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Original Research Article

SYNTHESIS, COMPUTATIONAL STUDY, *IN VITRO* AND *IN VIVO* ANTICHOLINESTERASE PROPERTIES OF GLYCINE DERIVED DIPEPTIDE BEARING SULPHONAMIDE CARBOXAMIDE SCAFFOLDS

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ABSTRACT

Cholinesterase is one of several key enzymes required for the normal functioning of the human neurological system. Acetylcholine is an organic compound (ester of acetic acid and choline) that functions in the brain and body of many animals. It is a neurotransmitter that plays a major role in memory, learning and involuntary muscle movements. Its primary role is to transmit signals between the central nervous system (CNS) and the peripheral nervous system (PNS). Insufficient levels of acetylcholine are associated with conditions such as Alzheimer's disease (AD), Parkinson's disease and myasthenia (muscle weakness). This study investigates the anti-cholinesterase activity of five newly synthesized glycine-derived dipeptides bearing sulphonamide and carboxamide functionalities. The nucleophilic substitution reaction of L- threonine and benzenesulphonyl chloride gave the sulphonamide derivative. The reaction of L-threonine sulphonamide with deprotected carboxamide derivatives gave the target products. Structural elucidation was performed using spectroscopic techniques (FT-IR, ^{1}H-NMR, ^{13}C-NMR and HR-MS). *In vitro* and *ex vivo* anti-cholinesterase evaluation was done with rat brains. Compound 8e had the highest percentage inhibition 0f 47.91% (IC_{50} = 8.91 mg/mL) for acetylcholinesterase (AChE) and compound 8c, 40.08% (IC_{50} = 28.11 mg/mL) for butyrylcholinesterase (BuChE), respectively. This is moderately comparable to the standard drug, galanthamine with a percentage inhibition of 82.02 ±2.87%. The computational and physicochemical studies revealed compound 8c having the highest binding energy of -11.0 kcal/mol for AChE and compound 8d exhibited the highest binding energy of -10.9 kcal/mol for BuChE. The result of this study showed that the newly synthesized compounds would not pose oral bioavailability, transport and permeability problems from the computational analysis. Due to the effective inhibitory biological properties of compounds 8e and 8c against AChE and BuChE, the two hit compounds are expected to serve as a promising therapy for Alzheimer's disease.

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INTRODUCTION

Alzheimer's dementia (AD) is a neurodegenerative and complex neurological disorder characterized by an insufficient level of acetylcholine. It is a leading cause of disability and mortality among older people. This condition is associated with memory loss, thinking disability, and muscle weakness, also known as myasthenia gravis [1]. Alzheimer's disease is expected to affect over 40 million people worldwide by 2050 [2]. Clinical evidence has shown that cholinesterase inhibitors are effective treatments for Alzheimer's disease cognitive symptoms. These medications prevent the brain's neurotransmitter acetylcholine (ACh) from being broken down by the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) [3, 4]. However, medications must pass the blood-brain barrier to work within the central nervous system and address AD's cognitive symptoms [5]. Drugs available in the treatment of AD such as galantamine, donepezil and rivastigmine, have shown to improve the symptomatic conditions by increasing the acetylcholine levels and inhibiting acetylcholinesterase (AchE). These drugs also have side effects like hepatotoxicity, gastrointestinal tract disorders and periphery disorders. Hence, there is a need to develop new effective pharmacophores that can reduce toxicity and reduce the underlying cause of AD [1]. BuChE inhibition has been reported as a viable therapy approach for advanced AD in some studies [6,7]. Developing multi-target inhibition, such as inhibiting the activities of acetylcholinesterase (AChE) and butyryl cholinesterase (BuChE), is the prevailing strategy for the discovery of new anti-Alzheimer's disease (AD) drugs [8,9]. In clinical studies, selective BuChE inhibitors were found to have neuroprotective benefits on cognitive impairment recovery and reduced amyloid pathology in transgenic AD [10]. The current findings demonstrate the viability of BuChE as a supplementary therapeutic target for anti-AChE. In clinical studies, rivastigmine, a dual inhibitor of AChE/BuChE, proved greater cognitive improvement when compared to the AChE selective inhibitor [11]. To achieve a balance between AChEI and BuChE inhibition that will result in excellent treatments alongside fewer side effects, further research is crucial to solve this problem [12].

