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RESEARCH ARTICLE

Synthesis, characterization, molecular docking evaluation, antidepressant, and anti-Alzheimer effects of dibenzylidene ketone derivatives

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Abstract

Novel bioactive compounds as synthetic analogs of the potent herbal medicines can be optimized as potential drug candidates for various neurologic disorders. This study was performed to investigate the newly synthesized dibenzylidene ketone derivatives: (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1) and (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and evaluate its potential anti-Alzheimer's and anti-depressant properties. Both the derivatives are chemically characterized by using HNMR and CNMR techniques. Auto Dock Vina program was used to investigate ligand-protein affinity. Forced swim test, tail suspension test, open field test, Y-maze test, and Morris water maze test (MWM) models were employed to evaluate anti-depressant and anti-Alzheimer's activity of dibenzylidene ketone derivatives in mice. Both A1K1 and A2K2 showed high binding affinities against various proteins involved in depression and Alzheimer's mechanisms like monoamine oxidase B, acetylcholinesterase, norepinephrine transporter 2, serotonin transporter, dopamine receptor, serotonin receptor modulator, and beta-amyloid targets. A1K1 and A2K2 dose-dependently (0.1–1 mg/kg) decreased immobility time, while increased swimming and climbing time of mice in forced swim test (FST). A1K1 and A2K2 decreased animal immobility time in TST. In the open field test, both A1K1 and A2K2 increased the number of ambulations and rearings. A1K1 and A2K2 dose-dependently (0.5–1.0 mg/kg) increased spontaneous alternation behavior (%) and the number of entries of mice in Y-maze test. In the MWM test, A1K1 and A2K2 decreased escape latency time. Overall, both *in-silico* and in-vivo investigations of A1K1 and A2K2, report their therapeutic potential for antidepressant and anti-Alzheimer properties. Hence, these compounds possess potent neuroprotective properties and may be further evaluated for their therapeutic potential in various neurological disorders.

KEYWORDS

anti-Alzheimer, anti-depressant, dibenzylidene ketone derivatives

1 | INTRODUCTION

Depression is a neuro-psychological disease that affects interest, thoughts, behavior, mental, and even physical health of a person. Some common symptoms may include change in actions, loss of self-confidence, loss of appetite, lack of interest in routine works, and disturbed sleep (Rang, Dale, Ritter, & Moore, 2003). Depression can be either develop primarily or secondarily to some serious conditions like cancer, diabetes, heart diseases, and Alzheimer (Cassano & Fava, 2002). A number of mechanisms have been involved in depression, like alteration in serotonin and dopamine levels, release of acetylcholine and increase concentration of monoamine oxidases. It has been stated that most of the available pharmacological treatments for depression work through altering monoaminergic transmission (Berton & Nestler, 2006). Furthermore, according to Krishnan and Nestler (2011), the understandings about the pathogenesis of altered mood, impaired concentration, and neurovegetative symptoms in major depression have come from animal models. Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by progressive loss of memory, deterioration of virtually all intellectual functions, increased apathy, decreased speech function, disorientation, and gait irregularities (Akina, Thati, & Puchchakayala, 2013). AD is associated with neuronal loss and the deposition of abnormal proteins in the form of amyloid plaques and neurofibrillary tangles. Research studies have shown that deposition of the soluble oligomers of β-amyloid harm excitatory synaptic functions which cause learning and memory defects. In addition, it is also believed that amyloid β peptide stimulates hyperphosphorylation of Tau protein and causes excessive neuronal cell death (Kontsekova, Zilka, Kovacech, Skrabana, & Novak, 2014). Different studies suggest that β-amyloid induces oxidative stress in cells which cause neurodegenerative disorders. RNA oxidation, a by-product of glycation, oxidation of lipid and free carbonyls induce some changes in the cell which are responsible for cognitive decline (Cioanca, Hritcu, Mihasan, & Hancianu, 2013).

The present study was conducted to investigate the potential therapeutic properties of the newly synthesized dibenzylidene ketone derivatives. These derivatives have been determined as a synthetic analogue of curcumin. A number of research bodies have reported potential pharmacologic activities of curcumin including antitumor, antiviral, anti-inflammatory, antibacterial, antifungal, and antioxidant properties (Shen & Ji, 2007). Ishrat et al., 2009 reported that curcumin inhibuits oxidative damage and improve cognitive dysfunction (Ishrat et al., 2009). In view of previous literature, the dibenzylidene ketone derivatives, that is, (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1) and (1E, 4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) were synthesized and chemically characterized by the spectroscopic method. Furthermore, both A1K1 and A2K2 were investigated for in-silico and in-vivo antidepressant and anti-Alzheimer's potential, using different computational tools and pharmacological assays. Chemical structures of A1K1 and A2K2 in the 2D format are shown in Figure 1a.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Donepezil and fluoxetine hydrochloride were obtained from Sigma Chemicals., St. Louis, MO. Benzaldehyde, cyclohexanone, dimethyl sulfoxide (DMSO), and ethanol were purchased from Merck Millipore., Billerica, MA. All chemicals were of analytical grade.

2.2 | Animals

Balb-C mice of either sex (25–30 g) housed at controlled temperature (22–25°C) and maintained on a 12 hr light/dark cycle with standard diet and water ad libitum. All the animals were randomly divided into five groups: a saline-treated group, three test-drugs treated groups (each receiving the various dose of the test compounds that is, A1K1 and A2K2) and a reference-drug treated group. Respective behavioral studies were performed to different groups to observe the antidepressant and anti-Alzheimer effects of A1K1 and A2K2. The experiments were performed according to the rulings of Institute of Laboratory

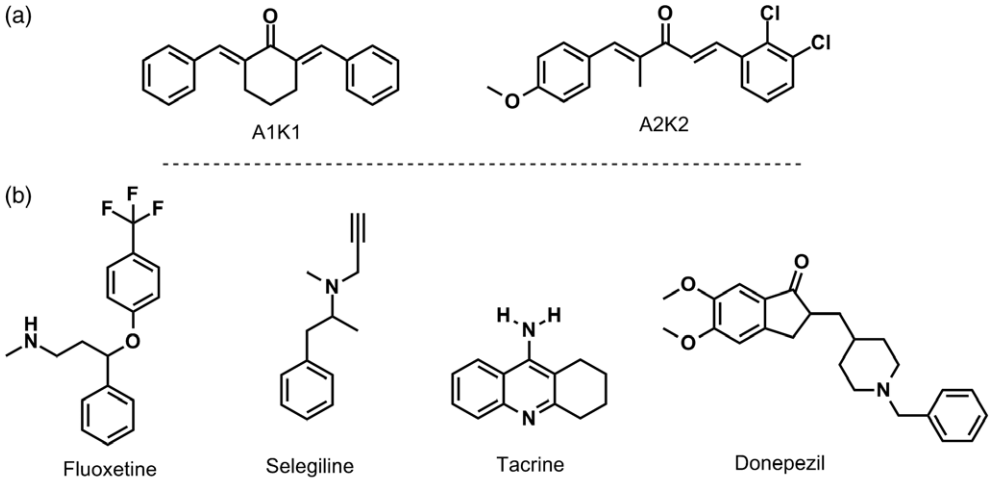

FIGURE 1 Chemical structures of compound (a) (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2), (b) chemical structures of reference drugs: Fluoxetine, selegiline, tacrine and donepezil in 2D format

Animal Resource, Commission on Life Sciences University, National Research Council (1996), approved by Ethical Committee of Riphah Institute of Pharmaceutical Sciences, Riphah International University (Ref No. REC/RIPS/2016/11).

