

# Synthesis of new 2-{2,3-dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl) sulfonyl]amino}-N-(un/substituted-phenyl) acetamides as $\alpha$ -glucosidase and acetylcholinesterase inhibitors and their *in silico* study

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The aim of the present research work was to investigate the enzyme inhibitory potential of some new sulfonamides having benzodioxane and acetamide moieties. The synthesis was started by the reaction of *N*-2,3-dihydrobenzo[1,4]-dioxin-6-amine (**1**) with 4-methylbenzenesulfonyl chloride (**2**) in the presence of 10% aqueous Na<sub>2</sub>CO<sub>3</sub> to yield *N*-(2,3-dihydrobenzo[1,4]-dioxin-6-yl)-4-methylbenzenesulfonamide (**3**), which was then reacted with 2-bromo-*N*-(un/substituted-phenyl)acetamides (**6a-1**) in DMF and lithium hydride as a base to afford various 2-{2,3-dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl)sulfonyl]amino}-*N*-(un/substituted-phenyl)acetamides (**7a-1**). All the synthesized compounds were characterized by their IR and <sup>1</sup>H-NMR spectral data along with CHN analysis data. The enzyme inhibitory activities of these compounds were tested against  $\alpha$ -glucosidase and acetylcholinesterase (AChE). Most of the compounds exhibited substantial inhibitory activity against yeast  $\alpha$ -glucosidase and weak against AChE. The *in silico* molecular docking results were also consistent with *in vitro* enzyme inhibition data.


Keywords: Benzodioxane. Acetamide. Spectral analysis.  $\alpha$ -Glucosidase. Acetylcholinesterase. Molecular docking.

## INTRODUCTION

Sulfonamides are chemotherapeutic compounds consisting of a -SO<sub>2</sub>NH<sub>2</sub> functional group that are of biological importance in the fight against pathogenic microbes and they have brought on a revolution in the field of pharmaceutical chemistry. Pathogenic microbes are a great threat to human health and are a cause of growing concern among people across the world. These compounds were firstly used as antibacterial agents (Reddy *et al.*, 2012; Alsughayer *et al.*, 2011; Mahdi *et al.*, 2015). Many sulfa drugs are widely used against various diseases as antifungal (Rathod *et al.*, 2012), antimicrobial (Ajeet, Kumar, 2014), antitumor agents (Huang, Lin Huang,

2001) and antihypertensive (Bhagwat *et al.*, 2014) agents. Sulfonamides act as antiviral and antitumor agents and they are used to inhibit cancer cell growth and to block tumor invasion (Huang, Lin, Huang, 2001; Ghorab *et al.*, 2016). Some sulfonamides act as good anti-proliferative agents and show promising broad-spectrum antitumor activity as compared to that of the commonly used anticancer drugs (Kasimogullari *et al.*, 2015). Many sulfonamide derivatives are effective inhibitors of butyrylcholinesterase (BChE), acetylcholinesterase (AChE) for the treatment of Alzheimer's disease (AD) (Abbasi *et al.*, 2014a) and also show a good inhibitory potential against  $\alpha$ -glucosidase and lipoxygenase (Abbasi *et al.*, 2014b).

The compounds that have a 1,4-benzodioxane moiety exhibit a wide range of attractive biological activities, and their synthesis has received considerable attention over the years. Compounds with this moiety have biological activities such as anti-hepatotoxic (Ahmad,

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Khan, Alam, 2003),  $\alpha$ -adrenergic blocking (Chapleo *et al.*, 1983) and anti-inflammatory (Vazquez, Rosell, Pujol, 1997). The 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.*, 2014). Flavonolignans were first discovered in the seeds of *Silybum marianum*, commonly known as milk thistle. The original flavonolignan to be discovered was silybin, and it is still the most studied flavonolignan and 1,4-benzodioxane lignin (Abouzid, Ahmad, 2013; Pelter, Hansel, 1968). Silybin has shown a range of different biological activities including hepatoprotective, anticancer and antioxidant, among others (Kren, Walterova, 2005; Gazak, Walterova, Kren, 2007; Kaspaday *et al.*, 2009).

