and other neurotransmitters in brain (37,38), making them attractive as potential therapeutics for dementia. These findings have spurred considerable research efforts to develop ligands selective for nAChRs, such as ABT-418 (3), ABT-594 (4), ABT-089 (11), SIB-1508Y (13), SIB-1553 (9), TC-2403 (7,25), and TC-2559 (6). Thus, there is abundant preclinical evidence that nAChR modulators may be capable of alleviating cognitive impairment in demented states.

In addition to cognition through enhanced attention and vigilance, a large body of research implicates nAChRs with neuroprotection under a variety of insults *in vitro* and *in vivo* suggesting potential for disease modification (1,6,15,16,19–21,26,27,32,34,36,39). Modulators of nAChR pharmacology have been reported to prevent neurite loss during NGF deprivation in PC12 cells, protect cortical, hippocampal and striatal neurons against glutamate mediated neurotoxicity or following surgical lesions (10,28), attenuate or reverse β-amyloid induced neurotoxicity (35), and protect against MPP^+ induced toxicity in mesencephalic cultures (17). Thus, there is a scientific rationale for the impact of nAChR agonists on disease progression, which would provide an advantage over currently available symptomatic treatments for memory loss.

The lack of appropriate selectivity, manifested as limiting side effects in animals and humans, has hampered the development of previous clinical candidates. The profile of several clinical candidates (poor oral bioavailability, side effects, or lack of efficacy) has not been adequate to warrant further development. We have demonstrated *in vitro* and in animal studies and established the proof of concept in humans that a marked separation between beneficial effects and side effects can be accomplished. The reduction of unde-

sirable effects due to activity at nAChRs in the central and peripheral nervous systems (CNS and PNS), including cardiovascular side effects and emesis, and elimination of effects mediated by certain nAChRs in the CNS, such as seizures and hypothermia, is necessary to create a well-tolerated drug.

The present review describes the pharmacological and safety profile of TC-1734, a safe, novel, orally active modulator of neuronal nicotinic receptors, with broad efficacy at end points representing the cardinal symptoms in dementia.

SUBJECTS

All studies involving animals were conducted in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

CHEMISTRY

(S)-(E)-N-Methyl-5-[3-(5-isopropoxypyridinyl)]-4-penten-2-amine (TC-1734; Fig. 1A) was synthesized as outlined in Fig. 1B. The palladium-catalyzed coupling ($Pd(OAc)_2$, $P(o\text{-tol})_3$, Et_3N, DMF) (Heck reaction) of 3-bromo-5-isopropoxypyridine and (S)-N-methyl-N-(*tert*-butoxycarbonyl)-4-penten-2-amine, followed by treatment of the resulting intermediate, (S)-(E)-N-methyl-N-(*tert*-butoxycarbonyl)-5-[3-(5-isopropoxypyridin)yl]-4-penten-2-amine with 6 M HCl in DMF yielded TC-1734 free base. The required 3-bromo-5-isopropoxypyridine was prepared by reaction of 3,5-dibromopyridine (Lancaster Synthesis) with sodium isopropoxide in N-methylpyrrolidinone (NMP). The protected olefinic amine, (S)-N-methyl-N-(*tert*-butoxycarbonyl)-4-penten-2-amine was prepared by reaction of (R)-4-penten-2-ol p-toluenesulfonate with an excess of 40% aqueous methylamine in DMF to produce (S)-N-methyl-4-penten-2-amine and subsequent protection of the amine with di-*tert*-butyl dicarbonate. The (R)-4-penten-2-ol was made by ring opening of (R)-propylene oxide (Rhodia Chirex) with vinyl magnesium chloride in the presence of cuprous chloride, and then converted to the corresponding *p*-toluenesulfonate ester by reaction with *p*-toluenesulfonyl chloride in THF. For pharmacological evaluation, (S)-(E)-N-methyl-5-[3-(5-isopropoxypyridin)yl]-4-penten-2-amine was converted to its hemigalactarate salt by reaction with half an equivalent of galactaric acid in methanol/water (5).

PRECLINICAL PHARMACOLOGY

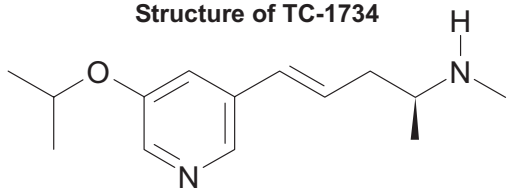
Biochemical Pharmacology

TC-1734 binds with high affinity (K_i = 11 nM) to nAChRs ($\alpha_4\beta_2$ subtype) labeled with [^{3}H]nicotine in membranes from rat cerebral cortex. By contrast, TC-1734 was not able to displace [^{125}I]α-bungarotoxin binding to α_7 nAChRs in rat hippocampal membranes (K_i > 50,000 nM), demonstrating selectivity for the $\alpha_4\beta_2$ subtype.

The agonist activity of TC-1734 was determined in a $^{86}Rb^+$ flux assay in rat thalamic synaptosomes ($\alpha_4\beta_2$ subtype) with an EC_{50} of 220 nM (E_{max} = 57% vs. tetramethylammo-

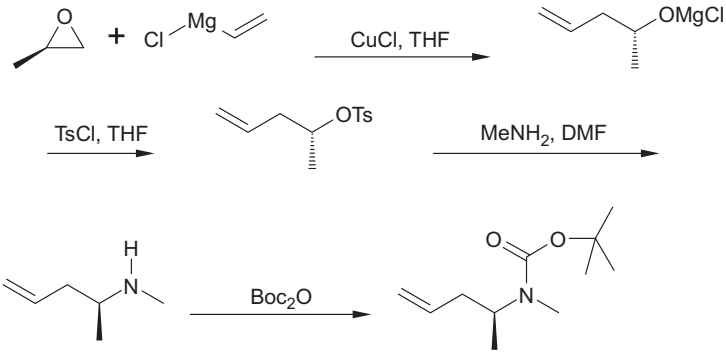
A

Structure of TC-1734

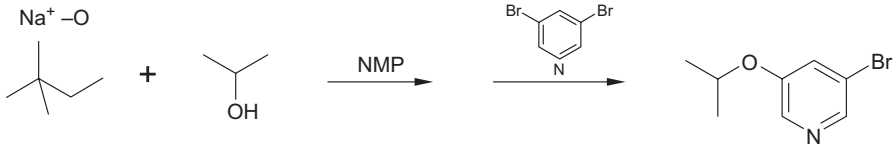


B

Stage 1. Preparation of chiral sidechain



Stage 2. Preparation of isopropoxy-bromo-pyridine



Stage 3. Preparation of TC-1734

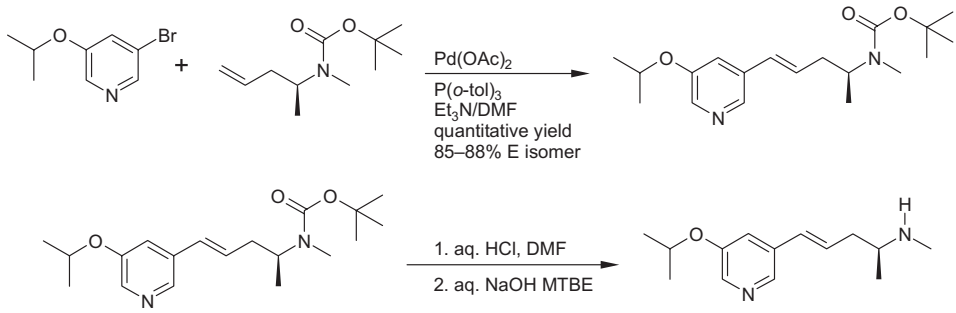


Fig. 1. A. Structure of TC-1734; **B.** Preparation of TC-1734.

