Empirical and molecular docking-based screening of heterocyclic compounds to identify potential acetylcholinesterase inhibitors to treat Alzheimer's disease and its histology

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Background
Alzheimer's disease (AD) is characterized by neuropathological symptoms, there has been no proper cure in recent era. It was linked to a deficiency in the brain neurotransmitter acetylcholine. Acetylcholinesterase is an enzyme that breaks down acetylcholine to an inactive form and the death of cholinergic neurons.
Objective
Therefore, there is a crucial need to identify alternative compounds with potential anti-cholinesterase agents and minimal undesirable effects. Fluoroquinolones and benzimidazole-benzothiazole derivatives offer antimicrobial, anti-inflammatory, anti-oxidant, anti-diabetic, and anti-Alzheimer activities.
Materials and methods
A series of fluoroquinolones and benzimidazole-benzothiazole derivatives were evaluated against acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) enzymes. For this purpose, molecular docking and adsorption, distribution, metabolism, excretion, and toxicology ADMET models were used for *in-silico* studies in addition to *in-vitro* studies, Fluoroquinolones (Z, Z3, Z4, Z6, Z8, Z12, Z15, and Z9) and benzimidazole-benzothiazole compounds (TBIS-16, TBAF-1, TBAF-2, TBAF-3, TBAF-4, TBAF-5, TBAF-6, TBAF-7, TBAF-8, and TBAF-9) passed through the AChE inhibition assay and their IC_{50} values were calculated.
Results and conclusion
The compound 1-ethyl-6-fluoro-7-(4-(2-(4-nitrophenylamino)-2-oxoethyl)piperazin-1-yl) –4-oxo-1, 4 di-hydroquinoline-3-carboxylic acid and 2-((1H-benzo[d]imidazol-2-yl)methyl)-N'-(3-bromobenzyl)-4-hydroxy-2H-thiochromene-3-carbohydrazide 1, 1-dioxide (Z-9 and TBAF-6) showed the lowest IC_{50} values against AChE/BChE (0.37±0.02/2.93±0.03 μM and 0.638±0.001/1.31±0.01 μM, respectively) than the standard drug, donepezil (3.9±0.01/4.9±0.05 μM). During the *in-vivo* investigation, behavioral trials were performed to analyze the neuroprotective impact of Z-9 and TBAF-6 compounds on AD mouse models. Hematological and histopathological parameters revealed that compounds have a safer aptitude. However heterocyclic compounds dramatically corrected the loss of neurons, neuroinflammation, neurofibrillary tangles, and degenerative changes in the brain's architecture. Also, Z-9 and TBAF-6 compounds improve behavioral and biochemical parameters hence treating neurodegenerative disorders effectively.

Keywords:
Alzheimer's diseases, heterocyclic compounds, neurofibrillary tangles, neuroinflammation, neuronal loss

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Introduction

Alzheimer's disease (AD) is a more severe neurological disorder, which steadily robs the patients and eventually kills them. Alzheimer's disease accounts for 60–70% of patients with increasing cognitive dysfunction in the elderly. In females than males, a ratio of 1.2 : 1.5 is frequent [1]. Positive lesions like amyloid plaques and angiopathies, neutral lesions like cord failure, neurofibrillary tangles, and glial responses are all neuropathological indicators of Alzheimer's disease, and negative lesions such as cord failure. Despite its intrinsic cross-sectional presence, post-mortem investigations have helped establish and improve the diagnostic criteria currently used globally to progress amyloid and invasive diseases [2,3].

Based on the starting age, AD was classified into two clinical categories. AD was a term reserved for the form of 'presenile' dementia caused by people under 65 years of age because of its initial diagnosis in a relatively young woman. In the elderly, the same disease was referred to as Alzheimer's type senile dementia, i.e., for people over 65 [4]. An estimated 3,60 000 new cases are reported each year, which equates to 980 new cases every day or 40 new cases for each hour [1]. According to dementia, AD can be classified into the following types vascular dementia, Lewy dementia, Parkinson's disease with dementia, frontal dementia, and reversible dementia [5]. Senile plates and neurofibrillary tangles (NFTs) are the most prominent and distinctive lesions within the diseased brain [6,7].

Regardless of the etiology, all people who have been diagnosed with dementia are not able to do everyday activity and take care of themselves [8]. This has a significant influence on the lives of both of their families and friends in AD patients. Patients are also highly living in incapacity because of the length of the condition [9]. The frequency of AD depends upon a variety of factors, such as age, genetics, and education. Therefore, there is no means of definitely diagnosing AD without an autopsy [10]. Likewise, although promising research and development are under progress in early identification and therapy, there is no cure for AD. The neurotransmitter acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) is primarily an inhibitor and are known to be of use in AD pathology, and secondly BChE. Both enzymes are present in the brain and are found in neurofibrillary tangles and neurotic plagues [11].

AChE catalyzes the hydrolysis of acetylcholine neurotransmitter to choline and acetic acid, a step that is required to re-activate a cholinergic neuron. AChE and cholinesterase butyryl are two cholinesterase types (BChE) [12]. Both forms vary from one substrate to another: former hydrolysis of acetylcholine quicker; butyrylcholine hydrolyzing quicker. The elevation of acetylcholine through inhibition of AChE enzymes was accepted as the most effective strategy for treatment against AD despite the unknown etiology of AD [13]. The inhibitors of AChE and BChE have therefore become the remarkable options in AD therapy. However, current medicinal products (tacrine, rivastigmine, and donepezil) with AChE-inhibiting activity have some side effects and are only effective against mild AD, and no BChE-inhibiting drugs are available to date [14].

Therefore, in order to combat AD, new drugs are compulsory to develop. AD reduces AChE enzyme activity levels, increases BChE activities, and the normal BChE and AChE ratio in the brain could change from 0.6–11% [15]. Based on these facts, it is being proposed to strengthen the treatment strategy's efficacy and extend its indications with a dual inhibition strategy for these enzymes. Since ancient times [16], heterocyclic compounds have been employed to cure many ailments. In various departments of inquiry, somewhat novel heterocyclic combinations were generated to find novel bioactive atoms [17].

Precisely, a few examination bunches have planned and combined different ligands containing, in any event, one heterocyclic framework utilizing the multi-target approach and investigating new conceivable natural targets [18]. AD is well-known as a multifactorial illness, and these mental origins imply a robust system to novel therapies can be found through the so-called 'multi-target ligands.' [19–22]. It is centered on identifying multifunctional compounds to concurrently accomplish two or more goals so that synergistic activities can be achieved and treatment efficiency improved [23]. In addition, another research group has demonstrated two series of N-heterocyclic compounds for biological activities (triazolothiadiazoles and triazolothiadiazines) [17]. These molecules showed good AChE and butyryl cholinesterase inhibition, fascinatingly (BChE). Fluoroquinolones and benzimidazole-benzothiazole derivatives offer antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and anti-Alzheimer activities. Keeping in view the importance of these heterocyclic compounds, the current research work was designed to find out potential acetylcholinesterase/butyryl cholinesterase inhibitors to treat Alzheimer's disease. The selection of these compounds based upon their novelty as there was no activity reported against these compounds.

Our unifying hypothesis was that, Empiricaland molecular docking-based screening of heterocyclic compounds to identify potential acetylcholinesterase inhibitors to treat Alzheimer's disease. Additional causes of dementia have been identified in addition to Alzheimer's disease, such as vascular dementia, which may develop after a stroke, dementia with Lewy bodies, frontotemporal lobar degeneration, Parkinson's disease (PD) dementia, and Creutzfeldt-Jakob disease [24–26]. Whatever the etiology, all people living with dementia eventually become unable to do everyday tasks and care for themselves,

necessitating assistance with all elements of daily living [27,28]. This gives people living with Alzheimer's a lot of power and has a significant influence on the lives of their family and friends. Similarly, due to the extended course of the condition, individuals are disabled for much extended periods [29].

The frequency of Alzheimer's disease varies according to various factors, including age, heredity, and educational level. There is no method to identify Alzheimer's disease without an autopsy definitively [30]. There is no cure for Alzheimer's disease, although promising early detection and therapy research and development is underway.

Materials and methods

The current study has been developed to find out the heterocyclic compound empirical and molecular modelling to locate possible acetylcholinesterase inhibitors to treat Alzheimer's disease. All animal experiments for this study performed at basic science labs of Shaqra University. This study aims to identify potent inhibitors of Alzheimer's acetyl cholinesterase/ butyryl cholinesterase.

Chemical and reagents

Most of the chemicals used in this research were purchased. Dimethyl sulfoxide (DMSO) is a strong solvent that was used to dissolve the heterocyclic compounds. PBS was used as a buffering agent as well as to prepare the enzyme and substrate. Acetylcholinesterase (C3389-500UN)/ Butyryl cholinesterase (C7512-1.2KU) enzyme, and Acetylcholine iodide (A5751)/ S-Butyryl thiocholine iodide (20820-1G) substrate were purchased from Sigma-Aldrich (USA). DTNB was used as a coloring reagent in an enzyme inhibition assay. Donepezil was used as a positive control. Ethanol, Chloroform, Aluminum chloride, Isopropanol, The following items were acquired: primers (Thermo Fisher Scientific – US), cDNA kit (Thermo Scientific), cyber green (SYBR Green master mix of Bio-Rad), and triazole (Invitrogen TM). The ELISA reader analyzed all chemicals at a different wavelength and UV spectrophotometer.

