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Synthesis, Molecular Docking and Biological Evaluation of Novel Chlorobenzenesulfonamide Analogues of Ibuprofen as Anti-Bacterial, Anti-Diabetic, and Anti-Alzheimer Agents

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Abstract

In this work, we have synthesized the novel chlorobenzenesulfonamide tertiary amide-based analogues of ibuprofen (Ibu) and investigated them for their anti-bacterial, anti-diabetic, and anti-Alzheimer activities. In the first step, Ibu was converted to amide derivatives by incorporating different aromatic amines. These were further converted into sulfonamide analogues (Sul-Ibu, 1–9) by treating them with p-chlorobenzenesulfonyl chloride. Sul-Ibu (1–9) were screened against Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Actinomyces odontolyticus, Bacillus halodurans, and Micrococcus luteus. The analogues 4, and 7–9 showed significant results as compared to Ibu due to the presence of the sulfonamide moiety. The anti-diabetic efficacy was also determined by testing the inhibitory potency against diabetes causing enzymes (α-amylase and α-glucosidase) and found 4 (IC_{50} 61.80±0.85 µg/mL) and 5(IC_{50} 53.75±0.79 µg/mL) as the most promising agents. Sul-Ibu analogues also exhibited impressive inhibition profiles as anti-Alzheimer agents. Compound 7 appeared to be the most potent one, providing the percentage inhibition of 95.8±0.31 against acetylcholinesterase (AChE) and 96.7±0.16 against butyrylcholinesterase (BChE). Additionally, this analogue also provided a K_i value of 27 µmol. Molecular docking study with α-amylase, α-glucosidase, AChE, and BChE was also in good accordance with that of experimental results. These results indicate that Sul-Ibu analogues appeared promising anti-bacterial, anti-diabetic, and anti-Alzheimer agents and should be considered for clinical studies.

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INTRODUCTION

Ibuprofen (NSAID) has been widely used as an analgesic [1], anti-pyretic [2], and anti-inflammatory agent, and for the treatment of rheumatoid arthritis [3, 4]. Adverse effects are well established, and its prolonged use is attributed to kidney damage [5], gastric ulcer [6], and hepatotoxicity [7]. Common toxic effects include gastrointestinal erosion, bleeding, and cardiovascular disturbance [8]. It was observed that the presence of a carboxylic acid group (–COOH) is responsible for gastric toxicity, as evaluated by NSAID prodrugs. NSAID derivatives lacking acidic mobility retain their anti-inflammatory activity but have no gastric toxicity [9]. Many amides, hydrazides, ansd hybrid derivatives of Ibu have been synthesized and evaluated for their efficacy

and reduction of side effects. Ibuprofen derivatives have been explored for their platelet protective efficacy and platelet aggregation inhibitory properties [10]. Oxadiazole derivatives of ibuprofen and naproxen were synthesized and tested for anti-bacterial potential [11]. Thiadiazole derivatives of ibuprofen were investigated for their DNA-binding abilities and to study their anti-cancer activities against human hepatocellular carcinoma (Huh-7) cell lines and were found to be potential anti-cancer drug candidates [12].

Sulfa drugs (drugs containing a sulfonamide moiety) are broadly effective anti-bacterial drugs and have brought about an antibiotic revolution in medicine. Many commercial sulfa drugs, *i.e.*, sulfanilamide, sulfadiazine, and sulfamethoxazole, have been used efficiently [13]. Many studies have explored the efficiency of sulfonamide moieties (pharmacophores) in addition to their anti-bacterial potential. It provides important insights into diverse biological responses, including anti-Alzheimer [14], anti-bacterial [15], anti-cancer, anti-viral [16], and anti-diabetic [17] potencies. Besides the medicinal importance of sulfonamides, a major drawback associated with them is low membrane permeability and most likely allergic reactions in patients [18].

Another serious challenge faced by researchers is the tendency of microorganisms to develop resistance against drugs [19]; therefore, there is an urgent need to discover new antibiotics. Modification of existing drugs by incorporating different pharmacophores is a suitable strategy to obtain better drugs. To the best of our knowledge, tertiary amide-based *N*-sulfonamide analogues of Ibu have not yet been reported. Therefore, we synthesized sulfonamide derivatives of Ibu and evaluated their biological potential. These molecules were also docked to compare the experimental results with computational findings and to understand protein-ligand interactions at the molecular level.

MATERIAL AND METHOD

Chemistry

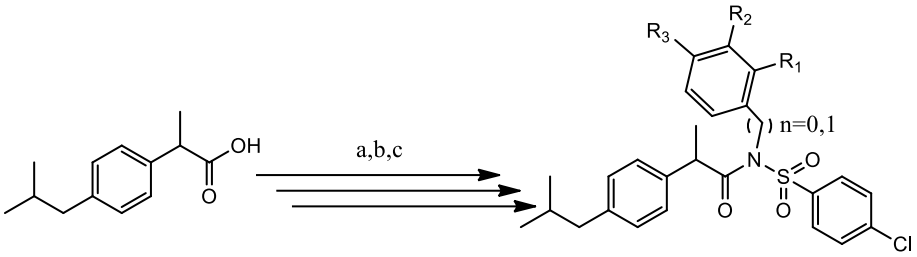
All chemicals were obtained from Sigma-Aldrich and Van Waters and Rogers (VWR) and used without further purification. Silica-coated Al-TLC cards (silica gel 60 F254) were used to monitor reactions. The synthesized derivatives (1–9) were purified by silica gel (70–230 mesh) column chromatography (EMD Millipore Corporation, Darmstadt, Germany). An electrothermal melting point apparatus was used to examine the melting points of the compounds. Fourier transform infrared (FTIR) spectra were recorded using a Bio-Rad spectrophotometer, and the data are represented in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker ARX-300 and ARX-500 instruments with Tetramethylsilane (TMS) as an internal standard. The compounds were dissolved in DMSO-d_6 and $CDCl_3$.

General Method for the Synthesis of N-sulfonamide Analogues of Ibu (1–9)

2-(4-Isobutylphenyl)propanoic acid (Ibu) (0.2 g, 1 mmol) was treated with thionyl chloride (362.7 µL, 5 mmol) and refluxed for 6 h to obtain 2-(4-Isobutylphenyl)propanoyl chloride. Excess SO_2 and HCl gases were removed under a vacuum using a rotary evaporator. Separately prepared equimolar solutions of different aromatic amines (a variety of anilines and benzylamines) in THF were added dropwise to the mixture. The mixture was refluxed for another 6 h, and the progress of the reactions was monitored by TLC. The crude product was purified via column chromatography using ethyl acetate/n-hexane (1:3). The amide derivatives of Ibu (0.3 g, 1 mmol) were dissolved in dichloromethane, and *p*-chlorobenzenesulfonyl chloride (0.2 g, 1 mmol) was added dropwise. The obtained mixture was refluxed for 12 h, and the progress of the reaction was monitored by TLC. The final products were purified via column chromatography using ethyl acetate/n-hexane (1:3). (Scheme 1) (^{1}H and ^{13}C NMR spectra).