nium) as compared to nicotine (EC_{50} = 591 nM, E_{max} = 87%). Activity was also demonstrated in dopamine-release assays in striatal synaptosomes (a mixed $\alpha_4\beta_2/\alpha_6/\alpha_3\beta_2$ preparation), which yielded an EC_{50} of 106 nM (E_{max} = 55%) as compared to an EC_{50} of 100 nM for nicotine (E_{max} = 113%). Results are summarized in Table 1.

TC-1734 did not display efficacy at muscle-type and ganglion-type nAChRs, as measured by an isotopic ion efflux assay. Specifically, TC-1734 had no detectable effects at concentrations up to 100 μM at the muscle-type ($\alpha_1\beta_1\delta\gamma$) nAChRs, expressed in the human clonal cell line TE671/RD, or at the rat or human ganglion-like nAChRs ($\alpha_3\beta_4$) expressed in the rat pheochromocytoma PC12 cell line or the human clonal line SH-SY5Y). In contrast, nicotine activated nAChRs in these preparations with an EC_{50} of 60 μM and E_{max} of 60% (compared to acetylcholine) in TE671/RD cells and EC_{50} of 20 μM and E_{max} of 65% (compared to acetylcholine) in PC12 cells. These results indicate that TC-1734 has a markedly enhanced CNS-PNS selectivity ratio. The interaction of TC-1734 at non-nAChR binding sites was also investigated. Of the 135 receptor and enzyme systems tested, TC-1734 (10 μM) partially displaced binding at the adrenergic α_2 (59%), imidazoline I_2 (67%), peripheral muscarinic (60%), serotonin transporter (86%), and σ_1 (88%) sites. At 0.1 μM, no significant inhibition was observed at any of these receptors.

Behavioral Pharmacology

The enhancing effect of TC-1734 on memory was investigated in mice and rats using standard animal models of learning and memory.

Step-Through Passive Avoidance Test in rats

In the Step-Through Passive Avoidance test (6), the ability of TC-1734 (0.6, 1.0, and 3.0 μmol/kg) to reverse scopolamine-induced cognitive deficits was compared with two prototypical acetylcholinesterase inhibitors (AChEIs), tacrine (Cognex®, THA; 1, 4, 12, and 30 μmol/kg) and donepezil (Aricept ®; E-2020; 0.1, 0.3, 1, and 3 μmol/kg). Scopolamine-induced memory loss was partially reversed by subcutaneous administration of tacrine, donepezil, or TC-1734 (Fig. 2). In this assay, typical inverted U-shaped dose-response curves were observed for all compounds. Maximal effects for tacrine, donepezil and TC-1734 in antagonizing cognitive deficits were observed at doses of 12, 1, and 1 μmol/kg, respectively. Based on the mechanism of action of TC-1734 (partly through

TABLE 1. Summary of TC-1734 in vitro *pharmacology*

Receptor Affinity		
K_i $\alpha_4\beta_2$ (nM)		11 nM
K_i α_7 (nM)		>50,000 nM
Functional Activity		
Ion flux, thalamus	EC_{50}	220 nM
	E_{max}	57%
DA release, striatum	EC_{50}	106 nM
	E_{max}	55%
Muscle (% of E_{max}) at 100 μM		0%
Ganglion (% of E_{max}) at 100 μM		0%

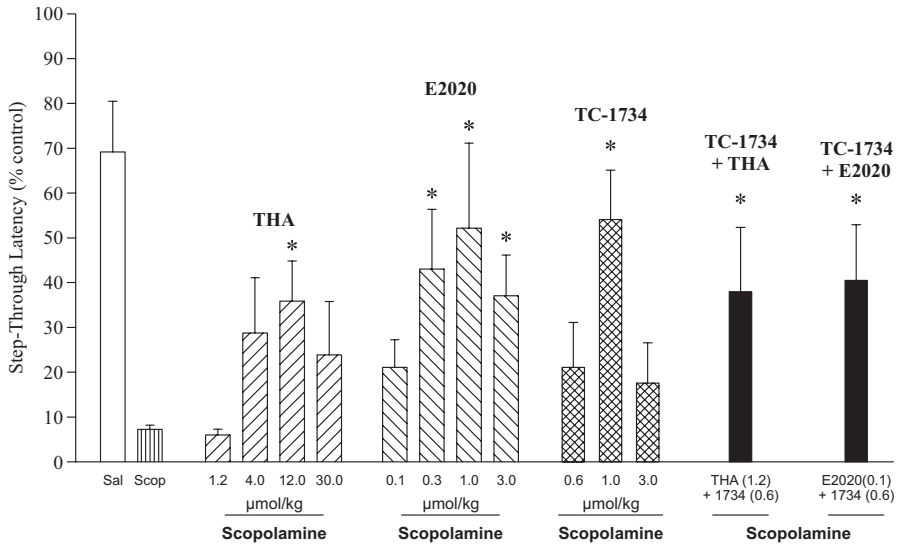


Fig. 2. Combining submaximal doses of TC-1734 (0.6 μmol/kg) with ineffective doses of tacrine (THA, 1.2 μmol/kg) or donezepil (E2020, 0.1 μmol/kg) produced enhanced cognitive performance similar to the passive avoidance behavior achieved at higher doses of tacrine (12.0 μmol/kg) or donezepil (0.3 μmol/kg) when administered alone. Kruskal–Wallis analysis of variance on ranks followed by Dunn's test: $*P < 0.05$ vs. scopolamine-control.

enhanced ACh release; see below) and that of AChE inhibitors, it was postulated that additivity or synergy between both pathways should be expected. Doses that resulted in no detectable or non-significant effects of AChE inhibitors were combined with similar submaximal (producing no significant effects) doses of TC-1734. Combination of ineffective doses of TC-1734 (0.6 μmol/kg) with tacrine (1.2 μmol/kg) or with donepezil (0.1 μmol/kg) resulted in significant effects on the reversal of scopolamine-induced memory loss. Similar effects were observed with other compounds sharing TC-1734 receptor selectivity (data not shown; see ref. 6).

