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PASS assisted prediction and pharmacological evaluation of novel nicotinic analogs for nootropic activity in mice

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ABSTRACT

The aim of present study is to predict the probable nootropic activity of novel nicotine analogues with the help of computer program, PASS (prediction of activity spectra for substances) and evaluate the same. Two compounds from differently substituted pyridines were selected for synthesis and evaluation of nootropic activity based on their high probable activity (Pa) value predicted by PASS computer program. Evaluation of nootropic activity of compounds after acute and chronic treatment was done with transfer latency (TL) and step down latency (SDL) methods which showed significant nootropic activity. The effect on scopolamine induced amnesia was also observed along with their acetylcholine esterase inhibitory activity which also showed positive results which strengthened their efficacy as nootropic agents through involvement of cholinergic system. This nootropic effect was similar to the effect of nicotine and donepezil used as standard drugs. Muscle coordination and locomotor activity along with their addiction liability, safety and tolerability studies were also evaluated. These studies showed that these compounds are well tolerable and safe over a wide range of doses tested along with the absence of withdrawal effect which is present in nicotine due to its addiction liability. The study showed that these compounds are true nicotine analogs with desirable efficacy and safety profile for their use as effective nootropic agents.

1. Introduction

In recent years, it has become clear that the neuronal nicotine acetylcholine receptors are a valid target against Alzheimer's disease (Bontempi et al., 2003; Newhouse et al., 2004) and a variety of other central nervous system disorders. It has been reported that cognition and memory deficits observed in normal aging individuals and individuals who are affected by neurodegenerative disorders like Alzheimer's disease are, at least in part, due to decrease in cholinergic transmission (Perry, 1986). Alzheimer's disease is a form of dementia affecting about 5–15% of the population over 65 years of age (Collerton, 1986). It affects more than 15 million people worldwide and grows as the proportion of elderly persons increases (Palmer, 2002).

Study of the effects of cholinergic drugs has provided information which reflects that anti-cholinergic drugs impair cognition, while cholinergic drugs often enhance cognition (Levin, 1992). This pharmacologic role of cholinergic and anti-cholinergic drugs suggests that there is an involvement of cholinergic system in the pathogenesis of Alzheimer's disease (Birtwistle and Hall, 1996; Collerton, 1986).

Several controlled studies have tested this cholinergic hypothesis in animals. Nicotine has been found to improve learning and memory on a variety of tasks. It has also been found to be effective in attenuating memory deficits resulting from lesions of the septohippocampal pathway or aging in experimental animals. Nicotinic receptors are decreased in the cortex of patients with Alzheimer's disease. Preliminary studies have found that some aspects of the cognitive deficit in Alzheimer's disease can be attenuated by nicotine (Levin, 1992). Generally, nicotinic agonists improve certain forms of memory, and nicotinic antagonists and cholinergic lesions impair memory (Jones et al., 1999; Levin et al., 2006; Newhouse et al., 1997; Picciotto et al., 1995). Jones et al. (1992) studied the effect of subcutaneous nicotine on humans with dementia of Alzheimer's type and on normal controls. The drug was shown to improve several measures of performance like improvement of rapid information processing in those with the disease (Levin, 1992).

The addiction liability and other undesirable side-effects of nicotine prohibit the use of this natural product for therapeutic applications (Lloyd and Williams, 2000). So there is a real need of development of novel nicotine analogs having improved pharmacological and safety profile along with lower toxicity profile. There are number of nicotine analogs developed and evaluated for such a profile and many of them are substituted pyridines. Examples of these include ABT-418 and SIB-1553A that were evaluated for nootropic

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activity (Bontempi et al., 2003; Lin et al., 1999). With this motive, we planned our study to virtually design, synthesize and evaluate some substituted pyridines as novel nicotine analogs which can be used for cognition enhancing activity as one of the pharmacological implication of nicotine acetylcholine receptors with better efficacy, high tolerability and low toxicity profile. As the nicotine and its analogs also have their effect on muscle coordination and locomotion (Bontempi et al., 2003; Buckley et al., 2004), we also evaluated these effects for the synthesized compounds.

2. Materials and methods

2.1. PASS computer program

We predicted the number of differently substituted pyridines by substituting different groups at R1 and R2 on Structure A (Fig. 1A) for the nootropic activity (Table 1). Prediction of these compounds for acetylcholine nicotinic agonistic activity, nootropic activity and for Alzheimer's disease treatment was done with the help of computer program, PASS (http://195.178.207.233/PASS/). PASS is a computer program which can be used for the prediction of different types of pharmacological activities for different substances (Anzali et al., 2001; Goel et al., 2005; Lagunin et al., 2000; Marwaha et al., 2007). Probable activity values of these compounds for these activities were given in Table 2. An attempt was made to synthesize a number of compounds from these on the basis of high probable activity value and feasibility of the synthesis of compounds in our laboratory. Compounds **1** and **2** were finally synthesized from these 28 compounds (Singh et al., 2005) which are structure analogs of nicotine (Fig. 1B, C, D) and evaluated these compounds for nootropic activity along with other pharmacological actions.

2.2. Animals

Young (3–4 months old) Female Swiss albino mice (*Mus musculus*), weighing 20–30 g obtained from Central Research Institute Kausali, Himachal Pardesh, were used for the present study. Female mice were used because they are more sensitive than male mice for the similar type of nicotine related behavior studies. The animals were housed in standard cages and maintained at room temperature with natural day and night cycles. Animals were allowed free access to food (standard laboratory rodent's chow) and water during study. All experiments were carried out between 07:00 and 16:00 h. Animals were grouped, one to five per cage and were allowed a 1-week habituation period to the animal room before testing. They were acclimatized with the laboratory conditions by handling them at least once a day during this period. All procedures were conducted as per guidelines of committee for the purpose of control and supervision of experimental animals. The protocol for the use of animals for this study was approved by the Institutional Animal Ethical committee. In our study, 6–8 mice were used for each group.

