Synthesis of some new *N*-(alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenesulfonamides as possible therapeutic agents for Alzheimer's disease and Type-2 Diabetes

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Abstract: In the current research work, a series of new *N*-(alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenesulfonamides has been synthesized by reacting 1,4-benzozzdioxan-6-amine (1) with 4-chlorobenzenesulfonyl chloride (2) to yield *N*-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenesulfonamide (3) which was further reacted with different alkyl/aralkyl halides (4a-n) to afford the target compounds (5a-n). Structures of the synthesized compounds were confirmed by IR, ^{1}H-NMR, EI-MS spectral techniques and CHN analysis data. The results of enzyme inhibition showed that the molecules, *N*-2-phenethyl-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5j) and *N*-(1-butyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5d), exhibited moderate inhibitory potential against acetylcholinesterase with IC_{50} values 26.25±0.11 μM and 58.13±0.15 μM respectively, whereas, compounds *N*-benzyl-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5i) and *N*-(pentane-2-yl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5f) showed moderate inhibition against α-glucosidase enzyme as evident from IC_{50} values 74.52±0.07 and 83.52±0.08 μM respectively, relative to standards Eserine having IC_{50} value of 0.04±0.0001 μM for cholinesterases and Acarbose having IC_{50} value 38.25±0.12 μM for α-glucosidase, respectively.

Keywords: 1,4-Benzodioxan-6-amine, 4-Chlorobenzenesulfonyl chloride, Alkyl/aralkyl halides, IR, ^{1}H-NMR, EI-MS, enzyme inhibition.

INTRODUCTION

The sulfa drugs are a significant class of compounds exhibiting tremendous biological activities and have been vastly employed to treat a number of microbial diseases after the discovery of Prontosil (fig. 1) since 70 years (Alsughayer *et al*., 2011).

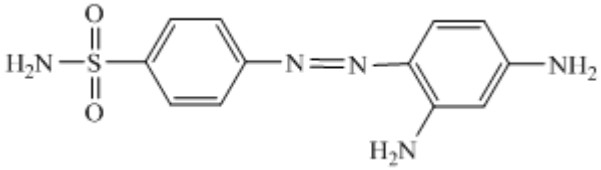

Fig. 1: Structure of Prontosil.

Sulfonamides having general formula R–SO_2NH_2 (Irshad *et al*., 2014a) are also utilized as anti-bacterial (Abbasi *et al*., 2014b), diuretics, anti-convulsants (Abbasi *et al*., 2014a), anti-microbials, and anti-inflammatory agents (Unsalan, *et al*., 2010). Many aromatic derivatives of sulfonamides showed good anti-cancer activity (Abbasi *et al*., 2013a). It is also reported that sulfonamides represent a group of inhibitors of the zinc carbonic anhydrase (CA) enzyme which is used in anti-glaucoma therapy. Sulfonamide core as anions attaches itself to the Zn^{2+} ion within the enzyme active site and block its function (Remko and Lieth, 2004; Supuran *et al*., 1999). These pharmaceutical compounds are frequently used in animal medicines to cure different infectious diseases of dairy cattle and also used as additives in food to promote growth in farm animals (Granja *et al.,* 2012). Several hydrophobic derivatives of sulfonamides are present in different naturally active therapeutics, e.g. anti-thyroid, insulin-releasing and anti-tumor drugs (Abbasi *et al*., 2013b). Sulfonamides are also bacteriostatic in nature. Their mechanism of action is initiated by the introduction of 4-aminobenzoic acid which participates in the synthesis of folic acid and blocks the dihydropetroate synthetase enzyme thus inhibiting the folic acid synthesis in bacteria which indirectly prevent the production of purines nucleotides (Abbasi *et al*., 2014c). Sulfonamides containing 1,4-benzodioxane-6-amine moiety exhibit profound biological activities as anti-hepatotoxic, α-adrenergic blocking, anti-inflammatory agents *etc.* This 1,4-benzodioxane ring system is also a part of many pharmaceutically important compounds such as Silybin, Americanin A6 and Haedoxan A7 which show anti-hepatotoxic and insecticidal activities(Irshad *et al*.,2014b)

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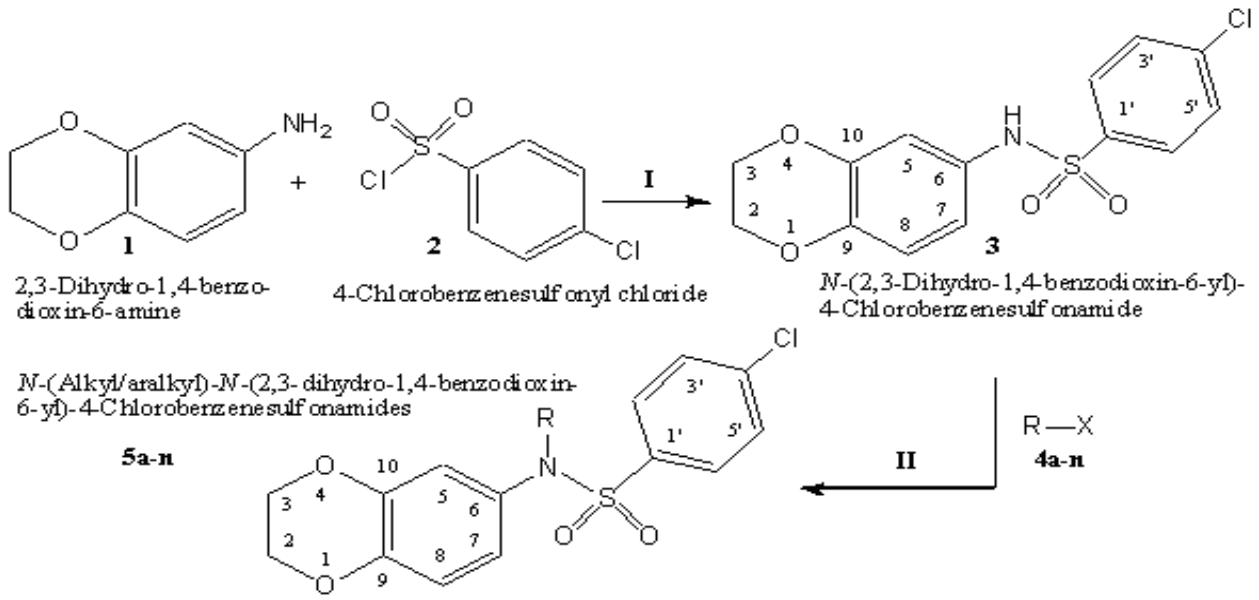


