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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 novel chlorobenzensulfonamide 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 it with p-chlorobenzensulfonyl chloride. Sul-Ibu (1-9) were screened against S. aureus, B. subtilis, S. mutans, E. coli, P. aeruginosa, S. epidermidis, A. odontolyticus, B. halodurans, and M. luteus. The analogues 4, 7-9 showed significant results as compared to Ibu due to the presence of 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 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 95.8±0.31 against AChE and 96.7±0.16 against BChE. Additionally, this analogue also provided K_i value 27 µmol. Molecular docking study with α-amylase, α-glucosidase, AChE and BChE were also in good accordance to that of experimental results. These results clearly indicate that Sul-Ibu analogues appeared promising antibacterial, antidiabetic and anti-Alzheimer agents and should be considered for clinical studies.

Keywords: Synthesis, anti-cholinesterase, anti-bacterial, anti-diabetic, α-amylase, α-glucosidase

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INTRODUCTION

Ibuprofen (NSAID) has been widely used as analgesic [1], anti-pyretic [2], anti-inflammatory agent and also cure rheumatoid arthritis [3, 4]. The adverse effects are well established and its prolonged use is attributed to kidney damage [5], gastric ulcer [6] and hepatotoxicity [7]. The common toxic effect are gastrointestinal erosion, bleeding and cardiovascular disturbance [8]. It was observed that the presence of carboxylic acid group (–COOH) is responsible for the gastric toxicity as evaluated by NSAID prodrugs. NSAID derivatives lacking the acidic moieties, retain the anti-inflammatory activity but no gastric toxicity [9]. A large number of amides, hydrazides and hybrid derivatives of Ibu have been synthesized and evaluated for their efficacy and reduction of the side effects. Ibuprofen derivatives were explored for the platelet protective efficacy and platelet aggregation inhibitory property [10]. Oxadiazole derivatives of

Ibuprofen/naproxen were synthesized and tested for their antibacterial potential [11]. Thiadiazole derivatives of Ibuprofen were investigated for their DNA binding abilities and to study their anticancer activities against human hepatocellular carcinoma (Huh-7) cell lines and were found to be potential anti-cancer drug candidates [12].

Sulfa drugs (drugs containing sulfonamide moiety) are broadly effective antibacterial drugs and brought antibiotic revolution in medicine. Many commercial sulfa drugs *i.e*. sulfanilimide, sulfadiazine, sulfamethoxazole etc. have been efficiently used since long [13]. A lot have been studied to explore the efficiency of sulfonamide moiety (pharmacophore) other than its antibacterial potential. It provides an important insight in 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, is low-membrane permeability and most likely allergic reactions aroused in patients [18].

Another very serious challenge faced by the researchers is the tendency of micro-organism to develop resistance against the drugs [19], it is therefore the need of hour to discover new antibiotics. The modification in the existing drugs by incorporating different pharmacophore is a suitable strategy to get better drugs. To the best of our knowledge nothing is available yet about the tertiary amide based *N*-sulfonamide analogues of Ibu. Therefore we worked on the synthesis of sulfonamide derivatives of Ibu and evaluated them for their biological potential. These molecules were also docked to compare the experimental results with computational findings and to understand protein-ligand interactions at molecular level.

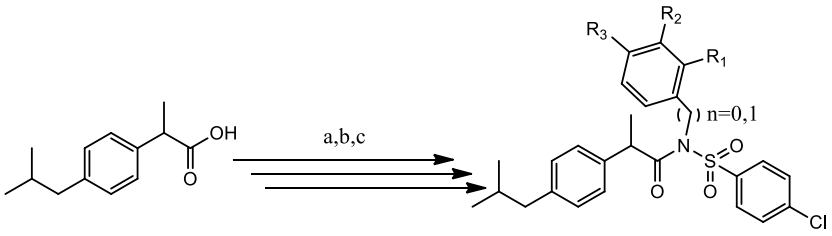
MATERIAL AND METHOD

Chemistry

All chemicals were obtained from Sigma Aldrich and VWR and used without further purification. Silica coated Al-TLC cards (silica gel 60 F254) were used to monitor the reactions. The synthesized derivatives (1-9) were purified by silica gel (70-230mesh) column chromatography from EMD Millipore Corporation (Darmstadt, Germany). Electrothermal melting point apparatus was used to examine the m.p. of the compounds. FTIR spectra were recorded by Bio-Rad spectrophotometer and the data was represented in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker ARX-300, ARX-500 with TMS as an internal standard. 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 hours to obtain 2-(4-Isobutylphenyl)propanoyl chloride. The excess of SO_2 and HCl gases were removed under vacuum on rotary evaporator. Separately prepared equimolar solutions of different aromatic amines (variety of anilines and benzylamines) in THF and dropwise added to above mixture. The mixture was refluxed for another 6 hours and progress of reactions was monitored through TLC. The crude product was purified by column chromatography with ethylacetate: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 drop wise. The obtained mixture was refluxed for 12 hours and progress of reaction was monitored through TLC. The final products were purified by column chromatography with ethylacetate:n-hexane (1:3). (Scheme 1) (^{1}H and ^{13}C NMR spectra).



