ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

SYNTHESIS OF SUBSTITUTED PHENYLETHYLAMINE AND ETHYLAMINE DERIVATIVES AS COGNITION ENHANCERS

Narinderpal Kaur*[1, 2], Jagdeep Singh Dua[3]

[1]*LBR college of Pharmacy, Golpura, Panchkula, Haryana, India*

[2]*I. K. Gujral Punjab Technical University, Jalandhar, Punjab, India*

[3]*Shivalik College of Pharmacy, Naya-Nangal, Punjab, India*

**Corresponding author: narinder.sonia@gmail.com*

ABSTRACT

The objective of the present study was to synthesize phenylethylamine and ethylamine derivatives and their effects on brain were studied by using passive avoidance paradigm and Morris water maze (MWM). Synthesized compounds were effective to reverse the memory deficits induced by scopolamine and synthesized compounds showed the neuroprotective effect but locomotor effects were not significantly affected. This shows that the synthesized compounds are effective to control the motor coordination.

Keywords: β-PEA, Lipolysis, Neuroprotective effect, Locomotor effects, Passive avoidance Paradigm, Morris water maze.

1. INTRODUCTION

Phenethylamine (PEA), commonly known as β-phenylethylamine (β-PEA) has psychoactive and stimulant effects. This is a natural monoamine alkaloid, a well-known trace amine which regulates the physical energy, mood, and attention [1]. Currently in the age group of 15-44, depression is the second leading cause of disability. It is a very common and sometimes serious disease which can shorten a person's life by 25-30 Years [2]. Depression can also be caused by deficiency of Phenylethylamine [3]. PEA can be effective in treating the depression, even in those which were unresponsive to standard treatments [4]. PEA may also stimulate lipolysis because of this some weight loss supplements, *e.g.* Fasting included PEA [5] and so can be used as an appetite suppressant. The urinary output of PEA was found to be lower in individuals with Attention deficit hyperactivity disorder [6, 7]. A form of PEA known as D-phenylalanine, increases endorphin levels which possess powerful painkilling properties [8]. PEAs are popular substances in sports nutrition supplements [9].

The objective of present study was to synthesize phenylethylamine and ethylamine derivatives which can improve cognitive behavior.

2. MATERIAL AND METHODS

2.1. Instrumentation

The Melting points of derivatives were determined with the help of sonar melting point apparatus in open glass capillaries which are uncorrected and the progress of reaction was observed with the help of TLC by using silica gel sheets. For recording proton NMR and C13-NMR spectra, Bruker Avance II 400 NMR spectrometer was used and tetramethylsilane (TMS) was used as an internal standard. For IR spectra, Perkin Elmer Spectrum RXI FTIR spectrophotometer was used with KBr phase. Mass spectra were taken on Bruker Compass Data Analysis 4.0 Mass spectrometer (Analysis outsourced from SAIF, Punjab University, Chandigarh). All chemicals were purchased from Merck company. Photoactometer (INCO, Ambala, India) was used to measure the locomotor activity of control and drug-treated animals. UV spectrophotometer (DU 640 B series, Beckman, USA) was used for spectrophotometric analysis.

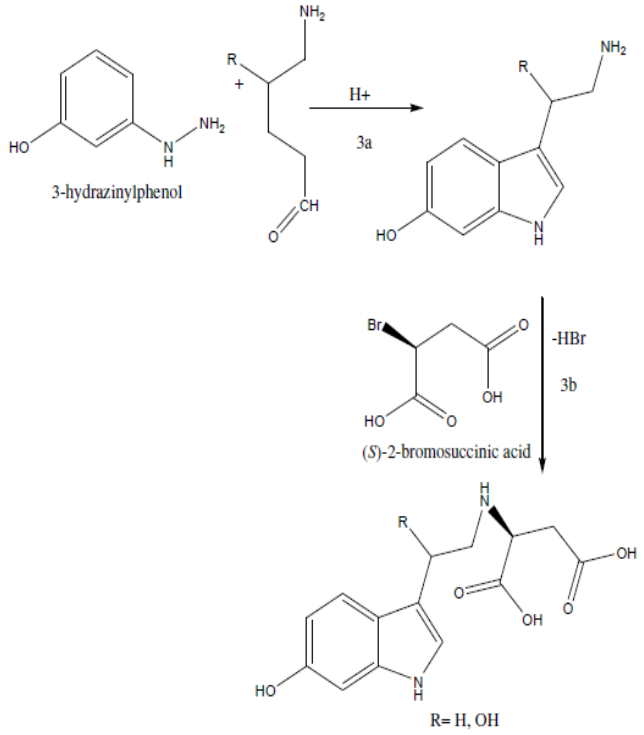
2.2. Synthesis of 3-(2-aminoethyl)-1H-indol-6-ol derivatives

An equimolar mixture (0.01mol) of 3-hydrazinyl phenol and substituted aldehydes in absolute ethanol (20 ml) was refluxed for 2 h in the presence of 2-3 drops of glacial acetic acid. The cooling of reaction mixture was

done at room temperature and then the mixture was poured into ice-cold water. The separated product was filtered, washed with cold water, dried and recrystallized from the appropriate solvent.