Sample collection

Heterocyclic compounds were synthesized by the Medicinal Chemistry laboratory in the Department of chemistry. Structures of the heterocyclic compounds were drawn by CHEMDRAW Ultra 12.0 software. A total of 300 heterocyclic compounds (fluoroquinolones, benzimidazole-benzothiazole, and benzodiazines, etc.) were screened out against the acetylcholinesterase/butyryl cholinesterase enzyme. However, this research included only the most powerful series data (Tables 1 and 2).

In-silico screening of heterocyclic compounds

Molecular docking

To find the best binding between ligand and receptor, the Molecular Docking technique was used. A ligand and receptor molecule were prepared for this purpose, as described below.

Preparation of ligand library

CHEMDRAW Ultra 12.0 software was used to design the structures of all the ligands, which were then saved as MDL files (SDF) to be opened in MOE software . The standard donepezil two-dimensional structure was retrieved from NCBI PubChem and saved as an SDF format. Finally, all of the ligands and donepezil structures were three-dimensional protonated and energy minimized using default MOE parameters.

Receptor preparation

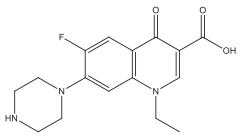
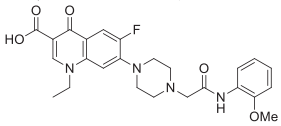
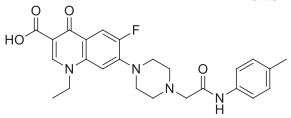
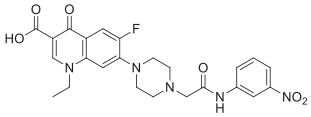
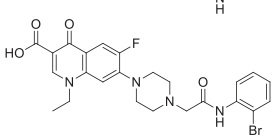
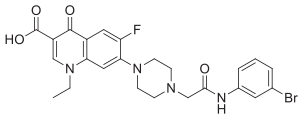
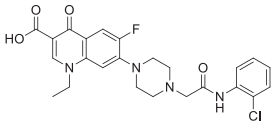
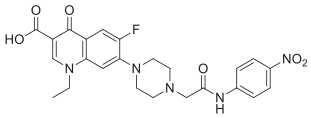
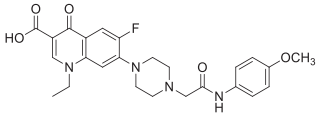
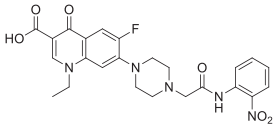
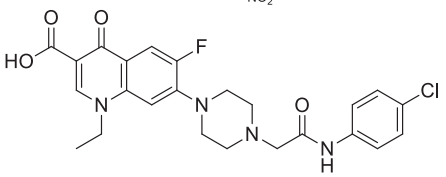
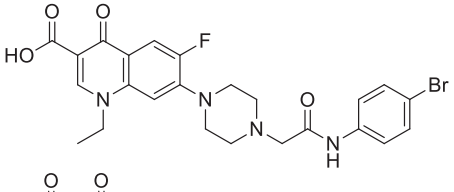
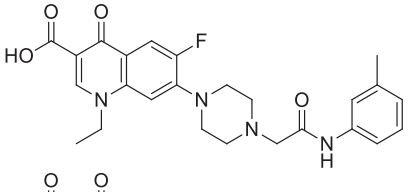
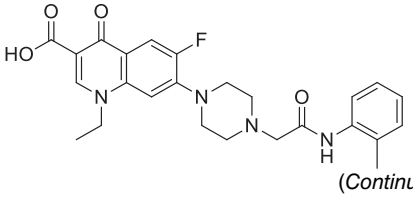
A PDB database (http://www.rcsb.org/pdb) with a PDB ID: 1EVE/PDB ID: 5DYW was used to generate the three-dimensional receptor's structure. The created structure was edited thoroughly via Molecular Operating Environment to get rid of ligand and solvent remains, three-dimensional protonation, and minimizing energy consumption.

Docking

The active site on acetyl cholinesterase/butyryl cholinesterase was identified using the docking software MOE (PDB ID: 1EVE/ PDB: 5DYW). The active site, which contains Tyr-121 and, Trp-84 has been selected for the acetyl cholinesterase enzyme. The MOE software docking algorithm was used to dock the ready-to-dock ligands library with interacting acetyl cholinesterase residues. The MOE software checks the unique ligand verification in order to generate a minimal energy structure. Using S-score and RMSD values, the highest and best ligand configurations were found after docking.

In-silico investigation of drug-likeness and ADMET features: Based on Lipinski's rule of five (Ro5), the phytochemical docking score with the greatest significance was then selected. Its physicochemical characteristics were ascertained using this via the Molinspiration service (http://www.molinspiration.com/cgibin/properties). The SwissADME program (http://www.swissadme.ch/) was used to assess

Table 1 List of heterocyclic compounds

Sr. no	Derivatives	Compounds codes	Structures
1	Fluoroquinolone	Z	
2	Fluoroquinolone	Z3	
3	Fluoroquinolone	Z4	
4	Fluoroquinolone	Z5	
5	Fluoroquinolone	Z6	
6	Fluoroquinolone	Z7	
7	Fluoroquinolone	Z8	
8	Fluoroquinolone	Z9	
9	Fluoroquinolone	Z11	
10	Fluoroquinolone	Z12	
11	Fluoroquinolone	Z13	
12	Fluoroquinolone	Z14	
13	Fluoroquinolone	Z15	
14	Fluoroquinolone	Z16	

(*Continued*)

Table 1 (*Continued*)

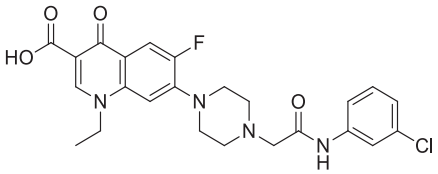
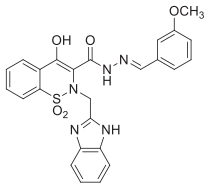
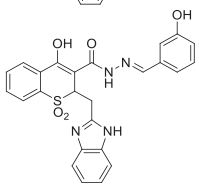
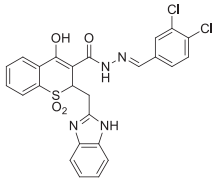
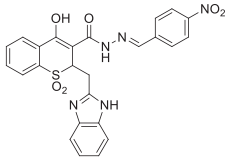
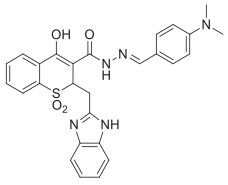
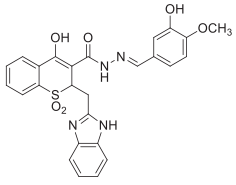
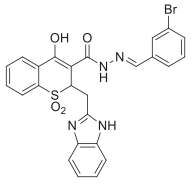
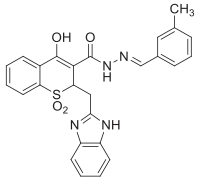
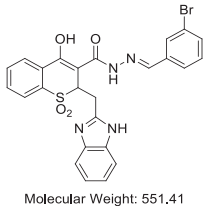
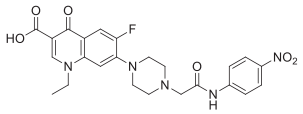
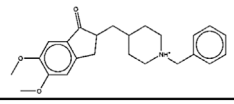
Sr. no	Derivatives	Compounds codes	Structures
15	Fluoroquinolone	Z17	
16	benzimidazole-benzothiazole	TB1S16	
17	benzimidazole-benzothiazole	TBAF1	 Molecular Weight: 488.52
18	benzimidazole-benzothiazole	TBAF2	 Molecular Weight: 541.41
19	benzimidazole-benzothiazole	TBAF3	 Molecular Weight: 517.51
20	benzimidazole-benzothiazole	TBAF4	 Molecular Weight: 515.58
21	benzimidazole-benzothiazole	TBAF5	 Molecular Weight: 518.54
22	benzimidazole-benzothiazole	TBAF6	 Molecular Weight: 551.41
23	benzimidazole-benzothiazole	TBAF7	 Molecular Weight: 486.54

Table 2 List of selective compounds

Sr. no	Compounds ID	Chemical structure	Docking score	RMSD value	Receptors
1	TBAF-6	 Molecular Weight: 551.41	−11.48	1.27	Thr-B120
2	Z-9		−12.37	1.50	Thr-B120
3	3152(Donepezil)Standard drug		−11.34	2.08	Gly- B116Tyr-B332 Thr-B120

In-silico screening of heterocyclic compounds

applicants' drug-like traits. An essential indicator of the drug candidate's behavior, fate, and toxicity in the human body is the calculation of the ADMET characteristics, which stand for Absorption, Distribution, Metabolism, Excretion, and Toxicity.