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, DCM, Et_3N, 12 hours.

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.

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.

Antibacterial Efficacy

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

α-amylase Inhibition Assay

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

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

α-glucosidase Inhibition Assay

glucopyranoside (PNPG) as a substrate [23]. Briefly, 10 µl enzyme (0.1U), 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 minutes at 37°C. After incubation 100 µl sodium bicarbonate solution (0.5 mM) was added and absorbance (Abs.) was measured at 405 nm. The experiment was performed in triplicate and Acarbose was used as positive control. Percentage inhibition was calculated using given formula;

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

AChE and BChE Inhibition Assay

The anti-Alzheimer potential of 1-9 was examined against AChE and BChE. Phosphate buffer solution was prepared with pH 7.6 (0.12 mol/L) and stock solution of enzymes were prepared in the above buffer solution. The stock solution of DTNB (5,5'-dithiobis(2-nitrobenzoic acid) was prepared by dissolving 0.4 mg of DTNB in 1 mL of buffer solution. The stock solutions of substrates acetylthiocholineiodide and butyrylthiocholineiodide for AChE and BChE respectively, were prepared at 1 mmol/L in buffer solution. The stock solutions of synthesized compounds (1-9) were prepared at 1

mM concentration in DMSO. The Elman's procedure was followed for the samples to be tested as reported earlier in our work [24]. The percentage inhibition was calculated by following equation:

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

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

Kinetic Study

The kinetic analysis of three most active compounds (5-7) was carried out to ensure their inhibition mode. The analysis was carried out by incubating 10 µL of enzyme solution with different concentrations (0.012 mM, 0.02 mM, 0.04 mM and 0.06 mM) of compounds 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 was measured for 20 min at 415 nm by using spectrophotometer. The values of K_m and V_{max} were determined from Lineweaver-Burk plots by equation (1):

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

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

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

Molecular Docking

The molecular docking was performed to determine the coupling mode and least binding energy of synthesized Ibu derivatives (1-9). The compounds were docked for *In silico* anti-diabetic and anti-Alzheimer study. The crystal structures of α-amylase (PDB ID: 4W93), α-glucosidase (PDB ID: 3WY1), AChE (PDBID: 4BDT) and BChE (PDB ID: 4BDS), were downloaded from https://www.rcsb.org/. The computational study was carried out by AutoDock v4.2 and MGL tools v1.5.6 [25]. ChemDraw ultra 12.0 was used to sketch the ligands, whereas, the energy minimization was carried out by Chem3D pro 12.0. Firstly, enzymes were prepared by removing water molecules and heteroatoms. The polar hydrogen atoms were added along with addition of charges and enzyme preparation was completed by assigning AD4 type atoms. The binding site dimensions for target enzymes were determined by Discovery Studio 4.0. To dock each ligand inside active pocket of enzymes, Lamarckian Genetic Algorithm was used to create 100 different poses of each ligand with grid box dimensions 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 the values are expressed as mean ± standard deviation (SD) of three replicates. Data was statistically analysed using two-way following Dunnett's test with $p>0.005$ in GraphPad Prism v8.0.

RESULTS

Synthetic Chemistry

Ibu was activated by heating with $SOCl_2$ for 6 hours to obtain chloroacyl derivative. The excess of gases were removed under reduced pressure. The equimolar solutions of variety of aromatic amines, (anilines and benzylamines) were added dropwise and refluxed for 6 hours. The crude product was purified by column to obtain clean amide derivatives of Ibu. The isolated derivatives were dissolved in DCM and stochiometric amount of Et_3N was added. The mixture was stirred for an hour followed by the addition of equimolar *p*-chlorobenzene sulfonyl chloride and stirred for 12 hours. The crude product was purified by column chromatography to obtain 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 small efficacy appeared against some bacteria at very high concentration (5 mg/ml) [26]. Therefore, Sul-Ibu (1-9) analogues were