Interestingly, dipeptide compounds possessing a sulfonamide and carboxamide scaffold as an inhibitor of AChE/BuChE have not been explored extensively by medicinal chemists. A report by [13] proved that dipeptide-derived compounds incorporating sulfonamide functionality hold promising efficacy as inhibitors of AChE and BuChE in the treatment of AD, and this prompted us to design glycine-derived dipeptide compounds possessing sulfonamide functionality as a potential dual inhibitor of AChE/BuChE with high activity, good penetration and favorable side effects. The sulfonamide scaffold has sparked the attention Uttah Pradesh, India.

of several synthetic organic chemists due to its broad pharmacological properties. Sulfonamides are unique chemical compounds with a wide spectrum of actions, according to synthetic and biological assessment studies which showed that they are antimalarial [14-17], antimicrobial [17, 20], antidiabetics [18], antioxidant [18,20], antitrypanosomal [14], anti-alzheimer [13,19], antiviral [21], anticonvulsant [22], anticancer [23,24] and anti-inflammatory [25,26] among others. The synthesis of new compounds and the discovery of cutting-edge medicinal medicines have been made possible by their distinct structural features and reactivity [27]. Sulfonamide molecules have a variety of actions that have led to the development of beneficial therapeutic agents. There are currently more than 150 sulfonamide drugs available for clinical use in the market. Glibenclamide, being one of the most often prescribed diabetic medications, is used to treat type-2 diabetes mellitus (T2DM). It works by initiating the secretion of insulin from pancreatic beta cells, lowering blood glucose [28]. Infections of the urinary tract and Crohn's disease symptoms are treated with sulfonamide-based drug, sulfamethoxazole [29]. When glaucoma occurs, one of the most often prescribed eye drop medications, dorzolamide, is used to treat the elevated intraocular pressure [30]. A protease-inhibiting drug, amprenavir, is recommended for the treatment of AIDS and HIV infection [31]. Sulfonamides are significant biologically active substances, as previously mentioned and they can be combined with compounds with pharmacological properties such as peptides and carboxamides. In the current effort, we sought to synthesize some new glycine-derived dipeptides possessing sulfonamide and carboxamide functionalities to expand our drug development research on their synthesis and biological evaluation.

MATERIALS AND METHODS

Instrumentation

All starting materials and reagents were obtained from commercial sources from Thomas Baker Chemical Pvt. Ltd., and Finar Limited Ahmedabad, India and were used without further purification. The purity of synthesized compounds was checked using Thin Layer Chromatography (TLC) with different solvent systems (methanol, n-hexane and ethyl acetate) giving single spot. The IR spectra were recorded in KBr pellet method (PerkinElmer spectrum version 10.03.06). Nuclear magnetic resonance spectra (^{1}H and ^{13}C) were recorded in CD_3OD/DMSO-d6 on varian new 500 MHz. Mass spectra were determined using micro TOF electrospray time flight (ESI-TOF) and sodium formate was used as the calibrant. All laboratory synthesis and spectral analysis were conducted in Prof Sandeep Verma's research laboratory, chemistry department of Indian Institute of technology, Kanpur,

General Procedure for Benzene Sulphonamide Bearing L-Threonine

L-Threonine (1.34g, 11.25mmol) was dissolved in 20ml distilled water. Na_2CO_3 (1.19g, 11.25 mmol) was added to the solution. And stirred until all solute dissolved. The clear solution was cooled to -20 °C. Benzene sulphonyl chloride (7.5 mmol) was added over a period of 1h in four portions. The slurry was allowed to stir overnight at room temperature and monitored for completion with TLC using CH_2Cl_2/CH_3OH solvent system (7:3). Upon completion, the reaction mixture was acidified to pH between (2 - 2.2) with 20% concentrated aqueous HCl. Aqueous workup was done with 50ml ethylacetate and 50ml water. The organic layer was washed with water (2X) and then washed with brine solution; it was then collected over sodium sulphate. The organic layer was then evaporated using a rotary evaporator and further dried in a desiccator for 12 hours. This gave the product benzene sulphonamide derivatives bearing L-Threonine in good to excellent yields (63.51% - 72.83%).

General Procedure for Carboxamide Synthesis

Boc-glycine (0.560g, 3.2 mmol) was dissolved in 20ml dichloromethane (DCM) and triethylamine TEA (6.4 mmol). Cooled to -2 0^0C, 1-(3- dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride (EDC.HCl) (1.226g, 6.4mmol), Hydroxybenzotriazole, HOBt (0.432g, 3.2 mmol), were added and stirred for 10 minutes. Amine (4.8 mmol) was added to the solution and stirred for 30 min. The Mixture was stirred for 20 - 22hrs at room temperature while being monitored with TLC. On completion, the reaction mixture was diluted with distilled water (50 mL) and an aqueous work up performed. The organic layer was washed with 1N HCl (50ml), 10%$NaHCO_3$ (50ml) and brine solution (50ml) and collected over sodium sulphate. The organic layer was collected over sodium sulphate and then evaporated in a rotary evaporator. The resulting concentrate was deprotected by mixing in (TFA) trifluoroacetic acid/dichloromethane (1:1) and stirred at room temperature for 1 hour. TLC was used to check the completion of the reaction and the mixture was evaporated in a rotary evaporator. 50ml of diethyl ether was added and evaporated, and methanol (50ml) was also added to remove traces of TFA. Purification by column chromatography was done to give the desired product in pure form.