2.3 | Synthesis of A1K1 and A2K2

Symmetrical diarylidene products (A1K1) was synthesized by the reaction of benzaldehyde with cyclohexanone in a 50 mL two-necked round-bottom flask in a ratio of 2:1 M. Dry HCl gas was passed from the content of the flask till it was saturated and turns to red color. The reaction mixture was stirred for 5–10 hr. The crude product was diluted with toluene and washed with $NaHSO_3$ solution. The organic layer was separated, dried with anhydrous Na_2SO_4 and evaporated under low pressure. The residue, when distilled under reduced pressure yielded pure compounds A1K1, which was recrystallized from ethanol as shown in Figure 2a. Chemical characterization was carried out based on the analysis of spectroscopic and crystallographic data. For the synthesis of A2K2, solution of intermediate compound 1 and 2,3-dichlorobenzaldehydes in ethanol (5 mL) was stirred for 5 min at room temperature after which sodium hydroxide solution in ethanol (4 mL, 50 mM) was added and stirring was continued until the reaction was complete. The solvent ethanol was evaporated under reduced pressure. The residue was dissolved in ethyl acetate extracted with $NaHSO_3$ solution and dried with Na_2SO_4, the solvent was evaporated using a rotary evaporator and the crude product was collected as a yellow precipitate which was further purified by column chromatography and recrystallized from ethanol as shown in Figure 2b. Chemical characterization was carried out based on the analysis of spectroscopic and crystallographic data.

2.4 | Docking studies

Auto Dock Vina program used to investigate ligand-protein affinity (Trott & Olson, 2010). 3D-structures of target proteins were taken from the protein data bank, RCSB PDB (http://www.rcsb.org/pdb/).The target proteins involved in depression pathways are serotonin transporter 2 (SERT2, PDB-ID: 5I6Z), serotonin transporter 3 (SERT3, PDB-ID: 5I6X), norepinephrine transporter 1 (NET1, PDB-ID: 5IPB), norepinephrine transporter 2 (NET2, PDB-ID: 4XJM), norepinephrine transporter 3 (NET3, PDB-ID: 4X8C), dopamine transporter (DAT, PDB-ID: 2Q73), monoamine oxidase B (MAO-B, PDB-ID: 2 V61), *N*-methyl-D-aspartate (NMDA, PDB-ID: 5VIH) receptor and serotonin receptor modulator (SRM, PDB-ID: 4IAQ). The target proteins involved in Alzheimer pathways include monoamine oxidase B (MAO-B, PDB-ID: 2V61), *N*-methyl-D-aspartate (NMDA, PDB-ID: 5VIH) receptor, beta-secretase (PDB-ID: 1M4H), butyrylcholinesterase (BChE, PDB-ID: 1P0M), beta-amyloid (PDB-ID: 2Z5Y), acetylcholinesterase (AChE, PDB-ID: 4PQE), and gamma-secretase (PDB-ID: 4R12). The structure of standard drug molecules were downloaded from pubchem data base (https://pubchem.ncbi.nlm.nih.gov/search/). Reference drugs used were fluoxetine (PubChem CID: 62857), selegiline (PubChem CID: 26758), tacrine (PubChem CID: 2723754), and donepezil (PubChem CID: 5741), as shown in Figure 1b. All these structures were downloaded in.xml format and converted to PDB format via Open Babel JUI software. PDB format of standard, a ligand as well as target proteins were converted to PDBQT via AutoDockTools (Version1.5.6 Sep_17_14). A1K1 and A2K2 along with protein targets were loaded in software named as PyRx and then docked against respective targets. For post docking interaction, Biovia Discovery Studio Visualizer (DSV) 2016 was used for a number of hydrogen bonds (classical and nonclassical) and binding amino acid residues: alanine (ALA), arginine (ARG), asparagine (ASN), aspartic acid (ASP), cysteine (CYS), glutamic acid (GLU), glycine (GLY), methionine (MET), serine (SER), threonine (THR), tyrosine (TYR), lysine (LYS), phenylalanine (PHE), and proline (PRO).

2.5 | Antidepressant activity

2.5.1 | Forced swim test (FST)

Antidepressant potential of A1K1 and A2K2 was determined by forced swim test (FST) model (Cryan, Valentino, & Lucki, 2005; Harquin Simplice, David Emery, & Hervé Hervé, 2014). The test model consists of a transparent glass cylinder (30 cm high × 20 cm diameter) filled with water up to 20 cm depth and maintained at 25 ± 2°C. Mice were divided into five groups (n = 5 each group) and were treated a single dose/day for seven days. Group I was used as negative control which received an intraperitoneal (i.p.) dose of normal saline (10 mL/kg). Group II, III, and IV were administered different doses of test compound (0.1, 0.5, and 1 mg/kg, respectively), while Group V

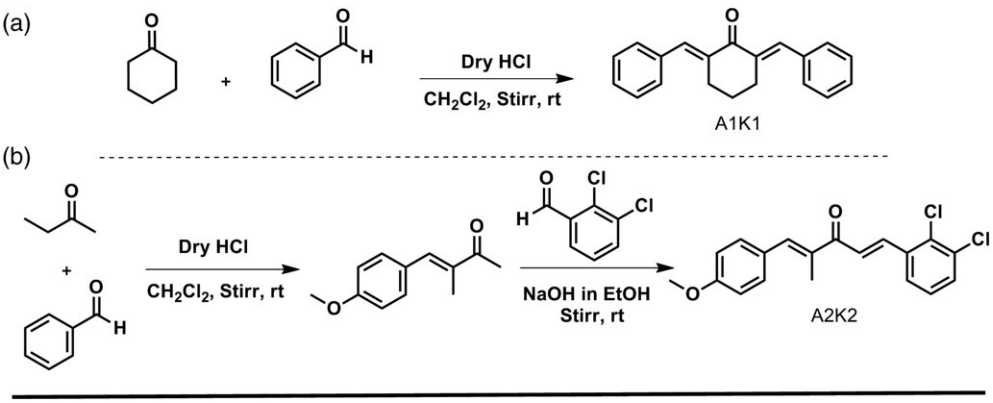

FIGURE 2 Synthesis of (a) (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1) and (b) (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2)

was assigned for the positive control (fluoxetine, 20 mg/kg). On 7th day, 30 min. Later to saline, test compounds and fluoxetine treatment, each mouse was allowed to swim in the water tank for 5 min and duration of immobility, swimming, and climbing were recorded via digital video camera. Mice were considered to be immobile when it remained floating over water without struggling, making only little movements to keep its head above water. Swimming is large horizontal movements of mice with forepaws, displacing mice body around the cylinder. Climbing is vigorous vertical movements of mice with forepaws, directed against the wall of tank, leading to the displacement of mice body around the cylinder. Decrease in immobility time and an increase in swimming and/or climbing behaviors showed antidepressant activity.