Scheme 1: Outline for the synthesis of *N*-(Alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamides (5a-n). Reagents & Conditions: (I) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 3 hrs. (II) DMF/LiH/stirring at RT for 2-3 hrs.

In literature, nucleophillic substitution reaction of 1,4-benzodioxan-6-amine with different cyclotri/tetraphos phazenes was also investigated to analyze their biological importance (Ibisoglu *et al*., 2014).

As reported, sulfonamides also act as competent cholinesterase and *α*-glucosidase inhibitors. Choline strases comprise a family of serine hydrolase enzymes. Butyrylcholinesterase (BChE,EC3.1.1.8) and acetyl cholinesterase (AChE, EC3.1.1.7) have different speci ficitication for inhibitors and substrates. This might have been due to the different sequences of amino acid residues in the active sites of these two enzymes. These enzymes are responsible for the termination of acetylcholine at cholinergic synapses which are the key components of cholinergic brain synapses and neuromuscular junctions. AChE and BChE catalyze the hydrolysis of acetylcholine which is a neurotransmitter that terminate the nerve impulse in cholinergic synapses (Abbasi *et al*., 2013a; Abbasi *et al*., 2013b). Meanwhile, it has been reported that BChE is more potent for the treatment of Alzheimer's disease (Abbasi *et al*., 2014d). It is produced in the liver and found in intestine, adipose tissue, white matter of the brain, smooth muscle cells, and in many other tissues (Abbasi *et al*., 2014b; Abbasi *et al*., 2013a). *α*-Gluco sidase (EC 3.2.1.20) belongs to a family of hydrolase enzymes and found in the surface membrane cells of small intestine. This enzyme system hydrolyses the 1,4-glycosidic linkage from the non-reducing end of the different substrates to yield *α*-D-glucose and other monosaccharides and cause postprandial hyperglycemia, which leads to the development of type-2 *diabetic mellitus*. Sulfonamides based anti-*α*-glucosidase drugs are mostly employed for patients with type-2 *diabetic mellitus*. These drug inhibitors retard the hydrolysis of oligosaccharides and disaccharides from dietary complex carbohydrates. Thus the excretion of D-glucose is delayed which results to reduce the postprandial hyperglycemia. Hence it is thought that the inhibition of *α*-glucosidase enzyme may prove an important step in the treatment of type-2 *Diabetes mellitus* (Abbasi *et al*., 2014a).

Thus, to introduce new cholinesterase and *α*-glucosidase inhibitors drug candidates, for the treatment of Alzheimer's disease and type-2 diabetes is an important strategy. The previous work of our group (Irshad *et al*., 2014b; Ibisoglu, 2014; Abbasi *et al*., 2014d; Abbasi *et al*., 2015; Abbasi *et al*., 2013b) concludes that different structural changes in the molecule by substitution have a great influence on the biological activities, such as sulfonamides based on 1,4-benzodioxine and different substituted sulfanyl chlorides are good inhibitors of butyrylcholinestrase (Irshad *et al*., 2014a) a while presence of 1,4-benzodioxane moiety in *O*- and *N*-substituted derivatives of planetol made them effective inhibitors of cholinestrases (Irshad *et al*., 2014b). Likewise, reported literature reveals that fused ring amines when reacted with different sulfanyl chlorides show good anti *α*-glucosidase activity (Abbasi *et al*., 2015). On the basis of above work, we have designed a new series of sulfonamides in search of new inhibitors of cholinesterases and *α*-glucosidase enzymes as possible therapeutic agents for the Alzheimer's disease and type-2 diabetes.

Table 1: Different Alkyl/aralkyl halides (4a-n) utilized in the synthesis of *N*-alkyl/aralkyl-*N*-(2,3-dihydro-1, 4-benzodioxin-6-yl)-4-chlorobenzenesulfonamides (5a-n)

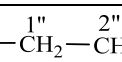
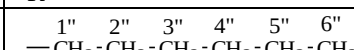
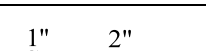
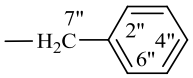
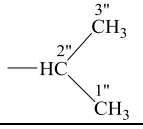
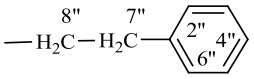
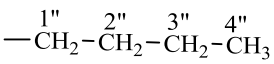
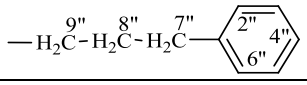
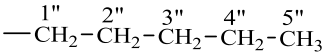
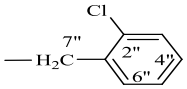
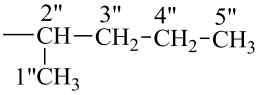
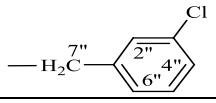
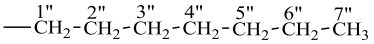
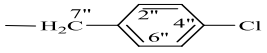
Code	-R	Code	-R
4a, 5a		4h, 5h	
4b, 5b		4i, 5i	
4c, 5c		4j, 5j	
4d, 5d		4k, 5k	
4e, 5e		4l, 5l	
4f, 5f		4m, 5m	
4g, 5g		4n, 5n	

Table 2: Enzyme inhibition results of *N*-(Alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamides (5a-n).