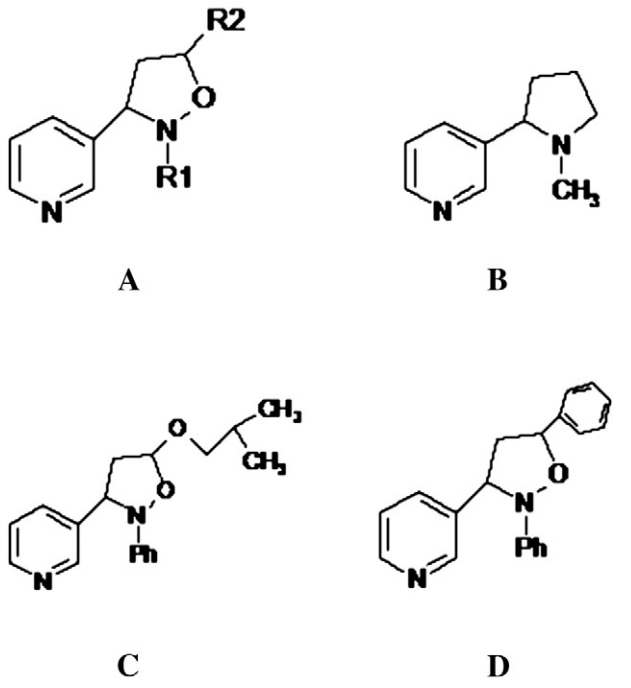

Fig. 1. Chemical structures; A: Structure A (pyridine moiety), B: nicotine, C: Compound **1** (syn-5-isobutoxy-2-phenyl-3-(3-pyridyl)-isoxazolidine), D: Compound **2** (syn-2,5-diphenyl-3-(3-pyridyl)-isoxazolidine).

Table 1
Prediction of various structures of differently substituted pyridine moiety (Structure A) for nootropic activity.

Structure no.	R1	R2	Structure no.	R1	R2
1	—C_6H_5	—O*iso*Butyl	15	—CH_3	—CH_3
2	—C_6H_5	—C_6H_5	16	—CH_3	—C_2H_5
3	—C_6H_5	—CH_3	17	—CH_3	—C_6H_5
4	—C_6H_5	—C_2H_5	18	—CH_3	—OCH_3
5	—C_6H_5	—OCH_3	19	—CH_3	—OC_2H_5
6	—C_6H_5	—OC_2H_5	20	—CH_3	—OC_6H_5
7	—C_6H_5	—OC_6H_5	21	—CH_3	—O*iso*Butyl
8	—C_2H_5	—CH_3	22	—H	—CH_3
9	—C_2H_5	—C_2H_5	23	—H	—C_2H_5
10	—C_2H_5	—C_6H_5	24	—H	—C_6H_5
11	—C_2H_5	—OCH_3	25	—H	—OCH_3
12	—C_2H_5	—OC_2H_5	26	—H	—OC_2H_5
13	—C_2H_5	—OC_6H_5	27	—H	—OC_6H_5
14	—C_2H_5	—O*iso*Butyl	28	—H	—O*iso*Butyl

Table 2
Prediction of different probable biological activities (Pa) of compounds 1–28.

Structure	Probable activity values (Pa)		
	Acetylcholine nicotinic agonist activity	Nootropic activity	Alzheimer's disease treatment
1	0.537	0.779	–
2	0.603	0.779	0.779
3	0.581	–	–
4	0.563	–	–
5	0.592	–	–
6	0.569	–	–
7	0.601	–	–
8	0.580	–	–
9	0.568	–	–
10	0.588	–	–
11	0.584	–	–
12	0.574	–	–
13	0.587	–	–
14	0.541	–	–
15	0.600	–	–
16	0.581	–	–
17	0.615	–	–
18	0.610	–	–
19	0.587	–	–
20	0.613	–	–
21	0.554	–	–
22	0.614	0.628	–
23	0.593	0.565	–
24	0.631	0.666	–
25	0.610	0.622	–
26	0.586	0.567	–
27	0.613	0.652	–
28	0.551	–	–

2.3. Drugs and chemicals

Acetylcholine chloride (Lancaster, England), scopolamine hydrobromide trihydrate (ACROS organics), dimethylsulfoxide (DMSO), monobasic sodium phosphate (s.d. Fine Chem Ltd), donepezil hydrochloride (QP Pharm. Chem.), nicotine hydrogen tartrate, physostigmine (Sigma Chemical Company U.S.A), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) (Sisco Research Laboratories Pvt Ltd), olive oil (Fegero company), dibasic sodium phosphate (Qualigens Fine chemicals), total protein test kit (Span Diagnostics Ltd, Surat, India) and nicotinic analogs; Compound **1** (syn-5-isobutoxy-2-phenyl-3-(3-pyridyl)-isoxazolidine) and Compound **2** (syn-2,5-diphenyl-3-(3-pyridyl)-isoxazolidine). Acetylcholine, scopolamine, donepezil, nicotine hydrogen tartrate and physostigmine were dissolved in distilled water. Compounds **1** and **2** were oily in nature; so they were dissolved in DMSO and olive oil solution (1:9).

2.4. Drug treatment

The nicotinic analogs were synthesized at the Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, India (Singh et al., 2005). They were reconstituted freshly before use. The different doses of compounds **1** (2.5, 5, 7.5, 25, 50 and 75 mg/kg) and **2** (5, 10, 15, 50, 100 and 150 mg/kg) used for evaluating their single dose effect on muscle coordination, locomotor activity and cognition performance were selected on the basis of nicotine doses in literature reports according to the molecular weight basis (Bontempi et al., 2003; Newhouse et al., 2004; Oishi et al., 1993; Tizabi et al., 1999). Vehicle (1: 9, DMSO and olive oil) and nicotine (1 mg/kg of free base) were also administered for comparison purposes for these activities along with donepezil administration (0.5 mg/kg of free base) as a standard drug for comparison of cognition performance. All the drugs were administered using intraperitoneal (i.p.) route. Because of the i.p. route of administration, all the parameters were evaluated 30 min after the drug administration. Volume of i.p. injection was 1 ml/100 g of mouse.