In-vitro screening of heterocyclic compounds

In-vitro acetylcholinesterase assay/In-vitro butyryl cholinesterase assay

AChE catalyzes the hydrolysis of acetylcholine neurotransmitter to choline and acetic acid, a step that is required to re-activate a cholinergic neuron. Acetylcholinesterase (AChE) and cholinesterase butyryl are two cholinesterase types (BChE). Both forms vary from one substrate to another: former hydrolysis of acetylcholine quicker; butyrylcholine hydrolyzing quicker. Enzyme preparation: Acetylcholinesterase enzyme (C3389-500UN) vial contains 500 units/vial. Therefore, to prepare 233 U/ml stock solution, 1 ml of PBS was added into 1 mg. Furthermore, to prepared 0.015 U/ml working solution, 64.37 µl of stock solution was mixed in 936 µl of PBS and stored at −20°C for further use. Butyryl cholinesterase enzyme (C7512-1.2KU) vial contains 1.2KU/vial. To prepare 8.7 U/ml (0.087 U/µl) working solution, 1ml of PBS was added into 1 mg and stored at −20°C for further use. Substrate preparation: the molecular weight of Acetylcholine iodide (A5751) is 289.18 g/mol. Therefore, 0.1445 mg of the substrate was mixed in 1 ml of PBS to make the 0.5 mM working solution. The molecular weight of S-Butyryl thiocholine iodide (20820-1G) is 317.23 g/mol. Therefore, 0.158 mg of the substrate was mixed in 1 ml of PBS to make the 0.5 mM working solution. DTNB (Coloring reagent) preparation: The molecular weight of DTNB is 391.85 g/mol. 0.1981 mg of coloring reagent was mixed in 1 ml of PBS to make the 0.5 mM working solution.

Inhibition assay

A spectrophotometric technique established was used to assess the in-vitro inhibitory activity of AChE/BChE. In brief, the reaction mixture contained 65 µl of phosphate buffer saline, 5 µl of test compound dissolved in DMSO, and 10 µl of enzyme solution (0.015 unit/well for AchE/0.0087 unit/well for BChE). During the preincubation stage, the reaction contents were thoroughly mixed and kept at 37°C for 10 min. Following the preincubation period, to start the enzyme-substrate reaction, a 10 ul solution of acetylthiocholine iodide/S-Butyryl thiocholine iodide (0.5 mM) was incorporated to the relevant AchE/BChE solution. The mixture also added 10 µl of (0.5 mM) 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) as coloring reagent. The mixture of the reaction was incubated 20 min at 37°C, and the absorbance with a microplate reader was measured at 405 nm. Experiments with their respective controls were performed in triplicates. A positive control was performed using the reference standard medication donepezil (1 mM/well). The following formula was used to compute the percentage of acetylcholinesterase/butyryl cholinesterase inhibition:

$$\%\ \text{of inhibition n=1} - \left(\frac{\text{At}}{\text{Ac}}\right) \times 100$$

Where 'At' and 'Ac' denote the absorbance acquired with and without inhibitors, respectively, after removing the appropriately matched background.

In-vivo evaluation

Experimental animals

Healthy Wistar Rats were purchased from the experimental animal house of the University, in the weight of 100–150 g, and placed seven days before the start of the experimentation in animal house to acclimatize the animals. The animals were kept in

separate cages in groups. All mice were fed for 1 week under standard environmental conditions (12 h dark/12 h light, temperature 20–25°C and 30–60% humidity).

Induction of AD in Rats: wistar rats were administered aluminum chloride 300 mg/kg p.o. and D-galactose 150 mg/kg p.o. for 21 days, causing behavioral, biochemical, and molecular defects and chronic aluminum accumulation and disposition in brain tissues.

Study design wistar rats in good health (n=7) were split up into seven groups for this investigation. Group 1: they received 1 ml/kg of vehicle (distilled water) as a control group. Group 2: as a preventative measure, treated with D-galactose 150 mg/kg p.o. and aluminum chloride 300 mg/kg p.o. Group 3: the standard group was subjected to D-galactose 150 mg/kg p.o., donepezil 1.5 mg/kg p.o., and aluminum chloride 300 mg/kg p.o. D-galactose 150 mg/kg p.o., Z-9 1.5 mg/kg p.o., and aluminum chloride 300 mg/kg p.o. were administered to group 4. D-galactose 150 mg/kg p.o., Z-9 0.75 mg/kg p.o., and aluminum chloride 300 mg/kg p.o. were administered to group 5. Treatment for Group 6 included the oral administration of D-galactose 150 mg/kg, TBAF-6 1.5 mg/kg, and aluminum chloride 300 mg/kg. Group 7: Received treatment with D-galactose 150 mg/kg p.o., aluminum chloride 300 mg/kg p.o., and TBAF-6 0.75 mg/kg p.o. in each group Animals received treatments for 21 consecutive days. At the beginning and end of the 21 days, behavior and weight were recorded. The animals were slaughtered by cervical dislocation after 1 day of receiving the highest dose under light anesthesia. For biochemical and histopathological studies, brains were isolated, washed with phosphate buffer, and stored at −80°C in triazole.

Behavioural studies

The Morris water maze is a test used to look at how long it takes for rats to learn, remember, and move around in an AD rodent model. It is a popular tool for neuropharmacology's to check for changes in neurocognition in rodent models. To test it, a circular plastic tub of 1 m diameter and 1.5 ft depth was used. The tub was filled with tap water and kept at a temperature of 26°C. A platform was placed at the middle of the pool, about an inch below the surface, and was exposed during training. To make the forum non-transparent, milk powder was mixed with water, so it was invisible to the rats. The pool was partitioned into four quadrants, and rats were taught about the platform by immersion in water and immersion in the tub for a period of 90 s. Four trials were conducted from four directions to locate a hidden platform at the beginning of the training. The rats were led up to the platform and remained on the platform for 20 s before leaving the pool. The duration of time it took to escape the submerged platform from the water was recorded. On the sixth day the forum was rendered undetectable to rats, and the duration of time to escape from the hidden platform was recorded for up to 120 s from four directions.

Open field test

In this test, the fear, the exploration and movement of animals were investigated. This test is sensitive to early indicators of memory loss associated with Alzheimer's disease in a mouse model. The regular conduct of rats is to find refuge and prevent the center's openness. Therefore, the early pathogenic change in AD models with increased memory and anxiety, which show the device's declining horizontal and vertical movement, is regarded as a sensitive test. It was composed of the floorboard, 72×72 cm in size and 36 cm walls in a hollow square, white resin chamber. One wall was built of Plexiglas to imagine the movement of rats within the gadget. The floor of this room has 16 identical squares with black lines (18×18 cm). A center square with red lines measuring 18×18 cm has been designed to separate the central region from other areas in its surroundings. The chamber was disinfected with 70% alcohol following each animal test. The rat was handled carefully with its tail and was put for 10 min in one corner of the appliance, while the total distance and lines were recorded, traversed by four paws. Time spent in the center region, extending posture, cleaning, faces, rearing and freezing have been noted.

Passive avoidance test

A shock motivated experiment was conducted to evaluate the associative memory decline in the short- and long-term memory in an AD-pathology animal. The apparatus used in this study was made of a Plexiglas with a diameter of 27 cm and a height of 27 cm. The floor grillwork consisted of stainless steel rods with a thickness of 3 mm spaced 8 mm apart. A 20 V power supply current battery sat on the grid floor at half-point. The rats were gently handled from their tails and placed on a platform. The rats had to maintain 15–22 s in the first trial to move from the platform to the floor. After 2 h, the second trial started. The test rats were heaped up with a wood rostrum. His step down was observed.

Wire hanging test

The objective of this test is to assess neuromuscular power and endurance. Equipment Horizontal grids of

stainless steel were used in this test. The grids were mounted on wooden walls of three inches wide and 50 cm high. Animals were handled gently from their tails and supported on the grid. They lifted the grid with front and rear paws. The animals had to stay on the wire for 30 s. The hang time was 30 s to 1 min.