2.3. Synthesis of phenyl ethyl indole derivatives

3-(2-aminoethyl)-1H-indol-6-ol derivatives (0.01 mol) and (S)-2-bromosuccinic acid (0.01 mol) were refluxed for about 30 min in the presence of DMF. The mixture was cooled and the solid obtained was separated by filtration and methanol was used for recrystallization to obtain the corresponding compounds.



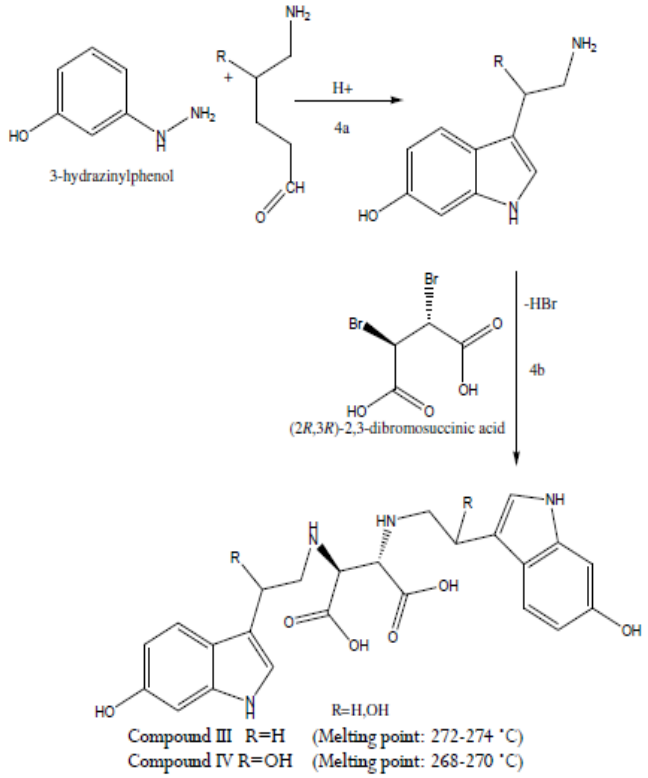
Compound I (R-H) - Melting point: 228-230 °C
Compound II (R-OH) - Melting point: 216-218 °C

Compound-I: (S)-2-((2-(6-hydroxy-1H-indol-3-yl) ethyl) amino) succinic acid, Compound-II: (2S)-2-((2-hydroxy-2-(6-hydroxy-1H-indol-3-yl)ethyl)amino)succinic acid

Fig 1: Synthesis of compound-I and compound-II

2.4. Synthesis of 3-(2-aminoethyl)-1H-indol-6-ol derivatives

An equimolar (0.01 mol) mixture of 3-hydrazinyl phenol and substituted aldehydes in absolute ethanol (20 ml) was refluxed for 2 h in the presence of 2-3 drops of glacial acetic acid. The cooling of reaction mixture was done at room temperature and then the mixture was poured into ice-cold water. The separated product was filtered, washed with cold water, dried and recrystallized from appropriate solvent.



Compound III R=H (Melting point: 272-274 °C)
Compound IV R=OH (Melting point: 268-270 °C)

Compound-III: (2S,3S)-2,3-bis((2-(6-hydroxy-1H-indol-3-yl)ethyl) amino) succinic acid, Compound-IV: 2S,3S)-2,3-bis((2-hydroxy-2-(6-hydroxy-1H-indol-3-yl)ethyl)amino) succinic acid

Fig 2: Synthesis of compound-III and compound-IV

2.5. Synthesis of phenyl ethyl indole derivatives

3-(2-aminoethyl)-1H-indol-6-ol derivatives (0.01 mol) and (2R,3R)-2,3-dibromosuccinic acid (0.01 mol) were refluxed in the presence of DMF for about 30 min. The mixture was cooled and the solid obtained was separated by filtration and recrystallized from methanol to get the corresponding compounds.