2.5.2 | Tail suspension test

Mice were divided and treated similar to that of the FST. On 7th day, 30 min later to saline, test compounds and fluoxetine treatment, mice were suspended 50 cm above floor on table's edge by adhesive tape placed about 1 cm from tip of tail. Duration of immobility was recorded for 5 min via digital video camera. Mouse was considered immobile when it becomes completely motionless and hanged passively. Decrease in immobility time showed antidepressant activity (Crowley et al., 2005).

2.5.3 | Open field test

For open field test, the apparatus was made of wooden base divided into 12 equal squares with glass walls of dimensions (50 × 25 × 50 cm). All the animals were randomly divided in five groups and treated similar to that of FST. On 7th day, 30 min later to saline, test compounds and fluoxetine treatment, each mouse was placed in corner square to explore test arena for 5 min and recorded number of ambulation's and rearings (Brown, Corey, & Moore, 1999; Jo et al., 2019; Maurmann, Reolon, Rech, Fett-Neto, & Roesler, 2011) via digital video camera. Increase in number of ambulation's and rearings showed antidepressant potential.

2.6 | Anti-Alzheimer's activity

2.6.1 | Y-maze test

In Y-maze test apparatus, each arm of the maze was 50 cm long, 20 cm high, and 10 cm wide at the bottom and top. Mice were divided into four groups, having five mice in each group and were administered a single dose/day for three days. Group I was used as negative control which received an i.p. dose of normal saline (10 mL/kg). Group II and III were treated with 0.5 and 1 mg/kg doses, respectively, of test compounds, while Group IV was assigned for positive control (donepezil, 3 mg/kg). Each mouse was placed at the center of the apparatus and allowed to move freely through the maze for three sessions (8 min each). The series of arm entries was observed via digital video camera. Spontaneous alternation was defined as the successive entry of the mice into the three arms in overlapping triplet sets. Alternation behavior percentage (%) was calculated as: (successive triplet sets [entries into three different arms consecutively]/total number of arm entries − 2) × 100 (Ali, Badshah, Kim, & Kim, 2015). Increase in the spontaneous alternation behavior (%) showed anti-Alzheimer potential.

2.6.2 | Morris water maze test (MWM)

Mice were divided into four groups (n = 5) and were treated similar to that of the Y-maze test except the treatment was prolonged to 5 days. Mice were exposed to swimming training for 60 s in the absence of the platform at day first. For four consecutive days the mouse was given trial session with the platform in place. Once the mouse located the platform, it was permitted to remain on it for 10 s. If the mice were unable to locate the platform within 120 s, it was placed on the platform for 10 s and then removed from the pool. On Day 5, mouse was individually subjected to a probe trial session in which the platform was removed from the pool. Mouse was allowed to swim for 120 s and the escape latency time was determined (Morris, 1984; Vorhees & Williams, 2006). Decrease in escape latency time showed anti-Alzheimer potential.

2.7 | Acute toxicity

Acute toxicity test of the dibenzylidene ketone derivatives is carried out according to the previously determined procedure (Adil et al., 2018; Chen et al., 2009). Mice were kept on overnight fasting and divided into four groups with each containing five animals. Group I was assigned as a saline group (10 mL/kg). Group II, III, and IV were utilized to administer each test compound at doses of 3, 5, and 10 mg/kg, respectively. All the animals were kept under observation for 24 hr to determine the symptoms of toxicity and mortality.

2.8 | Statistical analysis

Data expressed as Mean ± standard error of mean (*SEM*). The results were analyzed using one-way analysis of variance (ANOVA), followed by post hoc Tukey's test. $p < 0.05$ was noted as significantly different. The bar-graphs were analyzed using the Graph Pad Prism (Graph-Pad, San-Diego, CA).

3 | RESULTS

3.1 | Spectral analysis of A1K1

Mp: 118–119°C; 117–118.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 2H), 7.47 (d, J = 7.4 Hz, 4H), 7.45–7.38 (m, 4H), 7.38–7.31 (m, 2H), 2.94 (td, J = 6.6, 2.0 Hz, 4H), 1.84–1.74 (m, 2H);^{13}C NMR (400 MHz, $CDCl_3$) δ 190.47(1C), 137.04(2C), 136.03(2C), 136.09(2C), 130.48(2C), 128.70(2C), 128.49(2C), 28.56(6C), 23.12 (2C).

3.2 | Spectral analysis of A2K2

Percent yield: 79. m.p.: 119–121°C. 1H NMR (400 MHz, CDCl3) δ ppm: 8.02 (d, J = 15.7 Hz, 1H); 7.61 (dd, J = 7.9, 1.1 Hz, 1H); 7.55 (s, 1H), 7.51–7.42 (m, 3H); 7.35 (d, J = 15.7 Hz, 1H); 7.24 (d, J = 8.0 Hz, 1H); 6.96 (d, J = 8.8 Hz, 2H); 3.86 (s, 3H); 2.21 (d, J = 1.2 Hz, 3H). 13C NMR (400 MHz, CDCl3) δ ppm: 192.42 (1C, CO); 160.26 (1C, ArC); 139.91 (1C, =C); 138.89 (1C, =C); 136.49 (1C, =C); 136.30 (C, ArC); 134.15 (1C, ArC); 131.88 (2C, ArC); 131.34 (1C, =C); 128.47 (1C, ArC); 127.56 (1C, ArC); 127.47 (1C, ArC); 126.27 (1C, ArC); 125.96 (C, ArC); 114.23 (2C, ArC); 55.51 (1C, OCH3); 13.89 (1C, CH3). HRMS ESI(+): calcd for C19H16Cl2O2Na (M + Na) 369.0527, found 369.0381.