General Procedure for Sulphonamide-Dipeptide Derivatives.

Aryl sulphonamide derivative (3.2 mmol) was dissolved in 20ml DMF, triethylamine TEA (9.6 mmol) was added and the solution was cooled to -2^0C. EDC.HCL (6.4 mmol) and HOBt (6.4 mmol) were added and allowed to stir for 10 minutes. Carboxamide derivatives (4.8 mmol) were added to the mixture and stirred for 30 minutes. It was allowed to stir at room temperature for 20 – 22 hours while being monitored with TLC. On completion, the reaction mixture was diluted with H_2O (50 mL) and ethyl acetate EtOAc (50mL) and Aqueous work up performed. The organic layer was washed with 1N HCl, 10% $NaHCO_3$ and brine solution and then collected over sodium sulphate. The organic layer was evaporated in a rotary evaporator to give the desired product which was then purified by column chromatography to provide the pure compound.

3-hydroxy-N-(2-oxo-2-(phenylamino)ethyl)-2-(phenylsulfonamido)butanamide.

4-Yield (1.0753g, 68.61%), yellow liquid. FTIR (KBr, cm-1): 3533, 3415 (C-OH), 3364, 3315 (NH), 2971, 2882 (C-H of Alkanes), 1731, 1702 (C = O), 1599, 1539 (C = C Aromatic), 1346 (S =O), 1166 (S – N), 746 (mono substitution). ^{1}H NMR (500 MHz, METHANOL-D_4) δ 7.90 (d, 2H), 7.61 – 7.59 (m, 1H), 7.55 (dd, *J* = 3.1 Hz, 2H), 7.53 (dd, *J* = 1.3 Hz, 2H), 7.29 (d, 1H), 7.09 (d, 2H), 4.10 (d, *J* = 10.3 Hz, 2H), 3.97 – 3.82 (m, 1H), 3.68 (dd, 1H), 1.02 (d, 3H). ^{13}C NMR (126 MHz, METHANOL-D_4) δ 173.10, 169.42, 141.24, 139.26, 130.41, 130.21, 129.87, 129.81, 128.37, 127.82, 68.85, 63.76, 44.13, 19.55. ESI-MS (m/z): Found for $C_{18}H_{21}N_3O_5S$ 414.1095 (M + Na) $^+$; Calculated = 391.4469.

3-hydroxy-N-(2-oxo-2-(piperidin-1-yl)ethyl)-2-(phenylsulfonamido)butanamide.

4-Yield (1.0814g, 69.37%). Yellow liquid. FTIR (KBr, cm-1): 3356 broad (C-OH), 2858 (C-H of CH_3), 1634 (C = O), 1533, (C = C Aromatic, 1331 (S =O), 1165 (S – N), 757 (mono substitution). ^{1}H NMR (500 MHz, METHANOL-D_4) δ 7.87 (s, 2H), 7.60 (s, 1H), 7.54 (s, 2H), 4.06 (s, 1H), 3.89 (s, 2H), 3.74 (s, 1H), 3.44 (d, *J* = 78.2 Hz, 5H), 1.67 (s, 3H), 1.57 (s, 4H), 1.02 (s, 3H). ^{13}C NMR (126 MHz, METHANOL-D_4) δ 172.61, 167.98, 144.86, 138.77, 130.66, 128.40, 127.17, 69.02, 63.46, 46.88, 44.42, 41.87, 27.21, 25.33, 19.35. ESI-MS (m/z): Found for $C_{17}H_{25}N_3O_5S$:406.1407 (M + Na) $^+$; Calculated = 383.4671.