2.6. Evaluation of synthesized compounds on Brain (memory)

Male swiss mice of weight 20-25 g were used in the present study. They had free access to food, water and were maintained under standard laboratory conditions. The Institution Animals Ethics Committee (IAEC) of Chitkara College of pharmacy under registration number 1181/PO/ReBi/S/08/CPCSEA, had approved the experimental protocol (Reference number: IAEC/

CCP/19/02/PR-017) and care of animals were taken as per guidelines of CPCSEA, Department of Animal Welfare, and Government of India.

2.7. Drug protocol

Synthesized derivatives (2%, 4% and 8% *w/w*), Normal saline (vehicle, *p.o.*), Piracetam (400 mg/kg, *i.p.*), Donepezil (1 mg/kg, *i.p.*) were administered for 10 successive days to mice. Scopolamine (0.4 mg/kg, *i.p.*) and Diazepam (1 mg/kg, *i.p.*) were administered on 9th day. Biochemical studies were carried on 10th day after drugs/vehicle/Synthesized derivatives administration. Statistical analysis was performed using Graphpad Prism 6. Values were expressed as mean±SEM and One-way Analysis of Variance (ANOVA) was used for statistical analysis. ANOVA was followed by Tukey's as post hoc multiple comparison test.

2.8. Passive avoidance task

For examining the long-term memory, Passive avoidance behavior was used. The step-down latency (SDL) was recorded. The Animals which were used for the second session and the retention test, were the animals which show SDL in the range (2-15 sec) during the first test. The second-session was carried out after 90 min of the first test. The electric shocks were delivered for 15 sec when stepped down period of animals were before 60 sec. If animals did not step down for a period of 60 sec then they were removed from shock free zone, during the second test. After 24 h, retention was tested in a similar manner. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 sec [10, 11].

2.9. Morris Water Maze (MWM)

MWM test was carried out according to standard procedure [12]. As an index of learning escape latency time was noted. Each day, each animal was subjected to training trials for four consecutive days and synthesized derivatives were administered on fifth day. Time spent in all the three quadrants, that is, Q1, Q2 and Q3 was recorded and the time spent in the target quadrant (TSTQ) in search of the missing platform provided as an index of retrieval.

2.10. Interoceptive models

Diazepam and Scopolamine induced amnesia were used as interoceptive behavioral models. [13]

2.11. Biochemical Estimations

The collection of brain samples was done by cervical decapitation under light anesthesia after administration of the last doses of synthesized derivatives or standard drugs or vehicle. Then, whole brain was carefully removed from the skull. The weighing of fresh whole brain was done and homogenized in an ice bath. The homogenate was centrifuged at 3000 rpm for 10 mins and for the for biochemical estimations, the resultant cloudy supernatant liquid was used.

2.12. Estimation of Brain Acetylcholinesterase

A test tube containing 2.6 ml of phosphate buffer was taken and 0.4 ml of brain homogenate was added to it. Then to this mixture 0.1 ml 5,5'-dithiobis-(2-nitrobenzoic acid) reagent was added and absorbance was noted using UV spectrophotometer at 412 nm. Again absorbance was noted by adding 0.02 ml of acetylcholine iodide solution after 15 min. Change in absorbance per min was calculated [14].

2.13. Estimation of thiobarbituric acid reactive substances level (TBARS)

TBARS, are a measure of peroxidation of lipid, which were spectrophotometrically estimated. Homogenization of Brain tissues were done with 0.1 M sodium phosphate buffer having pH 7.4 The reagents, thiobarbituric acid, sodium Lauryl sulphate and acetic acid were added to 0.2 ml of processed tissue sample. The volume made up to 4.0 ml of this mixture was done with distilled water and heated the mixture for 60 min at 1000 °C. The cooling of mixture was done under tap water and 5 ml of *n*-butanol: pyridine (15:1% *v/v*), 1 ml of distilled water was added and shaken vigorously. The organic layer separated out by centrifugation for 10 min at 4000 rpm, and the absorbance was observed by using UV-Visible Spectrophotometer at 532 nm and the expression of results are in the form of µmol/g of tissue protein [15, 16].

2.14. Estimation of brain glutathione levels

UV spectrophotometer was used to measure Glutathione level. Homogenization of Brain tissues were done with 0.1 M sodium phosphate buffer having pH 7.4. This homogenate mixture was centrifuged by using 5% trichloroacetic acid so that the proteins could centrifuge out. To 0.1 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double distilled water was added. The mixture was shaken

vigorously by vortex shaker and within 15 min the absorbance measured at 412 nm and the expression of results are in the form of µmol/g of tissue protein [16, 17].

3. RESULTS

Rectified spirit was used for recrystallisation of all the compounds. The synthesized compounds were characterized on the basis of their IR, ^{1}H NMR, ^{13}C NMR and Mass spectral analysis.