3.3 | Docking evaluation

The 3D pattern of the derivative compounds showing the maximum binding interactions formed between ligands and amino acid residues are shown in Figures S1–S14. Docking against SERT2, A1K1 showed *E*-value −10.1 kcal/mol and formed two-hydrogen bonds with THR-497 and TYR-175. A2K2 showed *E*-value of −7.3 kcal/mol and formed 1 hydrogen bond with ALA-150 and SER-142. Fluoxetine showed *E*-value of −6.8 kcal/mol and formed one-hydrogen bond with ARG-104. The hydrogen bonding of A1K1, A2K2, and fluoxetine with SERT2 are shown in Figure S1. Against SERT3, A1K1 showed *E*-value of −9.5 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −9.1 kcal/mol and formed two hydrogen bonds with THR-497, TYR-175, and GLU-493. Fluoxetine showed *E*-value of −7.1 kcal/mol and formed no hydrogen bond. The hydrogen bonding of A1K1, A2K2, and fluoxetine with SERT3 are shown in Figure S2. Against NET1, A1K1 showed *E*-value of −8.4 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value −7.0 kcal/mol and formed one-hydrogen bond with SER-107. Reboxetine showed *E*-value of −6.5 kcal/mol and formed no hydrogen bond. The hydrogen bonding of A1K1, A2K2, and fluoxetine with NET1 are shown in Figure S3. Against NET2, A1K1 showed *E*-value of −10.9 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −9.8 kcal/mol and formed no hydrogen bond. Reboxetine showed *E*-value of −9.2 kcal/mol and formed three-hydrogen bonds with GLY-93, PHE-92, and PRO-401. The hydrogen bonding of A1K1, A2K2, and fluoxetine with NET2 are shown in Figure S4. Against NET3, A1K1 showed *E*-value of −8.5 kcal/mol and formed one-hydrogen bond. A2K2 showed *E*-value of −8.2 kcal/mol and formed one hydrogen bond with ASN-585 and ALA-581. Fluoxetine showed *E*-value of −7.3 kcal/mol and formed one-hydrogen bond with ALA-581. The hydrogen bonding of A1K1, A2K2, and fluoxetine with NET3 are shown in Figure S5. Against DAT, A1K1 showed *E*-value of −8.9 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −8.9 kcal/mol and formed two-hydrogen bonds with TYR-6 and TYR-65. Fluoxetine showed *E*-value of −8.5 and formed two-hydrogen bonds with LYS-84 and TYR-60. The hydrogen bonding of A1K1, A2K2, and fluoxetine with DAT are shown in Figure S6. Against MAO-B, A1K1 showed *E*-value of −10.8 kcal/mol and formed one-hydrogen bond with GLY-58 and MET-436. A2K2 showed *E*-value of −10.4 kcal/mol and formed no hydrogen bond. Selegiline showed *E*-value of −7.4 kcal/mol and formed no hydrogen bond. The hydrogen bonding of A1K1, A2K2, and selegiline with MAO-B are shown in Figure S7. Against NMDA, A1K1 showed *E*-value of −7.9 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −8.3 kcal/mol and formed one-hydrogen bond with TYR-214. Tacrine showed *E*-value of −7.4 kcal/mol and formed no hydrogen bond. The hydrogen bonding of A1K1, A2K2, and tacrine with NMDA are shown in Figure S8. Against SRM, A1K1 showed *E*-value of −9.8 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −8.9 kcal/mol and formed one-hydrogen bond with TYR-359. Fluoxetine showed *E*-value of −7.9 kcal/mol and formed one-hydrogen bond with SER-212 and THR-209. The hydrogen bonding of A1K1, A2K2, and fluoxetine with SRM are shown in Figure S9. Against beta-secretase, A1K1 showed *E*-value of −8.4 kcal/mol and formed one-hydrogen bond with ASP-62. A2K2 showed *E*-value of −7.7 kcal/mol and formed one-hydrogen bond with ASP-62. Donepezil showed *E*-value of −8.3 kcal/mol and formed one-hydrogen bond with TYR-124. The hydrogen bonding of A1K1, A2K2, and donepezil with beta-secretase are shown in Figure S10. Against BChE, A1K1 showed *E*-value −9.1 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −8.6 kcal/mol and formed no hydrogen bond. Donepezil showed *E*-value of −9.5 kcal/mol and formed no hydrogen bond. The hydrogen bonding of A1K1, A2K2, and tacrine with BChE are shown in Figure S11. Against beta amyloid, A1K1 showed *E*-value of −9.1 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −7.9 kcal/mol and formed no hydrogen bond. Donepezil showed *E*-value of −11.3 kcal/mol and formed one-hydrogen bond with CYS-406. The hydrogen bonding of A1K1, A2K2, and donepezil with beta amyloid are shown in Figure S12. Against AChE, A1K1 showed *E*-value of −9.3 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −10.2 kcal/mol and formed one-hydrogen bond with TYR-341. Donepezil showed *E*-value of −8.3 and formed one-hydrogen bond with TYR-124. The hydrogen bonding of A1K1, A2K2, and donepezil with AChE are shown in Figure S13. Against gamma secretase, A1K1 showed *E*-value of −8.4 kcal/mol and formed no hydrogen bond. A2K2 showed *E*-value of −7.3 kcal/mol and formed one-hydrogen bond with ASN-132. Donepezil showed *E*-value of −8.2 kcal/mol and formed two-hydrogen bonds with ASN-132 and ASP-127. The hydrogen bonding of A1K1, A2K2, and donepezil with gamma secretase are shown in Figure S14. The results of *E*-value, hydrogen bonds and binding residues of A1K1 and A2K2 with target proteins involved in depression pathways (SERT2, SERT3, NET1, NET2, NET3, DAT, MAO-B, NMDA, and SRM) along with standard drugs are shown in Table 1. The results of *E*-value, hydrogen bonds and binding residues of A1K1 and A2K2 with target proteins involved in Alzheimer's process (MAO-B, NMDA, beta secretase, BChE, beta amyloid, AChE, and gamma secretase) along with standard drugs are shown in Table 2.

3.4 | Effect on immobility, swimming, and climbing time

A1K1 dose-dependently (0.1–1 mg/kg) decreased immobility time, while increased swimming and climbing time in FST. The immobility

time noted for saline (10 mL/kg), A1K1 (0.1 mg/kg), A1K1 (0.5 mg/kg), A1K1 (0.1 mg/kg) and fluoxetine (20 mg/kg) treated groups were 150.4 ± 4.10 s, 64.80 ± 6.36 s ($p < 0.001$), 47.60 ± 3.26 s ($p < 0.001$), 23.80 ± 2.81 s ($p < 0.001$) and 63.60 ± 5.19 s ($p < 0.001$), respectively. The swimming time recorded for saline (10 mL/kg), A1K1 (0.1 mg/kg), A1K1 (0.5 mg/kg), A1K1 (1 mg/kg), and fluoxetine (20 mg/kg) treated groups were 90.0 ± 3.58 s, 170.2 ± 8.43 s ($p < 0.001$), 180.4 ± 4.96 s ($p < 0.001$), 194.6 ± 3.07 s ($p < 0.001$), and 168.60 ± 5.27 s ($p < 0.001$), respectively. Similarly the climbing time noted for saline (10 mL/kg), A1K1 (0.1 mg/kg), A1K1 (0.5 mg/kg), A1K1 (1 mg/kg), and fluoxetine (20 mg/kg) treated groups were 59.6 ± 4.90 s, 67.2 ± 4.46 s ($p > 0.05$), 72.0 ± 3.61 s ($p > 0.05$), 82.40 ± 4.17 s ($p < 0.01$), and 68.4 ± 3.15 s ($p > 0.05$), respectively (Figure 3a).