Y-maze test

It is used to identify spatial, short-term, and cognitive defects of the rodent in behavioral neuro-sciences. Rats were placed at the start of each arm. The apparatus was made of wooden three-arm apparatus with a Y-shaped arm of 120° in the middle. The arms were 35 cm in length, 25 cm in height, and 10 cm in width with trigonal median zone in the center. The exploratory behavior of the rats was recorded in the y-maze for 8 min. The number of entries in the apparatus and the number of triads were recorded to determine the spontaneous change. Only when the rear paws ultimately entered an arm it called ingress. The following formula calculated the spontaneous alternation:

$$\text{Spontaneous alteration \%} = \frac{\text{Total No. of triads}}{\text{Total No. of arm entries} - 2} \times 100$$

The laterality index used to examine the animal's side preference was calculated using the following equation [31].

$$\text{Laterality index} = \frac{\text{Movements toward left arm} - \text{Movements toward right arm}}{\text{Movements toward left arm} - \text{Movements toward right arm}}$$

Elevated plus-maze task

This test is a popular way to check for anxiety-like behavior and unpredictable behavior patterns in AD rodent models. It was also used in an outside behavioral model to check memory and exploration. It was set up with two open arms and two wooden platforms. The first day (the 20th day of the research) was spent gently positioning animals on the base of one open arm in a diverted direction to a platform. The latency transfer was noticed because it was their natural way of hiding from the investigation (how long it took them to go from one open arm to the other). After 24 h, the same process was done and the transfer delay was recorded to check for cognitive, comprehension, and memory deficits.

Hole board test

A hole board test is primarily used in mice and rats to evaluate multiple dimensions of unconditioned actions. Therefore, this task is an essential behavioral test in the AD rat model for estimating neophilia, emotion, stress, and anxiety. The floor was split into 16 uniformly divided holes with a ground height of 1.5 m and Plexiglas material of 25 cm × 25 cm, and a wall height of 30 cm. The rat was placed on the pitch with compassion and allowed 8 min to investigate. The number of heads descending into troughs and the distance travelling in the peripheral and central regions of the device has been documented. In addition, when both eyes of the animals vanished in the hole was head dipping scored.

Analysis of different markers to elucidate the molecular mechanisms of neurodegeneration in Alzheimer's disease.

The RNA Extraction Kit (PureLink TM, RNA Minikit, Invitrogen by Thermo Fisher Scientific, Cat No. 1218301 8A) was used to extract RNA from the corresponding tissues. Total RNA from the tissue was isolated from about 30–40 mg of the tissue. Using a high-capacity cDNA synthesis kit (Thermo Fischer Scientific, RevertAid First Strand cDNA Synthesis Kit, USA), isolated RNA (1 g) was reverse transcribed into cDNA. Thermo Scientific, USA's Maxima SYBR Green/ROX qPCR Master Mix (2X) was used in conjunction with the MyGo Pro PCR system (MyGo PCR systems, IT-IS Life Sciences) to determine the relative abundance of mRNA levels. To examine the hepatic gene expression of –amyloid, TREM2, Neuregulin 1 (NRG1), Choline Acetyltransferase (ChAT), and Homeobox 9 (HB9) in rat brain, specific primers were utilized for RT-PCR. The housekeeping gene β-actin was employed, and each sample was conducted in triplicate. The ΔΔCt technique was used to examine the data, and the mRNA fold change was determined.

Histopathology study

The materials were dehydrated in a progressive increase in ethanol, xylene, and paraffin. It was cut using a microtome and then stained with hematoxylin/eosin. Image software was used to determine the amount of collagen deposition in each group of fibrous lesion sites, which were detected using staining. A microscope, Germany, and an Am Scope microscope digital camera were used to take all of the section photographs.

Statistical analysis

IBM-SPSS tool evaluated the data. The experimental study data for intergroup variation were recorded at 5% mean ±SEM, one-way and two-way ANOVA, subsequent to the appropriate Tukey and Bonferroni post-tests. Statistically significant P less than 0.05 results were deemed.

Results

In-silico screening of heterocyclic compounds

In-silico with a molecular docking approach, the compounds' binding energy and RMSD value has been determined. MOE was used to conduct molecular docking. MOE is a drug research software platform for integrated visualization, modeling, and simulation by biologists.

Molecular docking

ADMET/Drug scan results

In the Molinspiration server, the drug-like characteristics of the suggested inhibitors of acetylcholinesterase/ Butyrylcholinesterase were anticipated using the ADMET based drug scan tool (Tables 3 and 4). No violation of the five-pill rule and appropriate drug-like features, such as molecular weight, has been identified by all selected candidates. SwissADME screened all the candidate compounds to evaluate their drug-like qualities, which provided a basis for additional drug-like potential validation (Tables 5 and 6).

In-vitro screening of heterocyclic compounds

In-vitro acetylcholinesterase assay results

The in vitro acetylcholinesterase assay was performed using different compound treated groups. Amon the different compound the Z-9 and TBAF-6 were found to have higher inhibition percentage (97±0.16, 96.35 ±0.45) and lowest IC_{50} values (0.37±0.02, 0.638 ±0.001), respectively (Table 7).

In-vitro butyryl cholinesterase assay

Butyryl cholinesterase assay is used to check the inhibitory potential of compounds against the butyryl cholinesterase enzyme. The acetylcholinesterase enzyme was used to screen a vast number of heterocyclic compounds. Regarding the screening, 2 Heterocyclic compounds were chosen for further evaluation. These 2 compounds have potent inhibitory activity against the Butyrylcholinesterase enzyme. Table 8 shows the % inhibition and IC_{50} of Heterocyclic compounds against the butyryl cholinesterase enzyme.

In-vivo analysis

Behavioral studies

Contiguous spatial learning, memory, and task strategy were examined in behavioral investigations using AD rodent models. Neuropharmacology tests were commonly used to validate neurocognitive alterations in rodent models. The outcomes of these behavioral tasks are as follows.

Test of Morris's water maze

Test of the Morris Water Maze reveals a reduction in escape latency per second in Z-9 and TBAF-6 treated groups as compared with control and disease control groups (Table 8.1. This data shows that our compound treated groups have improved behavioral parameters as compared with other groups.

Open field test

The open field test shows improved of movement of rats in Z-9 and TBAF-6 treated groups as compared with control and disease control groups (Table 9). This data shows that our compound treated groups have improved behavioral parameters as compared with other groups.

Table 3 Heterocycliccompounds analyzed for lipinski rule

Compound ID	Molecular weight (g/mol)	Number of HBA (nON)	Number of HBD (nOHNH)	LogP
Z-4	417.10	8	2	1.89
Z-3	422.55	11	2	0.99
Z	424.76	9	2	1.13
Z-12	417.10	8	2	1.84
Z-8	424.76	9	2	1.08
Z-6	415.77	8	2	1.48
Z-15	412.75	8	2	1.71
Z-9	415.77	8	2	1.52
TBIS-16	417.48	10	3	4.26
TBAF-1	404.00	9	4	3.94
TBAF-2	423.05	8	3	5.73
TBAF-3	419.32	11	3	4.40
TBAF-4	441.89	9	3	4.55
TBAF-5	429.55	10	4	3.78
TBAF-6	413.87	8	3	5.23
TBAF-7	412.54	8	3	4.87
TBAF-8	409.52	8	3	5.10
TBAF-9	427.47	9	4	3.69
3152(Donepezil) Standard drug	380.51	4	1	1.14

*HBA, hydrogen bond acceptance; HBD, Hydrogen bond Donor.

Table 4 ADMET profiling incorporates drug-like parameters related to absorption, metabolism and toxicity of candidate compounds

Compounds ID's	Blood-brain barrier	Gastro-intestinal absorption	P-glycoprotein substrate	CYP450 1A2 inhibitor	CYP450 2C9 inhibitor	CYP450 2D6 inhibitor	CYP450 2C19 inhibitor	CYP450 3A4 inhibitor
Z-4	No	High	Yes	No	Yes	Yes	Yes	Yes
Z-3	No	Low	Yes	No	Yes	Yes	Yes	Yes
Z	No	High	Yes	No	Yes	Yes	Yes	Yes
Z-12	No	High	Yes	No	Yes	Yes	Yes	Yes
Z-8	No	High	Yes	No	Yes	Yes	Yes	Yes
Z-6	No	High	Yes	No	Yes	Yes	Yes	Yes
Z-15	No	High	Yes	No	Yes	Yes	Yes	Yes
Z-9	No	High	Yes	No	Yes	No	No	No
TBIS-16	No	Low	No	No	Yes	No	Yes	Yes
TBAF-1	No	Low	No	No	Yes	No	Yes	No
TBAF-2	No	Low	No	Yes	Yes	No	Yes	No
TBAF-3	No	Low	No	No	Yes	No	No	No
TBAF-4	No	Low	No	Yes	Yes	No	Yes	No
TBAF-5	No	Low	No	No	Yes	No	No	No
TBAF-6	No	Low	No	Yes	Yes	No	Yes	No
TBAF-7	No	Low	No	Yes	Yes	No	Yes	No
TBAF-8	No	Low	No	Yes	Yes	No	Yes	No
TBAF-9	No	Low	No	Yes	Yes	No	Yes	Yes
3152 (Donepezil) Standard drug	Yes	High	Yes	No	No	No	No	No

*CYP, Cytochrome.