3.1. Spectral Data of some of synthesized compounds:

3.1.1. (S)-2-((2-(6-hydroxy-1H-indol-3-yl)ethyl) amino)succinic acid (Compound I)

IR (KBr, cm^{-1}): 3616 (OH), 3587 (NH), 3005 (C-H, Ar), 2877 (CH), 1610 (C=C), ^{1}H NMR (DMSO-d_6, 400 MHz): 11.93 (s, 3H, OH), 7.55-8.03 (m, 3H, ArH), 7.54 (s, 1H, CH indole), 2.94-3.18 (t, 4H, CH_2-CH_2), 3.04 (q, 1H, CH), 2.20-2.38 (d, 2H, CH_2), ^{13}CNMR (DMSO-d_6, δ ppm):161.68, 160.59, 150.51,133.69, 125.94, 122.80, 112.54, 64.25, 41.34, 39.66, , MS ES+ (ToF): *m/z* 290.

3.1.2. (2S)-2-((2-hydroxy-2-(6-hydroxy-1H-indol-3-yl)ethyl)amino)succinic acid (Compound II)

IR (KBr, cm^{-1}): 3619 (OH), 3542 (NH), 3027 (C-H, Ar), 2797 (CH), 1740 (C=O), 1618 (C=C), ^{1}H NMR (DMSO-d_6, 400 MHz): 10.09 (s, 4H, OH), 6.44-7.74 (m, 3H, ArH), 7.29 (s, 1H, CH indole), 4.44 (t, 1H, CH-OH), 2.38-2.73 (d, 2H, CH_2), 3.80 (q, 1H, CH), 2.07-2.50 (d, 2H, CH_2), ^{13}CNMR (DMSO-d_6, δ ppm):161.76, 160.73, 152.05, 149.44, 127.14, 124.65, 112.86, 110.32, 57.09, 55.06, 40.77.

3.1.3. (2S,3S)-2,3-bis((2-(6-hydroxy-1H-indol-3-yl)ethyl)amino)succinic acid (Compound III)

IR (KBr, cm^{-1}): 3623 (OH), 3549 (NH), 3146 (C-H, Ar), 2973 (CH), 1733 (C=O), 1650 (C=C), ^{1}H NMR (DMSO-d_6, 400 MHz): 11.13 (s, 4H, OH), 7.22-8.39 (m, 6H, ArH), 6.93 (s, 2H, CH indole), 2.21-2.59 (m, 8H, CH_2-CH_2), 4.41 (m, 2H, CH), ^{13}CNMR (DMSO-d_6, δ ppm):166.63,154.67, 149.61, 146.19, 146.19, 135.55, 132.19, 128.46, 127.65, 125.16, 112.71, 115.51, 41.02, 40.08, 39.08, MS ES+ (ToF): *m/z* 465.

3.1.4. (2S,3S)-2,3-bis((2-hydroxy-2-(6-hydroxy-1 H-indol-3-yl)ethyl)amino)succinic acid (Compound IV)

IR (KBr, cm^{-1}): 3609 (OH), 3561 (NH), 3029 (C-H, Ar), 2897 (CH), 1742 (C=O), 1617 (C=C), ^{1}H NMR (DMSO-d_6, 400 MHz): 10.03 (s, 6H, OH), 6.84-7.39 (m, 6H, ArH), 6.82 (s, 2H, CH indole), 4.65 (t, 1H, CH-OH), 2.50-3.37 (m, 4H, CH_2-CH_2), 3.88 (m, 2H, CH), ^{13}CNMR (DMSO-d_6, δ ppm):162.00, 149.51, 148.22, 140.56, 136.07, 133.86, 131.46, 130.44, 129.01, 128.36, 122.18, 40.76, MS ES+ (ToF): *m/z* 500.