N-((4-chlorophenyl)sulfonyl)-2-(4-isobutylphenyl)-N-phenylpropanamide (1)

Yield: 66%; White solid; Rf: 0.70; M.P.: 121–123°C; FTIR: 2954 (CH-arom), 1706 (C=O), 1580 (C=C) cm-1; 1H-NMR: δ 7.97–7.73 (4H, dd, J=8.7 Hz, Ar-H), 6.96–6.54 (6H, dd, J=8.1, 7.8 Hz, Ar-H), 3.44 (1H, q, J= 6.6 Hz, CH), 2.38 (2H, d, J= 6.9 Hz, CH2), 1.77 (1H, m, CH), 1.14 (3H, d, J= 6.6 Hz, CH3), 0.85 (6H, d, J= 6.6 Hz, 2CH3) ppm; 13C-NMR : δ 173.8, 140.4, 139.6, 137.9, 137.2, 135.2, 131.0, 130.9, 130.4, 129.7, 129.5, 127.1, 45.2, 44.5, 30.0, 22.5, 19.9 ppm.



Scheme 1. Synthetic scheme for sulfonamide derivatives of Ibu. (a) $SOCl_2$, overnight stirring; (b) R-NH_2/R-CH_2NH_2 THF, Et_3N, overnight stirring; (c) R-SO_2Cl, Dichloromethane (DCM), Et_3N, 12 hours.

N-((4-chlorophenyl)sulfonyl)-2-(4-isobutylphenyl)-N-(2-methoxyphenyl) propanamide (2)

Yield 79%; White solid, Rf: 0.71; M.P.: 92–96°C; FTIR: 2961 (CH-arom), 1697 (C=O), 1580 (C=C) cm-1; 1H-NMR: δ 7.87–7.74 (4H, dd, J=8.7 Hz, Ar-H), 6.95–6.52 (5H, dd, J=7.8 Hz, Ar-H), 3.61 (3H, s, OCH3), 3.56 (1H, q, J= 6.6 Hz, CH), 2.35(2H, d, J= 7.2 Hz, CH2), 1.67 (1H, m, CH), 1.18 (3H, d, J= 6.6 Hz, CH3), 0.87 (6H, d, J= 6.6 Hz, 2CH3) ppm; 13C-NMR: δ 174.1, 159.0, 141.3, 140.6, 138.2, 137.1, 136.8, 130.8, 130.3, 128.5, 127.8, 126.5, 124.5, 117.5, 114.6, 57.6, 47.8, 46.4, 30.3, 22.3, 20.6 ppm.

N-((4-chlorophenyl)sulfonyl)-2-(4-isobutylphenyl)-N-(3-methoxyphenyl) propanamide (3)

Yield: 74%; White solid; Rf: 0.42; M.P.: 194–197°C; FTIR: 2957 (CH-arom), 1710 (C=O), 1598 (C=C) cm-1; 1H-NMR :δ 7.98–7.76 (4H, dd, J=8.7Hz, Ar-H), 6.97–6.54 (5H, dd, J=7.8 Hz, Ar-H), 3.64 (3H, s, OCH3), 3.56 (1H, q, J= 6.6 Hz, CH), 2.38 (2H, d, J= 7.2 Hz, CH2), 1.77 (1H, m, CH), 1.13 (3H, d, J= 6.9 Hz, CH3), 0.84 (6H, d, J=6.6 Hz, 2CH3) ppm; 13C-NMR: δ 173.7, 160.0, 140.4, 139.6, 137.9, 137.5, 136.1, 131.0, 130.3, 129.5, 127.4, 127.1, 123.0, 116.4, 116.3, 55.6, 45.2, 44.5, 30.0, 22.5, 20.1 ppm.

N-((4-chlorophenyl)sulfonyl)-2-(4-isobutylphenyl)-N-(4-methoxyphenyl) propanamide (4)

Yield: 68%; White solid; Rf: 0.72; M.P.: 131–133°C; FTIR: 2951 (CH-arom), 1703 (C=O), 1600 (C=C)cm-1; 1H-NMR: δ 7.94–7.73 (4H, dd, J= 6.9, 6.6Hz, Ar-H), 6.98–6.59 (6H, dd, J= 8.1 Hz, Ar-H), 3.80 (3H, s, OCH3), 3.51 (1H, q, J= 6.9 Hz, CH), 2.39 (2H, d, J= 6.9 Hz, CH2), 1.78 (1H, m, CH), 1.14 (3H, d, J= 6.9 Hz, CH3), 0.86 (6H, d, J=6.6 Hz, 2CH3) ppm; 13C-NMR: δ 174.2, 160.5, 140.3, 139.5, 137.9, 137.3, 132.0, 130.9, 129.7, 129.5, 127.5, 127.1, 114.9, 55.9, 45.1, 44.5, 30.0, 22.5, 22.5, 19.9 ppm.

N-((4-chlorophenyl)sulfonyl)-N-(3-fluorophenyl)-2-(4-isobutylphenyl) propanamide (5)

Yield 78%; White solid, Rf: 0.73; M.P.: 174–179°C; FTIR: 2990 (CH-arom), 1711 (C=O), 1657 (C=C) cm-1; 1H-NMR: δ 7.96–6.59 (12H, Ar-H), 3.50 (1H, q, J=6.6 Hz, CH), 2.37 (2H, d, J= 6.9 Hz, CH2), 1.76 (1H, m, CH), 1.12 (3H, d, J=6.6 Hz, CH3), 0.83(6H, d, J= 6.6 Hz, 2CH3) ppm; 13C-NMR: δ 173.7, 163.2, 161.2, 141.6, 139.7, 137.8, 133.1, 130.7, 130.4, 129.1, 116.7, 45.4, 44.5, 30.0, 22.5, 20.0ppm.

N-((4-chlorophenyl)sulfonyl)-N-(4-fluorophenyl)-2-(4-isobutylphenyl) propanamide (6)

Yield 81%; White solid, Rf: 0.72; M.P.: 125–128°C; FTIR: 2952 (CH-arom), 1716 (C=O), 1577 (C=C) cm-1; 1H-NMR:δ 7.97–7.73 (4H, dd, J=8.4 Hz, Ar-H), 7.18 (3H, s (broad), Ar-H), 6.97–6.58 (5H, dd, J= 7.5Hz, Ar-H), 3.48 (1H, q, J=6.6 Hz, CH), 2.38 (2H, d, J= 6.9 Hz, CH2), 1.77 (1H, m, CH), 1.14 (3H, d, J=6.6 Hz, CH3), 0.84 (6H, d, J=6.3Hz, 2CH3) ppm; 13C-NMR: δ 173.7, 164.5, 161.2, 140.4, 139.7, 137.2, 133.2, 131.4, 129.7, 127.1, 116.7, 45.4, 44.5, 30.0, 22.5, 20.0 ppm.

N-benzyl-N-((4-chlorophenyl)sulfonyl)-2-(4-isobutylphenyl) propanamide (7)

Yield 83%; White solid, Rf: 0.59; M.P.: 101–104°C; FTIR: 2953 (CH-arom), 1707 (C=O), 1506 (C=C) cm-1; 1H-NMR: δ 7.96–7.26 (12H, Ar-H), 4.41 (2H, s, CH2), 3.53 (1H, q, J= 6.6 Hz, CH), 2.37 (2H, d, J=6.9 Hz, CH2), 1.72 (1H, m, CH), 1.13 (3H, d, J=6.6Hz, CH3), 0.87 (6H, d, J=6.3 Hz, 2CH3) ppm; 13C-NMR: δ 173.8, 141.2, 140.2, 138.5, 136.3, 135.2, 129.7, 127.1, 45.2, 44.8, 29.7, 22.5, 19.8 ppm.