Table 5 The Lipinski rule analyses of heterocyclic organic compounds are presented below

Compound ID	Molecular weight (g/mol)	Number of HBA (nON)	Number of HBD (nOHNH)	LogP
TBAF-6	371.01	4	1	1.14
Z-9	422.55	11	2	1.03
3152(Donepezil) Standard drug	413.87	8	3	5.23

Table 6 ADMET profiling enlisting absorption, metabolism and toxicity related drug-likeparameters of candidate compounds

Compounds ID's	Blood-brain barrier	Gastro-intestinal absorption	P-glycoprotein substrate	CYP450 1A2 inhibitor	CYP450 2C9 inhibitor	CYP450 2D6 inhibitor	CYP450 2C19 inhibitor	CYP450 3A4 inhibitor
TBAF-6	Yes	High	Yes	No	No	No	No	No
Z-9	No	Low	Yes	No	Yes	No	Yes	Yes
3152 (Donepezil) Standard drug	No	Low	No	Yes	Yes	No	Yes	No

Y-maze test

The Y-maze test shows improved behavior and intellect in rats in Z-9 and TBAF-6 treated groups as compared with control and disease control groups (Table 10). This data shows that our compound treated groups have improved behavioral parameters as compared with other groups.

Analysis of passive avoidance test, on hole board test, wire hanging test, elevated plus-maze

The rats in Z-9 and TBAF-6 treated groups showed improvement in their ability of performing Passive avoidance test, On hole board test, Wire hanging test, Elevated plus-maze as compared with control and disease control groups (Figs. 1–4). This data shows that our compound treated groups have improved behavioral parameters as compared with other groups.

The histopathological findings of control and treated groups were compared. $AlCl_3$ brain sections exhibited considerable damage in motor neurons. The treatment groups showed varying degrees of improvement due to the preceding change (Figs. 5–7).

Table 7 Percent inhibition and IC_{50} of heterocyclic compounds against acetylcholinesteraseenzyme

Sr. No	Compounds ID	% of inhibition	$IC_{50(\mu M)}$
1	Z-8	25±0.007	7.15±0.015
2	Z-14	87.76±0.16	0.8±0.43
3	Z-3	10±0.55	119.75±0.48
4	Z-11	29.19±0.14	8.05±0.30
5	Z-7	45±0.70	4.87±0.07
6	Z-16	1±0.57	−0.9±0.05
7	Z-15	35±0.61	6.67±0.02
8	Z	75±0.17	2.93±0.29
9	Z-9	97±0.16	0.37±0.02
10	Z-5	75±0.53	9.87±0.08
11	Z-6	17±0.55	0.87±0.01
12	Z-4	29±0.32	11.06±0.015
13	TBAF-1	27.68±0.30	−0.348±0.001
14	TBAF-2	39.76±0.43	18.96±0.56
15	TBAF-3	28.64±0.45	−22.58±0.01
16	TBAF-4	60.15±0.59	−0.34±0.01
17	TBAF-5	17.89±0.41	7.9±0.05
18	TBAF-6	96.35±0.45	0.638±0.001
19	TBAF-7	0±0.7	170.95±0.01
20	TBAF-8	41.21±0.07	−31.58±0.01
21	TBAF-9	55±0.07	15.82±0.03
22	TB1S-9	0±0.61	−0.9±0.09
23	TB1S-6	86.45±0.31	4.87±0.009
24	TB1S-4	27.60±0.007	33.93±0.015
25	TB1S-16	39.41±0.02	23.05±0.03
26	TB1S-14	32.29±0.06	11.06±0.03
27	3152 (Donepezil) Standard drug	68.74±0.07	3.90±0.01

Table 8 % inhibition and IC_{50} of heterocyclic compounds against butyrylcholinesterase enzymeTable 81 Heterocyclic compounds' effects on the Morris water maze in induced model of AD by $AlCl_3$

Sr. No	Compounds ID	% of inhibition	$IC_{50(\mu M)}$
1	TBAF-6	66.15±0.10	1.31±0.01
2	Z-9	72.30±0.41	2.93±0.03
3	3152(Donepezil) Standard drug	81.53±0.37	4.9±0.05

Table 8.1 Heterocyclic compounds' effects on the Morris water maze in induced model of AD by $AlCl_3$

Groups	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Escape latency in seconds				
Control	20.98±0.91	19.56±0.91	19.29±0.91	19.74±0.91
Disease control	42.33±0.45*	41.99±0.31*	42.12±0.31*	41.99±0.31*
Standard Drug	21.22±0.21*	21.25±0.21*	21.07±0.29*	19.56±0.21*
Z-9 (Higher)	32.82±0.37*	32.77±0.52*	32.30±0.51*	32.36±0.37*
Z-9 (Lower)	19.88±0.31**	19.38±0.45**	19.41±0.31**	20.58±0.31**
TBAF-6 (Higher)	35.95±0.15*	36.52±0.21*	35.48±0.15*	35.85±0.15*
TBAF-6 (Lower)	19.80±0.13***	20.1±0.18***	20.22±0.13***	19.32±0.13***

In comparison to the control, values are expressed as mean±SEM, (n=6). ***(*P* 0.001). **(*P* less than 0.01).

Analysis of different neuronal markers for elucidation of molecular pathway in brain tissues

It is a well-established phenomenon that $AlCl_3$ treatment leads to brain damage, especially to cholinergic/motor neurons due to which the animals exhibits the pathophysiology of AD. We further performed reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to check the expression of neuronal specific markers. We have found an increase expression of β- amyloid in diseased group while its expression was significantly downregulated in Z-9 and TBAF-6 treated groups, respectively (Fig. 8). Similarly, the expression of *TREM2* has been found elevated in disease control group; however, a significant decline was observed in Z-9 and TBAF-6 treated groups, respectively (Fig. 9).

Table 9 Heterocyclic compounds' impact on the open field test in the induced model of Alzheimer's disease by $AlCl_3$

Groups	Total no. of lines crossed	Freezing (seconds)	Rearing /10 min	Grooming/10min
Control	8.33±0.88	79.33±0.57	0.00±0.00	0.00±0.00
Disease control	27.12±0.58***	0.00±0.00***	4.38±0.41***	3.33±0.39***
Standard Drug	24.44±0.38****	19.33±0.38****	4.41±0.27****	3.74±0.17****
Z-9 (Higher)	24.04±0.27*	24.43±0.27*	4.88±0.19*	5.45±0.11*
Z-9 (Lower)	19.04±0.57***	21.22±0.57***	2.95±0.40***	2.66±0.50***
TBAF-6 (Higher)	25.30±0.21****	23.03±0.02****	5.00±0.21****	4.90±0.22****
TBAF-6 (Lower)	18.56±0.19*	19.26±0.27*	3.10±0.27*	2.75±0.17*

In reference to the control, values are expressed as mean±SEM, (n=6). *** (*P* less than 0.001). **(*P* less than 0.01), and ns(nonsignificant).

Table 10 Heterocyclic substances' impact on the Y-maze test in the $AlCl_3$-inducedAlzheimer's disease model

Groups	Total no. of arm entries	Total no. of triads	% spontaneous alteration	Laterality index
Control	10.00±0.5	2.00±0.0	37.00±0.5	0.14
Disease control	2.35±0.33***	0.00±0.0***	0.00±0.0***	−0.33
Standard Drug	10.00±0.5^{ns}	2.33±0.3^{ns}	36.00±0.5^{ns}	0.14
Z-9 (Higher)	15.00±1.7^{ns}	3.00±0.0^{ns}	38.18±0.49^{ns}	0.1
Z-9 (Lower)	8.66±0.33***	2.99±0.0^{ns}	35.44±0.4***	0
TBAF-6 (Higher)	16.78±1.8^{ns}	3.33±0.2^{ns}	35.85±1.75^{ns}	0.2
TBAF-6 (Lower)	7.00±0.5***	1.90±0.0**	22.33±0.8***	−0.2

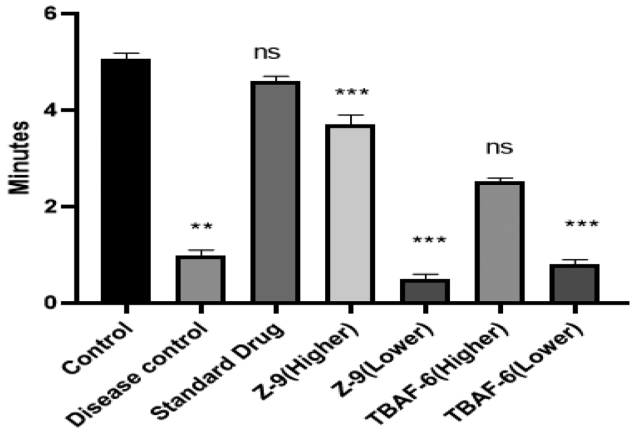
Values are expressed as mean±SEM, (*n*=6). ***(*P* less than 0.001). **(*P* less than 0.01) and ns(nonsignificant) as compare with control.

Furthermore, we found significantly increased expression of NRG1, ChAT and HB9 in Z-9 and TBAF-6 treated groups compared with disease control groups (Figs. 10–12). These results substantiate our hypothesis that our compounds (Z-9, TBAF-6) can relieve the AD pathophysiology by down regulated the disease causing genes while upregulating the beneficial markers.