2.5. Tolerability studies of the compounds

2.5.1. Neurotoxicity test

Neurotoxicity of different doses of compounds and nicotine were determined using rotarod apparatus. Mice which were able to remain on the rotating rod at 10 rpm for 5 min or more were selected and divided into different groups and received varying doses of Compounds **1** (2.5, 5, 7.5 and 75 mg/kg), **2** (5, 10, 15 and 150 mg/kg) and nicotine (1 and 10 mg/kg). All animals were placed on the rotating rod after 30 min of treatment with different doses of compounds. Neurotoxicity was assessed as inability of the animal to maintain equilibrium on rotating rod for at least 3 min. The less speed (10 rpm) of rotating rod was set to evaluate the neurotoxic effect of compounds affecting normal minimum muscle movement required to maintain the posture on rotating rod.

2.5.2. Seizurogenic potential and acute toxicity test

The compounds were administered at different doses, up to ten times of the effective doses i.e. 25, 50 and 75 mg/kg of Compound **1** and 50, 100 and 150 mg/kg of Compound **2**, to different groups of mice. A separate group of mice was administered with 10 mg/kg dose of nicotine. Occurrence of seizure, percent mortality and gross behavioral changes were observed during 24 h after administration of compounds and nicotine (Bontempi et al., 2003).

2.6. Muscle coordination test

To evaluate the effect of compounds on muscle coordination of mice, standard mouse rotarod apparatus (Model KI-9616-4) was used that measures the time, a mouse remains on a rotating rod. The rubber rotating rod (diameter 2.5 cm, length 65 cm divided into 5 equal sections) was raised 20 cm above the bottom of the rotarod enclosure and rotated at a fixed speed of 20 rpm. Automatic digital counter at the bottom automatically count the latency to fall in seconds. This apparatus was also used to evaluate neurotoxicity as described in the earlier section with different parameters.

Mice were pretrained on rotarod 1 to 3 h prior to testing. Training consisted of placing a mouse on the rotating rod and measuring latency to fall, up to a maximum of 120 s. Mice that did not remain on the rod for 120 s by the end of third trial were excluded from the study. Training trials were separated by 60 s. Mice were returned to their home cage between trials.

For drug testing, mice were distributed across treatment groups on the basis of the number of trials required to reach the 120 s criterion during training. Different groups of mice were tested on rotarod, 30 min after the treatment with varying doses of Compound **1** (2.5, 5, 7.5, 25, 50 and 75 mg/kg), Compound **2** (5, 10, 15, 50, 100 and 150 mg/kg), nicotine (1 mg/kg of free base) and vehicle (control group). The time from placement onto rod to falling off the rod (latency to fall in seconds) served as the measure of performance and it was compared between treated and control groups (Bontempi et al., 2003).

2.7. Locomotion test

Locomotor activity was assessed in actophotometer (NSW India), consisting of a standard plastic rodent cage (24×45.5) surrounded by a stainless-steel frame. Locomotor activity frames consisted of seven infrared photocell beams located across the axis of the frame and raised 2 cm above the floor and 5.5 cm apart. The number of beam crosses (crossovers, i.e. consecutive interruption of one beam followed immediately by interruption of adjacent beam) was recorded by the instrument during 10 min was used as a measure of spontaneous locomotion. The animals were injected with varying doses as given above of Compounds **1**, **2** or nicotine and vehicle. Then each mouse was placed in the actophotometer after 30 min of i.p. injection for 10 min. Locomotor activity was recorded in treated groups and compared with control group to determine significant change in locomotor activity (Bontempi et al., 2003).

2.8. Evaluation of nootropic activity

2.8.1. Transfer latency test

Elevated plus-maze served as the exteroceptive behavioral model to evaluate spatial long term memory in mice. The procedure, technique and end point for testing memory were followed using the parameters described by the earlier investigators with little modification (Itoh et al., 1990; Patil et al., 2006; Sharma and Kulkarni, 1990). The elevated plus maze for mice consisted of two open arms (16 cm×5 cm) and two covered arms (16 cm×5 cm×12 cm) extended from a central platform (5 cm×5 cm), and the maze was elevated to a height of 25 cm from the floor. After 30 min of the administration of varying doses as given above of Compounds **1**, **2**, nicotine and donepezil (0.5 mg/kg of free base) along with vehicle group, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs and was recorded on the first day (training session) for each animal. Cut-off time observed was 90 s. The mouse was allowed to explore the maze for another 2 min and then returned to its home cage. Retention of this learned-task (memory) was examined 24 h after the treatment. Significant reduction in TL value of retention indicated improvement in memory.

2.8.2. Step-down task

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The step-down paradigm

apparatus consisted of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of Plexiglass, featuring a grid floor (made up of 3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V, A.C.) was delivered to the grid floor. After 30 min of the administration of varying doses as given above of Compounds **1**, **2**, nicotine and donepezil along with vehicle group, training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped-down, placing all its paws on the grid floor, shocks were delivered for 15 s and the step-down latency (SDL), which was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor, was recorded. Animals showing SDL in the range of 2–15 s during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. During second session, if the animals stepped down before 60 s, electric shocks were delivered once again for 15 s. During the second test, animals were removed from shock free zone, if they did not step down for a period of 60 s and were subjected to retention test. Retention (memory) was tested after 24 h of the treatment in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut off time of 300 s. Significant increase in SDL value indicated improvement in memory (Sharma and Kulkarni, 1990).