Similarly, A2K2 dose-dependently (0.1–1 mg/kg) decreased immobility time, while increased swimming and climbing time. In saline (10 mL/kg) treated group, immobility, swimming and climbing time was 172.0 ± 4.37, 87.8 ± 4.00, and 40.2 ± 4.32 s, respectively. In A2K2 (0.1 mg/kg) treated group, immobility time was 99.20 ± 4.87 s ($p < 0.001$), swimming time was 154.2 ± 6.01 s ($p < 0.001$), and climbing time was 46.6 ± 4.92 s ($p > 0.05$ vs. saline group). In A2K2 (0.5 mg/kg) treated group, immobility time reduced to 54.60 ± 7.46 s ($p < 0.001$ vs. saline group), while swimming and climbing time increased to 181.6 ± 4.66 s ($p < 0.001$ vs. saline group) and 61.8 ± 6.82 s ($p < 0.001$ vs. saline group), respectively. In A2K2 (1 mg/kg) treated group, immobility time reduced to 39.80 ± 7.65 s ($p < 0.001$ vs. saline group), while swimming and climbing time increased to 191.0 ± 7.12 s ($p < 0.001$ vs. saline group), and 69.20 ± 5.48 s ($p < 0.001$ vs. saline group), respectively. In fluoxetine (20 mg/kg) treated group, immobility, swimming and climbing time were 63.60 ± 5.19, 181.60 ± 4.19, and 54.8 ± 3.87 s ($p < 0.001$ vs. saline group), respectively (Figure 3b).

3.5 | Effect on immobility time

Similar to FST, our tail suspension test (TST) results also showed dose-dependent decrease in immobility time. The immobility time noted for saline (10 mL/kg), A1K1 (0.1 mg/kg), A1K1 (0.5 mg/kg), A1K1 (0.1 mg/kg), and fluoxetine (20 mg/kg) treated groups were 154.8 ± 7.86 s, 72.6 ± 3.50 s, 42.60 ± 1.16 s, 30.40 ± 1.66 s, and 62.40 ± 3.12 s, respectively (Figure 4a). The immobility time recorded for saline (10 mL/kg), A2K2 (0.1 mg/kg), A2K2 (0.5 mg/kg), A2K2 (0.1 mg/kg), and fluoxetine (20 mg/kg) treated groups were 166.0 ± 6.30 s, 84.4 ± 3.97 s, 62.80 ± 4.38 s, 53.0 ± 8.03 s, and 60.60 ± 3.40 s, respectively (Figure 4b**)**. All the results were highly significant ($p < 0.001$ vs. saline group).

3.6 | Effect on the number of ambulations and rearings

Both A1K1 and A2K2 showed a significant increase in mice locomotor activity that is, number of ambulations and rearings in open field test. In saline (10 mL/kg) treated group, number of ambulations and rearings recorded were 57.4 ± 5.91 and 18.4 ± 0.81, respectively. Treatment with A1K1 (0.1 mg/kg), A1K1 (0.5 mg/kg), A1K1 (1 mg/kg), and fluoxetine (20 mg/kg) increase the numbers of ambulations to 83.40 ± 3.93, 102.80 ± 3.98, 115.6 ± 4.95, and 83.4 ± 3.47, respectively, while increase the number of rearings to 28.60 ± 2.76, 31.8 ± 2.67,

TABLE 1 *E*-value (kcal/Mol) and post-docking analysis of best pose of (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), (1E, 4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and standard drugs against serotonin transporter 2 (SERT2), serotonin transporter 3 (SERT3), norepinephrine transporter 1 (NET1), norepinephrine transporter 2 (NET2), norepinephrine transporter 3 (NET3), dopamine transporter (DAT), monoamine oxidase B (MAO-B), *N*-methyl-D-Aspartate (NMDA) receptor, and serotonin receptor modulator (SRM)

	A1K1			A2K2			Standard drugs			
Target	*E*-value	No of H-bonds	Binding residues	*E*-value	No of H-bonds	Binding residues	Standard	*E*-value	No of H-bonds	Binding residues
SERT2	−10.1	2	THR-197, TYR-175	−7.3	1	ALA-150, SER-142	Fluoxetine	−6.8	1	ARG-104
SERT3	−9.5	0	0	−9.1	2	THR-497, TYR-175, GLU-493	Fluoxetine	−7.1	0	0
NET1	−8.4	0	0	−7.0	1	SER-107	Fluoxetine	−6.5	0	0
NET2	−10.9	0	0	−9.8	0	0	Fluoxetine	−9.2	3	GLY-93, PHE-92, PRO-401
NET3	−8.5	1	0	−8.2	1	ASN-585, ALA-581	Fluoxetine	−7.3	1	ALA-581
DAT	−8.9	0	0	−8.9	2	TYR-6, TYR-65	Fluoxetine	−8.5	2	LYS-84, TYR-60
MAO-B	−10.8	1	GLY-58, MET-436	−10.4	0	0	Selegiline	−7.4	0	0
NMDA	−7.9	0	0	−8.3	1	TYR-214	Tacrine	−7.4	0	0
SRM	−9.8	0	0	−8.9	1	TYR-359, PHE-330	Fluoxetine	−7.9	1	SER-212, THR-209

Abbreviations: ALA, alanine; ARG, arginine; ASN, asparagine; GLU, glutamic acid; GLY, glycine; LYS, lysine; MET, methionine; PHE, phenylalanine; PRO, proline; SER, serine; THR, threonine; TYR, tyrosine.

TABLE 2 *E*-value (kcal/mol) and post-docking analysis of best pose of (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), (1E, 4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and standard drugs against monoamine oxidase B (MAO-B), *N*-methyl-D-aspartate (NMDA) receptor, beta secretase, butyrylcholinestrase (BChE), beta amyloid, acetylcholinestrase (AChE), and gamma secretase

	A1K1			A2K2			Standard drugs			
Target	*E*-value	No of H-bonds	Binding residues	*E*-value	No of H-bonds	Binding residues	Standard	*E*-value	No of H-bond	Binding residues
MAO-B	−10.8	1	GLY-58 MET-436	−10.4	0	0	Selegiline	−7.4	0	0
NMDA	−7.9	0	0	−8.3	01	TYR-314	Tacrine	−7.4	0	0
Beta secretase	−8.4	1	ASP-62	−7.7	01	ASP-62	Donepezil	−8.3	02	ARG-61, TYR-320
BChE	−9.1	0	0	−8.6	0	0	Donepezil	−9.5	0	0
Beta amyloid	−9.1	0	0	−7.9	0	0	Donepezil	−11.3	1	CYS-406
AChE	−9.3	0	0	−10.2	01	TYR-341	Donepezil	−8.3	1	TYR-124
Gamma secretase	−8.4	0	0	−7.3	01	ASN-132	Donepezil	−8.2	02	ASN-132, ASP-127

Abbreviations: ARG, arginine; ASN, asparagine; ASP, aspartic acid; CYS, cysteine; MET, methionine; TYR, tyrosine.

39.0 ± 1.41, and 29.0 ± 1.81, respectively (Figure 5a). In accordance with A1K1, treatment with A2K2 (0.1 mg/kg), A2K2 (0.5 mg/kg), A2K2 (1 mg/kg), and fluoxetine (20 mg/kg) increase the numbers of ambulations to 85.40 ± 13.4, 101.4 ± 8.28, 123.4 ± 4.61, and 88.2 ± 3.33, respectively, while increase the number of rearings to 26.4 ± 3.14, 32.4 ± 3.09, 42.6 ± 3.07, and 30.2 ± 3.44, respectively (Figure 5b). All the results were highly significant ($p < 0.001$ vs. saline group).