-Glucosidase (EC 3.2.1.20) belongs to the family of hydrolases and is located in the brush border surface membrane of small intestinal cells (Chiba, 1997). -Glucosidase inhibitors are used as oral anti-diabetic drugs for patients with type-2 diabetes mellitus (T2DM). Postprandial hyperglycemia plays a vital role in the development of T2DM and nephropathy, neuropathy, microangiopathy, macroangiopathy which are complications associated with this disease (Lebovitz, 1997). The inhibitors of this enzyme can delay the release of *D*-glucose from oligosaccharides and disaccharides from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial hyperglycemia. Hence, the inhibition of -glucosidase is an important step in the treatment of T2DM (Chapdelaine, Trembley, Dube, 1978; Abbasi *et al.*, 2016). Several marketed drugs including acarbose and voglibiose are famous inhibitors of -glucosidase.

Cholinesterases such as AChE (EC 3.1.1.7) and BChE (EC 3.1.1.8) belong to the serine hydrolase class. These are the best potential targets for the symptomatic treatment of AD and its related disorders. The specificities of the enzymes for their substrates and inhibitors depend on differences in the amino acid residues of the active sites of the two esterases. AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine, terminating the nerve impulse at cholinergic synapses (Tougu, 2001; Gauthier, 2001).

The revolutionary importance of sulfonamides in the field of pharmacology encouraged us to synthesize new sulfonamide-acetamide derivatives using 1,4-benzodioxane as central moiety. In the present work, a number of *N*-substituted derivatives starting from 2,3-dihydro-1,4-benzodioxin-6-amine were synthesized. The first, parent sulfonamide *N*-(2,3-dihydrobenzo[1,4]-dioxin-6-yl)-4-methylbenzenesulfonamide (**3**) was prepared by reacting 2,3-dihydro-1,4-benzodioxin-6-

amine (**1**) with 4-methylbenzenesulfonyl chloride (**2**) by stirring in aqueous alkaline medium at room temperature, and it was then further derivatized with different 2-bromo-*N*-(un/substituted-phenyl)acetamides (**6a-1**) to get targeted molecules (**7a-1**). These newly synthesized molecules were then screened against -glucosidase and acetylcholinesterase enzymes to explore their therapeutic potential for T2DM and AD.

## MATERIAL AND METHODS

### General

The chemicals utilized were procured from Sigma Aldrich/Alfa Aesar. The solvents used in reactions were of analytical grade and used as such. Pre-coated TLC silica gel G-25-UV<sub>254</sub> plates were used to monitor the reactions using various percentages of n-hexane and ethyl acetate as solvent system. Melting points of compounds were recorded using a Gallenkamp melting point apparatus with open capillary tube. A MIDAC M2000 photon spectrometer was used to record the FTIR spectra in KBr ( $\nu$ ,  $\text{cm}^{-1}$ ). A Burker spectrometer operating at 25 °C at 400/600 MHz was used to record the <sup>1</sup>H NMR spectra in CDCl<sub>3</sub>. The coupling constant (*J*) is given in Hz and chemical shift ( $\delta$ ) in ppm. The abbreviations used in the interpretation of <sup>1</sup>H NMR spectra are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br.t, broad triplet; q, quartet; quin, quintet; sex, sextet; sep, septet; m, multiplet.

### Synthesis

*Synthesis of N*-(2,3-dihydrobenzo[1,4]-dioxin-6-yl)-4-methylbenzenesulfonamide (**3**)

*N*-(2,3-Dihydrobenzo[1,4]-dioxin-6-amine (1 mL; 0.002 mol; **1**) was suspended in 25 mL distilled water and stirred for half an hour. Next, 10% aqueous Na<sub>2</sub>CO<sub>3</sub> was added to maintain the pH at 9-10, and the reaction mixture stirred for half an hour. 4-Methylbenzenesulfonyl chloride (0.47 g; 0.002 mol; **2**) was then added to the mixture along with gradual stirring, which was further stirred for several hours until completion of the reaction, which was monitored by TLC until a single spot was obtained. The product was precipitated at pH 2 using conc. HCl and filtered out, washed with distilled water, and air-dried to afford *N*-(2,3-dihydrobenzo[1,4]-dioxin-6-yl)-4-methylbenzenesulfonamide (**3**): a light brown amorphous powder; yield 80%; m.p. 129-130 °C; molecular formula: C<sub>15</sub>H<sub>15</sub>O<sub>4</sub>NS; molecular weight: 305.367 gmol<sup>-1</sup>; IR: 3248 (N-H stretching), 3045 (C-H stretching of aromatic ring), 2926 (-CH<sub>2</sub> stretching), 1633 (C=C stretching of aromatic