One best dose is selected for each compound (Compound **1**—7.5 mg/kg and Compound **2**—15 mg/kg) for evaluating its chronic dosing effect on cognition performance with the help of interoceptive and exteroceptive memory models. In exteroceptive memory model, selected doses of compounds were administered for 15 successive days to various groups. After 30 min of the administration of the last dose (on 15th day), mice were exposed to the training session using elevated plus maze and passive avoidance apparatus in the separate groups as done above. Retention (memory) was recorded after 24 h (on the 16th day). In interoceptive memory model, amnesia was induced in separate groups by scopolamine bromide (0.4 mg/kg) after 30 min of the last dose of compounds administration on the 15th day. Scopolamine control group received vehicle for 15 days and scopolamine, after 30 min of vehicle administration on the last day (15th day). The animals were exposed to the training session (on the 15th day) after 30 min of scopolamine bromide injection. The retention (memory) was measured after 24 h of last treatment (on the 16th day) in the same way as done previously in exteroceptive model. Donepezil (0.5 mg/kg) and nicotine (1 mg/kg) were used as standard drug for the above models and was administered for 15 days to positive control groups. Control group animals received vehicle (1: 9, DMSO and olive oil) for fifteen consecutive days.

2.8.3. Evaluation of acetylcholine esterase activity

The one best selected dose for each compound along with vehicle and standard drugs (nicotine and donepezil) were administered to different groups of mice for 15 days. On the 15th day after drug treatment, all the animals of the groups were sacrificed by cervical dislocation. The brains were removed and homogenized in phosphate buffer (pH 7.4, 10% w/v). The clear supernatant obtained after centrifugation at 3000 rpm for 15 min, was used for estimation of total protein content and acetylcholine esterase activity.

The brain total protein was determined by Lowry's method with slight modification (Lowry et al., 1951) using Total Protein Modified Biuret, End Point Assay Test Kit. The whole brain acetylcholine esterase activity was measured by the method of Ellman et al. with slight modification. 0.5 ml of clear supernatant liquid of the brain homogenate was pipetted out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB {5,5′-dithiobis (2-nitro benzoic acid)} solution (10 mg DTNB in 100 ml of Sorenson phosphate buffer, ph 8.0). From the volumetric flask, two 4 ml portions were pipetted out into two test tubes. Into one of the test tube, 2 drops of eserine solution was added. 1 ml of substrate solution (75 mg of acetylcholine chloride per 50 ml of distilled water) was pipetted out into both of the test tubes. The test tube containing eserine was taken as blank and the change in the absorption per minute of the test sample was read spectrophotometrically (Systronics spectrophotometer 2202) at 420 nm (Ellman et al., 1961; Voss and Sachsse, 1970).

2.9. Withdrawal effect

Effect of compounds and nicotine withdrawal after chronic dosing for 15 days on mice were evaluated by observing anxiogenic effect on elevated plus maze model. The one best selected dose for each compound along with vehicle and nicotine were administered to different groups of mice for 15 days. On the 16th day, vehicle was administered instead of nicotine and compounds to the various groups and mice were tested 30 min after this administration on the plus maze model for the number of entries and time spent in the open arm (Irvine et al., 2001; Jonkman et al., 2005).

2.10. Statistical analysis

All the results were expressed as mean ± standard error (S.E.M.). Data was analyzed using Student's *t*-test or one-way ANOVA followed by Tukey or Dunnett's *t*-test (SigmaStat Software, 2.03). P-values of <0.05 were considered as statistically significant for all comparisons.

3. Results

3.1. Tolerability studies of the compounds

In neurotoxicity test, the lower doses of Compounds **1** (2.5, 5 and 7.5 mg/kg), **2** (5, 10 and 15 mg/kg) and nicotine (1 mg/kg) were found to have no neurotoxic effects but at the higher dose of Compounds **1** (75 mg/kg), **2** (150 mg/kg) and nicotine (10 mg/kg) were found to be neurotoxic as indicated by the earlier fall off time of mice from the rod (Table 3).

No mortality and no occurrence of seizure was observed in mice treated with all doses of Compounds **1** (25, 50, and 75 mg/kg) and **2** (50, 100 and 150 mg/kg) but occurrence of seizure followed by death was observed in nicotine administered (10 mg/kg) group. There was no change in behavior of mice during 24 h of administration of the different doses of compounds (Table 3).

3.2. Effect on muscle coordination and locomotor activity

There was significant dose dependent first increase (at lower doses of compounds) and then decrease (at higher doses of compounds) in muscle coordination ($F_{(13,70)} = 230.62$, $P<0.001$) and locomotor ($F_{(13,70)} = 227.36$, $P<0.001$) activity in mice as compared to control group. The increase in these activities by compounds at lower doses (Compound **1:** 2.5, 5 and 7.5 mg/kg and Compound **2:** 5, 10 and 15 mg/kg) was similar to the effect of nicotine at 1 mg/kg dose but this effect was found to be significantly less in case of compounds as compared to nicotine. At higher doses of Compounds **1** (25, 50 and 75 mg/kg) and **2** (50, 100 and 150 mg/kg), the action was found to be reversed. (Figs. 2 and 3).

3.3. Effect of compounds on memory

3.3.1. Effect of acute administration on transfer latency and step down latency

Compound **1** showed dose dependent decrease in TL ($F_{(14,76)} = 10.85$, $P<0.001$) and increase in SDL ($F_{(14,76)} = 133.64$, $P<0.001$) of mice at different doses but significant change was observed at doses 5,

Table 3
Tolerability studies of compounds and nicotine.