Object Recognition in mice

The Object Recognition test measures the capacity to recognize an object presented at two occasions, some time apart. This assay is adapted from cognitive tests in humans, where a clear impairment in Alzheimer's patients has been demonstrated (30). Memory is assessed as the relative time spent by a mice exploring two objects A and B, one being familiar following a presentation 24 h before (object A), and the other (object B) being new. The recognition memory formed in this test is spontaneously labile over 24 h and does not require the use of pharmacological agents to induce deficits. Performance was measured by calculating a recognition index on the second of two trials as the ratio of time spent investigating object A to the total time spent investigating both objects A and B. Vehicle-treated control mice displayed a recognition index around 50%, reflecting the fact that object A is not recognized following a 24-h inter-trial interval and, hence, the equal exploration of both objects. When administered orally 1h before both the first and second (test) presentations, TC-1734 (2 and 4 μmol/kg) induced a dose-dependent facilitation of

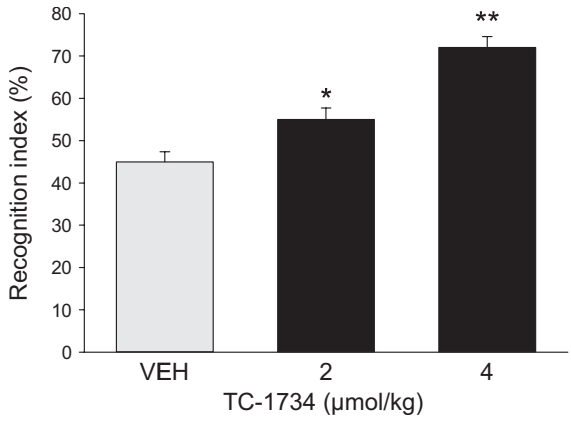


Fig. 3. The recognition index of the vehicle-treated mice 24 h after the training session was close to 50%, indicating absence of recognition of the familiar object after this delay. By contrast, animals treated orally with TC-1734 (2 and 4 μmol/kg) show recognition indexes significantly above controls, indicating recognition of the familiar object (*$P < 0.05$; **$P < 0.001$, Tukey–Kramer test).

the recognition memory in mice (Fig. 3). After 24 h, the recognition index was 45% in vehicle-treated animals (no memory of the previous presentation), whereas TC-1734 produced a recognition index of 55 and 72% in 2 and 4 μmol/kg treated groups, respectively. In addition, a single administration 1h before the first presentation led to a significant improvement in recognition in the second presentation 24 h later (recognition index 59%, $p < 0.001$, at 4 μmol/kg, data not shown), indicative of a persistence of memory retention. These effects are completely prevented by concomitant administration of mecamylamine, a brain-permeant non-competitive nAChR antagonist (see ref. 30).

Radial Arm Maze in rats

An additional cognitive study was conducted using the radial arm maze (6). The radial arm maze consisted of baiting four of eight arms to evaluate the effects of TC-1734 on working and reference memories in rats. Maze testing was conducted over a 6-day period. On days 1 and 6, the rat was orally administered saline or one of five doses of TC-1734 (0.1, 0.3, 0.6, 3.0, or 6 μmol/kg) and placed on the maze at 1 h after treatment. Testing was completed when the last baited arm was visited or 10 min elapsed. On days 2–5, the animal received daily administration of its appropriate dosing solution but was not tested on the maze. Two types of errors were scored: reference memory errors (the rat entered a non-baited arm for the first time) and working memory errors (rat re-entered a baited arm from which food had already been retrieved on that trial). Subsequent re-entries into non-baited arms were scored as working memory errors. On days 1 and 6, the animals were fed 1 h following maze testing. On days 2–5, the rats were fed 1 h after drug treatment.

TC-1734 is very effective and potent in improving memory performance in the radial arm maze (Fig. 4). Significant improvements were observed following acute oral administration at all doses tested (0.1, 0.3, 0.6, 3 or, 6 μmol/kg). Interestingly, effects on both working and reference memories were noted in our studies. Similar marked improvements were also observed following subacute administration (6 days daily administration, testing at day 6) demonstrating lack of tolerance. The oral effectiveness of TC-1734 contrasts with the limited bioavailability of previous candidates (ABT-418 and SIB-1553). Consistent with the long duration of effects observed in other cognitive tests (up to 2 days in the Lashley maze; not shown), at 0.6 μmol/kg TC-1734 induced improvements in

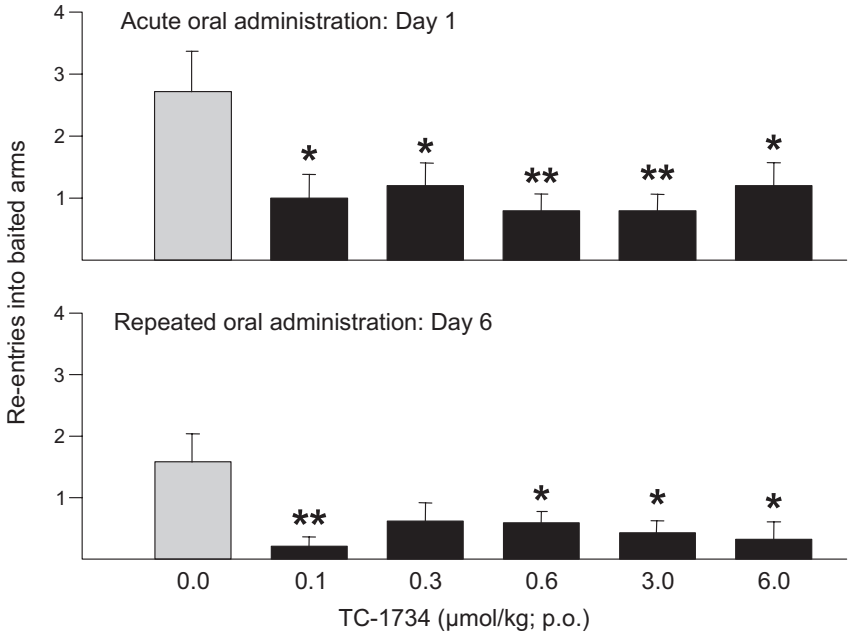


Fig. 4. Working memory improvements in the Radial Arm Maze following acute and repeated (6 days) oral administration of TC-1734 at the doses indicated on the *X*-axis. Significant decrease in errors was observed over a broad range of dose. One-way ANOVA followed by Dunnett's test, $*P < 0.05$; $**P < 0.01$.

working memory, they were detectable for up to 18 h after treatment in the Radial Arm Maze (Fig. 5). Dissociation between pharmacokinetics and the pharmacodynamics with effects lasting beyond the plasma levels observed with this compound is consistent with that of other procognitive compounds tested previously (12).