ring), 1383 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: δ (ppm) 10.12 (s, 1H, NHSO<sub>2</sub>), 7.57 (d, *J* = 7.8 Hz, 2H, H-2' & H-6'), 7.27 (d, *J* = 7.8 Hz, 2H, H-3' & H-5'), 6.62 (d, *J* = 8.4 Hz, 1H, H-8), 6.56 (d, *J* = 2.4 Hz, 1H, H-5), 6.47 (dd, *J* = 2.4, 8.4 Hz, 1H, H-7), 4.15 (br.s, 4H, CH<sub>2</sub>-2, CH<sub>2</sub>-3), 2.43 (s, 3H, CH<sub>3</sub>-7').

#### General procedure for the synthesis of 2-bromo-N-(un/substituted-phenyl)acetamides (6a-l)

2-Bromo-N-(un/substituted-phenyl)acetamides (6a-l) were prepared by vigorous manual shaking of un/substituted anilines (0.16 g; 0.55 mmol; 4a-l, one in each reaction) with bromoacetyl bromide (0.2 g; 0.50 mmol; 5). The pH of the solution was maintained by using 10% aqueous Na<sub>2</sub>CO<sub>3</sub>. The completion of the reaction was monitored by TLC till single spot. Afterwards, the reaction mixture was poured on crushed ice. The precipitated products were filtered, washed and dried to acquire the pure products (6a-l).

#### General procedure for the synthesis of 2-{2,3-dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl)-sulfonyl]amino}-N-(un/substituted-phenyl)acetamides (7a-l)

N-(2,3-Dihydrobenzo[1,4]-dioxin-6-yl)-4-methylbenzenesulfonamide (0.2 g; 0.57 mmol; 3) in 10 mL N,N-dimethyl formamide (DMF) was placed into a 50-mL round-bottomed flask along with lithium hydride (0.004 g) and the reaction mixture was stirred for 30 min at 25 °C. 2-Bromo-N-(un/substituted-phenyl)acetamides (0.60 mmol; 6a-l) were added to the reaction mixture, which was further stirred for 3-4 h. The reaction was monitored by TLC until a single spot. After completion, the reaction mixture was poured onto crushed ice and precipitated products were filtered out, washed and air dried to obtain the pure products (7a-l).

### Spectral characterization

#### 2-{2,3-Dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl) sulfonyl]amino}-N-phenylacetamide (7a)

Light pink amorphous powder; yield 82%; mp 177-178 °C; molecular formula C<sub>23</sub>H<sub>22</sub>O<sub>5</sub>N<sub>2</sub>S; molecular weight 438 g mol<sup>-1</sup>; IR (KBr) ν (cm<sup>-1</sup>): 3249 (N-H stretching), 3047 (C-H stretching of aromatic ring), 2929 (-CH<sub>2</sub> stretching), 1715 (C=O stretching), 1635 (C=C stretching of aromatic ring), 1387 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.31 (s, 1H, NHCO), 7.64 (d, *J* = 8.0 Hz, 2H, H-2' & H-6'), 7.52 (m, 2H, H-2''' & H-6'''), 7.33-7.28 (m, 4H, H-3', H-5', H-3''' & H-5'''), 7.11 (br.t, *J* = 7.2 Hz, 1H, H-4'''), 6.75 (d, *J* = 8.4 Hz, 1H, H-8), 6.66 (d, *J* = 2.4 Hz, 1H, H-5), 6.53 (dd, *J* = 2.4, 8.4 Hz, 1H, H-7), 4.22-4.21

(m, 4H, CH<sub>2</sub>-2 & CH<sub>2</sub>-3), 4.19 (br.s, 2H, CH<sub>2</sub>-2''), 2.43 (s, 3H, CH<sub>3</sub>-7'). Anal. Calc. for C<sub>23</sub>H<sub>22</sub>O<sub>5</sub>N<sub>2</sub>S (438.12): C, 63.00; H, 5.06; N, 6.39. Found: C, 63.31; H, 5.19; N, 6.53.