N-(4-chlorobenzyl)-N-((4-chlorophenyl)sulfonyl)-2-(4-isobutylphenyl) propanamide (8)

Yield 34%; White solid; Rf: 0.75; M.P.: 97–100°C; FTIR: 2955 (CH-arom), 1701 (C=O), 1583 (C=C) cm-1; 1H-NMR: δ 7.90–7.67 (4H, dd, J=8.7 Hz, Ar-H), 7.39–7.18 (4H, dd, J=8.4 Hz, Ar-H), 6.98–6.81 (4H, dd, J=8.1 Hz, Ar-H), 5.05 (2H, J = 18 Hz, CH2), 4.09 (1H, q, J= 6.9 Hz, CH), 2.37 (2H, d, J=6.9 Hz, CH2), 1.76 (1H, m, CH), 1.16 (3H, d, J= 6.6 Hz, CH3), 0.84 (6H, d, J=6.6 Hz, 2CH3) ppm; 13C-NMR: δ 174.6, 140.6, 139.6, 137.9, 136.9, 136.4, 132.4, 130.3, 129.7, 128.9, 127.2, 49.3, 44.5, 30.0, 22.6, 20.5 ppm.

N-((4-chlorophenyl)sulfonyl)-2-(4-isobutylphenyl)-N-(4-methoxybenzyl) propanamide (9)

Yield 46%; Yellow liquid, Rf: 0.66; FTIR: 3068 (CH-arom), 1680 (C=O), 1521(C=C) cm-1; 1H-NMR:δ7.84–7.63 (4H, dd, J=9.0, 6.0 Hz, Ar-H), 7.16–6.82 (8H, dq, J=8.7, 8.1 Hz, Ar-H), 4.97 (dd, J=15 Hz, CH2) 4.06 (1H, q, J= 6.9 Hz, CH), 3.74 (3H, s, OCH3), 2.39 (2H, d, J= 7.2 Hz, CH2), 1.77 (1H, m, CH), 1.14 (3H, d, J= 6.3 Hz, CH3), 0.84 (6H, d, J=6.6 Hz, 2CH3) ppm; 13C-NMR: δ 174.6, 159.1, 140.6, 139.4, 138.1, 137.0, 130.3, 129.6, 128.3, 127.2, 114.4, 60.21, 55.5, 49.2, 44.5, 44.3, 30.0, 22.6, 21.5, 20.4, 14.5 ppm.

Anti-Bacterial Efficacy

The anti-bacterial potential of Sul-Ibu (1–9) was tested using the disk diffusion method against *Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Actinomyces odontolyticus, Bacillus halodurans,* and *Micrococcus luteus*. The direct colony suspension method was used to prepare suspensions of organisms in saline (0.85% NaCl solution) to a density of 0.5 McFarland turbidity standard. The density of the suspension was determined using a spectrophotometer, and the absorbance (625 nm) was adjusted in the range of 0.08 to 0.13 [20]. Ciprofloxacin (standard drug) was used as a positive control. The bacterial strains were inoculated onto solid agar media. Filter paper discs were soaked in sample solutions in 80% dimethyl sulfoxide (DMSO) [21] at 200 and 500 µg/ml. Petri dishes were incubated for 24 h at 37°C. After 24 h of incubation, the zone of inhibition of each sample, along with the positive control, was measured in triplicate, and the average measurement was recorded in millimeters (mm).

α-Amylase Inhibition Assay

An α-amylase assay was performed to evaluate the enzyme inhibition potential of the newly synthesized compounds [22]. Briefly, 10 µL of enzyme (0.1 U), 40 µL of starch (0.5 mg/mL), 40 µL of phosphate buffer (pH 6.8), and 10 µL of each compound at three different concentrations (200, 100, and 50 µg/mL) were mixed in each well of a 96-well microtiter plate. The microtiter plate was then incubated for 30 min at 50 °C, followed by the addition of 20 µL of HCl (1 M) as a stopping reagent. Next, 100 µL of iodine reagent (5 mM KI+5 mM I_2) was added for color development and absorbance (Abs.) The absorbance was measured at 540 nm using a microplate reader (Elx800). The experiment was performed in triplicate, and acarbose was used as a positive control. IC_{50} (µg/mL) values were determined using Prism software, and the percentage inhibition was calculated using the following formula:

% inhibition = 100 – (((Abs. of control – Abs. sample) / Abs. control) x 100)

α-Glucosidase Inhibition Assay

p-Nitrophenyl-β-D-glucopyranoside (PNPG) as a substrate [23]. Briefly, 10 µL enzyme (0.1 U), 10 µl PNPG solution (20 mM), 70 µL phosphate buffer (pH 6.8), and 10 µL compound at three different concentrations (200, 100, and 50 µg/mL) were mixed and incubated for 30 min at 37°C. After incubation, 100 µL sodium bicarbonate solution (0.5 mM) was added, and the absorbance (Abs.) was measured at 405 nm. The experiment was performed in triplicate, and acarbose was used as a positive control. Percentage inhibition was calculated using the following formula:

% inhibition = [Abs. of control – Abs. of sample/Abs. of control] × 100

AChE and BChE Inhibition Assay

The anti-Alzheimer potential of 1–9 was examined against AChE and BChE. The phosphate buffer solution was prepared at pH 7.6 (0.12 mol/L), and a stock solution of enzymes was prepared in the above buffer solution. A stock solution of 5,5′-dithiobis(2-nitrobenzoic acid) was prepared by dissolving 0.4 mg in 1 mL of buffer solution. Stock solutions of acetylthiocholine iodide and butyrylthiocholine iodide substrates for AChE and BChE, respectively, were prepared at 1 mM in a buffer solution. Stock solutions of the synthesized compounds (1–9) were prepared at a concentration of 1 mM in DMSO. Ellman's procedure was followed for testing the samples, as previously reported in our work [24]. The percentage inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{A_s - A_t}{A_s} \times 100$$

Where, A_s is the absorbance of the standard solution, and A_t is the absorbance of the test samples.

Kinetic Study

Kinetic analysis of the three most active compounds (5–7) was performed to determine their inhibition mode. The analysis was performed by incubating 10 µL of enzyme solution with different concentrations (0.012 mM, 0.02 mM, 0.04 mM, and 0.06 mM) for 20 min at 37°C. After incubation, different concentrations of substrate (1 mM, 0.9 mM, 0.75 mM, 0.6 mM, 0.45 mM, 0.3 mM, and 0.25 mM) were added. The change in absorbance at 415 nm was measured for 20 min using a spectrophotometer. The values of K_m and V_{max} were determined from Lineweaver-Burk plots using equation (1):

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \quad (1)$$

Where, K_i was obtained by equation (2) from Dixon's plot:

$$K_i = \frac{\text{Intercept}}{\text{Slope}} \quad (2)$$

Molecular Docking

Molecular docking was performed to determine the coupling mode and the lowest binding energy of the synthesized Ibu derivatives (1–9). The compounds were docked for *in silico* anti-diabetic and anti-Alzheimer studies. The crystal structures of α-amylase (PDB ID: 4W93), α-glucosidase (PDB ID: 3WY1), AChE (PDB ID: 4BDT), and BChE (PDB ID: 4BDS) were downloaded from https://www.rcsb.org/. The computational study was carried out using AutoDock v4.2 and Molecular Graphics Laboratory (MGL) tools v1.5.6 [25]. ChemDraw Ultra 12.0 was used to sketch the ligands, and energy minimization was carried out using Chem3D Pro 12.0. First, enzymes were prepared by removing water molecules and heteroatoms. Polar hydrogen atoms were added along with the addition of charges, and the enzyme preparation was completed by assigning AD4-type atoms. The binding site dimensions for the target enzymes were determined using Discovery Studio 4.0. To dock each ligand inside the active pocket of enzymes, the Lamarckian Genetic Algorithm was used to create 100 different poses of each ligand with grid box dimensions of 60×60×60. The best conformer was selected by arranging the highest-frequency clusters over the energy spectrum, according to the binding energy.