Behavioral despair test in mice

Antidepressant activity was evaluated using the behavioral despair (i.e., Porsolt) task in mice. TC-1734 (1, 3, and 10 μmol/kg) was administered acutely i.p. at 30 min before the swimming session and duration of immobility (last 4 min of a 6-min session) was assessed. Imipramine (40 μmol/kg; i.p.), administered under the same experimental conditions, was used as the reference standard. In this paradigm TC-1734 had significant effects at 1 and 3, but not at 10 μmol/kg; this finding suggested a potential for additional beneficial effects of this drug on mood (Fig. 6).

Locomotor activity in rats

Behavioral sensitization (i.e., increase in locomotor activity following repeated administration) has been proposed to be a key component in drug addiction; TC-1734 was examined for its ability to induce locomotor sensitization following a 14-day long dosing regimen (6). Motor activity was measured in rats habituated in automated locomotor activity chambers for 60 min, at 72, 48, and 24 h prior to testing. Beginning on day four, rats received daily either saline or TC-1734 (3 μmol/kg s.c.). Immediately following the injection of the test compound, the animals were placed in the activity monitoring boxes for

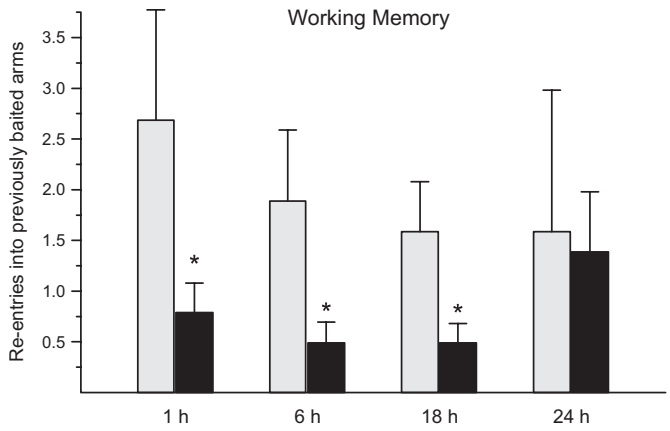


Fig. 5. Working memory improvements in the Radial Arm Maze at different pretreatment times following oral administration of TC-1734 (0.6 μmol/kg). At each time point, separate saline and TC-1734 groups ($n = 8$/group) were utilized. Each treatment group was tested once on the RAM. One-way ANOVA followed by Dunnett's test, $^*P < 0.05$.

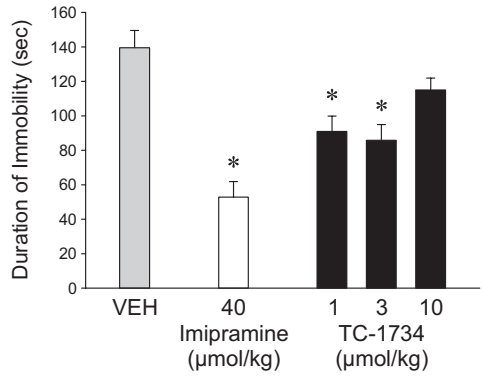


Fig. 6. Effects of acute dosing of TC-1734 on duration of immobility in the behavioral despair task in mice. TC-1734 was administered i.p. at 30 min before the swimming session. The total duration of immobility was measured during the last 4 min of a 6-min session. Imipramine was incorporated as the reference compound. Each column represents the mean value (±S.E.M.) from 10 mice per group. One-way ANOVA followed by Dunnett's test, $^*P < 0.05$.

60 min and horizontal counts were sampled every 5 min. The chronic dosing regimen of TC-1734 in combination with the 60-min locomotor activity session continued for two weeks. Nicotine (3 μmol/kg; s.c.), was administered under the same experimental conditions and used as the reference standard.

By acute administration nicotine produced an initial hypomotility followed by a distinct phase of hypermotility, whereas TC-1734 produced a monophasic change in locomotor behavior with an acute sustained hypolocomotion (data not shown). Sensitization to nicotine was apparent by the second day of the repeated dosing regimen and the locomotor response to nicotine was nearly 300% higher than the locomotor activity of the vehicle-control group by the end of the 14-day treatment period. (Fig. 7). In contrast, locomotor activity was not modified by repeated administration of TC-1734 and was indistinguishable from that in saline control animals (Fig. 7).

Drug discrimination in rats

Discriminative (cue) stimulus property is another component in which nicotine can induce drug-seeking behavior and craving. Therefore, a drug discrimination task was used to characterize the nicotine-like effects of TC-1734. Once the rats reliably discriminate

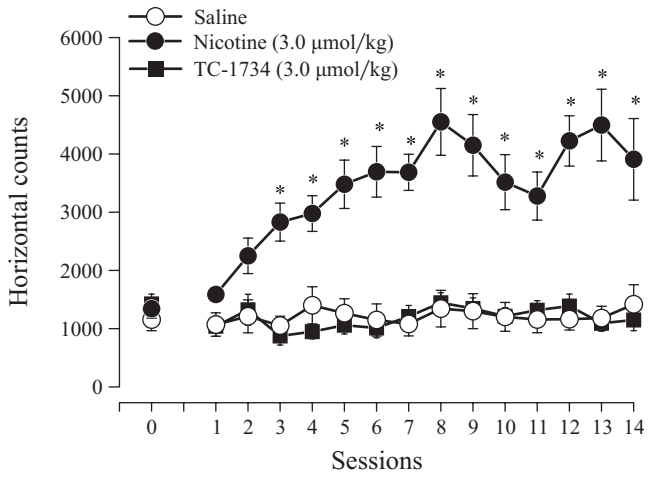


Fig. 7. Effect of repeated subcutaneous administration of TC-1734 on locomotor activity. For comparison, the effect of subcutaneous (–)-nicotine administration following repeated dosing was also examined. Results are presented as total horizontal counts over a 60-min period following repeated dosing (mean ± S.E.M.). One-way ANOVA followed by Dunnett's test, $*P < 0.05$ vs. saline-control.

(–)-nicotine (4 µmol/kg; p.o.) from saline, test sessions were conducted twice a week with training conditions present during the intermediate sessions. Test conditions were identical to training conditions, except 15 consecutive responses on either lever resulted in the delivery of food. The pretreatment time was 15 min for all compounds tested. After (–)-nicotine (0.6 to 6 µmol/kg; p.o.) dose-response determination, TC-1734 (2 to 40 µmol/kg; p.o.) was tested. Discriminative (cue) stimulus is a measure of a drug's ability to induce drug-seeking behavior and craving. Using the drug discrimination task, nicotine engendered appropriate responding of 70% at 1.9 µmol/kg whereas TC-1734 engendered nicotine-like responding at only 40 µmol/kg (Fig. 8).

Neuroprotection

In addition to the symptomatic properties, nicotinic agonists also display neuroprotective properties that offer a potential for disease-modification. The neuroprotective effects of TC-1734 were assessed initially using glutamate toxicity in mature cultures of rat forebrain neurons (Fig. 9). At 10 µM, TC-1734 reversed glutamate-induced toxicity as measured by LDH release (>95% inhibition, compared to control). Similar but less pronounced effects were observed with nicotine.