Treatment (dose)	Neurotoxicity test (time in seconds)	Seizurogenic potential and acute toxicity test		
		Occurrence of seizure	Percent mortality	Gross behavior changes
Compound **1** (2.5 mg/kg)	−(>180)	DNT	DNT	DNT
Compound **1** (5 mg/kg)	−(>180)	DNT	DNT	DNT
Compound **1** (7.5 mg/kg)	−(>180)	DNT	DNT	DNT
Compound **1** (25 mg/kg)	DNT	−	−	−
Compound **1** (50 mg/kg)	DNT	−	−	−
Compound **1** (75 mg/kg)	+(125.92 ± 3.7)	−	−	−
Compound **2** (5 mg/kg)	−(>180)	DNT	DNT	DNT
Compound **2** (10 mg/kg)	−(>180)	DNT	DNT	DNT
Compound **2** (15 mg/kg)	−(>180)	DNT	DNT	DNT
Compound **2** (50 mg/kg)	DNT	−	−	−
Compound **2** (100 mg/kg)	DNT	−	−	−
Compound **2** (150 mg/kg)	+(119.29 ± 3.4)	−	−	−
Nicotine (1 mg/kg)	−(>180)	DNT	DNT	DNT
Nicotine (10 mg/kg)	+(74.43 ± 2.7)	+	100%	+

−, negative test; +, positive test; DNT, do not test.

7.5, 25 and 50 mg/kg for TL and at all doses for SDL showing maximum effect at 7.5 mg/kg dose as compared to control group. This effect of 7.5 mg/kg dose of Compound **1** was comparable to the effect of donepezil and nicotine. Similarly, Compound **2** showed dose dependent change in TL and SDL of mice at different doses but significant change was observed at doses 10, 15 and 50 mg/kg for TL and at all doses for SDL showing maximum effect at 15 mg/kg dose as compared to control group which was comparable to the effect of donepezil and nicotine (Figs. 4 and 5).

3.3.2. Effect of chronic administration of selected dose of compounds and effect of scopolamine

There was significant decrease in TL ($F_{(4,25)} = 89.98$, $P<0.001$) and increase in SDL ($F_{(4,25)} = 109.17$, $P<0.001$) at 7.5 mg/kg dose of Compound **1** and 15 mg/kg dose of Compound **2** as compared to control group. This effect of chronic administration of selected doses of compounds was similar to the effect of donepezil and nicotine administration. A similar type of effect was seen in scopolamine treated groups in which scopolamine control group showed significant increase in TL ($t_{(10)} = 14.92$, $P<0.001$) and decrease in SDL ($t_{(10)} = 7.82$, $P<0.001$) as compared to vehicle control group. Selected dose of compounds showed significant decrease in TL ($F_{(4,25)} = 94.28$, $P<0.001$) and increase in SDL ($F_{(4,25)} = 65.73$, $P<0.001$) as compared to scopolamine control group. This effect of compounds was also comparable to the effect of donepezil and nicotine (Figs. 6 and 7).

3.3.3. Effect on acetyl cholinesterase activity

There was significant decrease in acetylcholine esterase activity of tissue homogenates obtained from the mice brains in Compound **1** (7.5 mg/kg dose) and Compound **2** (15 mg/kg dose) as compared to vehicle control ($F_{(4,25)} = 65.73$, $P<0.001$). This decrease in acetylcholine esterase activity was similar to the effect of donepezil and nicotine treated groups. There was slight increase in acetyl cholinesterase activity in Compound **1** (50 mg/kg dose) and Compound **2** (100 mg/kg dose) as compared to vehicle control but the increase was not significant (Fig. 8).

3.4. Effect of withdrawal

There was significant anxiogenic effect seen by withdrawal of chronic nicotine dosing as shown by decrease in the number of entries ($F_{(3,20)} = 4.35$, $P<0.05$) as well as time spent ($F_{(3,20)} = 7.59$, $P<0.05$) in open arm as compared to vehicle control group. In compounds treated groups, no such significant change was observed as compared to the control group (Table 4).

4. Discussion

Nicotine and its analogs have dose dependent varied actions on the muscle coordination and locomotor activity and their effectiveness as nootropic agent is also reported (Bontempi et al., 2003; Buckley et al.,

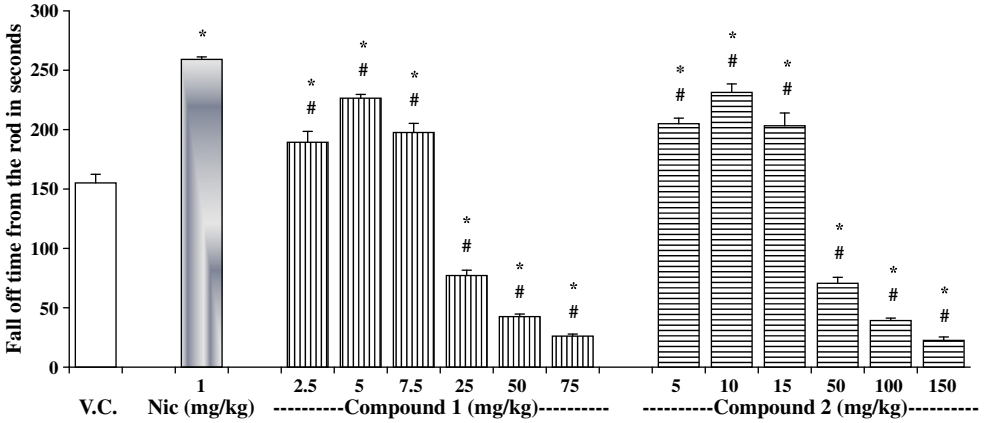

Fig. 2. Effect of varying doses of Compound **1** (2.5, 5, 7.5, 25, 50 and 75 mg/kg), **2** (5, 10, 15, 50, 100 and 150 mg/kg) and nicotine (Nic, 1 mg/kg) on muscle coordination in mice. * represents $P<0.05$ as compared to vehicle control (V.C.) group, # represents $P<0.05$ as compared to nicotine treated group. Data is expressed as mean ± S.E.M.