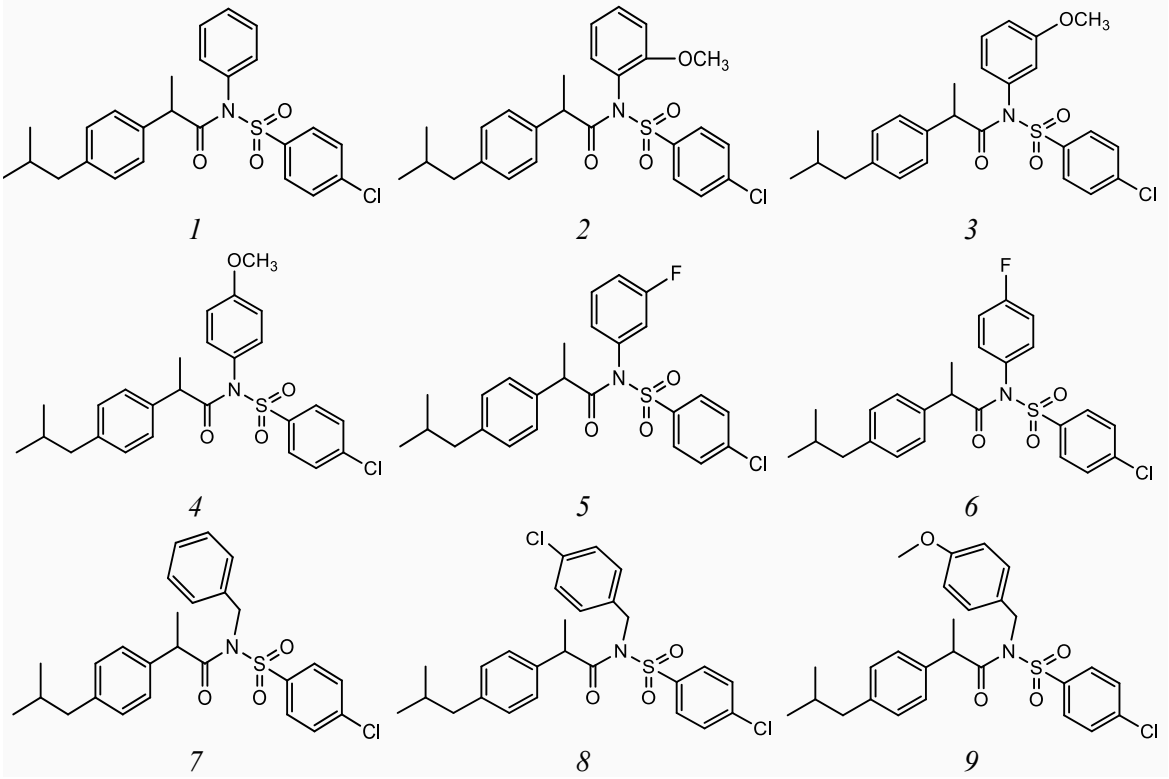


Figure 1. Chemical Structures of chlorobenzenesulfonamide analogues of Ibu (Sul-Ibu, 1-9).

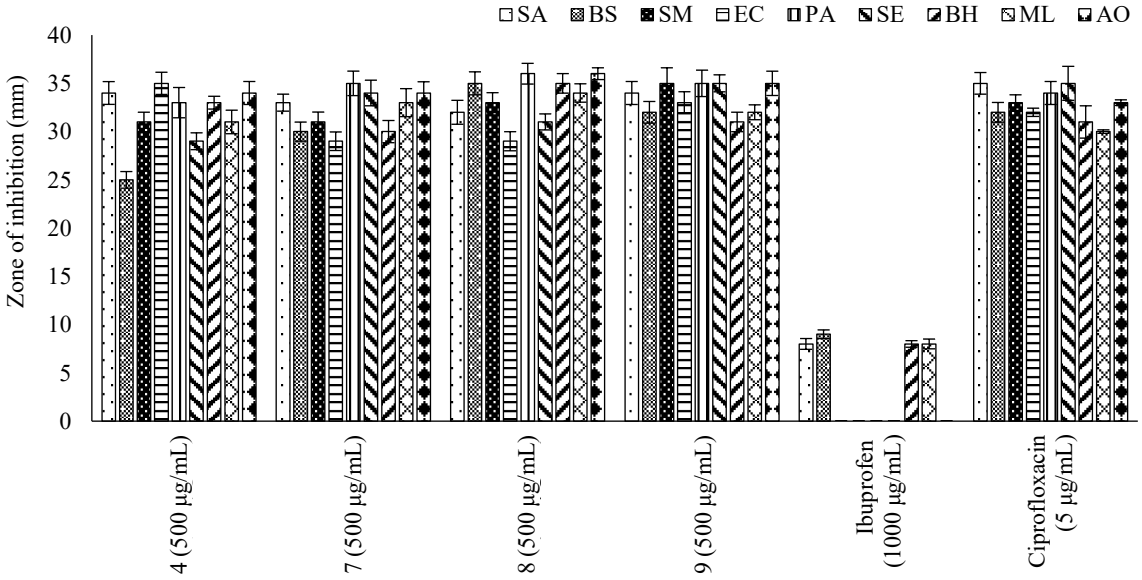


Figure 2. Potent anti-bacterial Sul-Ibu analogues (4,7,8,9) against nine bacterial strains.

screened for their antibacterial 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 positive control 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 activity was observed by inhibition zone diameter in mm. The results revealed that the synthesized derivatives exhibited significant activity against all the tested microbes as compared to that of Ibu (1000 µg/mL). It is assumed that the presence of the sulfonamide motif activated

the molecules against the microorganisms [27]. However, at higher dose (500 µg/mL), compounds (1-9) appeared as moderate to very good inhibitors. It was observed that compound 4, 7, 8 and 9 appeared promising antibiotics against different microbes. Compound 4 consist of *p*-OCH_3 phenyl substitution showed zone of inhibition (mm) higher than that of Ciprofloxacin against four microbes i.e. EC, BH, ML, AO [28]. Compound 7 having benzyl substitution exhibited very significant result against PA and ML. Compound 8 contained *p*-Cl benzyl moiety, appeared active against five microbes i.e. BS, PA, BH, ML, AO and 9 with *p*-OCH_3 benzyl motif provided 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 moiety on tertiary amide based Ibu analogues were more effective and have higher potential to inhibit the growth of the microbes [30, 31] (Figure 2).