Statistical Analysis

All values are expressed as mean ± standard deviation (SD) of three replicates. Data were statistically analyzed using two-way Dunnett's test with $p>0.005$ using GraphPad Prism v8.0.

RESULTS

Synthetic Chemistry

Ibu was activated by heating with $SOCl_2$ for 6 hrs to obtain a chloroacyl derivative. Excess gas was removed under reduced pressure. Equimolar solutions of various aromatic amines (anilines and benzylamines) were added dropwise and refluxed for 6 h. The crude product was purified by column

chromatography to obtain clean amide derivatives of Ibu. The isolated derivatives were dissolved in DCM, and a stoichiometric amount of Et_3N was added. The mixture was stirred for an hour, followed by the addition of equimolar *p*-chlorobenzene sulfonyl chloride and stirring for 12 h. The crude product was purified by column chromatography to obtain the tertiary amide-based sulfonamide derivatives of Ibu (1–9, Sul-Ibu) (Figure 1).

Anti-bacterial Efficacy

Ibu is ineffective against *E. coli* (EC) and *S. aureus* (SA); however, low efficacy was observed against some bacteria at very high concentrations (5 mg/ml) [26]. Therefore, Sul-Ibu (1–9) analogues were screened for anti-bacterial potential against *S. aureus* (SA), *B. subtilis* (BS), *S. mutans* (SM), *E. coli* (EC), *P. aeruginosa* (PA), *S. epidermidis* (SE), *B. halodurans* (BH), *M. luteus* (ML), and *A. odontolyticus* (AO). The activities of all compounds were compared to those of the positive controls, Ciprofloxacin (5 µg/mL) and Ibu (1000 µg/mL). The compounds were tested at two different concentrations (200 µg/mL and 500 µg/mL), and their activity was observed by the inhibition zone diameter in mm. The results revealed that the synthesized derivatives exhibited significant activity against all the tested microbes compared to that of Ibu (1000 µg/mL). It is assumed that the sulfonamide motif activates molecules against microorganisms [27]. However, at higher doses (500 µg/mL), compounds (1–9) appeared to be moderate-to-very good inhibitors. Compounds 4, 7, 8, and 9 appear to be promising antibiotics against different microbes. Compound 4, consisting of *p*-OCH_3 phenyl substitution, showed a zone of inhibition (mm) higher than that of ciprofloxacin against EC, BH, ML, and AO [28]. Compound 7, with a benzyl substitution, exhibited very significant effects against PA and ML. Compound 8 contained *p*-Cl benzyl moiety that was active against five microbes (BS, PA, BH, ML, AO, and 9) with *p*-OCH_3 benzyl motif, which provided a zone of inhibition greater than that of the standard drug against SM, EC, PA, ML, and AO [28, 29]. It was observed that a combination of sulfonyl and benzyl moieties on tertiary amide-based Ibu analogues was more effective and had a higher potential to inhibit the growth of microbes [30, 31] (Figure 2).

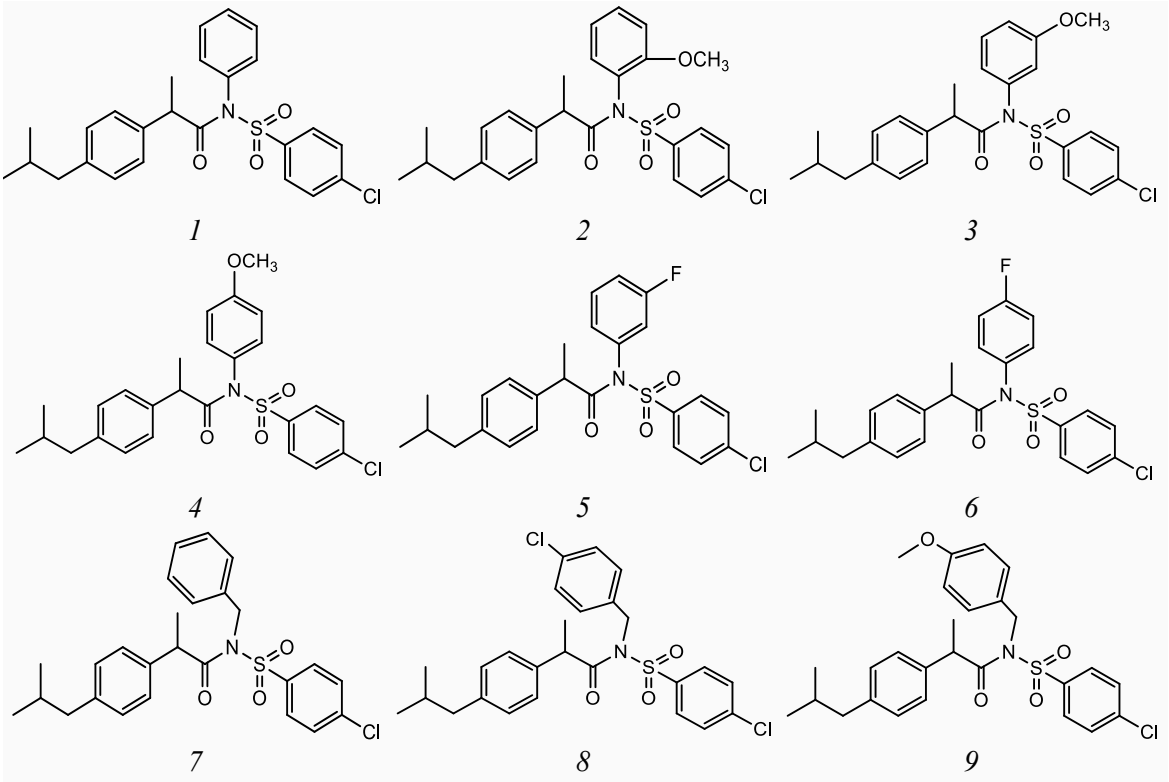


Figure 1. Chemical Structures of chlorobenzenesulfonamide analogues of Ibu (Sul-Ibu, 1–9).

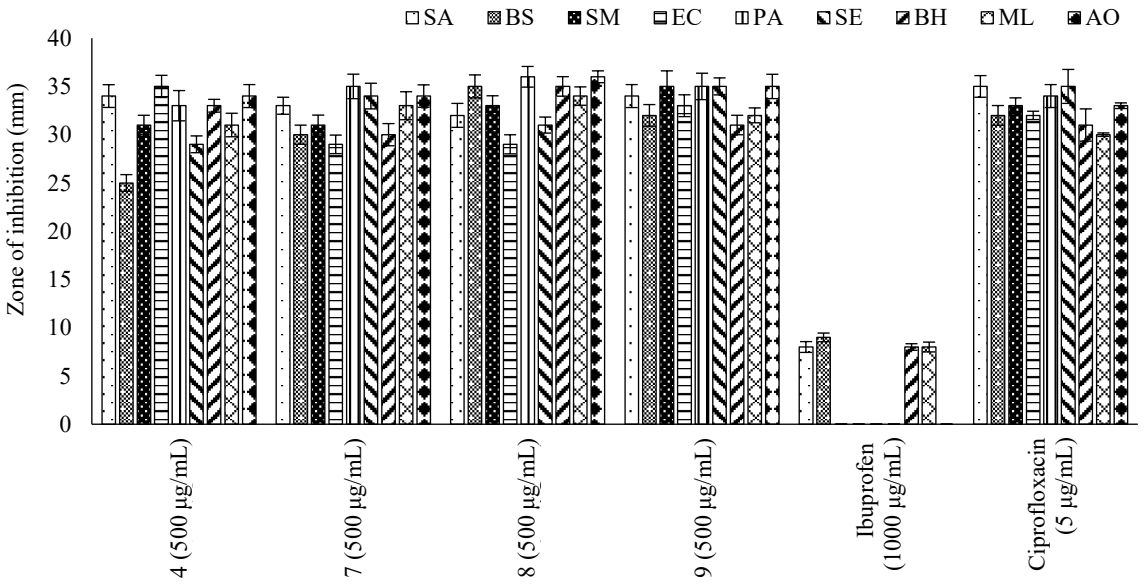


Figure 2. Potent anti-bacterial Sul-Ibu analogues (4, 7, 8, and 9) against nine bacterial strains.