Sr. No.	AchE		BChE		*α*-Glucosidase	
	% Inhibition 0.5 mM	IC50 (*μ*M)	% Inhibition 0.5 mM	IC50 (*μ*M)	% Inhibition 0.5 mM	IC_{50} (*μ*M)
5a	17.23±0.19	-	75.29±0.15	357.53±0.08	75.58±0.25	187.56±0.16
5b	81.74±0.19	89.12±0.11	85.56±0.11	242.52±0.08	82.56±0.28	-
5c	97.11±0.26	146.73±0.12	85.39±0.17	323.12±0.09	87.27±0.29	192.47±0.19
5d	97.28±0.29	58.13±0.15	12.37±0.17	-	98.14±0.36	156.31±0.27
5e	91.33±0.31	285.62±0.21	82.11±0.15	254.21±0.06	82.37±0.35	-
5f	93.43±0.24	136.42±0.12	87.51±0.15	279.28±0.06	47.67±0.27	83.52±0.08
5g	91.94±0.27	114.27±0.13	83.44±0.12	286.63±0.08	45.32±0.25	268.57±0.19
5h	36.45±0.18	-	33.27±0.16	-	48.76±0.26	331.65±0.23
5i	94.87±0.25	73.71±0.12	87.76±0.16	285.27±0.07	62.38±0.29	74.52±0.07
5j	95.83±0.21	26.25±0.11	91.67±0.12	283.27±0.09	46.23±0.27	-
5k	94.28±0.21	139.73±0.11	81.28±0.17	265.13±0.03	98.45±0.31	312.34±0.21
5l	89.72±0.21	128.17±0.13	86.27±0.12	286.13±0.09	75.25±0.29	-
5m	94.14±0.23	79.28±0.13	83.11±0.15	313.26±0.05	99.75±0.32	-
5n	95.72±0.34	213.54±0.21	82.57±0.18	241.24±0.05	19.63±0.23	153.43±0.16
Control	Eserine 91.27±1.17	0.04±0.0001	Eserine 82.82±1.09	0.85±0.0001	Acarbose 92.23±0.14	38.25±0.12

Note: AChE = Acetyl cholinesterase and BChE = Butyrylcholinesterase. IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

MATERIALS AND METHODS

General

Chemicals utilized in this research work were purchased from Sigma Aldrich and were of analytical grade. The reactions were monitored by TLC plates pre-coated by silica gel G-25UV_{254} and were visualized at 254nm in UV light using various percentages of *n*-hexane and ethyl acetate till single spot. A Jasco-320-A spectrophotometer was used to record IR spectra on KBr discs (ν=cm^{-1}) for functional group identification of these derivatives. ^{1H}-NMR: spectra were recorded on a Bruker spectrometer (at 500MHz in $CDCl_3$) and chemical shifts (δ) were given in ppm. Molecular masses of these derivatives were identified by Electron Impact Mass Spectrometry (EI-MS) technique at a JMS-HX-110 spectrometer. The melting points of the synthesized compounds were recorded by open capillary tube on a Griffin and George melting point apparatus.

Synthesis of N-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenesulfonamide (3)

1,4-Benzodioxan-6-amine (6.6mmol, 1) was suspended in distilled water (10mL) in a100mL round bottom flask under dynamic control of pH maintained at 9.0 by addition of 10% sodium carbonate at room temperature under stirring for 10minutes. Further 4-chlorobenzenesulfonyl chloride (8.14 mmol, 2) was added and stirred for 2-3 hours. At the completion of reaction, concentrated HCl was added dropwise to adjust the pH around 2.0. The precipitates thus formed were collected by filtration, washed with distilled water and air dried to get *N*-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-chloro-benzenesulfonamide (3) in 90 % yield.

Synthesis of different N-(alkyl/aralkyl)-N-(2,3-dihydro-1,4-benzodioxan-6-yl)-4- chlorobenzenesulfonamides (5a-n)

N-(2,3-Dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenes ulfonamide (0.0003 mmol, 3) solublized in 10mL *N*,*N*-dimethylformamide (DMF) in 50mL round bottomed flask and stirred with lithium hydride (0.0004mmol) for 30minutes at room temperature. Various alkyl/aralkyl halides (4a-n; 0.0003 mol) were then added to the reaction mixture and further stirred for 2-3 hours. The reaction was monitored by TLC till completion and then the products were precipitated out by adding ice cold distilled water with continuous shaking. Precipitates were filtered off, washed with cold distilled water and air dried to obtain *N*-(alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenesulfonamides (5a-n).

Biological evaluation enzyme inhibition studies (in vitro)cholinesterase inhibition assay

The AChE and BChE inhibition activities were performed accordind to the reported method (Ellman *et al*., (1961).

α-Glucosidase inhibition assay

α-glucosidase enzyme inhibition activity was conducted by method reported in literature (Chapdelaine *et al*., 1978).

STATISTICAL ANALYSIS

Statistical analysis was performed by Microsoft Excel 2010 and all the measurements were carried out in triplicate. The results are presented as mean±SEM with 90% CL.