α-amylase Inhibition

Sul-Ibu derivatives (1-9) were tested against 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 positive control in concentration dependant manner. For more accurate estimation of 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 compounds 5 with IC_{50} value 53.75±0.79 µg/mL (Figure 3) followed by the compounds **1** and **6** with IC_{50} value 75.47±0.53 µg/mL and 74.42±1.41 µg/mL respectively. Rest of the compounds also showed significant ($p>0.001$) IC_{50} response as compared with Acarbose. Compound **5** and **6**, both have fluoro group and exhibited relatively higher potential than other tested molecules. Previously in a similar study it was found that fluoro analogues were more active than other molecules of the series [32, 33].

α-glucosidase Inhibition

The glucosidase inhibition potential of compounds was determined by microplate-based method. All compounds showed very significant inhibition (70-90%) at 200 µg/mL as compared with Acarbose (95%) used as positive control in concentration dependant manner. IC_{50} values were determined and 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 $p>0.001$. IC_{50} response for rest of the compounds were also significant ($p>0.001$) as compared with Acarbose. The presence of -OCH_3 group in 4 was found favourable to inhibit the enzyme [34], whereas the p–Cl group on benzyl ring (8) appeared less effective than 4 [35]. The activity decreased further in the absence of any substituent (1) on the benzene ring.

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

Sample	α-amylase (µg/ml)	α-amylase ($Kcalmol^{-1}$)	α-glucosidase (µg/ml)	α-glucosidase ($Kcalmol^{-1}$)
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*

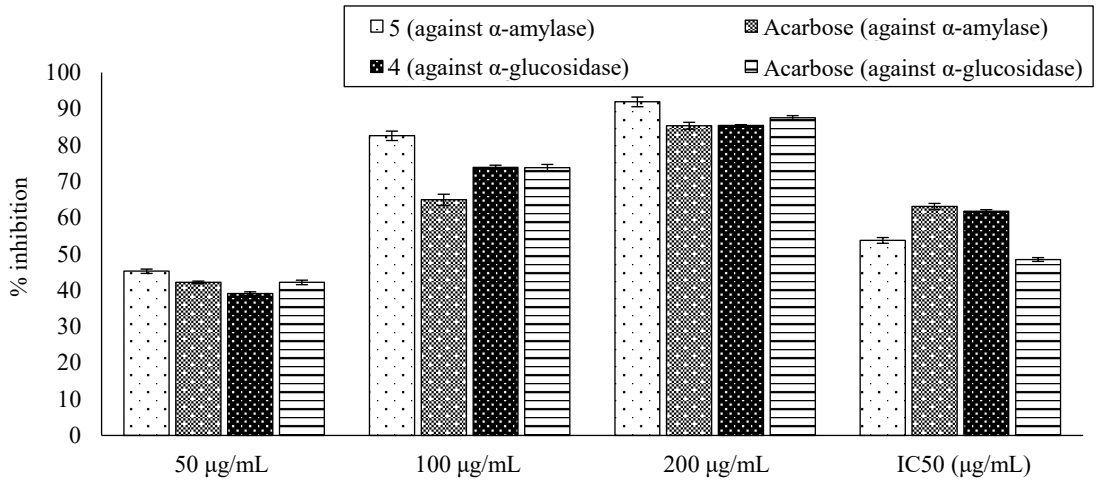


Figure 3. Graph of potent antidiabetic compounds 5 against α-amylase and 4 against α-glucosidase

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

Comp ID	Percentage inhibition (%)		Percentage inhibition (%)	
	AChE	***AChE (LBE in Kcalmol^{-1})***	***BChE***	***BChE (LBE in Kcalmol^{-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*

AChE and BChE Inhibition

The inhibitory potency of Sul-Ibu (1-9) against Alzheimer causing enzymes AChE and BChE was obtained by Elman's method and results obtained are presented in Table 2. Donepezil and tacrine were taken as reference drugs for AChE and BChE, respectively [36]. Percentage inhibition study revealed that all Sul-Ibu analogues were active against both enzyme with >80% inhibition. Three Sul-Ibu analogues (5-7) were very effective molecules against both enzymes with >90% inhibition. Compound 7 was found the most active analogue against both AChE (95%) and BChE (96%). The inhibitory potency of 7 could be due to presence of sulfonyl benzyl combination. The examination of anti-cholinergic effect of 1-9 revealed that sulfonyl substitution in addition to amide moiety could enhance the pharmacological response of molecules [37].