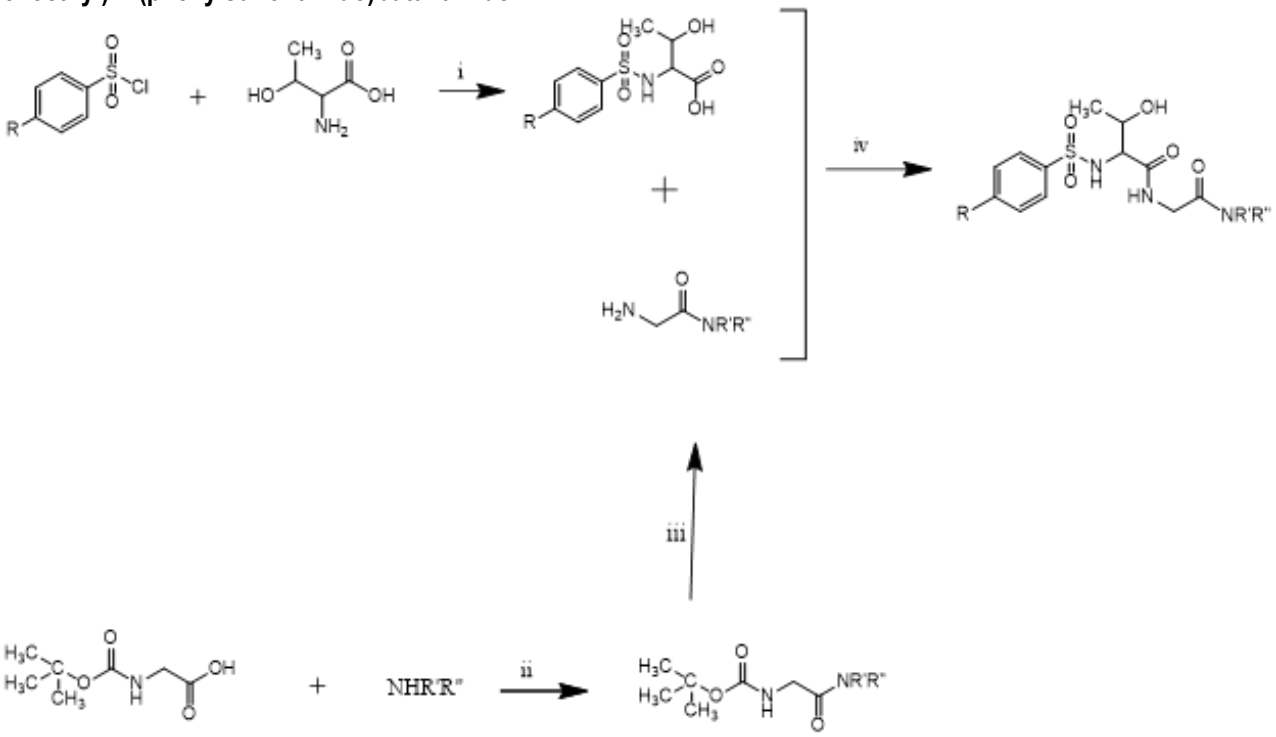
N-(2-((4-bromophenyl)amino)-2-oxoethyl)-3-hydroxy-2-phenylsulfonamido)butanamide. Yield (1.260g, 67.90%). Brown liquid. FTIR (KBr, cm-1): 3539, 3467 (C-OH), 3312, 3254 (N-H), 2932 (C-H of CH_3), 1827, 1730 (C = O), 1655 (C = C Aromatic), 1341 (S =O), 1166 (S – N), 753 (mono substitution). ^{1}H NMR (500 MHz, METHANOL-D_4) δ 7.90 (d, 2H), 7.69 – 7.62 (m, 1H), 7.57 (dd, 2H), 7.52 (d, 2H), 7.42 (d, 2H), 4.09 (d, 2H), 3.98 – 3.79 (m, 1H), 3.68 (dd, 1H), 1.01 (d, 3H). ^{13}C NMR (126 MHz, METHANOL-D_4) δ 173.10, 169.46, 134.05, 132.79, 130.41, 130.26, 128.35, 127.80, 127.07, 122.91, 68.82, 63.74, 62.79, 44.14, 19.54. ESI-MS (m/z): Found for $C_{18}H_{20}N_3O_5SBr$: 494.0338 (M + Na) $^+$; Calculated = 470.3431.

3-hydroxy-N-(2-morpholino-2-oxoethyl)-2-(phenylsulfonamido)butanamide. Yield (0.632g,

71.90%). Colourless liquid. FTIR (KBr, cm^{-1}): 3435 broad (C-O), 2983 (C-H of CH_3), 1726,(C = O), 1641 (C = C Aromatic), 1346 (S =O), 1167 (S – N), 750 (mono substitution). ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.18 (s, 1H), 7.16 (s, 1H), 7.10 (dd, *J* = 2.1, 1.0 Hz,2H), 7.09 (d, 1H), 6.83 (dd, *J* = 8.2 Hz, 2H), 3.95 (d, 2H), 3.65 (m, 1H), 3.59 (t, 4H), 3.38 (t, 4H), 0.32 (dd, 1H), 0.17 (d 3H). ^{13}C NMR (126 MHz, METHANOL-D_4) δ 167.14, 130.73, 130.41, 129.40, 128.56, 127.82, 83.09, 69.56, 63.87, 62.82, 19.79. ESI-MS (m/z): Found for $C_{16}H_{23}N_3O_6S$: 409.0021 (M + Na) $^+$; Calculated = 386.4483.

3-hydroxy-N-(2-((4-methoxyphenyl)amino)-2-oxoethyl)-2-(phenylsulfonamido)butanamide.