Spectral characterization

N-(2,3-Dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenesulfonamide (3)

Choclate brown amorphous powder, Yield 90%, m.p:104-106^{o}C, $C_{14}H_{12}ClNO_4S$, Mol. weight:325 g/mol;IR (υmax): 3498 (N-H), 3055 (C-HAr), 2985 (-CH2-), 1660 (C=C Ar), 1383 (-SO2), 1150 (C-O-C), 570 (C-Cl); ^{1}H-NMR: 10.01 (s, N-H, 1H), 7.88 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.63 (d,*J*=8.5Hz,2H,H-3′&H-5′), 6.80 (d,*J*=8.6Hz,1H,H-8), 6.55 (d,*J*=2.5Hz,1H,H-5), 6.49 (dd,*J*=2.5,8.6Hz,1H,H-7), 4.26-4.29 (m,4H,CH_2-2&CH_2-3); EI-MS:*m/z*327 [M+2], 325 [M^+], 260 [$C_{14}H_{11}ClNO_2]^+$; Anal. Calc. for $C_{14}H_{12}ClNO_4S$ (325.02):C,51.62;H,3.71;N,4.30. Found:C, 52. 14;H,393;N,4.88.

N-Ethyl-N-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenesulfonamide (5a)

Dark brown sticky solid, Yield 70%, $C_{16}H_{16}ClNO_4S$, Mol. weight:353 g/mol;IR (υmax):3052 (C-HAr), 2980 (-CH2-), 1656 (C=CAr), 1385 (-SO2), 1149 (C-O-C), 571 (C-Cl); ^{1}H-NMR: 7.91 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.63 (d,*J* =8.6Hz,2H,H-3′&H-5′), 6.66 (d,*J*=8.6Hz,1H,H-8), 6.60 (d,*J*=2.5Hz,1H,H-5), 6.04 (dd,*J*=2.5,8.6Hz,1H,H-7), 4.29-4.26 (m,4H,CH_2-2&CH_2-3), 3.20 (q,*J*=7.2Hz,2H, CH_2-1″), 1.15 (t,*J*=7.2Hz,3H,CH_3-2″); EI-MS:*m/z*355 [M+2], 32 5 $[C_{14}H_{12}ClNO_4S]^{+\cdot}$, 324 $[C_{14}H_{11}ClNO_4S]^+$, 260 [C_{14} $H_{11}ClNO_2]^+$, 178 $[C_{10}H_{12}NO_2]^+$; Anal. Calc. for C_{16} H_{16} $ClNO_4S$ (353.05):C, 54.31;H, 4.56;N, 3.96. Found: C,54. 98; H,4.76;N,4.34.

N-(2-Chloroethyl)-N-(2,3-dihydro-1,4-benzodioxan-6-yl)-4-chlorobenzenesulfonamide (5b)

Dark brown sticky solid, Yield 81%, $C_{16}H_{15}Cl_2NO_4S$, Mol.weight:387 g/mol;IR (υmax):3050 (C-HAr), 2979 (-CH2-), 1655 (C=CAr), 1380 (-SO2), 1150 (C-O-C), 568 (C-Cl); ^{1}H-NMR: 7.91 (d,*J*=8.6Hz, 2H,H-2′&H-6′), 7.63 (d,*J*=8.6Hz,2H,H-3′&H-5′), 6.83 (d,*J*=8.6Hz,1H,H-8), 6. 69 (d,*J*=2.5Hz,1H,H-5), 6.55 (dd,*J*=2.5,8.6Hz,1H,H-7), 4. 29-4.26 (m,4H,CH_2-2&CH_2-3,), 3.70 (t,*J*=7.2 Hz,2H, CH_2 -1″), 3.29 (t,*J*=7.4Hz,2H,CH_2-2″,); EI-MS: *m/z*389 [M+2], 387 [M^+], 352 $[C_{16}H_{15}ClNO_4S]^+$, 325 $[C_{14}H_{12}$ $ClNO_4S$ $]^+$, 324 $[C_{14}H_{11}ClNO_4S]^+$, 260 $[C_{14}H_{11}$ $ClNO_2]^+$, 178 $[C_{10}$ $H_{12}NO_2]^+$; Anal Calc for $C_{16}H_{15}$ Cl_2NO_4S (387.01): C,49. 49; H,3.89; N,3.61. Found: C, 50.06; H, 4.12; N,3.99.

N-(2-Propyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5c)

Dark brown sticky solid, Yield 77%, $C_{17}H_{18}ClNO_4S$, Mol. Weight:367g/mol;IR (υmax):3048 (C-HAr), 2978 (-CH2-), 1653(C=CAr), 1382(-SO2), 1148(C-O-C), 565(C-Cl); ^{1}H-NMR:7.92 (d,*J*=8.6Hz,H-2′&H-6′,2H), 7.55 (d,*J*=8. 6Hz,2H,H-3′&H-5′), 6.83 (d,*J*=8.6Hz,1H,H-8), 6.79 (d, *J*=2.5Hz,1H,H-5), 6.56 (dd, *J*=2.5,8.6Hz,1H,H-7), 4.27-4.24 (m,4H,CH_2-2&CH_2-3), 2.39-2.36 (m,1H,H-2″), 1.31 (d,*J*=7.3 Hz,6H,CH_3-1″&CH_3-3″); EI-MS:*m/z*369 [M+2], 367 [M^+],339 $[C_{15}H_{14}ClNO_4S]^{+\cdot}$,324 $[C_{14}H_{11}$ $ClNO_4S]$ $^+$,260 $[C_{14}H_{11}ClNO_2]^+$,192 $[C_{11}H_{14}NO_2]^+$; Anal. Calc. for $C_{17}H_{18}ClNO_4S$ (367.06):C,55.51;H,4.93; N,3.81. Found: C,55.77; H,515;N,3.94.