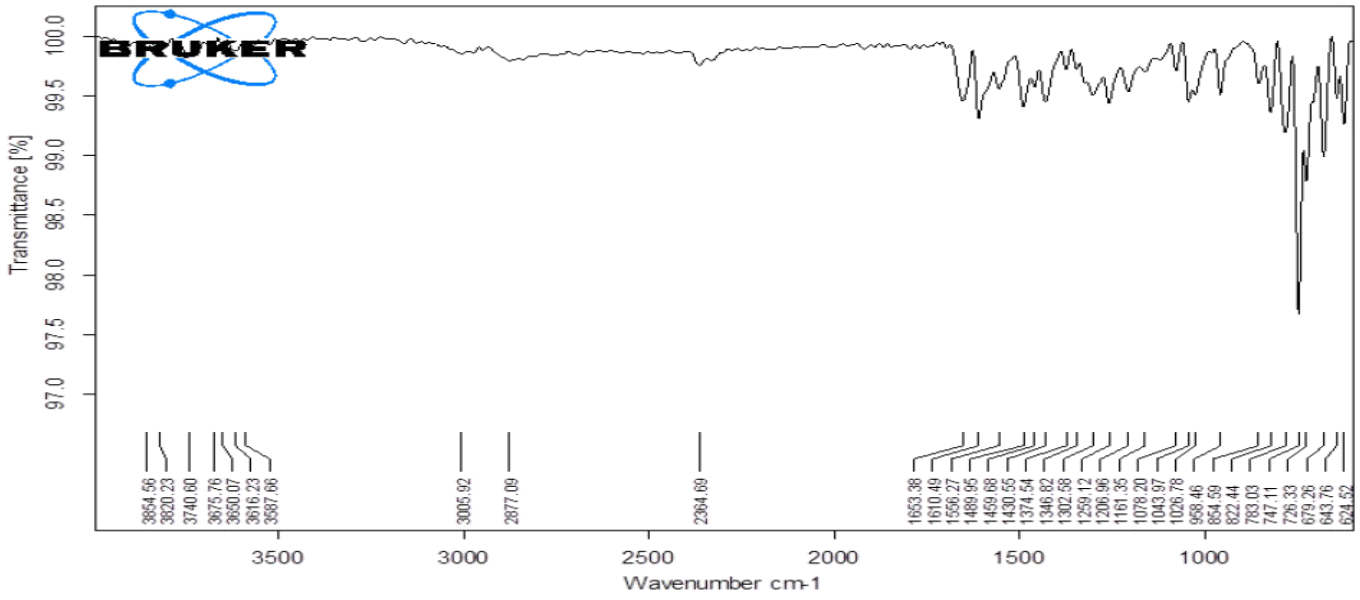


Fig. 3: IR Spectra of compound-I

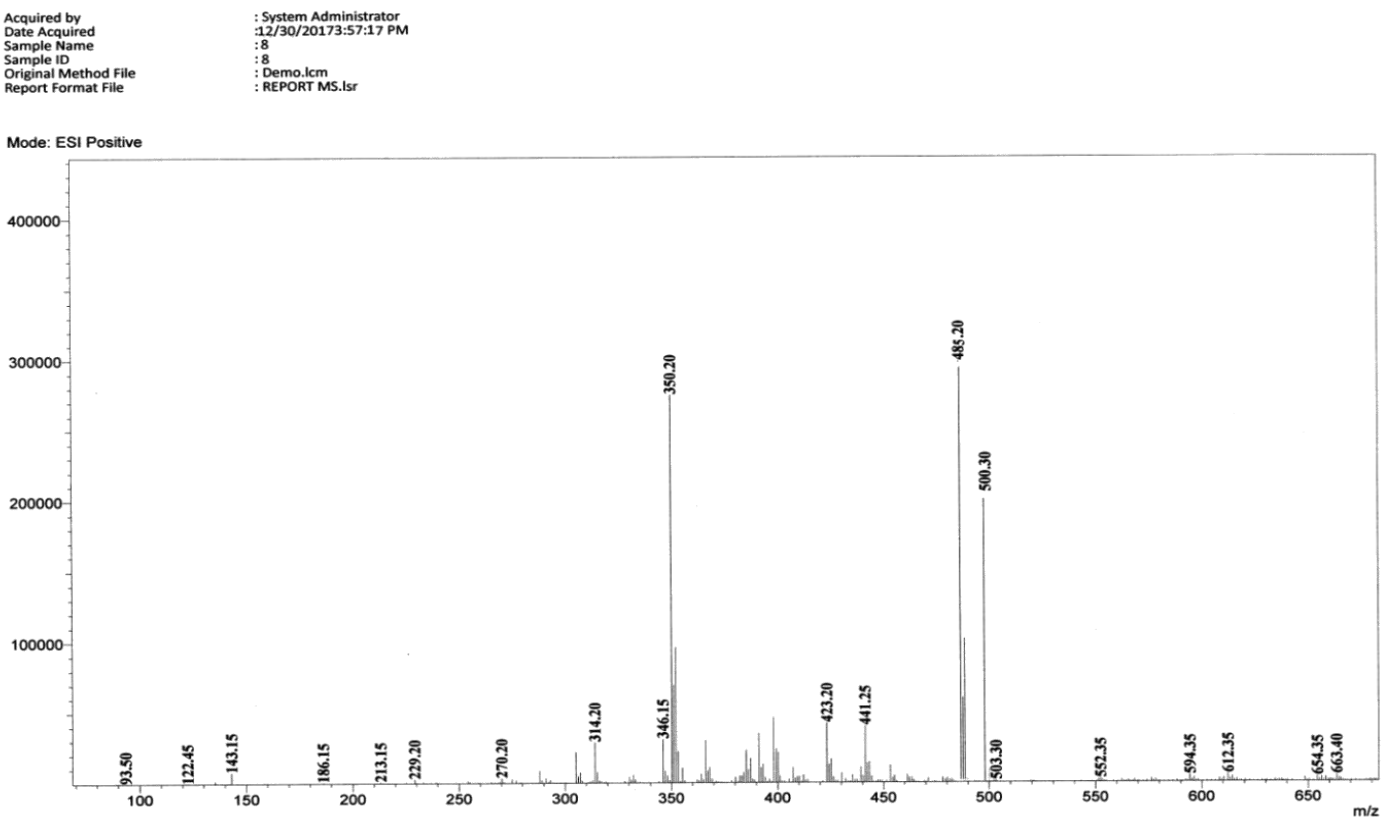


Fig. 4: Mass Spectra of compound-IV

Table 1: Physical properties of the synthesized compounds

Compound No.	Mol. Formula	Mol. Wt.	Rf Value	(%) Yield	Melting Point
1.	$C_{14}H_{16}N_2O_5$	292.29	0.88	65	228-230
2.	$C_{14}H_{16}N_2O_6$	308.29	0.81	68	216-218
3.	$C_{24}H_{26}N_4O^6$	466.49	0.74	74	272-274
4.	$C_{24}H_{26}N_4O_8$	498.49	0.72	67	268-270

3.2. Influence of CF on Step Down Latency (SDL) using Passive Avoidance Paradigm

Scopolamine (0.4 mg/kg, *i.p.*) decreased the SDL significantly ($p<0.01$), showing memory impairment. Synthesized compounds administered at 10mg/kg, *i.p.* concentration, reversed the memory deficits induced by Scopolamine. This memory improving effect of all the synthesized compounds was similar to Donepezil (anti-Alzheimer agent).

Table 2: Effect of synthesized derivatives on Step down latency (SDL) using Passive avoidance paradigm

Group	Treatment	Dose	10^{th} Day SDL (sec)
1.	Control	Normal saline	191.5±3.52
2.	Scopolamine	0.4 mg/kg, *i.p*	116.5±2.12*
3.	Scopolamine +Donepezil	1 mg/kg, *i.p*	251.36±4.39[a]
4.	Scopolamine +C-1	10 mg/kg, *i.p*	209.83± 9.04[a]
5.	Scopolamine +C-2	10 mg/kg, *i.p*	183.22±7.91[a]
6.	Scopolamine +C-3	10 mg/kg, *i.p*	219.83± 5.19[a]
7.	Scopolamine +C-4	10 mg/kg, *i.p*	170.83± 4.42[a]