In a rat hippocampal slice preparation, a more integrated system than primary cultures, TC-1734 prevented functional loss of interneuronal signaling due to cellular damage. When slices of the hippocampus, a region that is important for learning and memory both in rodents and man, are subjected to a hypoxia/glucose deprivation episode, there is an irreversible loss of synaptic transmission that can be measured electrophysiologically by the loss of the CA1 population spikes (Fig. 10A). TC-1734 prevented in a dose-dependent and mecamylamine-sensitive manner, this loss of synaptic transmission, with a >90% recovery rate at 100 µM. In the presence of 100 µM mecamylamine, a non-competitive nAChR an-

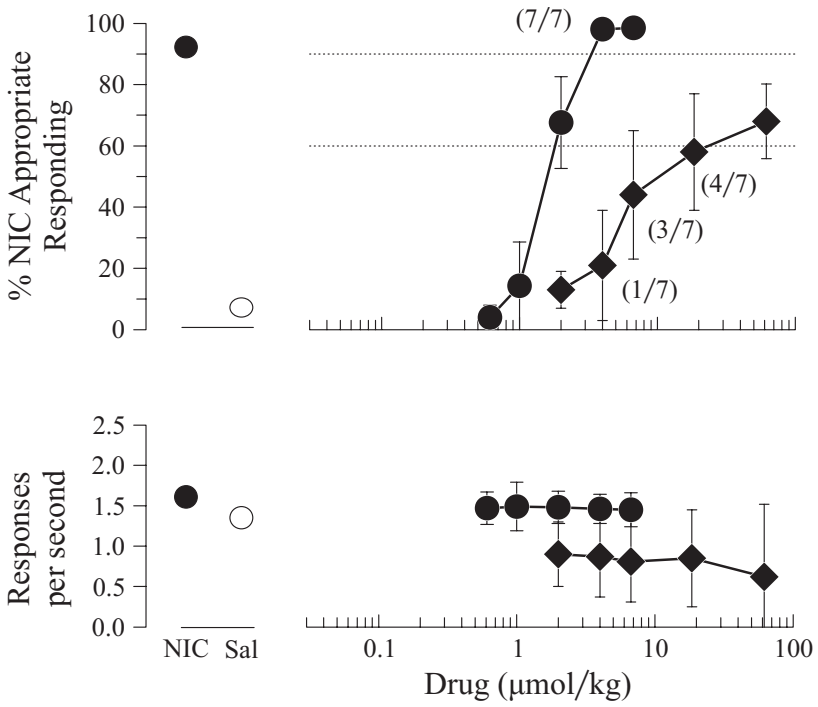


Fig. 8. Average percentage of (–)-nicotine-appropriate responding (top panel) and average responses rates (bottom panel) following different doses of (–)-nicotine in rats trained to discriminate 4 μmol/kg (–)-nicotine from saline. Numbers in parentheses indicate the number of rats that exclusively selected the (–)-nicotine lever. Dashed line at 80% in the top panel represents the operational definition of complete substitution, while the lower dash line indicates the operational definition of partial substitution. Legend: circles, nicotine; diamonds, TC-1734.

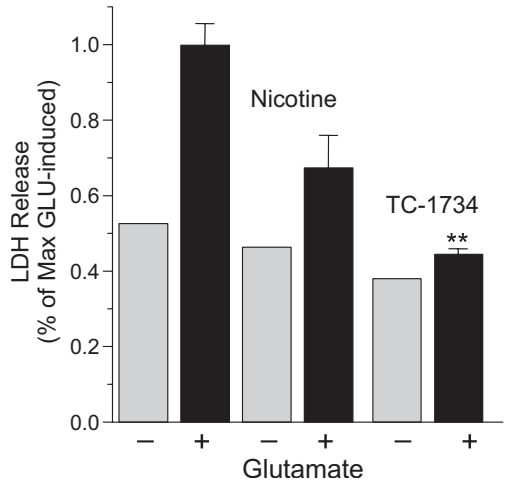


Fig. 9. Glutamate-induced neuronal cell death is prevented by TC-1734 (10 μM) and nicotine (10 μM). The neuroprotective effects of TC-1734 were assessed using glutamate toxicity (1 μM) in 3-week old cultures of rat forebrain neurons. At 10 μM, TC-1734 reversed ($p < 0.01$) glutamate-induced toxicity as measured by LDH release (>95% inhibition, compared to control). Similar but less pronounced effects were observed with nicotine (10 μM).