Yield (1.00g, 69.65%). Light purple solid, melting point = 186 – 188^{0}C. FTIR (KBr, cm^{-1}): 3543, 3314 (N-H), 2922 (C-H of CH_3), 1750, (C = O), 1537 (C = C Aromatic), 1344 (S =O), 1169 (S – N), 749 (mono substitution). ^{1}H NMR (500 MHz, METHANOL-D_4) δ 7.77 (d, 2H), 7.40 (d, 2H), 5.26 (s, 1H), 4.75 (d, 2H), 4.09 (m, 1H), 3.83 (dd, 1H), 3.41 (t, 4H), 2.43 (s, 3H), 1.88 (t, 4H), 1.19 (s, 3H). ^{13}C NMR (126 MHz, METHANOL-D_4) δ 173.13, 169.19, 141.19, 134.00, 132.20, 130.22, 128.38, 127.82, 123.01, 114.93, 68.82, 63.86, 55.84, 44.02, 19.79. ESI-MS (m/z): Found for $C_{19}H_{23}N_3O_6S$: 444.1200 (M + Na) $^+$; Calculated = 421.4735.



Scheme 1: Synthesis of sulphonamide dipeptide carboxamide derivatives. R = H

Computation of ADMET/drug-likeness of the compounds.

The molecular docking prediction of the drug-likeness of the synthesized compounds was done using the SwissADMET free online web. Computed descriptors include molecular weight, hydrogen bond donor, hydrogen bond acceptor, number of rotatable bonds, consensus Lop *p*, and topological polar surface area. The prediction of ADMET properties on a drug candidate plays an important role in drug design as it accounts for the failure of most drugs in the clinical phases. Drug candidates with good toxicity and pharmacokinetic properties often pass tests during clinical trials. The use of ADMET in the prediction of drug properties before embarking on expensive research or experimentation is pertinent as it helps in selecting a successful drug candidate. The present work used ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties to evaluate the toxicity profile of the ligands.

Determination of AChE and BChE inhibitory activities

Test Animals and Tissue Preparation

Swiss Albino mice of both sexes weighing 80-100 g were used; they were kept and maintained under laboratory conditions of temperature and humidity (12/12h light/dark cycle). They were exposed to free access to food (standard pellet diet) and portable tap water ad libitum and a 12 hr light/dark cycle. The animals were handled humanely in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals and Other Scientific Purposes ETS123. The Scientific and Bioethics

Committee at the University of Nigeria, Nsukka, approved these studies with ref no (UNN/FBS/24/PG/PHD/68316). The rats are decapitated, brains rapidly removed, corpora striata dissected free, weighed and homogenized in 19 volumes (approximately 7 mg protein/ml) of 0.05 M NaH_2PO4, pH 7.2 using a Potter Elvejhem homogenizer (Kontes, Vineland, NJ). A 25 µl aliquot of this suspension is added to 1 ml of the various concentrations of the test samples and preincubated for 10 min at 37 °C.

Method for acetylcholinesterase/ butyrylcholinesterase Assay

The assay for acetylcholinesterase was carried out according to Ellman *et al* (1961) method with slight modification. The total volume of the reaction mixture was 1000µL. It contained 60µL 0.1M Na_2HPO_4 buffer, pH 8.0. 20µL test compound (2.5, 5.0 and 10 mg/mL) was added, followed by the addition of 50µL of the enzyme source. The contents were mixed and preincubated for 15 min at room temperature. The reaction was initiated by the addition of 75µL of substrate (acetylthiocholine/ butyrylthiocholine iodide), followed by the addition of 450µL DTNB. After 30 min of incubation at room temperature, absorbance was measured at 412nm using a spectrophotometer. All experiments were carried out with their respective controls in duplicate. Eserine was used as a positive control (Standard).

Percentage inhibition was calculated as follows:

Inhibition (%) = $\frac{Ao-A1}{Ao} X100$

where Ao is the absorbance of Control (that is, the total enzyme activity without inhibitor) and A_1 is the absorbance of Test (that is, the enzyme activity in the presence of test compound or inhibitor). The principle of the method is the measurement of the rate of production of thiocholine as acetylthiocholine/ butyrylthiocholine iodide is hydrolyzed. This is accomplished by the continuous reaction of the thiol with 5-dithiobis-2-nitrobenzoate ion to produce the yellow anion of 5-thio-2-nitrobenzoic acid. The rate of colour production is measured at 412 nm in a spectrophotometer.

Data Analysis

Molecular docking simulations were performed on the studied compounds **8a-e** to determine their binding interactions and inhibitory properties with the target enzymes. The docking simulation result is presented in Table 1. From the results obtained, it was noticed that the compounds had strong interactions with the target proteins, thereby inhibiting their biochemical processes. Compound **8c** showed a very strong inhibition against the AChE enzyme, with a binding affinity of -11.0kcal/mol comparable with galantamine (11.4kcal/mol), a clinical drug used in the management of Alzheimer's disease (AD). The BChE enzyme studies revealed that compound **8d** (-10.9kcal/mol) displayed a more favorable interaction against the studied protein than the standard drug galantamine (-10.7kcal/mol). Compounds **8e, 8a** and showed a promising inhibitory action against AChE enzyme with a docking score ranging from -10.5 to -10.1kcal/mol and could be considered possible drug contenders for the treatment of AD while compound **8c**, **8a** and **8e** showed very good interaction with a binding affinity between the range of -10.0 to -9.4kcal/mol for BChE protein and are also considered contending anti-BChE drug candidates comparable to the standard drug, galantamine.