Values are mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's t-test.

**denotes $p<0.01$ as compared to control group, a denotes $p<0.01$ as compared to Scopolamine group.*

3.3. Influence on time spent in target quadrant (TSTQ) using (MWM)

The time spent in the target quadrant (TSTQ) in search of the missing platform provided an index of retention of memory. Scopolamine (0.4 mg/kg, *i.p*) decreased the time period in target quadrant significantly ($p<0.01$), showing memory impairment. Enhanced time period in target quadrant reflect better spatial memory of mice. Synthesized compounds administered at 10 mg/kg, *i.p.* concentration reversed the memory deficits induced by Scopolamine. This memory improving effect of all the synthesized compounds was similar to Donepezil (anti-Alzheimer agent).

Table 3: Effect of synthesized derivatives on time spent in target quadrant (TSTQ) using Morris water maze

Group	Treatment	Dose	10th Day TL (sec)
1.	Control	Normal saline	31.06 ± 0.64
2.	Scopolamine	0.4 mg/kg, *i.p*	19.80 ± 0.54*
3.	Scopolamine +Donepezil	1mg/kg, *i.p*	59.82 ± 2.70[a]
4.	Scopolamine +C-1	10mg/kg, *i.p*	43.05 ± 0.81[a]
5.	Scopolamine +C-2	10mg/kg, *i.p*	39.85 ± 3.81[a]
6.	Scopolamine +C-3	10mg/kg, *i.p*	49.25 ± 1.51[a]
7.	Scopolamine +C-4	10mg/kg, *i.p*	46.25 ± 2.12[a]

Values are mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's t-test.