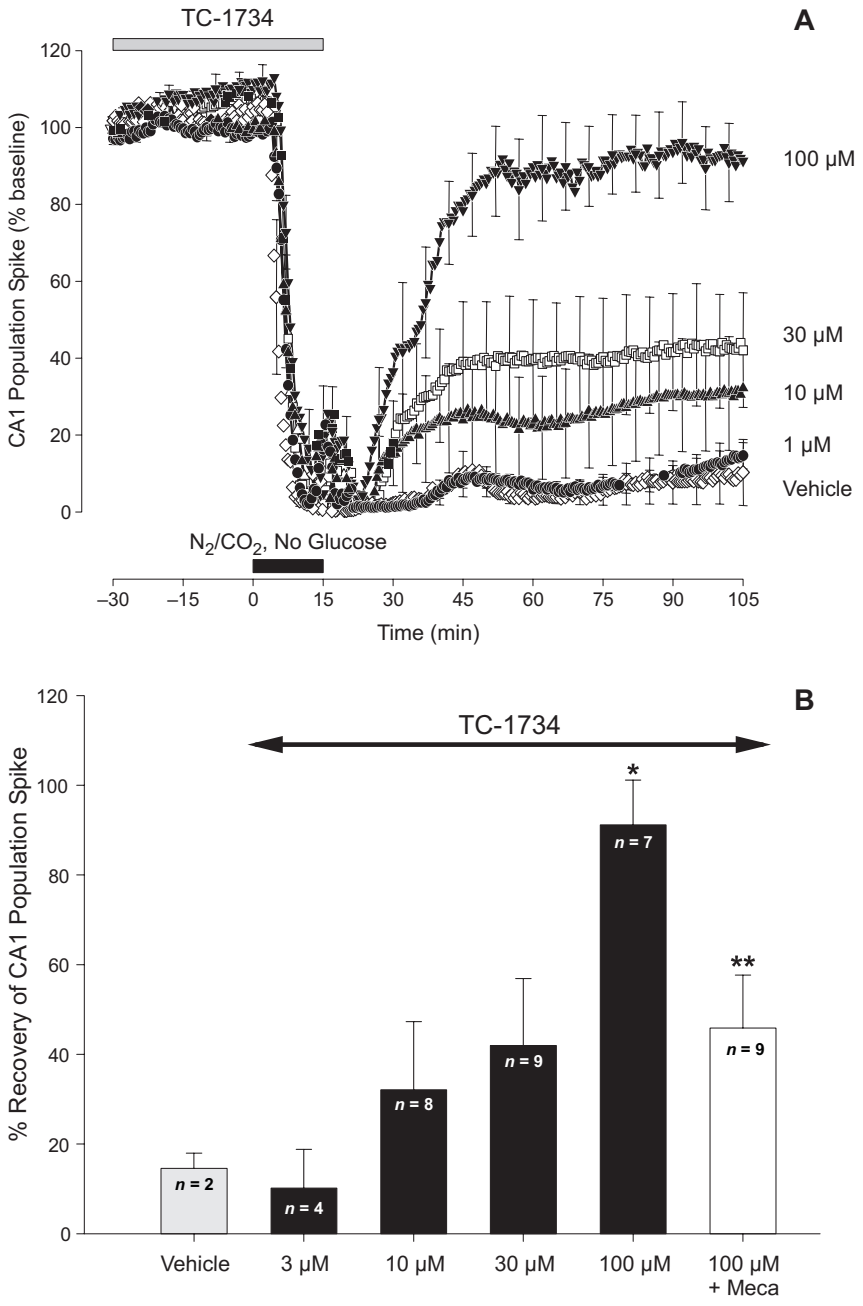


Fig. 10. A) TC-1734 prevents the loss of synaptic transmission upon oxygen/glucose deprivation in CA1 hippocampal slices. Data show time course of change in population spike size in the absence (circles) or in the presence of the indicated concentration of TC-1734. The black and gray bars below and above tracings indicate periods of drug application (30 min) and oxygen/glucose deprivation (15 min), respectively. **B**) TC-1734 produces a concentration-dependent increase in recovery of synaptic transmission following oxygen/glucose deprivation. The effect at 100 µM TC-1734 is partially blocked by 100-µM mecamylamine. *n*, number of experiments; $*P < 0.05$ vs. vehicle, Dunnett's test following significant ANOVA; $**P < 0.01$ vs. TC-1734 100 µM, unpaired *t*-test).

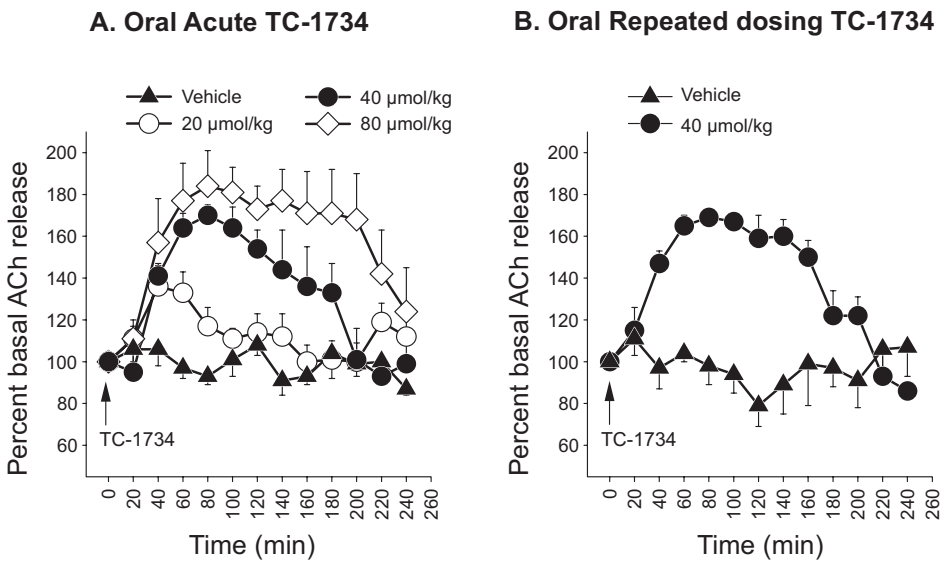


Fig. 11. **A**) Effect of TC-1734 on the extracellular concentration of ACh in the cerebral cortex of rats. **B**) ACh released after 4 days of treatment with TC-1734.

tagonist, the apparent neuroprotective effect afforded by 100 µM TC-1734 was significantly inhibited (Fig. 10B).

Microdialysis Studies

The transversal microdialysis technique was conducted in anesthetized rats implanted with a dialysis tube at the level of the frontoparietal cortex (coordinates A +1.0, V –2.0) (30). Ringer's solution was pumped through the dialysis probe at a constant rate of 2.2 µL/min and, to achieve a detectable amount of ACh in the dialysate, neostigmine (0.1 µM) was added to the Ringer's solution. ACh was measured in 40 µL samples of dialysate by high performance liquid chromatography with electrochemical detection. The detection limit for ACh was 0.05 pmol/injection. Experiments were started 24 h after the implantation of the dialysis tube. By single oral administration TC-1734 elicited a marked increase in the release of acetylcholine in the cerebral cortex, as measured by microdialysis (30; Fig. 11A). A clear dose-response effect was observed over the 20 to 80 µmol/kg dose range, with a more sustained stimulation at higher doses. Nicotine also stimulated acetylcholine release but little dose-dependence could be observed over the dose range tested. The ability of TC-1734 or nicotine to elicit acetylcholine release was antagonized by mecamylamine (6.0 µmol/kg s.c.), indicating that the effect was nAChR-specific with either drug (30).

After daily administration of TC-1734 for 4 days (40 µmol/kg/d, p.o.), the amplitude of the elicited ACh-release response was not significantly different from that observed after single dose of the drug (Fig. 11B) (30). This biochemical evidence indicates a lack of tachyphylaxis and is fully consistent with the lack of tolerance to the cognitive effects of TC-1734 following repeated administration.

DRUG METABOLISM AND PHARMACOKINETICS

Pharmacokinetics (PK) in the Rat

Preliminary PK parameters were determined in male Sprague–Dawley rats (3 animals/timepoint/dose route) after i.v. and p.o. administration of TC-1734. For oral administration at 10 mg/kg the drug was dissolved in water at 4 mg/mL; for the i.v. use it was dissolved in physiological saline (0.9% w/v).

Following i.v. administration TC-1734 was cleared rapidly with a terminal elimination half-life of 0.8 h at 1 mg/kg. A high volume of distribution at steady state (4.6 L/kg) indicated a large drug diffusion and is consistent with similar high volumes of distribution seen with other alkaline drugs. Total plasma clearance was high (7.1 L/h/kg at 1 mg/kg), markedly higher than hepatic plasma/blood flow (2.0/4.0 L/h/kg) in this species.

After oral administration at a dose of 10 mg/kg high systemic levels were obtained leading to a Cp_{max} value of 370 ng/mL at a T_{max} of 0.25 h. The parent compound was cleared rapidly with a terminal elimination half-life of 1.1 h at 10 mg/kg and quantifiable levels were present for up to 7 h in all 3 animals used. At this dose level the bioavailability was high (73.3%). Whole brain levels were measured after a dose of 10 mg/kg p.o. and proved to be high (mean Cb_{max} of 659 ng/g at T_{max} of 0.5–2.0 h) indicating appreciable brain penetration at this dose level.