α-Amylase Inhibition

Sul-Ibu derivatives (1–9) were tested against the α-amylase enzyme at three different concentrations (50, 100, and 200 µg/mL). All compounds showed prominent enzyme inhibition (200 µg/mL) in the range of 75–95% as compared with acarbose (90%) used as a positive control in a concentration-dependent manner. For a more accurate estimation of the enzyme inhibition response, IC_{50} values were calculated (Table 1). The results of IC_{50} represented that the highest enzyme inhibition was shown by the compound **5** with IC_{50} value of 53.75±0.79 µg/mL (Figure 3), followed by the compounds **1** and **6** with IC_{50} values of 75.47±0.53 µg/mL and 74.42±1.41 µg/mL, respectively. The rest of the compounds also showed a significant ($p>0.001$) IC_{50} response compared to acarbose. Compounds **5** and **6** both have a fluoro group and exhibit a relatively higher potential than the other tested molecules. Previously, in a similar study, fluoro analogues were found to be more active than other molecules in the series [32, 33].

α-Glucosidase Inhibition

The glucosidase inhibitory potential of the compounds was determined using a microplate-based method. All compounds showed very significant inhibition (70–90%) at 200 µg/mL compared with acarbose (95%) used as a positive control in a concentration-dependent manner. The IC_{50} values were determined and are tabulated in Table 1. The IC_{50} values represented that the highest enzyme inhibition was shown by the compound 4 (Figure 3), followed by 8 and 1 with IC_{50} values 61.80±0.85, 65.38±1.76 µg/mL, and 66.85±1.71, respectively, with statistical significance $p>0.001$. The IC_{50} response for the rest of the compounds was also significant ($p>0.001$) compared to acarbose. The presence of the-OCH_3 group in 4 was found to favorably inhibit the enzyme [34], whereas the p–Cl group on the benzyl ring (8) appeared to be less effective than 4 [35]. The activity further decreased in the absence of any substituent (1) on the benzene ring.

AChE and BChE Inhibition

The inhibitory potency of Sul-Ibu (1–9) against the Alzheimer causing enzymes AChE and BChE was determined using Ellman's method, and the results obtained are presented in Table 2. Donepezil and tacrine were used as reference drugs for AChE and BChE, respectively [36]. The percentage inhibition study revealed that all Sul-Ibu analogues were active against both enzymes, with >80% inhibition. Three Sul-Ibu analogues (5–7) were very effective against both enzymes, with >90% inhibition. Compound 7 was the most active analogue against both AChE (95%) and BChE (96%). The inhibitory potency of 7 was attributed to the presence of a sulfonyl benzyl combination. Examination

of the anti-cholinergic effect of 1–9 revealed that sulfonyl substitution in addition to the amide moiety could enhance the pharmacological response of molecules [37].

Table 1. The α-amylase and α-glucosidase inhibitory activity (IC50) was calculated using three different concentrations in GraphPad Prism. Values are as mean (n=3) ± standard deviation. (***p>0.001) represents statistical significance of the results as compared with the positive control (Acarbose). Docking results in the lowest binding energies docked onto α-glucosidase and α-amylase.

Sample	α-amylase (µg/ml)	α-amylase (kcal mol^{-1})	α-glucosidase (µg/ml)	α-glucosidase (kcal mol^{-1})
1	75.47±0.53***	-7.43	66.85±1.71***	-8.13
2	121.73±0.95***	-7.88	61.80±0.85***	-8.33
3	131.97±2.47***	-7.64	88.08±0.38***	-8.18
4	120.53±0.84***	-7.39	94.07±2.02***	-8.13
5	53.75±0.79***	-7.97	102.10±1.76***	-7.98
6	74.42±1.41***	-7.63	81.88±2.23***	-8.06
7	90.24±2.04***	-7.58	82.77±2.10***	-8.15
8	94.79±1.12***	-7.46	65.38±1.76***	-8.20
9	89.33±1.68***	-7.76	78.73±2.62***	-7.96
Acarbose	*63.09±0.38*	*-8.80*	*48.48±0.17*	*-8.83*

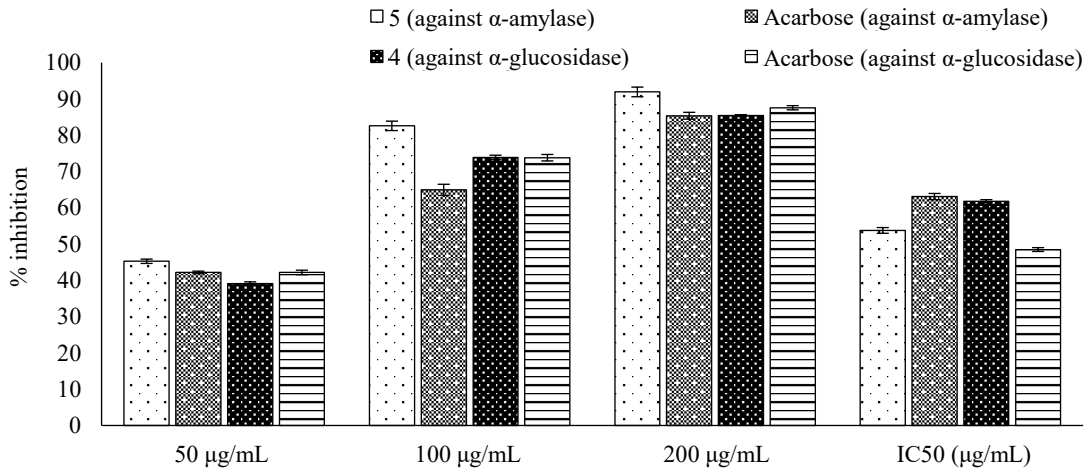


Figure 3. Graph of potent anti-diabetic compounds 5 against α-amylase and 4 against α-glucosidase.

Table 2. Percentage inhibition of compounds (1–9) against AChE and BChE.