Discussion

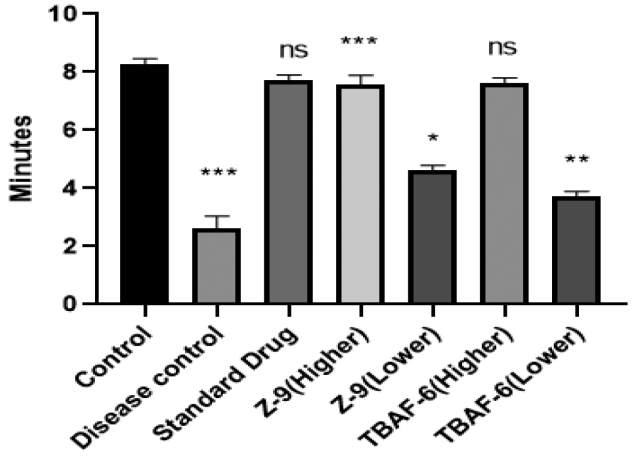
The most frequent neurodegenerative and predominant dementia form of the elderly is (AD) [1]. The rise in the synthesis and aggregation of amyloid-beta (Aβ), leading to oxidative stress, neuroinflammation and neurodegeneration, is one of the primary hallmarks of AD [32]. Heterocyclic compounds with acetylcholinesterase/butyryl cholinesterase inhibitory potential are commercially available [33]. For instance, donepezil, glutamine, and rivastigmine are available in local markets to restrict enzyme activity, still they have side effects that reduce their usefulness, necessitating developing a new inhibitor with lower toxicity and fewer adverse effects [10]. Fluoroquinolones and benzimidazole-benzothiazole derivatives offer antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and anti-

Figure 1



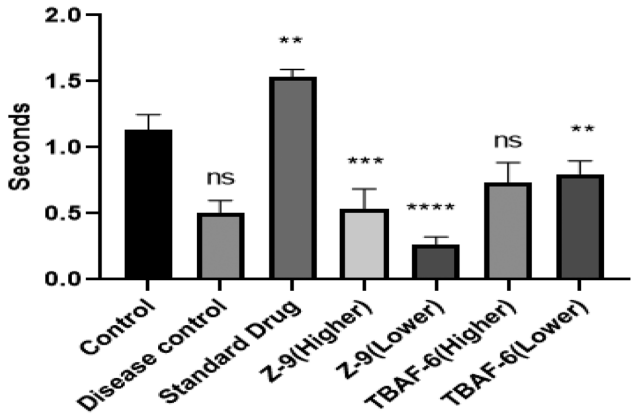
Effect of heterocyclic compounds on the $AlCl_3$-induced Alzheimer's disease model's passive avoidance test. Values are presented as the mean±SEM, with *n*=6 and ****P* less than 0.05 denoting statistically nonsignificant differences from the control.

Figure 2



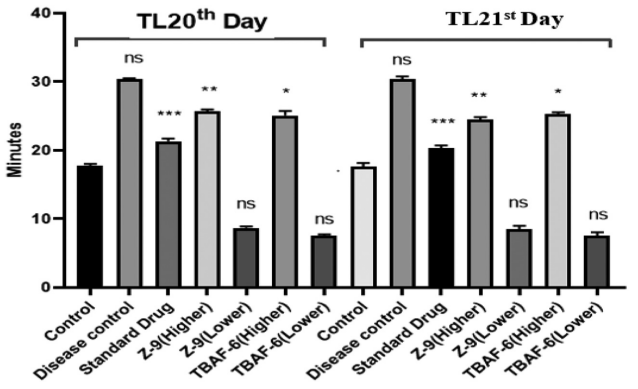
Effect of heterocyclic substances on the hole-board test in the Alzheimer's disease model driven by AlCl3. Head dipping in (HD) Values are presented as the mean±SEM, with *n*=6 and ****P* less than 0.05 denoting statistically non-significant differences from the control.

Figure 3



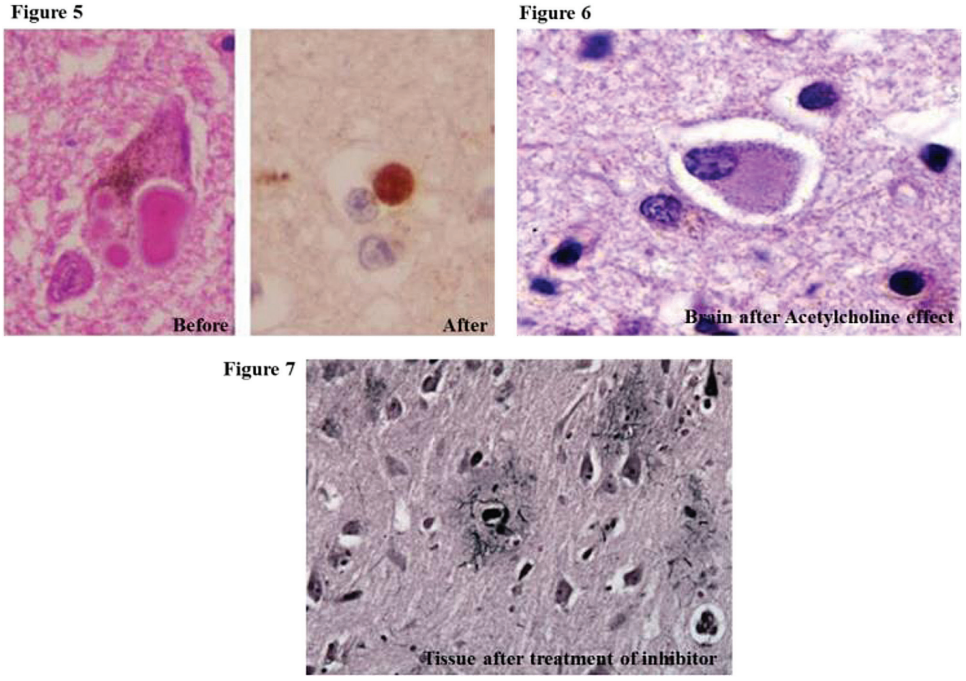
Effect of heterocyclic compounds on a wire hanging test in $AlCl_3$ induced Alzheimer's disease model HT: Hanging time, Values are expressed as mean±SEM, (n=6), ***P less than 0.001 and ns (nonsignificant) as compared with control.

Figure 4



Effect of heterocyclic chemical substances on enhanced plus-maze in $AlCl_3$-induced Alzheimer's disease model: transfer latency at day 20 ($TL20^{th}$), and ($TL21^{th}$) transfer latency at day 21, respectively. Values are shown as the mean standard error of the mean (n=6), with two-way ANOVA applied, and compared with the control group using ***P less than 0.001, **P less than 0.01, and ns (non-significant).

Figure 5–7



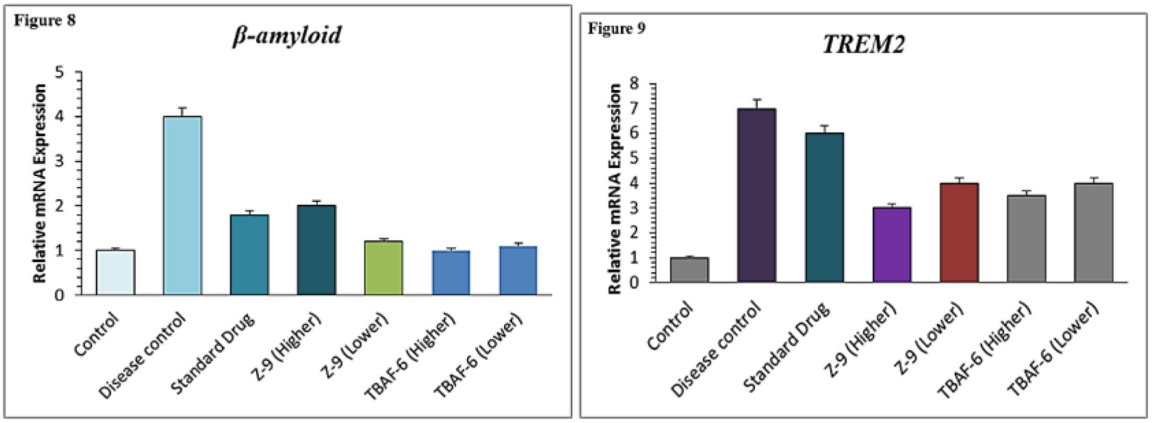
Histopathological comparison between treated and untreated animal brains by $AlCl_3$ compounds.

Alzheimer activities. This study was designed to check the *in-silico* anti-Alzheimer activity of synthetic heterocyclic compounds, *in-vitro* screening of these selected compounds as a potential inhibitor of acetylcholinesterase/butyryl cholinesterase (AChE/BChE), and also check their anti-Alzheimer activity and toxicity in a mouse model.