Pharmacokinetics in the Dog

Preliminary PK parameters were determined in male Beagle dogs following i.v. and oral administration of TC-1734 in a cross-over study design. The product was formulated as an aqueous solution at 1 mg/mL for the oral phase and in physiological saline (0.9% w/v) at 2 mg/mL for i.v. administration.

Following i.v. administration at 1 mg/kg TC-1734 was cleared rapidly with a terminal elimination half-life of 2.0 h. A high volume of distribution at steady state (6.2 L/kg) indicated a large drug diffusion. Total plasma clearance was high (3.2 L/h/kg), markedly higher than hepatic plasma/blood flow (1.2/2.4 L/h/kg) in this species.

After oral administration at 1 mg/kg low systemic plasma levels of TC-1734 were detected, giving rise to a mean Cp_{max} of 35 ng/mL at 0.5–2.0 h. The drug was thereafter cleared rapidly with a terminal elimination half-life of 1.3 h. Only occasional quantifiable levels just above the limit of detection were evident after 4 h in the 3 animals. However, in spite of low plasma levels, its estimated bioavailability was 31.4%.

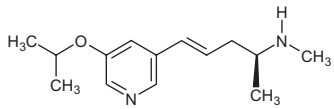
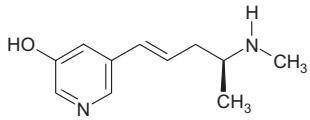
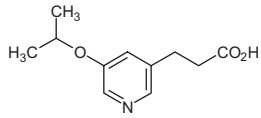
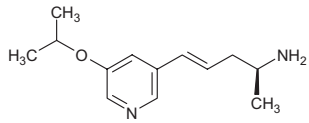
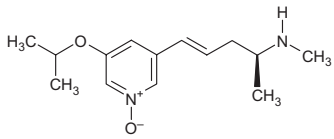
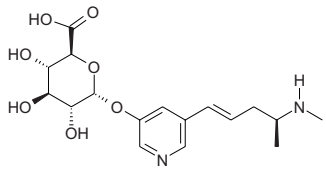
In Vitro Metabolism

[^{14}C]TC-1734 was used in liver microsome preparations from human and several animal species for evaluation and comparison of metabolic profiles. Both the mouse and rat had similar metabolic and gender profiles, while the rate of disappearance was highest in the female rabbit and male monkey and decreased in the following order: male dog > male and female mouse, male guinea pig > human > male and female rat. The M5 (O-dealkyl derivative endogenous conjugate) was the main metabolite in monkey and human, while M4 (N-oxide metabolite) was present in all species except human and monkey.

In Vivo Metabolism

Analysis of plasma, urine and feces from male Sprague-Dawley rats that received an oral dose of 20 mg/kg of TC-1734 hemigalactarate revealed extensive metabolism of the compound. Five metabolites were tentatively identified by mass spectrometry and by co-chromatography against authentic reference standards; including pyridine N-oxide (M4), 5-hydroxypyridyl (M1), 3-alkylcarboxyl (M2) and an N-desalkylated (M3) derivative as well as a glucuronide conjugate (M5) of the 5-hydroxypyridyl derivative. The M1 metabolite exhibited the highest affinity (K_i = 237 nM) to $\alpha_4\beta_2$ nAChR subtype of the five metabolites tested. The binding affinity of the M1 metabolite is approximately 20 fold less potent than the binding affinity of TC-1734 (K_i = 11 nM) (Table 2).

TABLE 2. In vivo *metabolic profile of TC-1734 in rats*

Metabolite #	Structure	Relative % plasma[1]	Relative % urine[2]	K_i[3] (nM)
Parent		58	24	11
M1		0	14	237
M2		0	6	6600
M3		22	6	462
M4		3	8	1400
M5		16	41	Not Tested

[1] The plasma sample analyzed for metabolites was from 2 animals taken 1 h after dosing. These animals were dosed orally with TC-1734 at 20 mg/kg. Samples from the dosed animals were compared with samples from animals treated with vehicle solution only.

[2] The urine sample analyzed for metabolites was from three rats taken at 6 h after dosing. These animals were dosed orally with TC-1734 at 20 mg/kg. Samples from the dosed animals were compared with samples from animals treated with vehicle solution only.

[3] Binding affinity studies.

TC-1734 was determined to be the major product in plasma, although appreciable levels of M3 and M5 were also evident. Metabolites M1 and M2 were not detected in plasma. Parent compound was also excreted unchanged in the urine, with all five metabolites found to be present, although metabolite M5 was the major product therein. None of the above metabolites were observed in fecal extracts, although a small amount of parent compound was detected.

[^{14}C]TC-1734 was administered as a single oral dose of 10 mg/kg to male and female rats in order to determine the metabolic profiles in plasma and urine. Unchanged TC-1734, accounting for 11% of the administered dose, was present in male rat plasma with a C_{max} of 1.4 μg eq/mL and a T_{max} of 0.25 h and in female rat plasma with a C_{max} of 0.74 μg eq/mL and a T_{max} of 0.5 h. Twelve metabolites were detected in the plasma with the main metabolite (M5) being the glucoronide conjugate of M1. Urinary excretion of the total radioactivity accounted for 61% of the administered dose.

In a separate study [^{14}C]TC-1734 was administered orally to male or female rats at a dose of 10 mg/kg. The following evaluations were conducted: blood, plasma, and brain total radioactivity kinetics, plasma protein binding, and mass balance excretion. [^{14}C]TC-1734 was rapidly absorbed and transferred to the brain. Total radioactivity concentrations in blood and plasma decreased rapidly from peak concentrations at 6 h. TC-1734 levels were not detectable at 24 h. The PK parameters for total radioactivity are presented below in Table 3.

TC-1734 had variable and little affinity for red blood cells. Cumulative excretion accounted for 95 to 96% of the dose for males and females, respectively, with urinary excretion as the primary route. After 72 h, there was no retention of drug-derived materials. Mean results are presented in Table 4.

PRECLINICAL SAFETY EVALUATION

In safety pharmacology studies in male rats, TC-1734 had no effect on locomotor activity, and on respiratory rate at the highest oral dose of 500 mg/kg; had no effect on

TABLE 3. Pharmacokinetic parameters for [^{14}C]TC-1734 in rats following single oral administration at 10 mg/kg

	Sex	C_{max} (μg eq/kg)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg eq · h/kg)	
					0–72 h	0–∞
Blood	Male	2.88	0.25	22.9 (24–72 h)	18.86	20.06
	Female	1.99	1	31.2 (24–72 h)	17.17	18.84
Plasma	Male	2.49	0.25	18.6 (24–72 h)	15.27	15.64
	Female	1.88	2	41.5 (24–72 h)	14.42	15.46
Brain	Male	5.02	0.5	0.7 (2–6 h)	12.52	12.59
	Female	3.80	1	1.2 (2–6 h)	14.76	15.66

The blood and plasma profiles of radioactivity were relatively similar.
The protein binding decreased with time from 0.25 to 6 h and ranged from 34.7 to 6.8% in males and from 35.2% to 9.3% in females.

gastric emptying at oral doses ≤250 mg/kg; and no effect on the contractile response of the rat diaphragm *in vitro* following direct or indirect stimulation at concentrations ≤50 mg/mL.