**denotes $p<0.01$ as compared to control group, a denotes $p<0.01$ as compared to Scopolamine group.*

Table 4: Effect of synthesized derivatives on locomotor activity

Group	Treatment	Dose	10th Day SDL (sec)
1.	Control	Normal saline	251.2 ± 3.21
2.	Scopolamine	0.4 mg/kg, *i.p*	267.1 ± 3.14
3.	Scopolamine +Donepezil	1 mg/kg, *i.p*	241.36 ± 4.21
4.	Scopolamine +C-5	10 mg/kg, *i.p*	249.13 ± 5.98
5.	Scopolamine +C-6	10 mg/kg, *i.p*	273.12 ± 7.11
6.	Scopolamine +C-7	10 mg/kg, *i.p*	269.33 ± 4.28[a]
7.	Scopolamine +C-8	10 mg/kg, *i.p*	249.77 ± 4.31[a]

Values are mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's t-test.

3.4. Effect of Synthesized Derivatives on Locomotor Activity

Locomotor activity was assessed to screen the effect of test drugs on motor coordination. There was no significant change found in the locomotor activity.

3.5. Effect on Brain Acetylcholinesterase Activity (AChE)

The most important neurotransmitter which is supposed to be involved in the regulation of cognitive functions is Acetylcholine. Acetylcholine is degraded by *AChE* enzyme which is responsible for controlling the concentration of acetylcholine in brain**.** Scopolamine (0.4 mg/kg, *i.p*) decreased the concentration of acetylcholine by increasing the activity of acetylcholinesterase activity**.** Synthesized compounds administered at 10 mg/kg, *i.p.* concentration reversed the memory deficits induced by decreasing the activity of acetylcholinesterase activity.

Table 5: Effect of synthesized derivatives on brain acetylcholinesterase activity

Group	Treatment	Dose	Acetylcholinesterase activity (nmol/min/g protein)
1.	Control	Normal saline	39.26 ± 1.94
2.	Scopolamine	0.4 mg/kg, *i.p*	79.81 ± 2.14*
3.	Scopolamine +Donepezil	1 mg/kg, *i.p*	42.82 ± 1.12[a]
4.	Scopolamine +C-5	10 mg/kg, *i.p*	61.32 ± 4.35[a]
5.	Scopolamine +C-6	10 mg/kg, *i.p*	59.35 ± 5.21[a]
6.	Scopolamine +C-7	10 mg/kg, *i.p*	49.21 ± 3.43[a]
7.	Scopolamine +C-8	10 mg/kg, *i.p*	55.28 ± 1.89[a]

Table 6: Effect of synthesized derivatives on Brain MDA level

Group	Treatment	Dose	MDA level (nmol/mg protein)
1.	Control	Normal saline	0.45±0.024
2.	Scopolamine	0.4 mg/kg, *i.p*	1.63±0.029*
3.	Scopolamine +Donepezil	1mg/kg, *i.p*	0.54 ± 1.12 [a]
4.	Scopolamine +C-5	10mg/kg, *i.p*	0.93±0.078[a]
5.	Scopolamine +C-6	10mg/kg, *i.p*	0.71 ± 0.072 [a]
6.	Scopolamine +C-7	10mg/kg, *i.p*	0.99 ± 0.011 [b]
7.	Scopolamine +C-8	10mg/kg, *i.p*	0.89 ± 0.063 [a]

Values are mean ± SEM (n = 6). One way ANOVA followed by Dunnett's t-test.

** denotes p<0.01 as compared to control group, [a]denotes p<0.01 and b denotes p<0.05 as compared to Scopolamine group*

3.6. Effect on Brain of MDA Level

TBARS was the form in which MDA level (a product of peroxidation of lipid) was estimated. Scopolamine (0.4 mg/kg, *i.p*) increased the concentration of MDA level**.** Synthesized compounds administered at 10 mg/kg, *i.p.* concentration reversed the memory deficits induced by decreasing the level of MDA level.

3.7. Effect On Brain Glutathione (Gsh) Levels

GSH is an endogenous free radical scavenger. Scopolamine (0.4mg/kg, i.p) increased the oxidative stress by decreasing the concentration of GSH level**.** Synthesized compounds administered at 10mg/kg, i.p. concentration reversed the memory deficits induced by increasing the level of GSH level.

Table 7: Effect of synthesized derivatives on Brain MDA level

Group	Treatment	Dose	GSH level (nmol/mg protein)
1.	Control	Normal saline	0.0061±0.00022
2.	Scopolamine	0.4 mg/kg, *i.p*	0.0019±0.00016*
3.	Scopolamine +Donepezil	1mg/kg, *i.p*	0.0052±0.00015[a]
4.	Scopolamine +C-5	10mg/kg, *i.p*	0.0031±0.00021[b]
5.	Scopolamine +C-6	10mg/kg, *i.p*	0.0047±0.00016[a]
6.	Scopolamine +C-7	10mg/kg, *i.p*	0.0049±0.00027[a]
7.	Scopolamine +C-8	10mg/kg, *i.p*	0.0033±0.00016[a]

Values are mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's t-test.