RESULTS

Table 1 shows the free binding energy (Kcal/mol) of compounds with protein target 4EY7 and 7AIY

Table 1: Free binding energy (Kcal/mol) of compounds with protein target 4EY7 and 7AIY

Compounds	AChE Binding Energies (kcal/mol)	BChE Binding Energies (kcal/mol)
8a	-10.2	-9.4
8b	-9.7	-8.6
8c	-11.0	-10.0
8d	-9.4	-10.9
8e	-9.3	-9.4
Galanthamine (standard drug)	-11.4	-10.7

Grades of binding energy: ≤ -4 = weak binding**,** -5 ≤ -7 = moderate binding**,** -8 ≤ - 9 = strong binding**,** ≥ -10 = very strong binding

Drug-Likeness Studies

A compound is considered a potential drug candidate for clinical trial or use if it possesses standard pharmacokinetics and safety characteristics and it must be biologically active for its intended purposes. The computed drug-likeness properties of the novel compounds are presented in Table 2. Lipophilicity accounts for how a drug

molecule dissolves in a nonpolar solvent or lipids and this property plays a crucial role in the overall ADMET property. Lipophilicity is a parameter that significantly affects drug absorption across cell membranes (Arnott and Planey, 2012). According to the Lipinski rule of five, a lipophilicity range of 0 to 5 is usually needed for optimal drug design. The computed result for lipophilicity studies ranges from -0.20 to 2.0, respectively. The reported compounds **8a-e** are therefore expected to be readily soluble and to exhibit good absorption across cell membranes upon administration. In drug design, molecular weight is a parameter that influences toxicity. The molecular weight of a drug candidate must not exceed 500 according to the rule of five for druglikeness. In this report, the molecular weight of the ten novel dipeptides ranges from 345.41-470.31g/mol. This implies that Compound **8a-e** passed the toxicity test; hence, they are considered as drug candidates for further clinical evaluation. As reported in this research, none of the compounds **8a-e** violated the Lipinski rule (Lipinski et al., 2001) and as such they are considered contending drug candidates for further examination.

Table 2: Computed ADMET/drug-likeness of synthesized compounds

Compound	**MW**	**donor HB**	**accept HB**	**Log P**	**RBC**	**Rule of five**	**tPSA [Å²]**	**B. A**
8a	391.44	4	6	0.93	10	0	132.98	0.55
8b	383.46	3	6	0.30	9	0	124.19	0.55
8c	470.31	4	6	1.62	10	0	132.98	0.55
8d	385.44	3	7	-0.36	9	0	133.42	0.55
8e	423.48	5	8	0.85	11	1	145.37	0.55
Recommended value	405.47	4	6	1.31	10	0	132.98	0.55

Abbreviations: MW- Molecular weight of the molecule, **donorHB -** number of hydrogen bond donor. **acceptHB-** number of hydrogen bond acceptor. **Rule Of Five-** Number of violations of Lipinski's rule of five, **Log P-** partition coefficient**, RBC -** number of rotatable bonds, **tPSA** – topological polar surface area**, B.A**- bioavailability score.

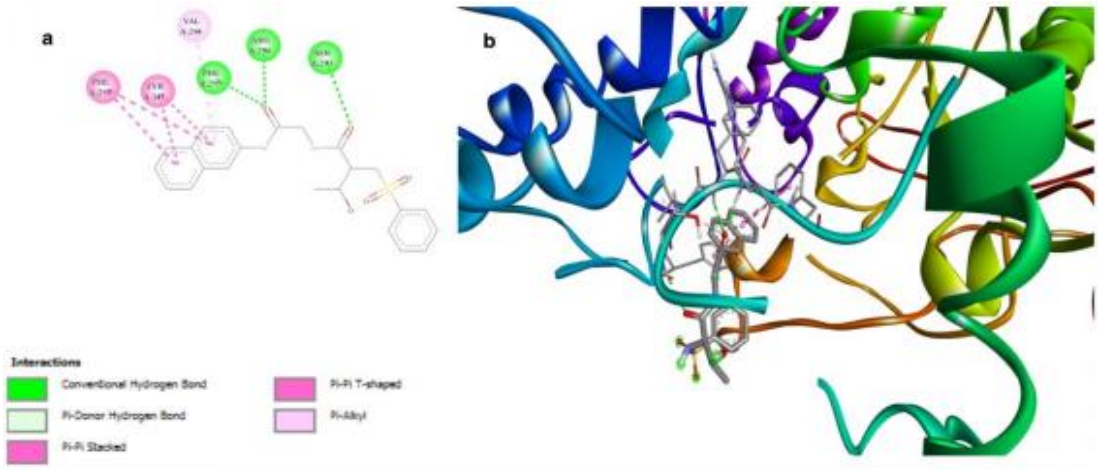


Figure 1. (a) 2d (b) 3d binding model of **compound 8c** in the binding cavity of 4ey7. Ligands, amino acid residues, and receptors are depicted in line, ball, and solid ribbon models respectively. Green and pink depict H-bond acceptor and donor respectively.