N-(1-Butyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5d)

Light brown sticky solid, Yield 74%, $C_{18}H_{20}ClNO_4S$, Mol. Weight:381 g/mol;IR (υmax):3047 (C-HAr), 2977 (-CH2-), 1658 (C=CAr), 1379 (-SO2), 1147 (C-O-C), 569 (C-Cl); ^{1}H-NMR: 7.91 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.63

(d,*J*=8.6Hz,2H,H-3′&H-5′), 6.80 (d,*J*=8.6Hz,1H,H-8), 6.75 (d,*J*=2.5Hz,1H,H-5), 6.54 (dd,*J*=2.5, 8.6Hz, 1H,H-7), 4.29-4.26 (m,4H,CH_2-2&CH_2-3), 3.16 (t,*J*=6.7Hz, 2H, CH_2-1″), 1.40-1.30 (m,4H,CH_2-2″&CH_2-3″), 0.90 (t,*J*= 6.7Hz,3H,CH_3-4″); EI-MS:*m/z*383 [M+2], 381 [M^+], 353 [$C_{16}H_{16}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 260 [$C_{14}H_{11}ClNO_2]^+$, 206 [$C_{12}H_{16}NO_2]^+$; Anal. Calc. for $C_{18}H_{20}ClNO_4S$ (381.08): C, 56.61;H, 5.28;N, 3.67.Found:C, 57.03;H, 5.20;N, 3.81.

N-(1-Pentyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5e)

Dark brown sticky solid, Yield 70%, $C_{19}H_{22}ClNO_4S$, Mol. Weight-395g/mol;IR (υmax):3046 (C-HAr), 2979 (-CH2-), 1657 (C=CAr), 1375 (-SO2), 1144 (C-O-C), 566 (C-Cl); ^{1}H-NMR:7.91 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.63 (d,*J*=8.6 Hz,2H,H-3′&H-5′), 6.76 (d,*J*=8.6Hz, 1H, H-8), 6.66 (d,*J*=2.5Hz, 1H,H-5), 6.56 (dd,*J*=2.5,8.6 Hz,1H, H-7,), 4.29-4.29 (m,4H,CH_2-2&CH_2-3), 3.14 (t,*J*=6.7Hz,2H, CH_2-1″), 1.49-1.29 (m,6H,CH_2-2″toCH_2-4″), 0.87 (t,*J*=6.7 Hz,3H,CH_3-5"); EI-MS: *m/z*397 [M+2], 395 [M]$^+$, 367 [$C_{17}H_{18}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 260 [$C_{14}H_{11}ClNO_2]^+$, 220 [$C_{13}H_{18}NO_2]^+$; Anal. Calc. for $C_{19}H_{22}ClNO_4S$ (395.10):C, 57.64;H, 5.60;N, 3.54.Found: C,58. 08; H,5 .82; N,3.76.

N-(Pentane-2-yl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5f)

Dark brown sticky solid, Yield 88%, $C_{19}H_{22}ClNO_4S$, Mol. Weight-395 g/mol;IR (υmax):3047 (C-HAr), 2977 (-CH2-), 1655 (C=CAr), 1374 (-SO2), 1146 (C-O-C), 570 (C-Cl); ^{1}H-NMR: 7.65 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.43 (d,*J*=8.6Hz,2H,H-3′&H-5′), 6.83 (d,*J*=8.6Hz,1H,H-8), 6.69 (d,*J*=2.5Hz,1H,H-5), 6.55 (dd,*J*=2.5,8.6Hz,1H,H-7), 4.29-4.29 (m,4H,CH_2-2&CH_2-3), 2.75 (m,1H,H-2″), 1.48-1.35 (m,4H,CH_2-3″&CH_2-4″), 1.22 (d,*J*=6.8Hz,3H,CH_3-1″), 0.88 (t,*J*=7.2Hz,3H,CH_3-5"); EI-MS:*m/z*397 [M+2], 395 [M]$^+$, 367 [$C_{17}H_{18}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 260 [$C_{14}H_{11}ClNO_2]^+$, 220 [$C_{13}H_{18}NO_2]^+$; Anal. Calc. for $C_{19}H_{22}ClNO_4S$ (395.10):C,57.64;H,5.60;N,3.54.Found:C, 58.2;H,5.88;N,3.82.

N-(1-Heptyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5g)

Dark brown sticky solid, Yield 75%, $C_{21}H_{26}ClNO_4S$, Mol. Weight:423g/mol;IR (υmax):3045 (C-HAr), 2974 (-CH2-), 1653 (C=CAr), 1372 (-SO2), 1145 (C-O-C), 565 (C-Cl); ^{1}H-NMR: 7.91 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.63 (d,*J*= 8.6Hz,2H,H-3′&H-5′), 6.76 (d,*J*=8.6Hz,1H,H-8), 6.66 (d,*J*=2.5Hz,1H,H-5), 6.56 (dd*J*=2.5,8.6Hz,1H,H-7), 4.29-4.26 (m,4H,CH_2-2&CH_2-3), 3.16 (t,*J*=6.7Hz,2H, CH_2-1″), 1.47 (quint,*J*=7.2Hz,2H,CH_2-2″), 1.25 (brs,6H, CH_2-3″ toCH_2-5″), 1.30 (m,2H,CH_2-6″), 0.87 (t,*J*=7.2Hz, 3H, CH_3-7″); EI-MS:*m/z*425 [M+2], 423 [M^+], 395 [$C_{19}H_{22}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 260 [$C_{14}H_{11}ClNO_2]^+$, 248 [$C_{15}H_{22}NO_2]^+$; Anal. Calc. for $C_{21}H_{26}ClNO_4S$ (423.13):C,59.49; H,6.8;N,3.30 Found: C, 60.01; H,6.44;N,3.68.