Molecular docking was performed to evaluate the inhibitory effect of fluoroquinolones and benzimidazole-benzothiazole substitution compounds against AChE/BChE. Molecular docking results showed that derivatives of fluoroquinolones (Z, Z3, Z4, Z6, Z8, Z12, Z15, and Z9) and benzimidazole-benzothiazole (TBIS-16, TBAF-1, TBAF-2, TBAF-3, TBAF-4, TBAF-5, TBAF-6, TBAF-7, TBAF-8, and TBAF-9) had better score and showed maximum interactions with active site of AChE compared with control.

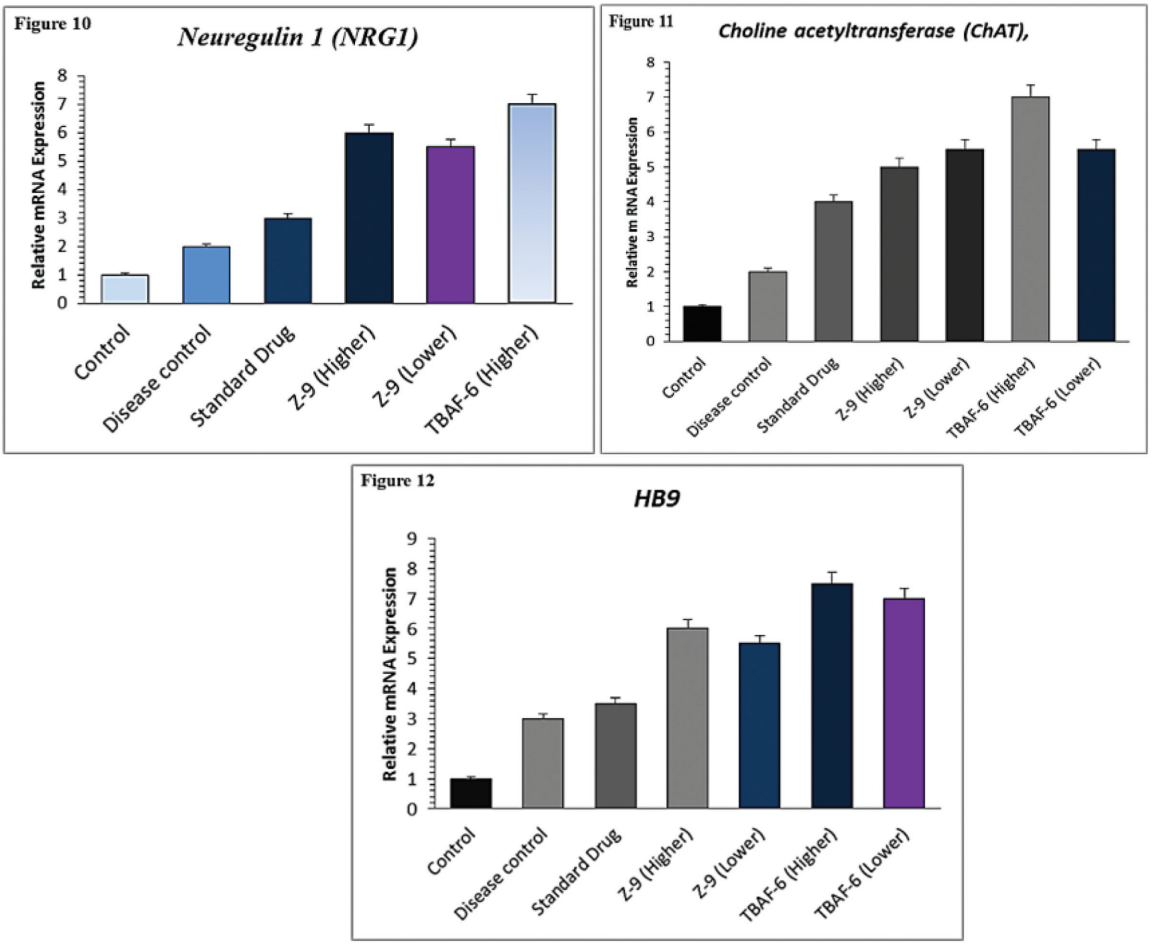
On the other hand, Z-9 and TBAF-6 showed significant docking results against BChE than

Figure 8–9



Histopathological comparison between treated and untreated animal brains by $AlCl_3$ compounds.

Figure 10–12



Histopathological comparison between treated and untreated animal brains by $AlCl_3$ compounds.

donepezil. also reported heterocyclic compounds as anti-Alzheimer's agents and more potent compounds than donepezil against AChE/BChE. To check the drug-like property of screened Z-9 and TBAF-6, it is founded that these compounds can exhibit high absorption and solubility compared with donepezil. Our findings also predicted that these AChE/BChE inhibitors have physiochemical properties within the reference range of Lipinski's rule and apposite drug-like compound. The inhibitory effect of docked heterocyclic compounds also verified *in-vitro* against AChE and BChE enzyme. Fluoroquinolones(Z, Z3,

Z4, Z6, Z8, Z12, Z15, and Z9) and benzimidazole-benzothiazole compounds (TBIS-16, TBAF-1, TBAF-2, TBAF-3, TBAF-4, TBAF-5, TBAF-6, TBAF-7, TBAF-8, and TBAF-9) passed through the AChE inhibition assay and their IC_{50} values were calculated. The compound Z-9 and TBAF-6 showed the lowest IC_{50} values against AChE/BChE (0.37±0.02/2.93±0.03 μM and 0.638±0.001/1.31 ±0.01 μM, respectively) compared with donepezil (3.9±0.01/4.9±0.05 μM). Also [18] proved that heterocyclic compounds have great potential to inhibit the AChE and BChE activity compared with commercially available medicines. Neurodegenerative disorders, especially AD, gain momentum in modern health care systems. Our planet's crust is largely made of aluminum, which has been related to the etiopathogenesis of neurodegenerative disorders marked by neuropsychiatric symptoms, behavioral abnormalities, and cognitive deficits such impaired working memory and semantic memory as well as a lack of interest in learning new information [34,35]. Aluminum chloride impairs glucose uptake, damages lipids and proteins by peroxidation, alters phosphoinositide metabolism, alters protein phosphorylation, and produces more free radicals and reactive oxygen species [35].

To examine the neuroprotective effects of fluoroquinolone (Z-9) and benzimidazole-benzothiazole (TBAF-6) compounds on AD mice models, behavioral studies were conducted. The results of the Morris water maze test were analogous to those of Petrasek's earlier investigations, which demonstrated improved latency in the treated groups and increased escape latency in the disease control group.

The findings from an open field study on exploration, learning, and movement were similar to previous research. In the Y-maze task, it was explored that the use of chronic aluminum chloride affected sustaining cognition, shorter-term and inherent ability of mice to alternative the arms or neophilia. The fluoroquinolone and benzimidazole-benzothiazole compounds treated groups had better cognitive behavior compared with standard drug. A study by corroborated our findings of the Y-maze test, which showed that long-term exposure to aluminum chloride impaired rodents' long-term cognitive function, short-term learning, and their natural ability to switch between arms or neophiles. Our findings show that fluoroquinolone (Z-9) and benzimidazole-benzothiazole (TBAF-6) compounds consumption increase muscular strength in rats due to their multifunctional pharmacological actions. The role of β-amyloid is well understood and these plagues are one of the main causes of AD [2]. We have found an increase in β-amyloid level in disease group while our treated groups showed significant down regulation suggesting decrease in disease severity. In addition, TREM2 (triggered receptor by myeloid cell 2) has been shown to play a critical role in AD pathogenesis [36]. TREM2 is one of the transmembrane receptors expressed in myeloid lineage cells. The association of TREM2 with Alzheimer's disease suggests that immune and inflammatory pathways are involved in AD pathogenesis rather than as a result of the disease itself. Here, we find a significant decrease in TREM2 expression in our group treated with Z-9 and in the group treated with TBAF-6, which is consistent with previously published reports.

Synaptic dysfunction is one of the early core features of AD, and is closely related to cognitive symptoms [37]. NRG1 (neuronegulin 1) is an important growth and differentiation factor that plays a critical role in the formation and maintenance of neurotransmitters. In our group treated with our compound, NRG1 has an increased expression compared with the group treated with AD disease. Choline acetyltransferase (*ChAT*) is the enzyme that caters to the biosynthesis of choline, which is the neurotransmitter responsible for regulating signal transduction at neuromuscular junctions and motor behavior and visceral stimulation underlying vegetative function in the autonomic nervous system. The expression level of *ChAT*in AD has been found to significantly down regulated thus decreasing the synthesis of acetylcholine; however, we have found a significant higher expression of *ChAT* in our compound treated groups. Finally, we also checked the expression of *HB9,* which is a cholinergic neuron specific marker and showed significant higher expression in our compounds treated groups compared with the disease control. From the transcriptomic analysis of these important markers we conclude that, our compounds efficiently relieve the AD pathophysiology and can be used in future for the possible treatment of AD.