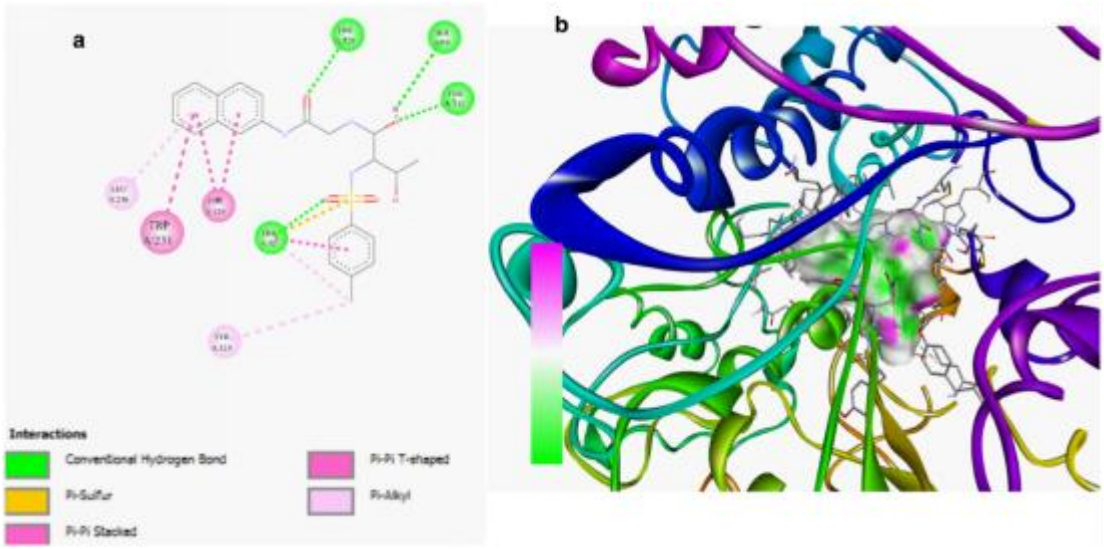


Figure 2. The binding interaction of compound **8d** with the enzymes 7aiy, visualized at the binding site cavities by Discovery studio. Compound **8d** is presented in line model, amino acid residues in ball, and receptor in solid ribbon. Hydrogen bond donor and acceptor are disappeared in green and pink respectively. (a) Displaying the 2D binding site interactions of **compound 8d** with 7aiy binding pocket, (b) 3D binding model of **compound 8d** with 7a binding pocket.

Anticholinesterase Study.

Two different enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) hydrolyze acetylcholine in vertebrates. Acetylcholinesterase (AChE) inhibition has been thought to be quite important for the reduction of activity of the cholinergic neurons detected in the brains of AD patients. Five compounds were screened for *in vitro* acetylcholinesterase and butyrylcholinesterase pharmacological properties and their potency in the treatment of Alzheimer's dementia was evaluated. The Ellman spectrophotometric method was adopted for this study with little modifications. The table below shows the result of the *in vitro* acetylcholinesterase and butyrylcholinesterase properties of the newly synthesized compounds.

Table 3: Percentage inhibition for Acetylcholinesterase Activity

Compound No	Inhibition (%)	IC_{50} (mg/ml)
8a	31.80	51.82
8b	38.29	1190
8c	45.98	9.95
8d	36.20	54.85
8e	38.00	13.57
Standard drug(Galanthamine)	82.02	--

Table 4: Percentage inhibition for Butyrylcholinesterase Activity

Compound No	Inhibition (%)	IC_{50} (mg/ml)
8a	29.07	49.12
8b	47.9	791.57
8c	40.08	28.11
8d	27.92	44.24
8e	35.34	21.72
Standard drug(Galanthamine)	86.02	--