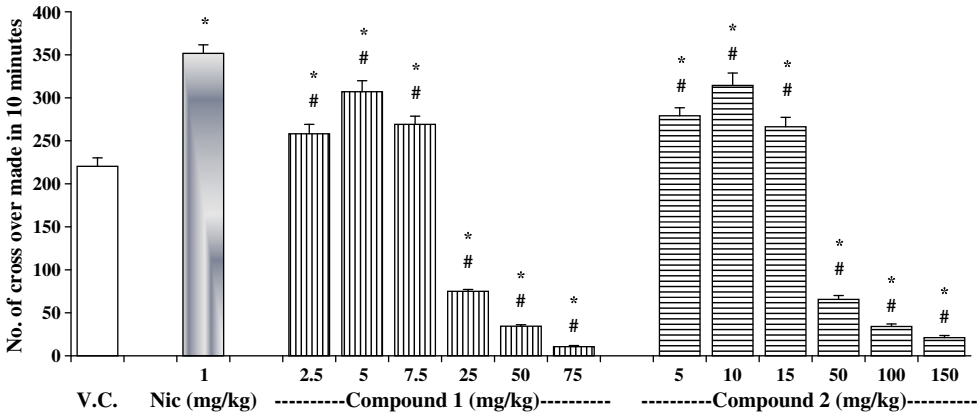

Fig. 3. Effect of varying doses of Compound **1** (2.5, 5, 7.5, 25, 50 and 75 mg/kg), **2** (5, 10, 15, 50, 100 and 150 mg/kg) and nicotine (Nic, 1 mg/kg) on locomotor activity in mice. * represents $P<0.05$ as compared to vehicle control (V.C.) group, # represents $P<0.05$ as compared to nicotine treated group. Data is expressed as mean ± S.E.M.

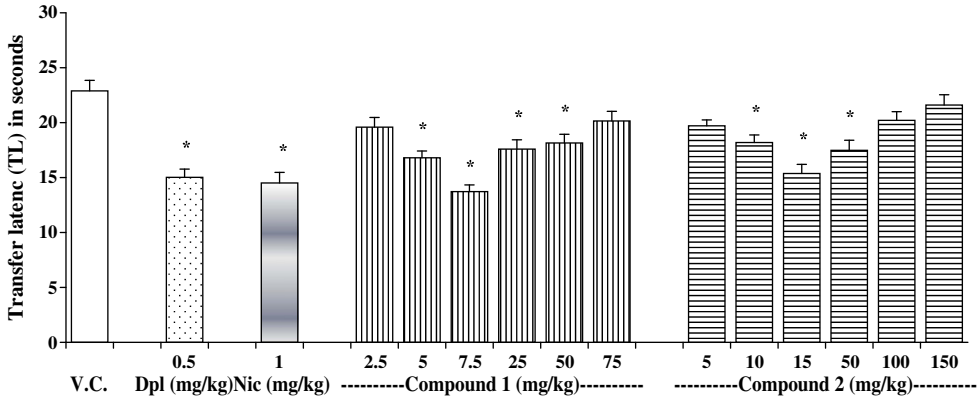

Fig. 4. Effect of acute treatment of varying doses of Compound **1**, **2**, donepezil (Dpl) and nicotine (Nic) on transfer latency (TL) in mice. * represents $P<0.05$ as compared to vehicle control (V.C.) group. Data is expressed as mean ± S.E.M.

2004; Newhouse et al., 2004; Picciotto et al., 2002). Experimental factors influence the cognition enhancing activity of nicotine and its agonists because activity of these agents are highly dose dependent, having no influence at the lowest concentrations and potentially producing seizures at the highest concentrations with a decline in cognition enhancing activity (inverse U shaped effect of most of memory enhancing agents). Furthermore, nicotinic manipulations can produce artifacts by altering autonomic and motor function. So these effects should also be considered during evaluation of memory enhancing effect of nicotinic analogs. That is why we have predicted the probable concerned activities of some novel nicotinic analogs with the help of computer program PASS and evaluated the dose dependent varied actions of these agents on cognition enhancing activity and other concerned pharmacological actions as discussed above.

Nicotine and most of its analogs show effect on the muscle coordination due to their action on the N_M receptors. So we evaluated the same and found dose dependent first increase (at lower doses) and then decrease (at higher doses) in muscle coordination. This first increase in muscle coordination by compounds may be due to the binding of these compounds with the N_M receptors of neuromuscular

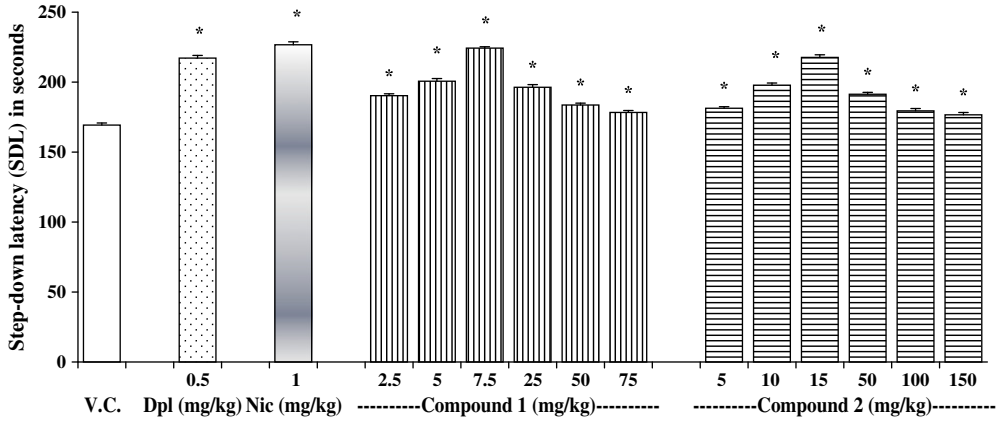

Fig. 5. Effect of acute treatment of varying doses of Compound **1**, **2**, donepezil (Dpl) and nicotine (Nic) on step down latency (SDL) in mice. * represents $P<0.05$ as compared to vehicle control (V.C.) group. Data is expressed as mean ± S.E.M.