Chronic Toxicity in Rats and Dogs

TC-1734 was administered orally to rats and beagle dogs in 13-week toxicology studies. In rats TC-1734 was administered at 5, 50, or 200 mg/kg, while in dogs oral capsules of 1, 3, 10, and 30 mg/kg were employed. During the conduct of the study, biochemical and pathologic examinations were performed on all animals, while ECG evaluations were conducted only in dogs. The compound was free of significant clinical, chemical, and histopathologic findings at doses as high as 50 mg/kg in the rat and 30 mg/kg in the dog. Only a slight and transient bradycardia was observed in dogs at the highest dose of 30 mg/kg.

TC-1734 produced no metabolic activation in a battery of *in vitro* genotoxicity assays, including the Ames test, CHO micronucleus test, and mouse lymphoma assay.

CLINICAL SAFETY

A double blind placebo controlled cross-over study was undertaken in healthy male volunteers aged 18–45 years. Routine biochemical, hematological and urinary safety assessments were made, vital signs measured and adverse events recorded. Twelve-lead ECGs were recorded along with 24-h Holter monitoring. TC-1734 was administered at doses of 2, 4, 10, 20, 40, 80, 160, and 320 mg, with 6 volunteers in each dose group.

No biochemical, hematological or urinary changes were detected. Vital signs were unchanged. Likewise, cardiovascular monitoring revealed no drug-induced effects. Adverse events were dose-related, being usually mild and transient. Headache, nausea, dizziness, feeling faint and asthenia were the most common adverse events. Impaired vigilance and somnolence were seen at the highest dose. Initial PK results (Fig. 12) indicate linear kinetics with increasing doses. Plasma T_{max} of TC-1734 occurred at 2 h and its elimination half-life was 4 h. At the dose of 320 mg the plasma C_{max} was 120 ng/mL; it represents a plasma concentration 5–10-fold higher than that expected at the maximal cognitive effects. At this C_{max} TC-1734 had no emetic effects. This is the first reported example of a nicotinic agonist achieving a very broad potential therapeutic index in humans. These results suggest TC-1734 is safe and well tolerated when given at single doses to healthy volunteers.

TABLE 4. Disposition at 72 h post-dosing of [^{14}C]TC-1734 in rats following single oral administration at 10 mg/kg

	Urine	Feces	Cage Wash	Digestive Tract	Skin	Carcass	Total
Male	61.3 ± 11.4	29.4 ± 6.4	4.2 ± 3.9	0.1 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	95.7 ± 6.4
Female	62.6 ± 5.1	26.8 ± 5.5	4.7 ± 2.5	0.1 ± 0.1	0.2 ± 0.1	0.6 ± 0.5	95.0 ± 3.2

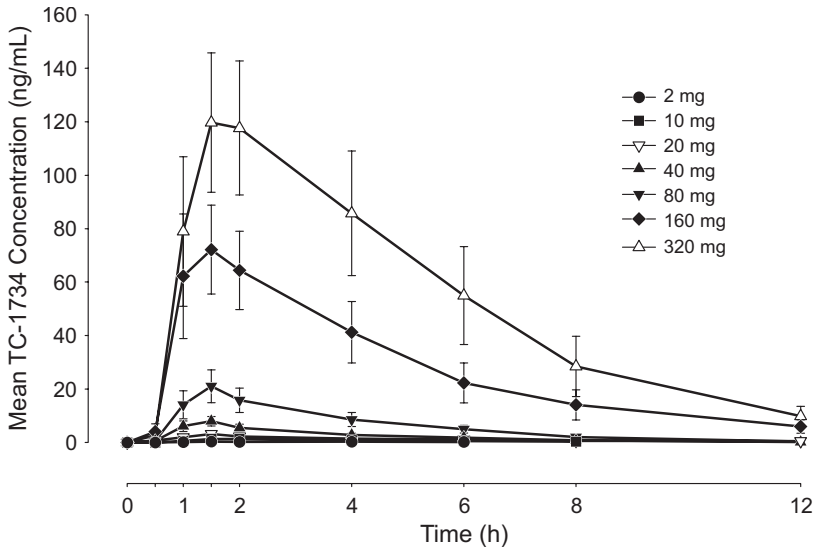


Fig. 12. Plasma concentration of TC-1734 vs. time in healthy human volunteers following oral administration of the drug at the indicated doses. Numbers represent the means ± S.E.M. of $n = 6$.

CONCLUSIONS

The potential therapeutic benefit of nicotinic cholinergic ligands in the management of dementia is based on a plethora of data from animal and human studies. This therapeutic opportunity has been hampered by the lack of suitable candidates due to poor bioavailability, unacceptable side effects, or poor efficacy resulting from limiting side effects. We have synthesized, characterized and developed a novel clinical candidate, TC-1734, that presents many of the desired features to become an effective therapy in humans. It is a partial agonist at the $\alpha_4\beta_2$ nAChR without interaction with other nAChRs or other receptor systems. TC-1734 is orally active, acutely and chronically, and potent in several models of cognition and attention with additivity or synergy with acetylcholinesterase inhibitors. TC-1734 induces long-lasting cognitive enhancement and exhibits neuroprotective effects in models with overt neuronal excitotoxicity or in models mimicking decreased perfusion (hypoxia/glucose deprivation). It exhibits antidepressant activity and is well tolerated in animals with no evidence of genetic or cellular toxicity, or reproductive toxicity. Athough the abuse potential of TC-1734 has not been directly examined, the fact that it failed to induce behavioral sensitization following repeated administration and was 25 times less potent e ngendering nicotine-like responding in a nicotine drug discrimination task indicate that the addiction liability of TC-1734 is low when compared to that of nicotine. TC-1734 was very well tolerated acutely and in chronic studies in rats and dogs, and acutely in humans at doses up to 320 mg. The bioavailability, pharmacological, pharmacokinetic, and safety profile of TC-1734 are providing the first example of a safe, potent and efficacious neuronal nicotinic modulator that holds promise for the management of the hallmark symptomatologies observed in dementia.

Addendum: SIB-1508Y, (S)-(–)-5-ethynyl-3-(1-methyl-2-pyrrolidinyl)pyridine; SIB-1553, 4-[[2-(1-methyl-2-pyrrolidinyl)ethyl]thio]-phenol; TC-2403, (E)-N-methyl-4-(3-pyridinyl)-3-butene-1-amine; TC-2559, (E)-N-methyl-4-[3-(5-ethoxypyridin)yl]-3-buten-1-amine; ABT-418, (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole; DMF, N,N-dimethylformamide; THF, tetrahydrofuran.

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