3.7 | Effect on alternation behavior

The Y-maze task was performed to analyze the spatial working memory using spontaneous alteration behavior (%). Both A1K1 and A2K2 dose-dependently (0.5-1 mg/kg) increased spontaneous alternation behavior (%) of mice in the Y-maze test. Percent spontaneous alternation behavior was recorded for saline (10 mL/kg), A1K1 (0.5 mg/kg), A1K1 (1 mg/kg), and Donepezil (3 mg/kg) treated groups as 48.0 ± 2.34%, 63.40 ± 1.86%, 72.4 ± 1.63, 65.0 ± 2.38%, respectively (Figure 6a). All the results were highly significant ($p < 0.001$ vs. saline group). Similarly A2K2 (0.5 mg/kg) and A2K2 (1 mg/kg) treated group showed increased alternation behavior of 57.0 ± 1.58% ($p > 0.05$ vs. saline group) and 78.6 ± 3.77% ($p < 0.001$ vs. saline group), respectively. Donepezil (3 mg/kg) treated group showed alternation behavior of 64.8 ± 1.90% ($p < 0.01$ vs. saline group; Figure 6b).

3.8 | Effect on number of entries

A1K1 dose-dependently (0.5–1 mg/kg) increased number of entries in Y-maze test. In saline (10 mL/kg) treated group, number of entries on Day 1, 2, and 3 were 21.4 ± 1.43, 18.4 ± 1.12, and 17.0 ± 0.70, respectively. In A1K1 (0.5 mg/kg) treated group, number of entries on Day 1, 2, and 3 increased to 28.2 ± 1.35, 25.8 ± 1.24, and 24.2 ± 1.39 ($p < 0.01$ vs. saline group), respectively. In A1K1 (1 mg/kg) treated group, number of entries on Day 1, 2, and 3 increased to 31.2 ± 1.02, 28.2 ± 1.39, and 26.4 ± 1.43 ($p < 0.001$ vs. saline group), respectively. In donepezil (3 mg/kg) treated group, number of entries on Day 1, 2, and 3 recorded were 26.0 ± 1.00, 23.0 ± 0.70, and 20.6 ± 0.51 ($p > 0.05$ vs. saline group), respectively (Figure 7a). A2K2 dose-dependently (0.5–1 mg/kg) increased number of entries. In saline (10 mL/kg) treated group, numbers of entries on Day 1, 2, and 3 were 20.4 ± 0.87, 18.0 ± 0.89, and 16.8 ± 0.73, respectively. In A2K2

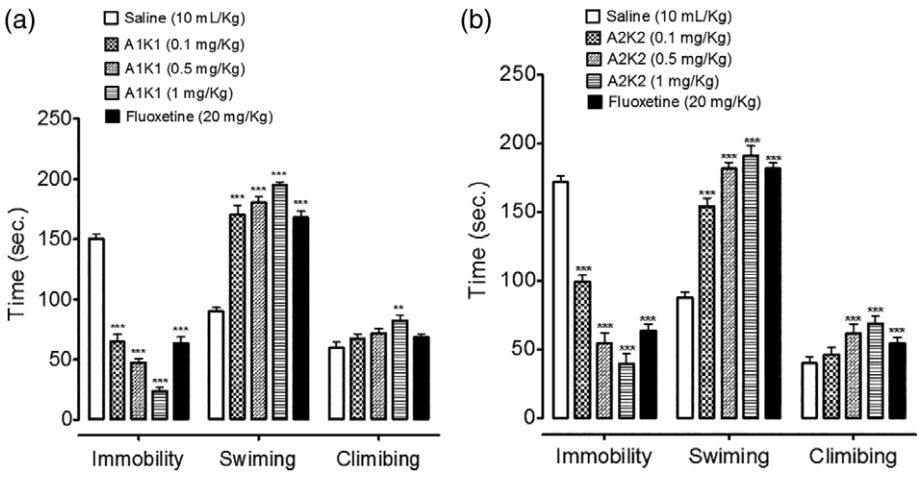

FIGURE 3 Bar-graph showing anti-depressant effect of (a) (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), (b) (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and fluoxetine on immobility, swimming and climbing time of mice in forced swim test. Data expressed as mean ± *SEM*, $n = 5$. $^{**}p < 0.01$, $^{***}p < 0.001$ versus saline group, one-way ANOVA followed by posthoc Tukey's test. ANOVA, analysis of variance

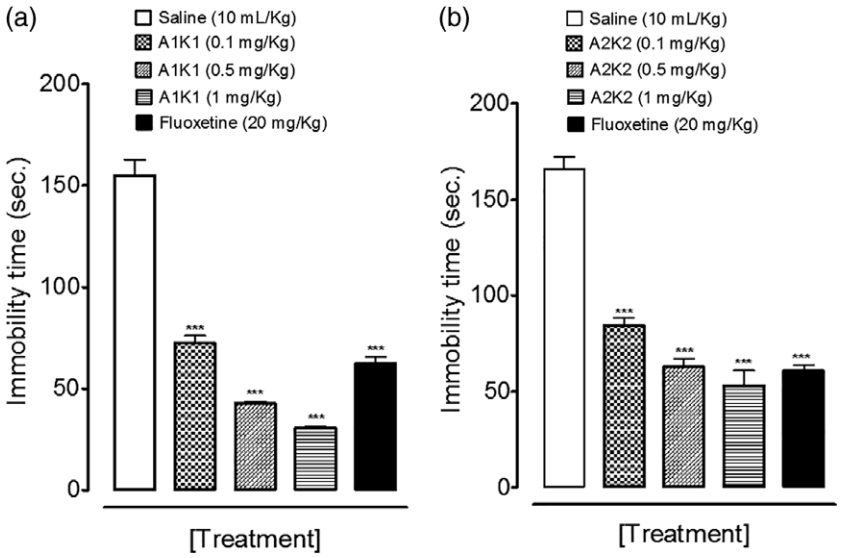

FIGURE 4 Bar-graph showing anti-depressant effect of (a) (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), (b) (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and fluoxetine on immobility time of mice in tail suspension test. Data expressed as mean ± *SEM*, $n = 5$. $^{***}p < 0.001$ versus saline group, one-way ANOVA followed by posthoc Tukey's test. ANOVA, analysis of variance

(0.5 mg/kg) treated group, number of entries on Day 1, 2, and 3 increased to 24.8 ± 1.15, 21.8 ± 0.86, and 20.2 ± 0.89 ($p > 0.05$ vs. saline group), respectively. In A2K2 (1 mg/kg) treated group, number of entries on Day 1, 2, and 3 increased to 33.2 ± 1.28, 30.0 ± 1.04, and 28.0 ± 1.43 ($p < 0.001$ vs. saline group), respectively. In donepezil (3 mg/kg) treated group, number of entries on Day 1, 2, and 3 recorded were 25.6 ± 1.07, 23.0 ± 0.89 and 21.2 ± 1.02 ($p < 0.05$ vs. saline group), respectively (Figure 7b).