#### 2-{2,3-Dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl) sulfonyl]amino}-N-(2-methoxyphenyl)-acetamide (7b)

Amorphous powder; yield 78%; m.p. 119-120 °C; molecular formula C<sub>24</sub>H<sub>24</sub>O<sub>6</sub>N<sub>2</sub>S; molecular weight 468 g mol<sup>-1</sup>. IR (KBr) ν (cm<sup>-1</sup>): 3247 (N-H stretching), 3049 (C-H stretching of aromatic ring), 2930 (-CH<sub>2</sub> stretching), 1713 (C=O stretching), 1637 (C=C stretching of aromatic ring), 1383 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.23 (s, 1H, NHCO), 7.57 (d, *J* = 7.8 Hz, 2H, H-2' & H-6'), 7.27 (d, *J* = 7.8 Hz, 2H, H-3' & H-5'), 6.96 (m, 2H, H-3''' & H-5'''), 6.70 (br.t, *J* = 7.2 Hz, 1H, H-4'''), 6.62 (d, *J* = 8.4 Hz, 1H, H-8), 6.56 (d, *J* = 2.4 Hz, 1H, H-5), 6.47 (dd, *J* = 2.4, 8.4 Hz, 1H, H-7), 6.14 (br.d, *J* = 8.5, 1H, H-6'''), 4.25 (br.s, 2H, CH<sub>2</sub>-2''), 4.15 (br.s, 4H, CH<sub>2</sub>-2 & CH<sub>2</sub>-3), 3.87 (s, 3H, 2-OCH<sub>3</sub>), 2.40 (s, 3H, CH<sub>3</sub>-7'). Anal. Calc. for C<sub>15</sub>H<sub>15</sub>O<sub>4</sub>NS (468.14): C, 61.52; H, 5.16; N, 5.98. Found: C, 61.74; H, 5.27; N, 6.19.

#### 2-{2,3-Dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl) sulfonyl]amino}-N-(4-ethoxyphenyl)-acetamide (7c)

Pinkish amorphous powder; yield 80%; m.p. 156-157 °C; molecular formula C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>6</sub>S; molecular weight 482 g mol<sup>-1</sup>; IR (KBr) ν (cm<sup>-1</sup>): 3251 (N-H stretching), 3047 (C-H stretching of aromatic ring), 2927 (-CH<sub>2</sub> stretching), 1717 (C=O stretching), 1635 (C=C stretching of aromatic ring), 1387 (-SO<sub>2</sub> stretching); <sup>1</sup>H NMR: (ppm) 8.25 (s, 1H, NHCO), 7.55 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.35 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.32 (d, *J* = 9.0 Hz, 2H, H-2''' & H-6'''), 6.82 (d, *J* = 9.0 Hz, 2H, H-3''' & H-5'''), 6.72 (d, *J* = 2.4 Hz, 1H, H-5), 6.70 (d, *J* = 8.4 Hz, 1H, H-8), 6.58 (dd, *J* = 2.4, 8.4 Hz, 1H, H-7), 4.32 (br.s, 2H, CH<sub>2</sub>-2''), 4.20-4.18 (m, 4H, CH<sub>2</sub>-2 & CH<sub>2</sub>-3), 3.98 (q, *J* = 7.2 Hz, 2H, 4-OCH<sub>2</sub>CH<sub>3</sub>), 2.43 (s, 3H, CH<sub>3</sub>-7'), 1.34 (t, *J* = 7.2 Hz, 3H, 4-OCH<sub>2</sub>CH<sub>3</sub>). Anal. Calc. for C<sub>25</sub>H<sub>26</sub>O<sub>6</sub>N<sub>2</sub>S (482.15): C, 62.23; H, 5.43; N, 5.81. Found: C, 62.41; H, 5.57; N, 5.93.

#### 2-{2,3-Dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl) sulfonyl]amino}-N-(2-methylphenyl)-acetamide (7d)

Dark brown amorphous powder; yield 80%; m.p. 95-96 °C; molecular formula C<sub>24</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S; molecular weight 452 g mol<sup>-1</sup>; IR (KBr) ν (cm<sup>-1</sup>): 3257 (N-H stretching), 3043 (C-H stretching of aromatic ring), 2930 (-CH<sub>2</sub> stretching), 1719 (C=O stretching), 1640 (C=C stretching of aromatic ring), 1389 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.13 (s, 1H, NHCO), 7.54 (d, *J* = 8.5 Hz, 2H, H-2' & H-6'), 7.36 (d, *J* = 8.5 Hz, 2H, H-3' & H-5'), 7.27 (br.d, *J* = 7.0 Hz, 1H, H-6'''), 7.18 (br.d, *J* = 7.5 Hz, 1H, H-3'''), 7.15-7.13 (m,