The results from the tables above showed that some of the synthesized compounds were highly active and moderate for anticholinesterase properties. The percentage inhibition recorded for the compounds ranged between 29.66% – 47.91% for acetylcholinesterase and 26.15% – 40.08% for butyrylcholinesterase respectively. The $IC_{50,}$ which is the half-maximal inhibitory concentration, was also evaluated. IC_{50} value is a measure of the concentration of a drug or compound required to inhibit a particular biological or biochemical process by 50%. It measures a drug's efficacy. The lower the IC_{50}, the more potent the drug or compound is. The IC_{50} was in the range of 8.91mg/ml to 9520 mg/ml for

the acetylcholinesterase analysis, and for butyrylcholinesterase, the range of IC_{50} is between 21.72mg/ml to 1620 mg/ml. Compound **8b** had the highest percentage inhibition of 47.91% with an IC_{50} value of 8.91mg/ml, which is moderately comparable to the standard drug Galanthamine with a percentage inhibition of 82.02% ±2.87. The compound with the second highest inhibition is compound **8c** with a percentage inhibition of 45.98% and an IC_{50} value of 9.95mg/ml. For the butyrylcholinesterase evaluation, compound **8c** recorded the highest percentage inhibition of 40.08% compared to the standard drug Galanthamine with a percentage inhibition of 86.02% ±1.18, with IC_{50} value of 28.11mg/ml. This was followed by compound **8e** with a percentage inhibition of 35.34% and the lowest IC_{50} value of 21.72mg/ml.

DISCUSSION

The synthetic pathway involves the reaction of L-Threonine with unsubstituted benezenesulphonyl chloride in the presence of sodium carbonate (base). This gave the benzenesulphonamido butanoic acid (**8a**) at 72.83% yield. N-Boc-protected glycine was treated with ten primary or secondary amines in the presence of trimethylamine (as the base) and the coupling reagents: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) and 1-hydroxybenzotriazole (HOBt), giving protected carbamates. The Boc-protecting group of the resulting carbamates **(6a-e)** was removed with TFA/DCM (1:1) to afford deprotected carboxamides. Further reaction of the deprotected carboxamides and benzenesulphonamide afforded the target products (**8a-e**) in good yields. The compounds were characterised using FTIR, ^{1}H NMR, ^{13}C NMR and high-resolution mass spectroscopy (HRMS). The data were in agreement with the proposed structure. For the IR spectra, the final compounds showed signals at 3533 – 3347 cm^{-1} (broad) attributed to the hydroxyl group, 3346 – 3314 cm^{-1} for NH of amide, 1731 – 1726 cm^{-1} for carbonyl, 1599 – 1530 cm^{-1} for C = C of aromatic and 1168 – 1165 cm^{-1} attributed to S – N group. In the ^{1}H-NMR spectra, the signal of the amide protons was dependent on the solvent used; it appeared at 8.80–8.40 in DMSO-d6 and was undetectable in methanol-d4. The three signals of the phenyl protons appeared at 7.90, 7.61 and d 7.55 ppm, which is indicative of aromatic mono-substitution. The signal of the methylene protons appeared at 4.10 – 4.09 ppm. The two signals of the methine protons appeared at 3.89 – 3.00 ppm. In the carbon-13 NMR spectra, the peaks between 173.10 - 164.9 ppm were assigned to the carbonyl of the carboxamide, 141.24 – 126.40 were assigned to C = C of aromatic carbons. . In high-resolution mass spectrometry, molecular ion peaks ranging from 366.3082 – 486.5054 $(M + Na)^{+}$ were found.

CONCLUSION

Acetylcholinesterase (AChE) inhibitors are an important class of medicinal agents useful for the treatment of AD, glaucoma, myasthenia gravis, and for the recovery of neuromuscular block in surgery. In the present study, *in vitro* AChE and BChE inhibitory activities of ten newly synthesized glycine derived dipeptide bearing sulphonamide carboxamide moieties were studied. The results of *in vitro and ex vivo* acetylcholinesterase and butyrylcholinesterase pharmacological properties as anti-Alzheimer's dementia were evaluated. The percentage inhibition recorded for the compounds ranges between 29.66% – 47.91% for acetylcholinesterase and 26.15% – 40.08% for butyrycholinesterase respectively. The IC_{50} recorded was in the range of 8.91mg/ml to 9520 mg/ml for the acetylcholinesterase analysis, and for butyrylcholinesterase, the range of IC_{50} is between 21.72mg/ml to 1620 mg/ml. Compound 8g had the highest percentage inhibition of 47.91% with an IC_{50} of 8.91mg/ml, which is moderately comparable to the standard drug Galanthamine with a percentage inhibition of 82.02% ±2.87. For the butyrylcholinesterase evaluation, compound 8c recorded the highest percentage inhibition of 40.08% with a low IC_{50} of 28.11mg/ml compared to the standard drug Galanthamine with a percentage inhibition of 86.02% ±1.18. This result correlates with the *in silico* studies which showed compound 8c as having the highest binding energy of -11kcal/mol for acetylcholinesterase activity. But for butyrylcholinesterase, there is a slight deviation as recorded in compound 8c, having the highest percentage inhibition of 40.08% with a low binding energy of -9.3kcal/mol. This can be attributed to the different pathways of enzyme metabolism.

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AUTHORS' CONTRIBUTION

JNO carried out the research, SIA proof read the manuscript UCO, BEE and DIU design the work, COE, TNO and RA did the molecular docking, FNI, VIO, BOE and FUE brought resource.

CONFLICT OF INTEREST

The authors have no conflict of Interest

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