3.9 | Effect on escape latency

In MWM, escape latency time for A1K1 and A2K2 was performed in three trials. Our results showed that both A1K1 and A2K2 dose-dependently (0.5–1 mg/kg) decreased escape latency time as compared to the saline-treated group. The escape latency time noted for A1K1 in the final trial in saline (10 mL/kg), A1K1 (0.5 mg/kg), A1K1 (1 mg/kg) and donepezil (3 mg/kg) treated groups was, 19.9 ± 1.10, 13.15 ± 1.14, 8.3 ± 0.63, and 15.2 ± 0.54, respectively (Figure 8a). Similarly the escape latency time recorded for A2K2 in the final trial in saline (10 mL/kg), A1K1 (0.5 mg/kg), A1K1 (1 mg/kg), and donepezil (3 mg/kg) treated groups was 20.8 ± 0.66, 14.4 ± 1.40, 07.8 ± 0.97, and 13.6 ± 0.81, respectively (Figure 8b). All the results were highly significant ($p < 0.01$ vs. saline group).

3.10 | Acute toxicity analysis

Compound A1K1 and A2K2 were found safe up to the dose of 10 mg/kg, as no mortality was caused at the given doses. Furthermore, there was not any kind of detrimental effect observed on overall health during the experimental studies.

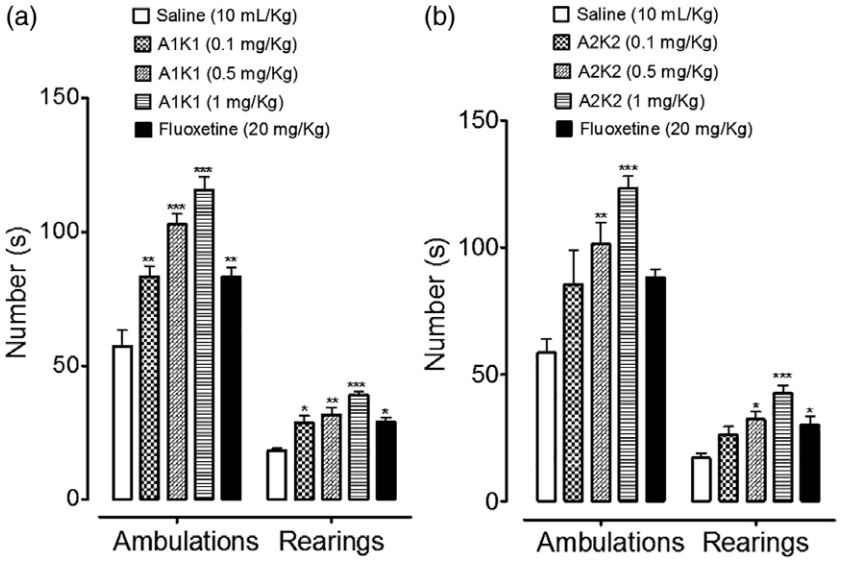

FIGURE 5 Bar-graph showing anti-depressant effect of (a) (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), (b) (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and fluoxetine on ambulations and rearings of mice in open field test. Data expressed as mean ± *SEM*, $n = 5$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus saline group, one-way ANOVA followed by posthoc Tukey's test. ANOVA, analysis of variance

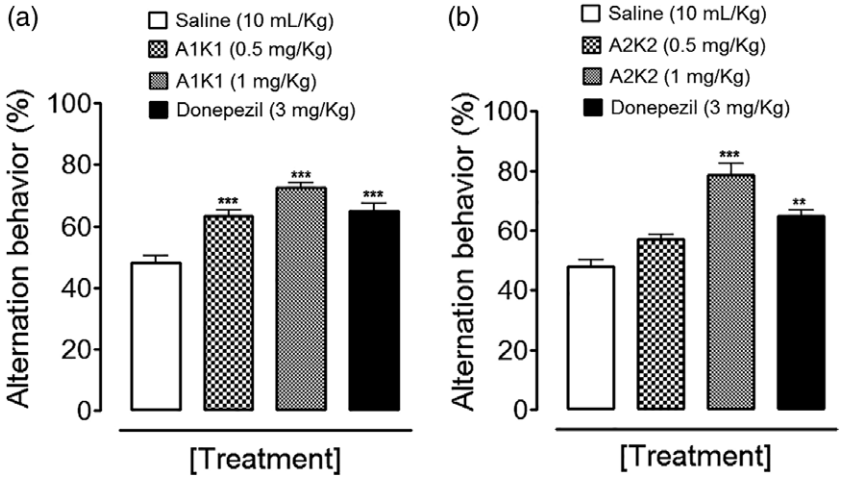

FIGURE 6 Bar-graph showing anti-Alzheimer's effect of **(a)** (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), **(b)** (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and donepezil on alternations behavior of mice in Y-maze test in mice. Data expressed as mean ± *SEM*, $n = 5$. $^{**}p < 0.01$, $^{***}p < 0.001$ versus saline group, one-way ANOVA followed by posthoc Tukey's test. ANOVA, analysis of variance

4 | DISCUSSION

In this study, we first assessed the affinity of dibenzylidene ketone derivatives (A1K1 and A2K2) through *E*-value and number of hydrogen bonds against protein targets that influence antidepressant and anti-Alzheimer effects. Docking tool was preliminary used to check the affinity of ligands to their respective protein targets. Hydrogen bonding is reported to be significant in formation of ligand protein complex (Wang, Lai, & Wang, 2002). The docking of the selected ligands was carried out by using Auto Dock Vina program through PyRx (Dallakyan & Olson, 2015). A1K1 order of binding affinity against target proteins was found as: NET2 > MAO-B > SERT-Ts2 > SRM > SERT-Ts3 > AChE > BChE > Beta amyloid > DAT > NET3 > NET1 > beta secretase > gamma secretase > NMDA. Similarly the order of binding affinity of A2K2 against target proteins was found as: MAO-B > AChE > NET2 > SERT-Ts3 > DAT > SRM > BChE > NMDA > NET3 > beta amyloid > beta secretase > SERT-Ts2 > gamma secretase > NET1. A1K1 and A2K2 showed highest binding affinity against NET2 and MAO-B, which can be correlated to its highest in-vivo antidepressant and anti-Alzheimer activities, respectively. Behavioral pharmacological techniques were employed to investigate the antidepressant and anti-Alzheimer effects of A1K1 and A2K2. FST, TST, and open field test models were used for screening of antidepressant potential (Brown et al., 1999; Maurmann et al., 2011; Simplice et al., 2014). Stressful condition plays a key role in depression. Substances that decreased immobility time in FST and TST, while increased swimming and climbing behavior in FST, showed antidepressant effects (Borsini & Meli, 1988). A1K1and A2K2, dose-dependently decreased immobility time in FST and TST, while increased swimming and climbing time in FST. Mice locomotor activity was also increased in the open field test. Swimming behavior is sensitive to serotonergic, while climbing behavior is sensitive to noradrenergic neurotransmissions (Detke, Rickels, & Lucki, 1995). So A1K1 and A2K2 have serotonergic as well as noradrenergic effects that reveal their antidepressant potential. Depression can result from reduction of monoamine neurotransmitters (norepinephrine, dopamine, and serotonin) in brain (Delgado, 2000). MAO-B is involved in catalyzing the oxidation of monoamines. Norepinephrine involved in attentiveness, learning, emotions, dreaming, and sleeping. Dopamine involved in pleasure, emotions, and locomotion regulation. Serotonin plays a major role in memory, learning, mood, and behavior. A1K1 and A2K2 modulate the levels of these neurotransmitters and reduce serotonin metabolism in mice brain, demonstrating their antidepressant potential. Antidepressant activity of A1K1 and A2K2 might be possible due to the involvement of these transporters as well as the involvement of different other pathways.