Kinetic Analysis

Kinetic studies were performed with 5, 6 and 7 to find out the inhibition mechanism. Straight lines were observed at different concentrations of inhibitors 5-7 in Lineweaver-Burk plots (double reciprocal plots) (Figure 4). The double reciprocal plots showed that V_{max} remains the same while, K_m increased with increasing concentrations of the inhibitors. This particular behavior indicated that 5-7 inhibited the enzymes in a competitive manner. The inhibitory constant values (K_i) for 5, 6 and 7 were calculated 47 µmol, 61 µmol and 27 µmol, respectively. K_i value is an important parameter to determine the binding affinity of any inhibitor inside active region of an enzyme. The greater the values of K_i lower the binding affinity and vice versa. The compound 7 displayed the lowest K_i value (27 µmol) which apparently means that it could inhibit the enzymes more effectively.

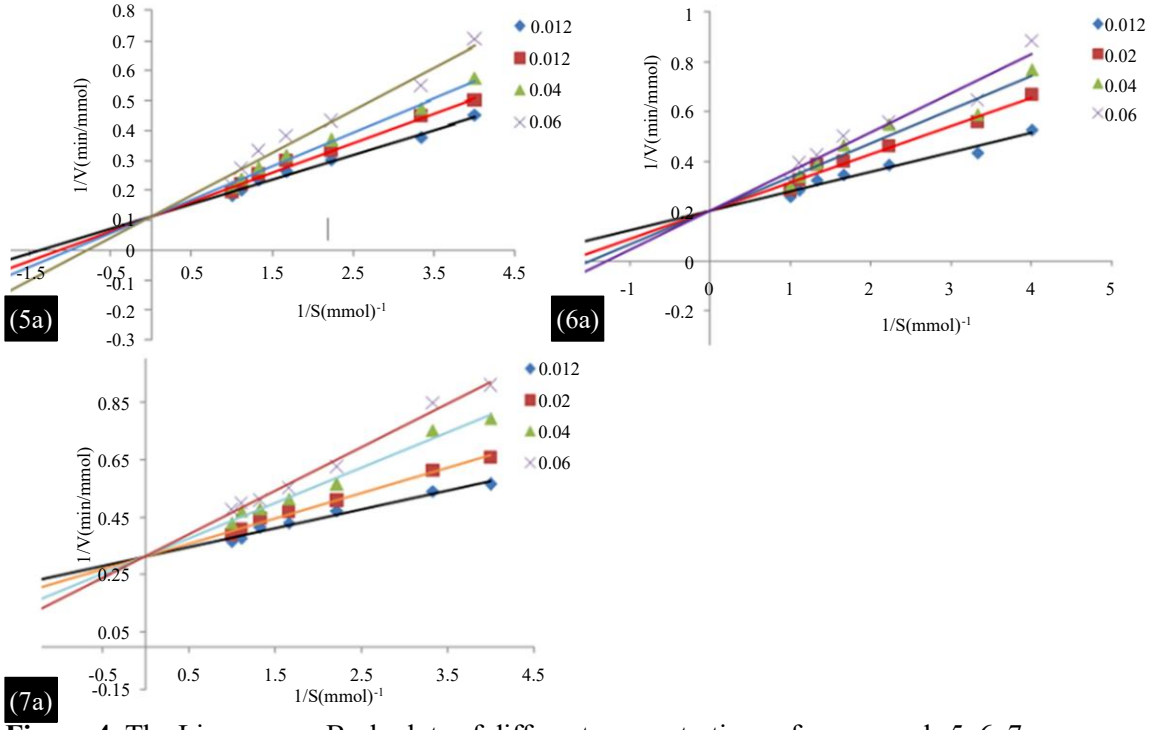


Figure 4. The Lineweaver-Burk plots of different concentrations of compounds 5, 6, 7.