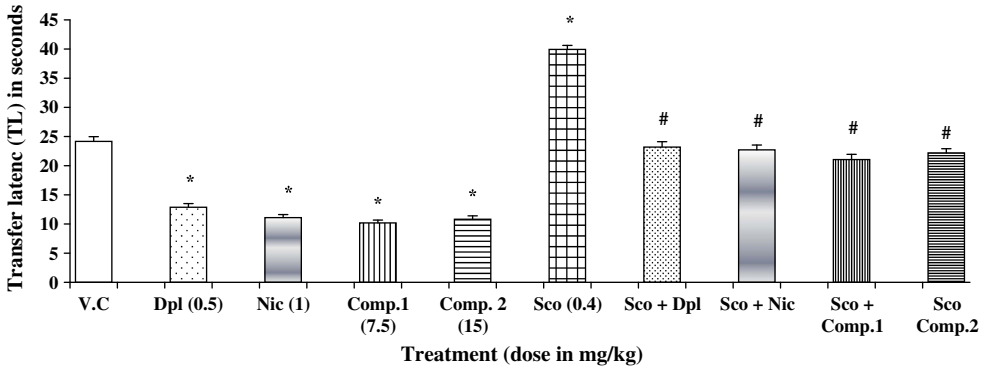

Fig. 6. Effect of chronic treatment of selected doses of Compound **1**, **2**, donepezil (Dpl) and nicotine (Nic) on transfer latency (TL) in normal and scopolamine (Sco) treated groups. * represents $P<0.05$ as compared to vehicle control (V.C.) group, # represents $P<0.05$ as compared to scopolamine control group. Data is expressed as mean ± S.E.M.

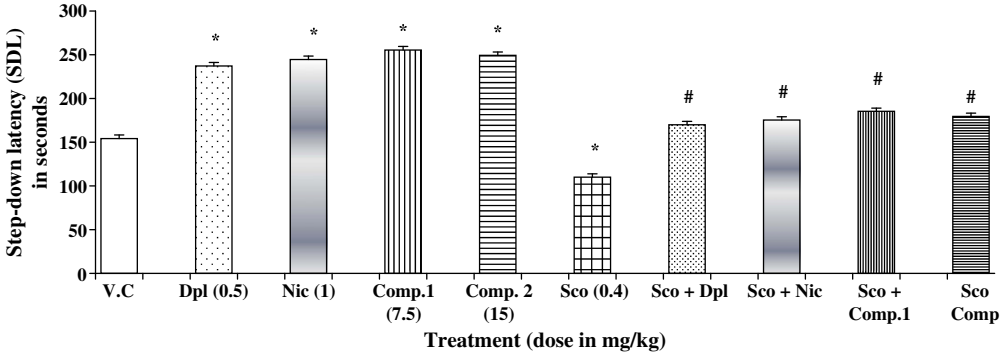

Fig. 7. Effect of chronic treatment of selected doses of Compound **1**, **2**, donepezil (Dpl) and nicotine (Nic) on step down latency (SDL) in normal and scopolamine (Sco) treated groups. * represents $P<0.05$ as compared to vehicle control (V.C.) group, # represents $P<0.05$ as compared to scopolamine control group. Data is expressed as mean ± S.E.M.

junction of somatic muscles. The data also shows that Compound **2** is more potent in increasing muscle coordination than Compound **1** but this effect is less than the nicotine effect. This shows that these compounds can be used over nicotine with lesser interference with muscle coordination. The decrease in muscle coordination at higher doses of the compounds may be due to the desensitization of these receptors at higher doses of compounds which is a common feature of nicotine acetylcholine receptors (Picciotto et al., 2008). This explains the biphasic response of these compounds on muscle coordination.

There are many evidences that show that nicotine and its analogs have dose dependent action on locomotor activity as well (Bontempi et al., 2003; Janhunen et al., 2005; Rauhut et al., 2008; Whiteaker et al., 1995). Due to their action on both N_N and N_M receptors, they show action on the locomotor activity. So we evaluated this action and found the same type of biphasic response as seen for muscle coordination activity. Both compounds show dose dependent first increase (at lower doses) and then decrease (at higher doses) in locomotor activity but the increase in activity by lower doses of compounds is less potent than the effect of nicotine on locomotor activity which encourages the use of these compounds over nicotine because of less interference of locomotor activity.

Nicotine and its analogs are reported to have a role in enhancing memory by different ways, some of which are well established and some are still under the stage of research or not well defined (Jones et al., 1999; Levin et al., 2006; Newhouse et al., 1997; Picciotto et al., 1995). For this reason we predicted the probable biological activity of these two compounds for nootropic activity and evaluated the same. We found dose dependent inverted U shaped effect of acute treatment

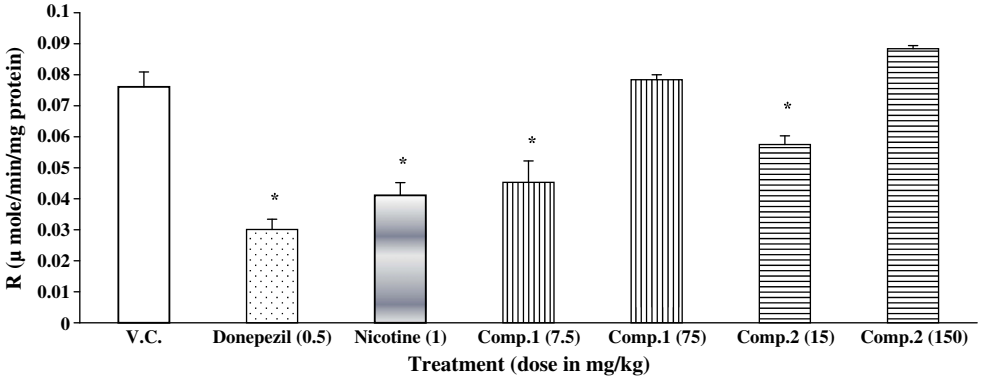

Fig. 8. Effect of chronic treatment of selected doses of Compound **1**, **2**, donepezil (Dpl) and nicotine (Nic) on acetylcholine esterase activity of mice brain. * represents $P<0.05$ as compared to vehicle control (V.C.) group. Data is expressed as mean ± S.E.M.