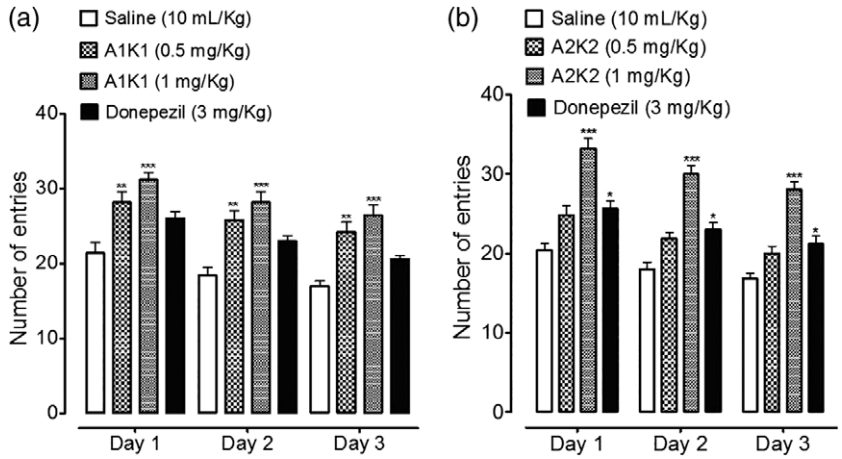

FIGURE 7 Bar-graph showing anti-Alzheimer's effect of **(a)** (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), **(b)** (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and donepezil on number of mice entries in Y-maze test on day 1, 2 and 3. Data expressed as mean ± *SEM*, $n = 5$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus saline group, one-way ANOVA followed by posthoc Tukey's test. ANOVA, analysis of variance

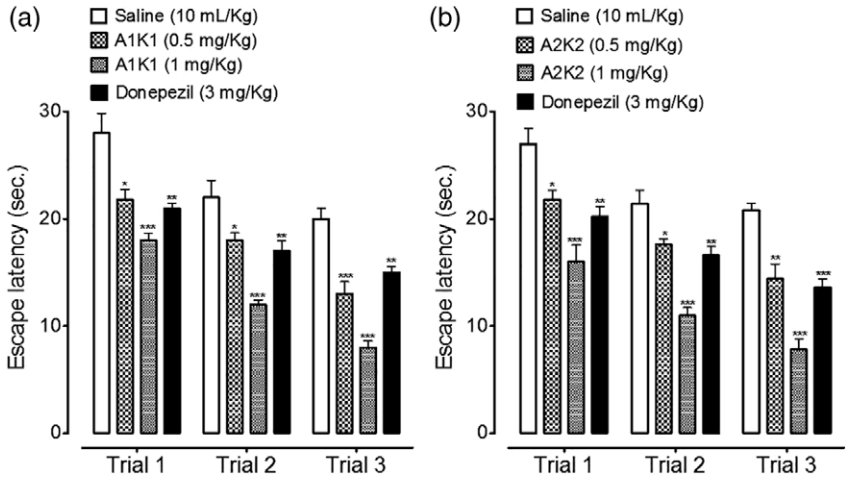

FIGURE 8 Bar-graph showing anti-Alzheimer's effect of **(a)** (2E,6E)-2,6-dibenzylidene cyclohexanone (A1K1), **(b)** (1E,4E)-5-(2,3-dichlorophenyl)-1-(4-methoxyphenyl)-2-methylpenta-1,4-diene-3-one (A2K2) and donepezil on escape latency time of mice in trial 1, 2 and 3 in Morris water maze test. Data expressed as mean ± *SEM*, $n = 5$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus saline group, one-way ANOVA followed by posthoc Tukey's test. ANOVA, analysis of variance

Next, in this study Y-maze test and MWM test models were used for the screening of anti-Alzheimer potential (Ali et al., 2015; Moris, 1984). Accumulation of amyloid plaques increased oxidative stress, neurofibrillary tangles in neurons and acetylcholine deficiency play role in AD (Hiremathad, 2017). The molecular docking results showed that these compounds possess good binding affinity against MAO-B, AChE, BChE, and beta amyloid targets, so it might be possible that the pharmacological potential of A1K1 and A2K2 is due to modulation of these targets. It has also been stated that AD is associated with progressive cognitive deterioration along with behavioral changes (Mishra & Palanivelu, 2008). Spontaneous alteration behavior (%) was tested to assess spatial working memory that is dependent upon the hippocampus (Holcomb et al., 1998; Lelong, Lhonneur, Dauphin, & Boulouard, 2003; Ohno et al., 2004). A higher percentage of spontaneous alteration behavior represents enhanced cognitive performance (Ali et al., 2015). A1K1 and A2K2 dose-dependently increased spontaneous alteration behavior (%) and a number of entries of mice in the Y-maze test. Learning and memory were also evaluated using the spatial reference version of the MWM, which is highly dependent on the hippocampus (Billings, Oddo, Green, McGaugh, & LaFerla, 2005). The MWM results showed that A1K1 and A2K2 dose-dependently decreased escape latency time which reveals their anti-Alzheimer's potential.

Overall, we can infer that our compounds have potential antidepressant and anti-Alzheimer's actions. The dibenzylidene ketone derivative A1K1 has been studied to possess lipophilic character and that they can cross the blood brain barrier (Sasaki et al., 2011). By improving bioavailability, A1K1 and A2K2 must have improved action against AD. It was observed that even at high doses all the compounds were safe, producing no mortality after 24 hr of administration. Further investigation is needed in order to confirm its permeability, stability, suitability for oral administration, pharmacodynamics and pharmacokinetic confirmation before launching these new moieties in the market.

5 | CONCLUSIONS

The present study reveals that dibenzylidene ketone derivatives, A1K1 and A2K2 showed binding affinities against different targets involved in depression and AD-like SERT2, SERT3, NET1, NET2, NET3, DAT, SRM, MAO-B, NMDA, beta-secretase, BChE, beta-amyloid, AChE, and gamma-secretase. Furthermore, it was also noted that A1K1 has more binding sites for targets involved in depression mechanism while A2K2 has more therapeutic potential to target Alzheimer's related proteins. Both A1K1 and A2K2 exhibited protective effect against Alzheimer's and depressive like behavior. Hence, our studies suggest that these dibenzylidene ketone derivatives could be intriguing therapeutic approaches for the treatment of various neurological disorders.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. M.A. carried out the computational studies, experimental work, analyzed the data and documentation. A-U.K and H.B. supervised the research project. E.F. and Z.D. synthesized dibenzylidene ketone derivatives. A.K. revised the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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