Comp ID	Percentage inhibition (%)		Percentage inhibition (%)	
	AChE	*AChE (LBE in kcal mol^{-1})*	*BChE*	*BChE (LBE in kcal mol^{-1})*
1	87.8±0.51	-8.98	88.2±0.67	-10.50
2	82.6±0.56	-9.87	81.7±0.62	-8.84
3	83.2±0.32	-10.84	81.9±0.51	-9.09
4	85.6±0.62	-10.61	84.3±0.64	-9.60
5	92.6±0.39	-11.12	91.5±0.31	-10.24
6	94.7±0.21	-11.59	93.4±0.12	-10.0
7	95.8±0.31	-11.73	96.7±0.16	-10.41
8	87.2±0.43	-8.34	89.7±0.34	-10.04
9	89.4±0.30	-8.67	89.7±0.15	-9.14
Reference	*98.6±0.13*	*-10.83*	*97.5±0.12*	*-6.90*

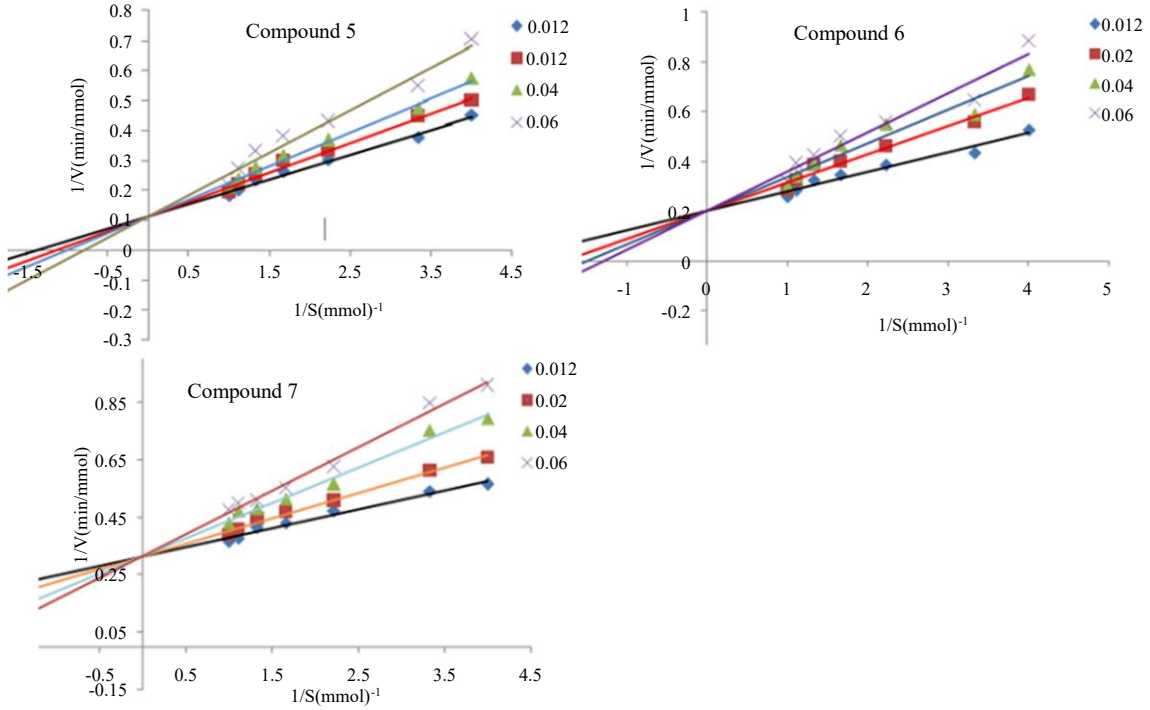


Figure 4. The Lineweaver-Burk plots of different concentrations of compounds 5, 6, and 7.

Kinetic Analysis

Kinetic studies were performed with compounds 5, 6, and 7 to determine the inhibition mechanism. Straight lines were observed at different concentrations of inhibitors 5–7 in the Lineweaver-Burk plots (double reciprocal plots) (Figure 4). The double reciprocal plots showed that V_{max} remained the same, whereas K_m increased with increasing concentrations of the inhibitors. This behavior indicated that 5–7 inhibited the enzymes competitively. The inhibitory constants (K_i) for 5, 6, and 7 were calculated as 47, 61, and 27 µmol, respectively. K_i is an important parameter for determining the binding affinity of any inhibitor inside the active region of an enzyme. The greater the value of K_i lower the binding affinity, and vice versa. Compound 7 displayed the lowest K_i value (27 µmol), suggesting that it could inhibit enzymes more effectively.

Molecular Docking Studies

Sul-Ibu molecules were docked into the corresponding enzymes to study their binding affinity and understand the structure-activity relationship. For this, the docking of compounds (1–9) with α-amylase and α-glucosidase was analyzed for anti-diabetic activity, while AChE and BChE were studied for anti-Alzheimer's activity.

Docking with α-Glucosidase and α-Amylase

Docking was performed using the crystal structures of α-amylase (PDB ID: 4W93) and α-glucosidase (PDB ID: 3WY1) [38]. The results are presented as the lowest binding energy (LBE). Ligand 5 showed -7.97 kcal mol-1 binding energy exhibiting hydrophobic interactions with LYS200, HIS201, LEU162, LEU 165, ALA98, GLU233, TYR62 and TRP59. The binding modes of the receptor-ligand complex are shown in Figure 5.

Ligand **2** exhibited the same binding energy (-8.33 kcal/mol) as that of the standard drug acarbose. The ligand interacted with PHE297, LYS398, PRO230, VAL335, LEU227, LEU300, TYR389, PHE147, ILE46, and PHE166 through hydrophobic interactions. The binding of ligands inside the active regions is mainly assisted by hydrophobic contacts with both α-amylase and α-glucosidase. 3D and 2D diagrams of the ligand-receptor complex are shown in Figure 6.

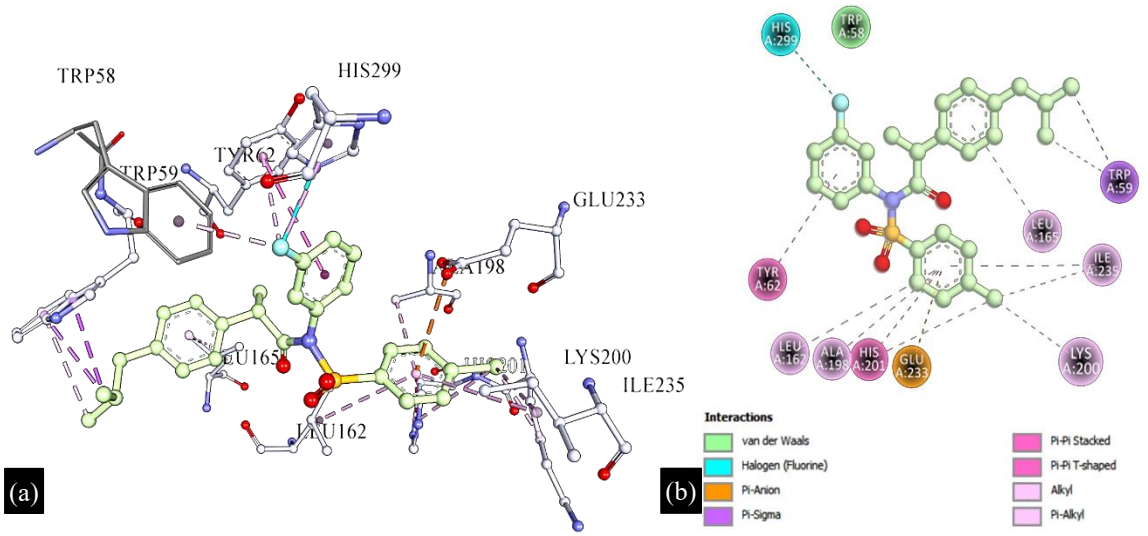


Figure 5. Interacting mode (a) and 2D-representation of 5 (b) inside the active pocket of α-amylase, visualized on Discovery Studio v20.