Table 4
Effect of compounds and nicotine withdrawal after chronic dosing on elevated plus maze model. Data are represented as mean values ± S.E.M.

Treatment (dose)	No. of entries in open arm	Time spent in open arm (in seconds)
Vehicle control	17.3 ± 2.1	138 ± 3.4
Nicotine (1 mg/kg)	6.5 ± 1.5*	109 ± 3.9*
Compound **1** (7.5 mg/kg)	15.6 ± 3.2	135 ± 5.2
Compound **2** (15 mg/kg)	16.1 ± 2.4	133 ± 6.3

* Represents $P<0.05$ as compared to vehicle control (V.C.) group.

of compounds on nootropic activity. These results must be interpreted in light of nicotine's inverted-U curve effect; although at lower doses a positive relationship exists between dose and performance, beyond a certain dose, the drug increasingly hinders performance. This is a common feature of most of the memory enhancing agents (Bontempi et al., 2003; Collerton, 1986).

Chronic treatment with the most effective selected dose of Compounds **1** and **2** showed better cognition performance than the acute treatment of compounds and this effect is comparable to the effect of chronic treatment of donepezil and nicotine. This encourages the use of these compounds for many cognition related disorders requiring chronic treatment.

Scopolamine, a postsynaptic blocker of cholinergic function, was known to cause impairment of memory (Oishi et al., 1993). The most effective selected dose of compounds successfully reversed scopolamine induced amnesia in a similar manner to the effect of nicotine and donepezil. This suggests that Compounds **1** and **2** may have induced acetylcholine release by activating presynaptic nicotine acetylcholine receptors to a degree sufficient enough to displace scopolamine from postsynaptic muscarinic sites and to restore memory function. This is consistent with the proposed role of presynaptic nicotine acetylcholine receptors in modulating neurotransmitter release (Kaiser and Wonnacott, 1998). Alternatively, the cognitive improvements induced by compounds may also result from enhanced activation of postsynaptic nicotine acetylcholine receptors sufficient to overcome the effects of decreased muscarinic tone caused by scopolamine. This shows that these compounds show its nootropic effect through nicotine acetylcholine receptors by directly acting on these receptors as well as by acting presynaptically to induce more release of acetylcholine for acting on these receptors and showing nootropic effect. This type of effect of compounds is already well established with nicotine which is used as a standard for this study to compare the effectiveness of compounds as nootropic agents.

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions (Kaur et al., 2010). This is the major reason for the use of acetylcholine esterase inhibitors for the symptomatic treatment of diseases related to the impaired cognition like Alzheimer's disease in its early stages. There are extensive evidences linking the central cholinergic system to memory (Ghelardini et al., 1998; Peng et al., 1997). Cognitive dysfunction has been shown to be associated with impaired cholinergic function and the facilitation of central cholinergic activity with improved memory (Bhattacharya et al., 1993). It has been reported that nicotine can inhibit acetylcholine esterase to some level (Terry et al., 1998) whereas donepezil is a well known acetylcholine esterase inhibitor. For this reason, they are used as the standards for comparison with compounds for acetylcholine esterase activity. Acetylcholine esterase activity was observed to be decreased by most effective lower dose of compounds which shows that a part of its nootropic activity is due to the inhibition of acetylcholine esterase causing increase in the level of acetylcholine in brain. This decrease in acetylcholine esterase activity was similar to the decrease in activity due to donepezil and nicotine which shows the rational of using these compounds as effective nootropic agents with having a part of its nootropic action through inhibition of acetylcholine esterase. Higher doses of compounds have no effect on acetylcholine esterase activity and thus have very less nootropic action. It is reported that the higher dose of nicotine can cause increase in the secretion acetylcholine esterase from the adrenal chromaffin cells which can be responsible for the slight increase in the level of acetylcholine esterase in brain at higher doses of compound (Mizobe and Livett, 1983).

It predicts its possible mechanism of action through nicotine acetylcholine receptors and acetylcholine esterase inhibition for its nootropic effect. In this way, these agents can be used in different diseases associated with nicotine acetylcholine receptors and memory like Alzheimer's disease in which defective cholinergic function is observed (Coyle et al., 1983; Perry, 1986). There has been a steady rise in the number of patients suffering from Alzheimer's disease all over the world. There are around 35 million patients suffering from Alzheimer's disease all over the world, out of which United States of America alone has around 4.5 million patients (Hebert et al., 2003).

The effective doses of the compounds did not show any neurotoxicity. The compounds also did not show seizure and mortality at all the doses tested where nicotine showed seizure followed by death at a dose which is around 1/10th times lesser than the doses of compounds tested. The compounds did not show withdrawal induced anxiogenesis showed by nicotine due to its addiction liability which strengthens the further prospects of use of these compounds over nicotine for nootropic activity.

5. Conclusions

We can conclude that the nootropic effect of these compounds can be attributed through the involvement of activation of postsynaptic or presynaptic nicotinic acetylcholine receptors on neurons, with the latter resulting in release of acetylcholine and non-cholinergic neurotransmitters (Kaiser and Wonnacott, 1998) which show the above pharmacological actions. Further, it is observed that the compounds are less potent than nicotine for showing the side effects and equipotent for nootropic activity which can be used over more wide range of doses. Many agents have been developed and a number of agents are being developing for this purpose, and this is a small step to enlighten the path for developing an effective and safer agent for nootropic activity.

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