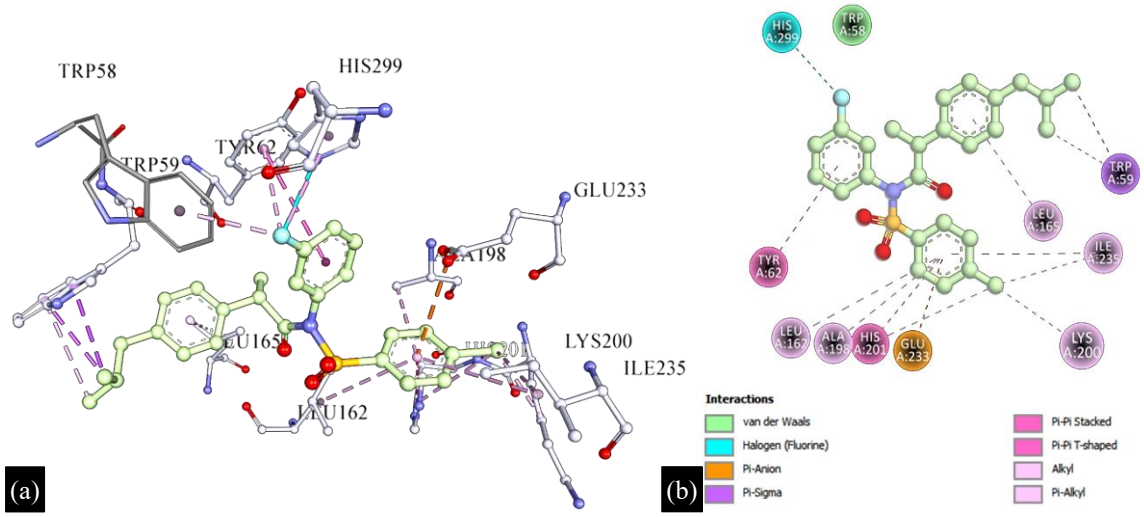


Figure 5. Interacting mode (a) and 2D-represntation of 5 (b) inside active pocket of α-amylase, visualized on Discovery Studio v20

Molecular Docking Studies

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

Docking with α-glucosidase and α-amylase

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

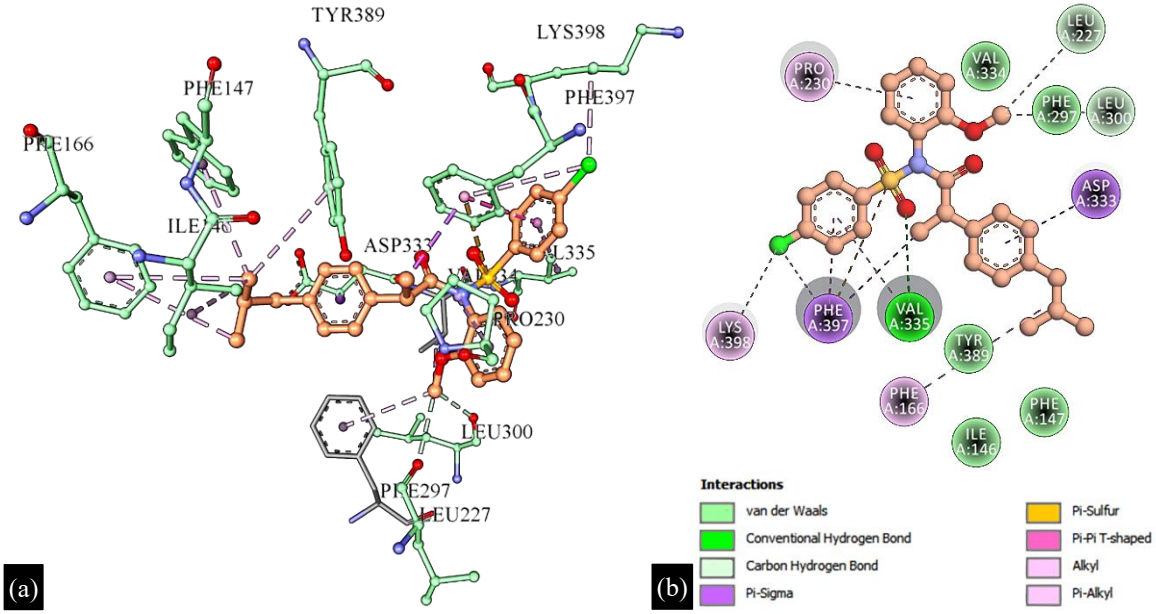


Figure 6. Interacting mode (a) and 2D-represntation of 2 (b) inside active pocket of α-glucosidase, visualized on Discovery Studio v20.

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

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 carried out based on minimum energy values of docked inhibitor-enzyme complexes. The binding energy values gave better understanding of binding potential of synthesized compounds. Compound 7 was the most active compound among all the tested derivatives, against both the enzymes (Table 2). The binding energy value obtained was -11.73 and -10.41 Kcalmol-1, respectively. The active binding region of AChE and BChE has been reported [40]. The residues of active site of AChE (SER203, GLY122, TYR449, TRP86, TRP439, PRO446, HIS447, MET443, and TYR337) actively participate in π-π stacked, π-alkyl, hydrogen bonding, and hydrophobic interactions. Compound 7 having benzene and benzyl ring were responsible for pi-pi stacked interaction with TRP86 and TYR337, having bond distances 3.98Å and 4.60Å, respectively (Figure 7). The compound also showed hydrophobic interaction with PRO446, MET443 and TRP439.

The residues involved inside the active pocket of BChE are ALA328 and TRP82 with tacrine (THA) as co-crystallized ligand and it makes π-alkyl and π-π stacked interactions with ALA328 and TRP82. The docked ligand 7 displayed π-alkyl interaction with ALA328 (4.14 Å) but pi-pi T-shaped interaction with TRP82 (4.39Å). Therefore, on the basis of our docking results, we can hypothesize that compound 7 has potential in the treatment of Alzheimer disease, as it actively binds in the active region of target enzymes (Figure 8).