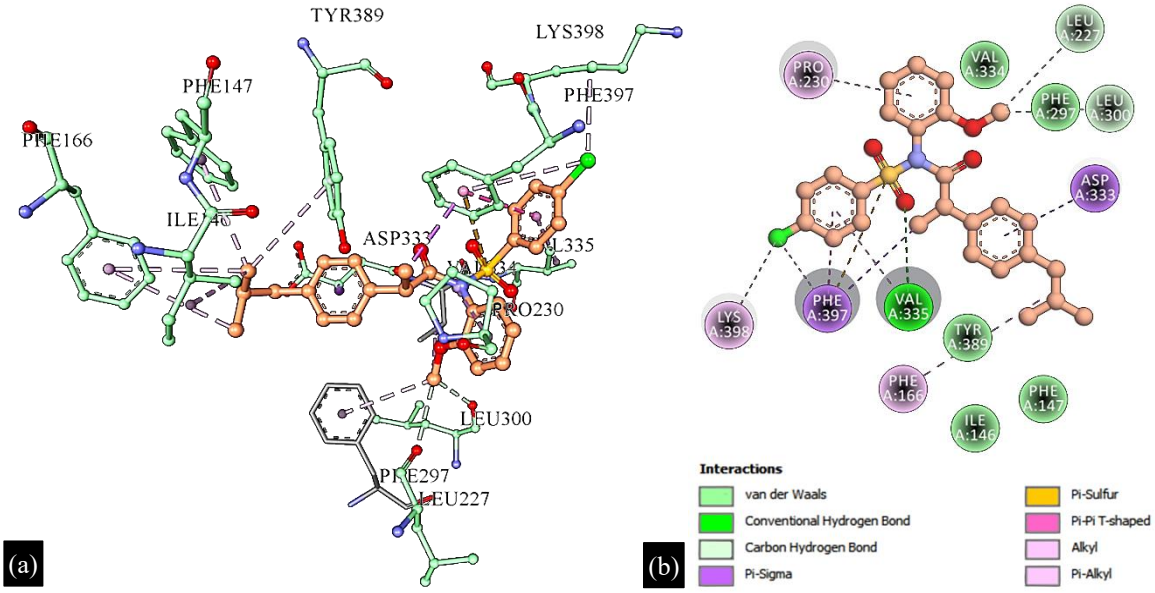


Figure 6. Interacting mode (a) and 2D-representation of 2 (b) inside the active pocket of α-glucosidase, visualized on Discovery Studio v20.

Docking with Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE)

Sul-Ibu (1–9) analogues were investigated for their anti-Alzheimer efficacy by docking against AChE and BChE, as reported in our previous work [39]. The analysis was performed based on the minimum energy values of the docked inhibitor-enzyme complexes. The binding energy values provided a better understanding of the binding potentials of the synthesized compounds. Compound 7 was the most active compound among all the tested derivatives against both enzymes (Table 3). The binding energy values obtained were -11.73 and -10.41 kcal mol-1, respectively. The active binding regions of AChE and BChE have also been reported [40]. The residues in the active site of AChE (SER203, GLY122, TYR449, TRP86, TRP439, PRO446, HIS447, MET443, and TYR337) actively participate in π-π stacking, π-alkyl interactions, hydrogen bonding, and hydrophobic interactions. Compound 7, having a benzene and benzyl ring, was responsible for pi-pi stacked interaction with TRP86 and TYR337, having bond distances 3.98Å and 4.60Å, respectively (Figure 7). This compound also showed hydrophobic interactions with PRO446, MET443, and TRP439.

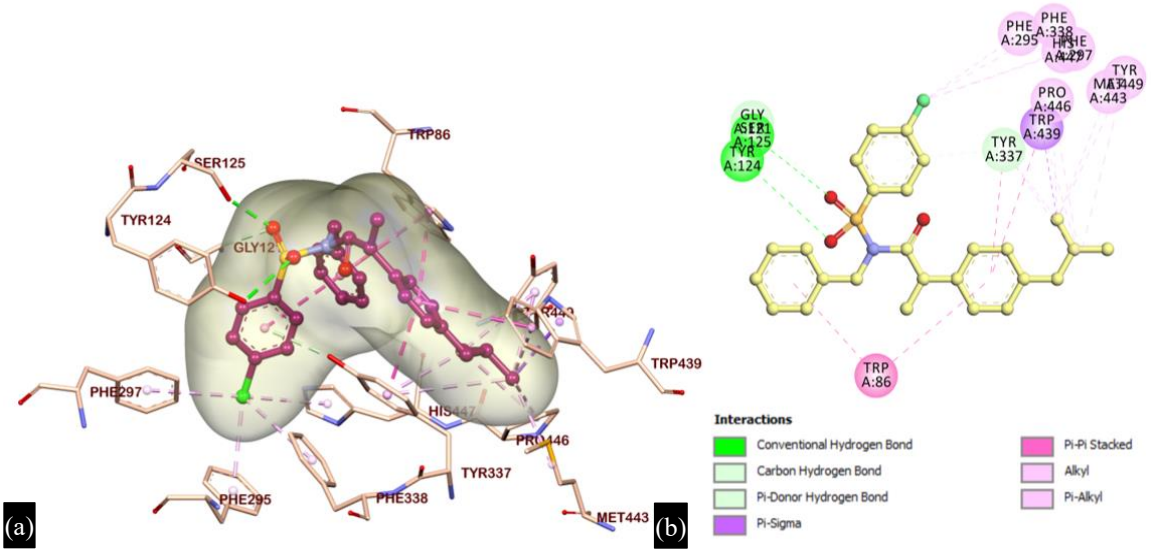


Figure 7. Interacting mode (a) and 2D-representation (b) of 7 inside the active pocket of AChE, visualized on Discovery Studio v20.

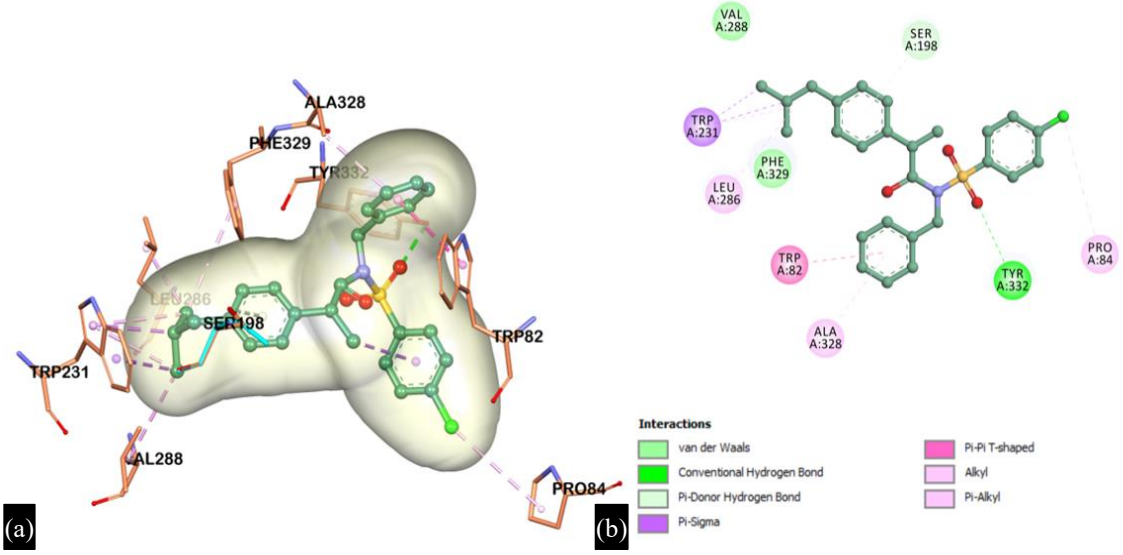


Figure 8. Interacting mode (a) and 2D-representation (b) of 7 inside the Active Pocket of BChE, visualized on Discovery Studio v20.