**denotes p<0.01 as compared to control group, [a]denotes p<0.01 as compared to Scopolamine group.*

4. DISCUSSION

In the present study, four compounds were synthesized and their effects on brain were studied. Scopolamine was used to induce memory loss in laboratory animals. A memory enhancing agent, Donepezil was used as standard drug in the present study. Synthesized compounds were found to reverse the memory deficits induced by scopolamine. But no significant effect was seen in the locomotor effect, this shows that these agents are effective to control the motor coordination. Cognitive functions are thought to be regulated by acetylcholine, which is an important neurotransmitter. In the present study, synthesized compounds produced significant inhibition of AChE activity. Synthesized compounds inhibited the AChE activity leading to increased accumulation of Ach at the synapse and facilitation of cholinergic transmission. TBARS are one of several products of damage produced by oxidative stress. Pathogenesis of neurodegenerative disorders like Parkinsonism disease, Alzheimer's disease, apoptosis *etc.* are greatly affected by Oxidative stress. Thus, increase in TBARS or MDA levels corresponds to increase oxidative stress leading to brain damage and decrease in their level reflects neuroprotection. In the present study all the synthesized compound reduced the TBARS levels, ultimately providing a neuroprotective. GSH (Glutathione) is a major endogenous antioxidant produced by the cells. It participates directly in the neutralization of reactive oxygen compounds and free radicals and help in preventing damage to important cellular components. Thus, Glutathione (GSH) is the major free radical scavenger in the brain. Increase in its level indicates neuroprotection. In the current study, there was a significant rise of GSH levels in the brain of synthesized compounds treated mice which suggests the neuroprotective effect of synthesized compounds. These

observations may be further explored for the design and synthesis of more potent cognition enhancers.

5. ACKNOWLEDGEMENTS

The authors are thankful to I.K. Gujral Punjab Technical University, Jalandhar (Punjab) for their support and encouragement for this research work.

6. REFERENCES

1. Kaur N, Kumari B. *World J Pharm Pharma Sci.,* 2016; **5(4):**743-750.
2. Voinov B, Richie WD, Bailey RK. Prim Care Companion CNS Disord. 2003; 15: doi: 10.4088/PCC.12r01468. Epub ahead of print.
3. Sabelli H, Borison RL, Diamond BI, Havdala HS, Narasimhachari N. *Biochem Pharmacol.*, 1978; **27:**1707-1711.
4. Grimsby J, Toth M, Chen K, Kumazawa T, Klaidman L, Adams JD, et al. *Nat Genetics*, 1997; **17:**206-210.
5. Paterson IA, Juorio AV, Boulton AA. *J Neurochem*. 1990; **55:**1827-1837.
6. Baker GB, Bornstein RA, Rouget AC, Ashton SE, Van Muyden JC, Coutts RT. *Biol Psychiatry*., 1991; **29:**15-22.
7. Kusaga A, Yamashita Y, Koeda T, Hiratani M, Kaneko M, Yamada S, Matsuishi T. *Ann Neurol.*, 2002; **52:**372-374.
8. Walsh NE. *Arch Phys Med Rehabil*., 1986; **67(7):**436-439.
9. Zhao J, Wang M, Avula B, Khan IA. *J Pharm Biomed Anal.*, 2018, 20; **151:**347-355.
10. Joshi H, Parle M. *Ind J Expt Biol.,* 2006a; **44:**133-136.
11. Venault P, Chapouthier G, de Carvalho LP, Simiand J, Morre M, Dodd RH, Rossier J. *Nature*, 1986, **321:**864-866.
12. Parle M, Bansal N, *Evid Based Complement Alternat Med.*, 2011; **2011:**898593.
13. Rahman H, Sindhura S, Eswaraiah MC, Elumalai A. *Int J Pharm Sci Rev Res.,* 2011; **11(1):**23-28.
14. Ellman GL, Courtney KD, Andres V Jr. *Biochem Pharmacol,* 1961; **7:**88-95.
15. Okhawa H, Ohishi N, Yagi K. *Anal Biochem*, 1979; **95:**351-358.
16. Kapil Soni, Milind Parle. *Neurochemical Research*, 2017
17. Ellman G. *Arch Biochem Biophys* 1959; **82:**70-73.