DISCUSSION

The sulfonamides act as substitute for *p*-aminobenzoic acid (PABA) and responsible for the blocking of nucleic acid synthesis in bacteria and exhibited broad spectrum of activity. Therefore, the sulfonamide derivatives of Ibu were more active than Ibu. The promising results of compound **4** against

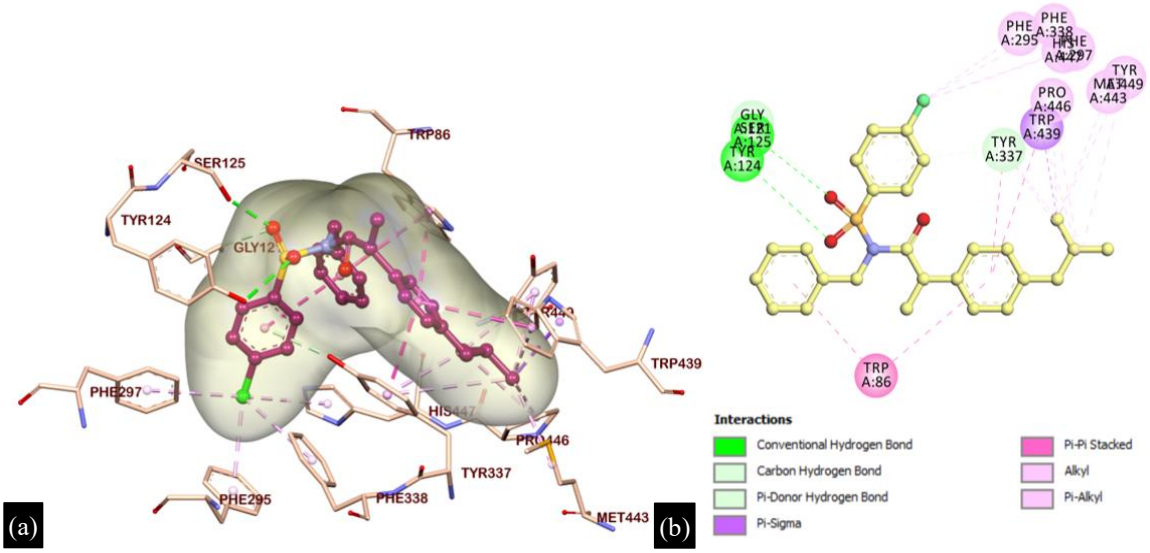


Figure 7. Interacting mode (a) and 2D-representation (b) of 7 inside active pocket of AChE, visualized on Discovery Studio v20

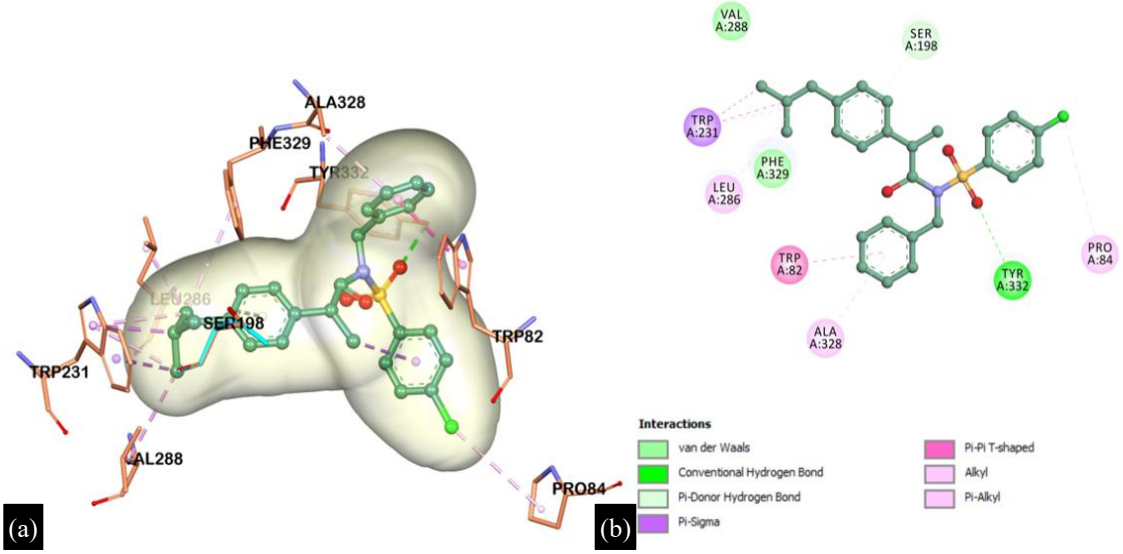


Figure 8. Interacting mode (a) and 2D-representation (b) of 7 inside Active Pocket of BChE, visualized on Discovery Studio v20