The residues involved in the active pocket of BChE are ALA328 and TRP82 with tacrine (THA) as co-crystallized ligands, which form π-alkyl and π-π stacked interactions with ALA328 and TRP82. The docked ligand **7** displayed a π-alkyl interaction with ALA328 (4.14Å), but a pi-pi T-shaped interaction with TRP82 (4.39Å).

Therefore, on the basis of our docking results, we hypothesized that compound **7** has potential in the treatment of Alzheimer's disease, as it actively binds to the active region of target enzymes (Figure 8).

DISCUSSION

Sulfonamides act as substitutes for *p*-aminobenzoic acid (PABA), are responsible for blocking nucleic acid synthesis in bacteria, and exhibit a broad spectrum of activity. Therefore, the sulfonamide derivatives of Ibu are more active than those of Ibu.

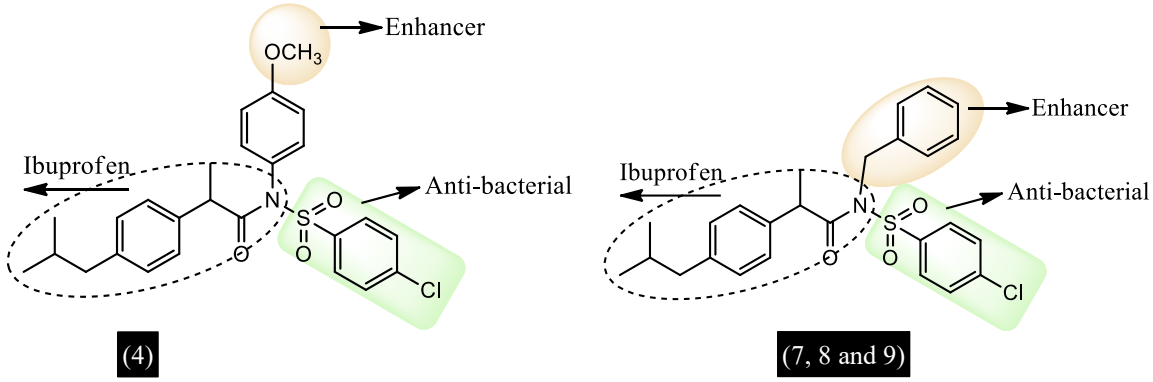


Figure 9. Structure–activity relationship **(**SAR) for the promising compounds 4, 7, 8, and 9.

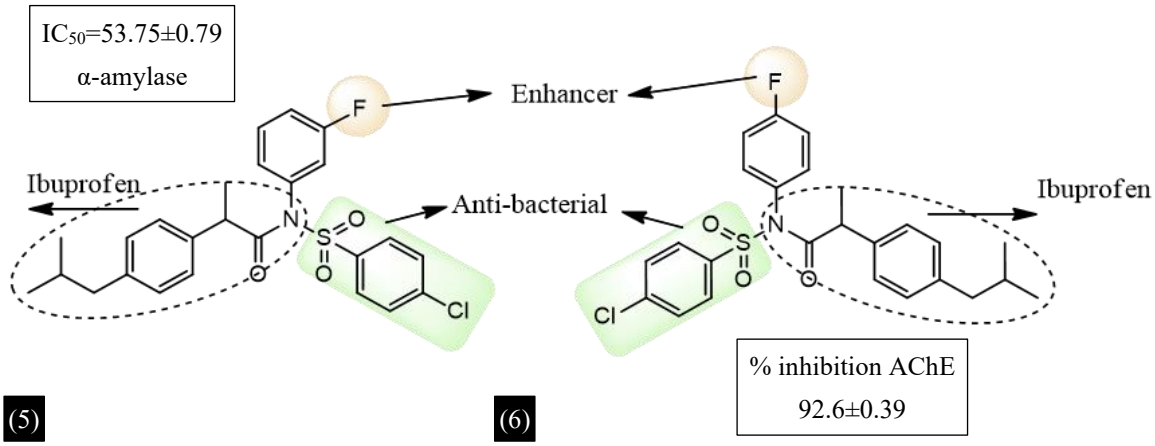


Figure 10. SAR for the promising compounds 5, and 6 against cholinesterases.

Promising results for compound 4 might be due to the methoxy group present at the para position of the phenyl group, which is marked as an enhancer in this case (Figure 9). Compounds 7, 8, and 9 had a benzyl group (activity enhancer), which provided very significant results. Compound 7, with a benzyl group, emerged as an efficient inhibitor of AChE (% inhibition = 95.8±0.31) and BChE (% inhibition = 96.7±0.16) (Figure 9).

The Sul-Ibu fluoro analogues (5 and 6) exhibited high percentages of inhibition against AChE (93% and 92%, respectively) and BChE (95% and 93%, respectively). In 5, the fluoro group is at the meta position, while in 6, it is at the para position. In the other analyses carried out in this study, these two compounds also showed good results. Surprisingly, the meta-substituted fluoro analogue was more active than its para analogue. In addition, 5 exhibited a zone of inhibition against Bacillus subtilis (BS) measuring 32±0.62 mm, which was comparable to the zone observed for ciprofloxacin (32±1.02 mm). This compound also provided a higher IC_{50} value, 53.75±0.79 µg/mL, as compared to 6 (IC_{50} value 74.42±1.41 µg/mL) against α-amylase (Figure 10).

CONCLUSION

In this study, novel tertiary amide-based sulfonamide analogues of ibuprofen were successfully synthesized and evaluated for their anti-bacterial, anti-diabetic, and anti-Alzheimer's potency. The present study concluded that compounds 4, 7–9 showed promising results against different bacterial strains. The combination of benzyl and sulfonyl moieties seemed very effective in catering to bacterial strains. The promising anti-diabetic potential of compounds 4 and 5 against α-glucosidase and α-amylase, respectively, could be associated with the presence of methoxy and fluoro groups, whereas derivatives 5–7 exhibited very significant results against cholinesterases. Compound 7 exhibited the best results for AChE and BChE activity. A kinetic study revealed that compounds 5–7 were all

competitive inhibitors of cholinesterase, with K_i values of 47, 61, and 27 μmol, respectively. The molecular docking study of the synthesized compounds was in good agreement with the experimental results. The results of this study allowed us to understand the key pharmacophoric units responsible for various biological activities using SAR analysis. This approach should be encouraged for further studies and the development of new analogues with anti-bacterial, anti-diabetic, and anti-Alzheimer potencies.

Author Contributions

Conceptualization—Shahzad Murtaza (S.M.), Adina Tatheer (A.T.); Methodology—S.M., A.T.; Software—A.T.; Validation—S.M., Naghmana Kausar (N.K.); Formal Analysis—A.T., N.K., Hammad Ismail (H.I.), Safeer Ahmed (S.A.), Syed Hafeez Shah (S.H.S.), Saba Durrani (S.D.), M. Junaid Akhtar (M.J.A.); Investigation—S.M., A.T., N.K., H.I.; Resources—S.M.; Data Curation—N.K., S.A.; Writing—Original Draft Preparation—A.T.; Writing—Review and Editing—S.M.; Supervision—S.M.; Project Administration—S.M. All authors have read and agreed to the published version of the manuscript.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Acknowledgments

None.

Conflicts of Interest

The authors declare no conflict of interest.

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