Conclusion

The passive avoidance test looked at the effects of aluminum chloride on memory retention. The disease control group had the worst effect on memory retention with lower step-through latency, as well as an increased sensitivity to electric shock, according to a previous study by Lakshmi. Our findings

of hole boards for neophilia assessment, curiosity, and exploratory behavior were similar. In the disease control group, treatment with aluminum chloride caused a significant drop in first-line antioxidant enzymes and an increase in malondialdehyde level, which was similar to previous research. The antioxidant potential of these compounds helped to restore the levels of CAT, SOD, GPx, and GSH in the treated groups, whereas the level of MDA was significantly reduced. Reduced levels of SOD have been linked to neurodegeneration and myocardial damage. According to previous research, several mental diseases are associated with CAT deficiency. From the transcriptomic analysis of important neurological markers we conclude that, our compounds efficiently relieve the AD pathophysiology. In this study, it is concluded that fluoroquinolone and benzimidazole-benzothiazole compounds. In future, the antioxidant properties of these compounds would be beneficial in the development of therapeutic strategies for the management of neurological degenerative diseases.

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Author contribution: Conceptualization: ZA and ZMM. Methodology: MM, ZB, HMA. Software and Visualization: RM, FK, and RM. Formal analysis: ZA, WSA, and ZMM. Investigation: RM, FK, and RM. Writing-original draft preparation: ZMM. Editing: WSA, RM, FK, MHS and RM. Supervision: WSA, MM, ZB, MHS, and HMA. Project administration: HMA. Funding acquisition, H M. A. Submission, M H.S. All authors have read and agreed to the published version of the manuscript.

Ethical Approval: This study was conducted in accordance with the guidelines of the International Ethical Committee of the Institutional Bioethics Committee. All study is reported in accordance with ARRIVE guidelines.

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Conflicts of interest

Authors declare no conflict of interest.

References

1 Cummings JL, Cole G. Alzheimer disease. Jama 2002; 287:2335–2338.

2 Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb perspect med 2011; 1: a006189.

3 Barker GR, Banks PJ, Scott H, Ralph GS, Mitrophanous KA, Wong LF, Warburton EC. Separate elements of episodic memory subserved by distinct hippocampal-prefrontal connections. Nat Neurosci 2017; 20:242–250.

4 Apostolova LG. Alzheimer disease. Continuum: Lifelong Learning in Neurology 2016; 22(2 Dementia):419.

5 Castellani RJ, Rolston RK, Smith MA. Alzheimer disease. Disease-a-month: DM 2010a; 56:484–546.

6 Berkowitz LE, Harvey RE, Drake E, Thompson SM, Clark BJ. Progressive impairment of directional and spatially precise trajectories by TgF344-Alzheimer's disease rats in the Morris Water Task. Sci Rep 2018; 8:1–14.

7 Bendheim PE, Poeggeler B, Neria E, Ziv V, Pappolla MA, Chain DG. Development of indole-3-propionic acid (OXIGON^{TM}) for alzheimer's disease. J Mol Neurosci 2002; 19:213–217.

8 Bethune K. Thesis: Diagnosis and Treatment of Alzheimer's Disease: Current Challenges 2010

9 Bhushan I, Kour M, Kour G, Gupta S, Sharma S, Yadav A. Alzheimer's disease: Causes & treatment-A review. Ann Biotechnol 2018; 1:1002.

10 Black S, Doody R, Li H, McRae T, Jambor K, Xu Y, *et al.* Donepezil preserves cognition and global function in patients with severe Alzheimer disease. Neurology 2007; 69:459–469.

11 De-Paula VJ, Radanovic M, Diniz BS, Orestes V. Forlenza. Protein Aggregation and Fibrillogenesis in Cerebral and Systemic Amyloid DiseaseEgypt. Pharm J 2012a; 65:329.

12 De-Paula VJ, Radanovic M, Diniz BS, Forlenza OV. Alzheimer's disease. SubcellBiochem 2012b; 65:329–352.

13 DeKosky ST, Abrahamson EE, Ciallella JR, Paljug WR, Wisniewski SR, Clark RS, Ikonomovic MD. Association of increased cortical soluble Aβ42 levels with diffuse plaques after severe brain injury in humans. Arch of Neurol 2007; 64:541–544.

14 Shah MS, Najam-ul-Haq M, Shah HS, Rizvi SUF, Iqbal J. Quinoline containing chalcone derivatives as cholinesterase inhibitors and their in silico modeling studies. Comput Biol Chem 2018; 76:310–317.

15 Harizal S, Mansor S, Hasnan J, Tharakan J, Abdullah J. Acute toxicity study of the standardized methanolic extract of Mitragyna speciosa Korth in rodent. J Ethnopharmacol 2010; 131:404–409.

16 Shrivastava SK, Sinha SK, Srivastava P, Tripathi PN, Sharma P, Tripathi MK, *et al.* Design and development of novel p-aminobenzoic acid derivatives as potential cholinesterase inhibitors for the treatment of Alzheimer's disease. Bioorg Chem 2019; 82:211–223.

17 Hiremathad A, Piemontese L. Heterocyclic compounds as key structures for the interaction with old and new targets in Alzheimer's disease therapy. Neural Regen Res 2017a; 12:1256–1261.

18 Hiremathad A, Piemontese L. Heterocyclic compounds as key structures for the interaction with old and new targets in Alzheimer's disease therapy. Neural Regen Res 2017b; 12:1256.

19 Chávez-Gutiérrez L, Bammens L, Benilova I, Vandersteen A, Benurwar M, Borgers M, *et al.* The mechanism of γsecretase dysfunction in familial Alzheimer disease. The EMBO J 2012; 31:2261–2274.

20 Doraiswamy PM, Leon J, Cummings JL, Marin D, Neumann XX, *et al.* Prevalence and impact of medical comorbidity in Alzheimer's disease. J. Gerontol 2002; 57:M173–M177.

21 Emilien G, Durlach C, Minaker KL, Winblad B, Gauthier S, Maloteaux JM. Alzheimer Disease: Neuropsychology and Pharmacology 2003; SSBM

22 Fraga VG, Magalhães CA, Loures Cd MG, de Souza LC, Guimarães HC, Zauli D, *et al.* Inflammatory and pro-resolving mediators in frontotemporal dementia and Alzheimer's disease. Neurosci 2019; 421:123–135.

23 Clare L, Linden DE, Woods RT, Whitaker R, Evans SJ, Parkinson CH, *et al.* Goal-oriented cognitive rehabilitation for people with early-stage Alzheimer disease: a single-blind randomized controlled trial of clinical efficacy. Am J Geriatr Psychiatry 2010; 18:928–939.

24 Fraga H, Malheiro AC, Moutinho-Pereira JJ. An overview of climate change impacts on European viticultureA. Santos, Food Energy Secur 2012; 1:94–110.

25 Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. Cell 2012; 148:1204–1222.

26 Parle M, Vasudevan M, Singh N. Swim everyday to keep dementia away. J Sports Sci Med 2005; 4:37.

27 Ravn MB, Petersen KS, Thuesen J. Rehabilitation for people living with dementia: A scoping review of processes and outcomes. J Aging Res 2019; 2019:4141050.

28 Roberts RO, Christianson TJ, Kremers WK, Mielke MM, Machulda MM, Vassilaki M, *et al.* Association between olfactory dysfunction and amnestic mild cognitive impairment and Alzheimer disease dementia. JAMA Neurol 2016; 73:93–101.

29 Scott KR, Barrett AM. Dementia syndromes: evaluation and treatment. Expert Rev of Neurother 2007; 7:407–422.

30 Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan A., *et al.* Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia 2011; 7:280–292.

31 Van der Borght K, Robbert H, Thomas B, Bart JL, Van der Zee EEA. Exercise Improves Memory Acquisition and Retrieval in the Y-Maze Task: Relationship With Hippocampal Neurogenesis. Behav Neurosci 2007; 121:324–334.

32 Barker I, Steventon A, Williamson R, Deeny SR. Self-management capability in patients with long-term conditions is associated with reduced healthcare utilisation across a whole health economy: cross-sectional analysis of electronic health records. BMJ Qual Saf 2018; 27:989–999. https://doi.org/10.1136/bmjqs- 2017-007635

33 Gong QH, Wu Q, Huang XN, Sun AS, Shi JS. Protective effects of Ginkgo biloba leaf extract on aluminum-induced brain dysfunction in rats. Life Sci 2005; 77:140–148.

34 Hoffman E, Winder SJ. A modified wire hanging apparatus for small animal muscle function testing. PLoS Curr 2016; 8:ecurrents.

35 Brown GR, Nemes C. The exploratory behavior of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia? Behav Processes 2008; 78:442–448.

36 Thenmozhi AJ, Raja TRW, Janakiraman U, Manivasagam T. Neuroprotective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats. Neurochem Res 2015; 40:767–776.

37 Sohanaki H, Baluchnejadmojarad T, Nikbakht F, Roghani M. Pelargonidin improves passive avoidance task performance in a rat amyloid beta 25-35 model of Alzheimer's disease via estrogen receptor independent pathways. Acta Med Iran 2016; 54:245–250.