N-(1-Octyl) -N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5h)

Dark brown sticky solid, Yield 84%, $C_{22}H_{28}ClNO_4S$, Mol. Weight:437 g/mol;IR (υmax):3044 (C-HAr), 2973 (-CH2-), 1654 (C=CAr), 1373 (-SO2), 1144 (C-O-C), 562 (CCl); ^{1}H-NMR: 7.91 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.63 (d,*J*=8.5 Hz,2H,H-3′&H-5′,), 6.76 (d,*J*=8.6Hz,1H,H-8), 6.66 (d,*J*= 2.5Hz,1H,H-5), 6.56 (dd,*J*=2.5,8.6Hz,1H,H-7), 4.29-4.26 (m,4H,CH_2-2&CH_2-3), 3.67 (t, *J*=6.8Hz,2H,CH_2-1″), 2.02 -1.97 (m,6H,CH_2-2″toCH_2-4″), 1.24-1.17 (m,6H,CH_2 -5″ to CH_2-7″), 0.83 (t,*J*=7.2Hz,3H,CH_3-8″); EI-MS:*m/z*439 [M+2], 437 [M^+], 409 [$C_{20}H_{24}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 262 [$C_{16}H_{24}NO_2]^+$, 260 [$C_{14}H_{11}ClNO_2]^+$; Anal. Calc. for $C_{22}H_{28}ClNO_4S$ (437.14): C, 60.33; H,6.44; N,3.20.Found: C,60.97; H,6.76; N,3.42.

N-Benzyl-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5i)

Brown sticky solid, Yield 86%, $C_{21}H_{18}ClNO_4S$, Mol. Weight:415g/mol;IR (υmax):3044 (C-HAr), 2975 (-CH2-), 1653 (C=CAr), 1371 (-SO2), 1142 (C-O-C), 565 (C-Cl); ^{1}H-NMR: 7.92 (d,*J*=8.5Hz,2H,H-2′&H-6′), 7.64 (d,*J*=8.6Hz,2H,H-3′&H-5′), 7.55 (d,*J*=8.6Hz,2H,H-2″&H-6″), 7.49-7.46 (m,3H,H-3″toH-5″), 6.68 (d,*J*=8.6Hz,1H,H-8), 6.54 (d,*J*=2.5Hz,1H,H-5), 6.46 (dd,*J* =2.5,8.6Hz,1H,H-7), 4.68 (s,2H,CH_2,-7″), 4.23-4.20 (m, 4H,CH_2-2&CH_2-3); EI-MS:*m/z*417 [M+2], 415 [M^+], 386 [$C_{19}H_{13}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 260 [$C_{14}H_{11}ClNO_2]^+$, 240 [$C_{15}H_{14}NO_2]^+$; Anal. Calc. for $C_{21}H_{18}ClNO_4S$ (415.06):C, 60.65;H, 4.36;N, 3.37. Found: C,61. 11;H,4.64;N,3.75.

N-(2-Phenethyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5j)

Dark brown sticky solid, Yield 76%, $C_{22}H_{20}ClNO_4S$, Mol. Weight:429 g/mol;IR (υmax):3043 (C-HAr), 2973 (-CH2-), 1651 (C=CAr), 1370 (-SO2), 1142 (C-O-C), 563 (C-Cl); ^{1}H-NMR: 7.91 (d,*J*=8.5Hz,2H,H-2′&H-6′), 7.63 (d,*J*= 8.5Hz,2H,H-3′&H-5′), 7.22 (brt,*J*=7.6Hz,2H,H-3″&H-5″), 7.17 (brt,*J*=8.6,1H,H-4″), 7.06 (brd,*J*=8.6Hz, 2H,H-2″&H-6″), 6.71 (d,*J*=8.6Hz,1H,H-8), 6.56 (d,*J*= 2.5Hz, 1H,H-5), 6.41 (dd,*J*=2.5,8.6Hz,1H,H-7), 4.29-4.26 (m,4H, CH_2-2&CH_2-3), 3.08 (t,*J*=6.8Hz,2H,CH_2-8″), 2.80 (t,*J*= 6.8Hz,2H,CH_2-7″); EI-MS:*m/z*431 [M+2], 429 [M^+], 401 [$C_{20}H_{16}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 260 [$C_{14}H_{11}ClNO_2]^+$, 254 [$C_{16}H_{16}NO_2]^+$; Anal. Calc. for $C_{22}H_{20}ClNO_4S$ (429.08): C,61.46; H,4.69; N,3.26. Found: C, 61.84;H, 4.90;N, 3.48.

N-(3-Phenylpropyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5k)

Dark brown sticky solid, Yield 73%, $C_{23}H_{22}ClNO_4S$; Mol. Weight:443 g/mol;IR (υmax):3042 (C-HAr), 2971 (-CH2-), 1650 (C=CAr), 1369 (-SO2), 1141 (C-O-C), 561 (C-Cl); ^{1}H-NMR: 7.91 (d,*J*=8.4Hz,2H,H-2′andH-6′), 7.63 (d,*J*=8.5Hz,2H,H-3′&H-5′), 7.21 (brt,*J*=7.6Hz,2H,H-3″& H-5″), 7.15 (brt,*J*=6.8,1H,H-4″), 7.07 (brd,*J*=7.2Hz, 2H, H-2″&H-6″), 6.71 (d,*J*=8.6Hz,1H,H-8,), 6.56 (d,*J*=2.6Hz,

1H,H-5), 6.41 (dd,*J*=2.5,8.6Hz,1H,H-7), 4.29-4.26 (m,4H, CH_2-2&CH_2-3), 3.73-3.68 (m,2H,CH_2-9″), 2.62-2.53 (m, 2H,CH_2-7″), 2.04 (quint., *J*=7.2Hz,2H,CH_2-8″); EI-MS: *m/z*445 [M+2], 443 [M^+], 415 [$C_{21}H_{18}ClNO_4$ S]$^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 268 [$C_{17}H_{18}NO_2]^+$, 260 [$C_{14}H_{11}$ $ClNO_2]^+$; Anal. Calc. for $C_{23}H_{22}ClNO_4S$ (443.10): C,62. 23;H,4.99; N,3.16. Found: C,62.56; H,5.23; N,3.42.