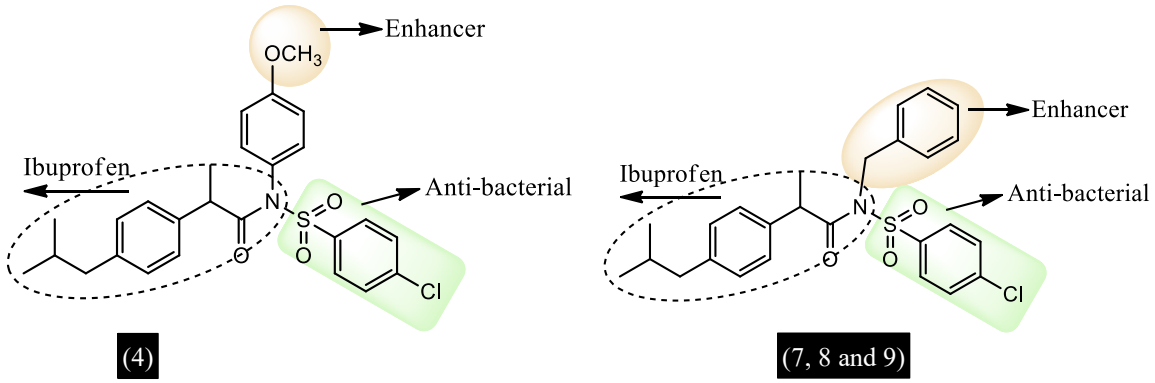


Figure 9. SAR for the promising compounds 4,7,8,9.

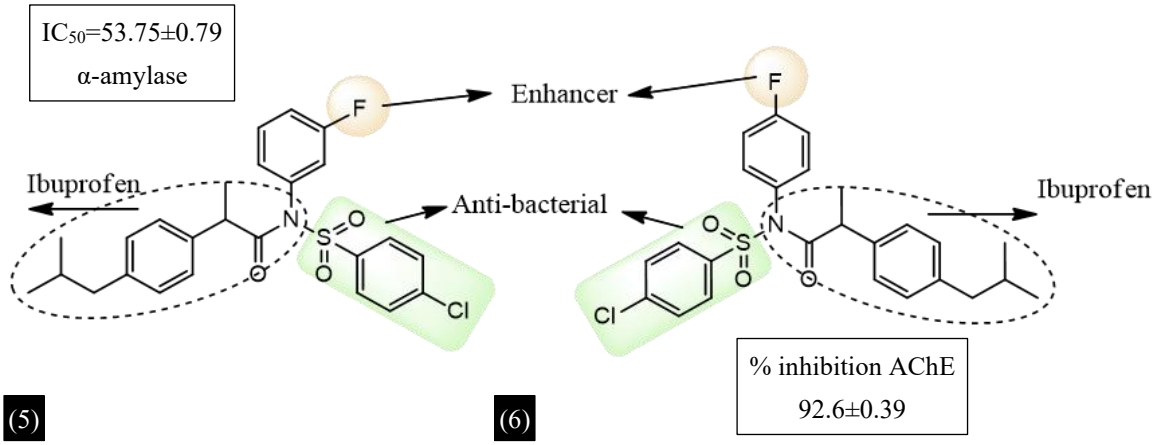


Figure 10. SAR for the promising compounds 5, 6 against cholinesterases.

tested bacteria might be due the methoxy group present on para position of the phenyl group which is marked as enhancer in this case (Figure 9). Compound 7, 8, and 9 all have benzyl group (activity enhancer) provided very significant results. Compound 7 with benzyl group emerged as efficient inhibitor of AChE (%inhibition = 95.8±0.31) and BChE (%inhibition = 96.7±0.16) (Figure 9).

Sul-Ibu fluoro analogues (5 and 6) exhibited high percentage of inhibition against AChE (93%, 92%) and BChE (95%, 93%) respectively. In 5, fluoro group is at meta position while in 6 it is at para position. In other analysis carried out in this work, these two compounds also showed good results. Surprisingly, 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 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 present research work tertiary amide based novel sulfonamide analogues of Ibuprofen were successfully synthesized and evaluated for their anti-bacterial, anti-diabetic and anti-Alzheimer potency. The present study concludes that compounds 4, 7-9 showed promising results against different bacterial strains. The combination of benzyl and sulfonyl moieties seemed very effective to cater with the 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 the derivatives 5-7 exhibited very significant results against cholinesterases. The compound 7 exhibited the best results with AChE and BChE. Kinetic study revealed that compounds 5-7 were all competitive inhibitors on cholinesterase with K_i of 47, 61 and 27 µmol, respectively. The molecular docking study of synthesized compounds is well aligned with the experimental results. The results of this research work allowed us to understand the key pharmacophoric units responsible for various biological activities via SAR study. This approach should be encouraged for further studies and development of new analogues with antibacterial, antidiabetic and anti-Alzheimer potencies.

Author Contributions

"Conceptualization, S.M., A.T.; methodology, S.M., A.T.; software, A.T.; validation, S.M., N.K.; formal analysis, A.T, N.K, H.I., S.A., S.H.S, S.D., 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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