N-(2-Chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5l)

Dark brown sticky solid, Yield 87%, $C_{21}H_{17}Cl_2NO_4S$, Mol. Weight:449 g/mol;IR (υmax):3041 (C-HAr), 2969 (-CH2-), 1651 (C=CAr), 1368 (-SO2), 1140 (C-O-C), 560 (C-Cl); ^{1}H-NMR: 7.91 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.63 (dd,*J*=2.5,8.6Hz,1H,H-3″), 7.54 (d,*J*=8.6Hz,2H,H-3′&H-5′), 7.34 (dd,*J*=2.5,8.6Hz,1H,H-6″), 7.12-7.10 (m,2H,H-4″&H-5″), 6.82 (d,*J*=8.6Hz,1H,H-8), 6.67 (d,*J*=2.5Hz ,1H,H-5), 6.45 (dd,*J*=2.5,8.6Hz,1H,H-7), 4.63 (s,2H, CH_2,-7″), 4.29-4.26 (m,4H,CH_2-2&CH_2-3); EI-MS:*m/z* 451 [M+2], 449 [M^+], 414 [$C_{21}H_{17}ClNO_4S]^+$, 386 [$C_{19}H_{13}$ $ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 274 [$C_{15}H_{13}ClNO_2]^+$, 260 [$C_{14}H_{11}ClNO_2]^+$; Anal. Calc. for $C_{21}H_{17}Cl_2NO_4S$ (449.03): C, 56.01; H,3.80; N,3.11. Found: C, 56.38; H,4.08; N,3.39.

N-(3-Chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide (5m)

Dark brown sticky solid, Yield 79%, $C_{21}H_{17}Cl_2NO_4S$, Mol. Weight:449 g/mol;IR (υmax):3040 (C-HAr), 2970 (-CH2-), 1649 (C=CAr), 1367 (-SO2), 1139 (C-O-C), 558 (C-Cl); ^{1}H-NMR: 7.91 (brd,*J*=8.5Hz,1H,H-4″), 7.63 (d,*J*= 8.4Hz,2H,H-2′&H-6′), 7.49 (brt,*J*=8.6Hz,1H,H-5″), 7.46 (d,*J*=8.5Hz,2H,H-3′&H-5′), 7.23-7.19 (m,2H,H-2″&H-6″), 6.71 (d,*J*=8.6Hz,1H,H-8), 6.56 (d,*J*=2.6Hz,1H,H-5), 6.41 (dd,*J*=2.5,8.6Hz,1H,H-7), 4.63 (s,2H,CH_2,-7″), 4.29-4.26 (m,4H,CH_2-2&CH_2-3); EI-MS:*m/z*451 [M+2], 449 [M^+], 414 [$C_{21}H_{17}ClNO_4S]^+$, 386 [$C_{19}H_{13}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}ClNO_4S]^+$, 274 [$C_{15}H_{13}ClNO_2]^+$, 260 [$C_{14}H_{11}$ $ClNO_2]^+$; Anal. Calc. for $C_{21}H_{17}Cl_2NO_4S$ (449.03): C,56. 01; H,3.80; N,3.11. Found: C,56.44; H,4.02; N,3.23.

N-(4-Chlorobenzyl)-N-(2,3-dihydrobenze-1,4-dioxin-6-yl)-4-chlorobenzenesulfonamide (5n)

Dark brown sticky solid; Yield: 80%; $C_{21}H_{17}Cl_2NO_4S$; Molecular weight:449 g/mol;IR (υmax):3042 (C-H Ar),2972 (-CH2-), 1648 (C=CAr), 1368 (-SO2), 1137 (C-O-C), 555 (C-Cl); ^{1}H-NMR: 7.91 (d,*J*=8.6Hz,2H,H-2′&H-6′), 7.63 (d,*J*=8.5Hz,2H,H-3′&H-5′), 7.49 (d,*J*= 8.6Hz ,2H,H-3″& H-5″), 7.18 (d,*J*=8.6Hz,2H,H-2″&H-6″), 6.83 (d,*J*=8.6Hz,1H,H-8), 6.52 (d,*J*=2.5Hz,1H,H-5), 6.46 (dd, *J*=2.5,8.6Hz,1H,H-7), 4.63 (s,2H,CH_2,-7″), 4.24-4.21 (m, 4H,CH_2-2&CH_2-3); EI-MS:*m/z*451 [M+2], 449 [M^+], 414 [$C_{21}H_{17}ClNO_4S]^+$, 386 [$C_{19}H_{13}ClNO_4S]^{+\cdot}$, 324 [$C_{14}H_{11}$ $ClNO_4S]^+$, 274 [$C_{15}H_{13}ClNO_2]^+$, 260 [$C_{14}H_{11}$ $ClNO_2]^+$; Anal.Calc. for $C_{21}H_{17}Cl_2NO_4S$ (449.03): C,56. 01; H,3.80; N,3.11. Found: C,56.52; H,4.14; N,3.29.

RESULTS

In the present research, the parent molecule (3) and its derivatives were synthesized according to the outline illustrated in (Scheme1; table1) An equimolar amount of 1,4-benzodioxan-6-amine (1) and 4-chlorobenzenesul fonyl chloride (2) was reacted in 10% Na_2CO_3 under stirring at room temperature to obtain the parent com pound (3). Further, *N*-alkylation / aralkylation of com pound 3 was done with different electrophiles (4a-n; table1) in the presence of DMF taken as solvent and lithium hydride which acts as an activator to yield various derivatives (5a-n) in good yields. The structural study of these compounds was done by spectral techniques such as IR, $^{1H\text{-}NMR:}$ and EI-MS. All the synthesized derivatives were evaluated for their *in vitro* enzyme inhibition studies against AChE, BChE and *α*-glucosidase enzymes and their % inhibition and IC_{50} values are given in table-2.

DISCUSSION

One of the synthesized compounds is discussed hereby in detail for the expediency of the readers. The molecule 5n was obtained as dark brown sticky solid with 80% yield. Its molecular formula was confirmed by assigning the number of protons in its ^{1}H-NMR spectrum. EI-MS spectrum revealed molecular ion peak $[M]^+$ at *m/z* 449 and an $[M+2]^+$ peak with the difference of two mass units at *m/z* 451 which definitely was rationalized for the presence of isotopic chlorine atom in the molecule. Base peak appeared at *m/z* 274 while other fragment ion peaks at *m/z* 414, 386 and 260 corroborated the structure of the molecule. The absorption band at 1368 cm^{-1} in its IR spectrum was characteristic for -SO_2 stretching in this molecule while other furnctional groups were assigned on the basis of observed peaks at 3042 cm^{-1} (C-H Ar), 1648 cm^{-1} (C=CAr),1137 cm^{-1} (C-O-C) and 555 cm^{-1} for C-Cl stretching. In the ^{1}H-NMR spectrum, two A_2B_2 spin systems were clearly observed, one for 4-chloro-benzenesulfanyl moiety represented by two *di-ortho* coupled doublets at *δ* 7.91 and *δ* 7.63 while other *di-ortho* coupled pattern represented by peaks at *δ* 7.49 and *δ* 7.18 along with a methylene singlet at 4.63 (CH_2-7″) was assignable to the 4-chlorobenzyl moiety substituted on the nitrogen atom in the molecule. Moreover, an AMX spin system represented by peaks as two doublets at chemical shift values of *δ* 6.83 and *δ* 6.52 along with a doublet of doublet at *δ* 6.46 ppm. A four proton multiplet resonating around *δ* 4.24-4.21 ppm (CH_2-2 & CH_2-3) indicated the aliphatic unit of 1,4-benzodioxane moiety in the molecule. Hence, on the basis of above collective spectral proofs, the structure of 5n was confirmed as *N*-(4-chlorobenzyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-chlorobenzenesulfonamide. Similarly, the structures of all other synthesized molecules were also confirmed by their spectral investigations.

Enzyme Inhibition and Structure-activity Relationship Studies

In search of new suitable therapeutic agents for one possible treatment of Alzeimers's disease and for the control the type-2 diabetes, the synthesized molecules (5a-n) were screened against acetyl/butyrylcholin-esterases and α-glucosidase enzymes, respectively. The IC_{50} values (table 2) of these molecules demonstrated that these might be utilized as moderate to weak inhibitors of the studied enzymes. Amongst these derivatives, some compounds exhibited better inhibitory potential for AChE as compared to BChE. The compounds 5j and 5d displayed relatively good inhibition against AChE, having IC_{50} values 26.25±0.11 μM and 58.13±0.15 μM, respectively. The better enzyme inhibitory potential of 5j and 5d against AChE might be attributed to the substitution of 2-phenethyl and 1-butyl groups, respectively at nitrogen atom, as discussed in previous literature (Abbasi *et al*., 2014d). Whereas, compounds 5n and 5b were found as better inhibitors of BChE among the studied series, as it was shown by their IC_{50} values as 241.24±0.05 and 242.52±0.08 μM, respectively. Their better inhibitory potential might be rationalized by fact that substitution of 4-chlorobenzyl or 2-chloroethyl on nitrogen atom of parent sulfonamide provides relatively suitable entities for the inhibition of BChE enzyme as compared with compounds having same *N*-substitution in literature (Abbasi *et al*., 2014d). Eserine used as standard for AChE as well as BChE, having IC_{50} value of 0.04±0.0001 and 0.85±0.0001 μM, respectively, against these enzymes. Similarly, the inhibitory potential of synthesized molecules was also evaluated against α-glucosidase enzyme. The results revealed that some compounds might be considered as moderate inhibitors of this enzyme as demonstrated by their IC_{50} values in table 2. The compounds 5i and 5f displayed convincing inhibitory potentials with IC_{50} values of 74.52±0.07 and 83.52±0.08μM, respectively, relative to the reference standeard, acarbose, having IC_{50} of 38.25±0.12 μM. The credible activity of these two compounds might be attributed to the presence of un-substituted aralkyl group i.e. benzyl group and medium sized branched aliphatic chain i.e. pentane-2-yl group, respectively, in these molecules as values are compared with reported literature (Abbasi *et al*., 2015).

The inhibition activity of these compounds against both enzymes showed that molecules containing small alkyl *N*-substitution like 2-chloroethyl, butyl group *etc*. were found to be better inhibitors of cholinestrases may be because they fit well into the active site of enzyme. Also *N*-substitution by benzyl and 2-phenethyl groups presented better result probably owing to their possible ability to generate better π-π interactions with the active site. Thus, the compounds 5b, 5d, 5j and 5i remained significantly efficient among the whole series of synthesized molecules.

CONCLUSION

The synthesized derivatives (5a-n) were synthesized in good yields with a facile method and their therapeutic potential was evaluated as inhibitors against acetyl/butyr ylcholinesterase and α-glucosidase enzymes. Amongst the series some molecules exhibited convincing enzyme inhibitory potential and might be considered as suitable entities for drug designing in future to explore new therapeutic agents for Alzheimer's disease and type-2 diabetes.

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