1H, H-5'''), 7.12-7.10 (m, 1H, H-4'''), 6.75 (br.s, 1H, H-5), 6.73 (d,  $J = 6.5$  Hz, 1H, H-8), 6.60 (dd,  $J = 2.5-8.5$  Hz, 1H, H-7), 4.34 (br.s, 2H, CH<sub>2</sub>-2''), 4.22-4.19 (m, 4H, CH<sub>2</sub>-2 & CH<sub>2</sub>-3), 2.43 (s, 3H, CH<sub>3</sub>-7'), 2.08 (s, 3H, CH<sub>3</sub>-2'''). Anal. Calc. for C<sub>24</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S (452.14): C, 63.70; H, 5.35; N, 6.19. Found: C, 63.83; H, 5.44; N, 6.31.

*2-(2,3-Dihydro-1,4-benzodioxin-6-yl[(4-methylphenyl)sulfonyl]amino)-N-(3-methylphenyl)-acetamide (7e)*

Amorphous powder; yield 80%; m.p. 114-115 °C; molecular formula C<sub>24</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S; molecular weight 452 gmol<sup>-1</sup>; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3251 (N-H stretching), 3045 (C-H stretching of aromatic ring), 2933 (-CH<sub>2</sub> stretching), 1717 (C=O stretching), 1641 (C=C stretching of aromatic ring), 1390 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.19 (s, 1H, NHCO), 7.55 (d,  $J = 8.4$  Hz, 2H, H-2' & H-6'), 7.35 (d,  $J = 8.4$  Hz, 2H, H-3' & H-5'), 7.30 (br.s, 1H, H-2'''), 7.26 (br.d,  $J = 7.8$  Hz, 1H, H-6'''), 7.15 (br.d,  $J = 7.8$  Hz, 1H, H-5'''), 6.91 (br.d,  $J = 7.2$  Hz, 1H, H-4'''), 6.71 (d,  $J = 9.0$  Hz, 1H, H-8), 6.56 (d,  $J = 2.4$  Hz, 1H, H-5), 6.47 (dd,  $J = 2.4, 8.4$  Hz, 1H, H-7), 4.34 (br.s, 2H, CH<sub>2</sub>-2''), 4.20-4.18 (m, 4H, CH<sub>2</sub>-2 & CH<sub>2</sub>-3), 2.29 (s, 3H, CH<sub>3</sub>-3'''), 2.43 (s, 3H, CH<sub>3</sub>-7'). Anal. Calc. for C<sub>24</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S (452.14): C, 63.70; H, 5.35; N, 6.19. Found: C, 63.88; H, 5.41; N, 6.27.

*2-(2,3-Dihydro-1,4-benzodioxin-6-yl[(4-methylphenyl)sulfonyl]amino)-N-(4-methylphenyl)-acetamide (7f)*

Of white amorphous powder; yield 80%; m.p. 134-135 °C; molecular formula C<sub>24</sub>H<sub>24</sub>O<sub>5</sub>N<sub>2</sub>S; molecular weight 452 gmol<sup>-1</sup>; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3258 (N-H stretching), 3047 (C-H stretching of aromatic ring), 2936 (-CH<sub>2</sub> stretching), 1711 (C=O stretching), 1643 (C=C stretching of aromatic ring), 1385 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.29 (s, 1H, NHCO), 7.51 (d,  $J = 8.0$  Hz, 2H, H-2' & H-6'), 7.36 (d,  $J = 8.5$  Hz, 2H, H-3' & H-5'), 7.28 (d,  $J = 8.0$ , 2H, H-2''' & H-6'''), 7.11 (d,  $J = 8.0$  Hz, 2H, H-3''' & H-5'''), 6.75 (d,  $J = 8.5$  Hz, 1H, H-8), 6.66 (d,  $J = 2.5$  Hz, 1H, H-5), 6.53 (dd,  $J = 2.5, 8.5$  Hz, 1H, H-7), 4.22-4.19 (m, 4H, CH<sub>2</sub>-2 & CH<sub>2</sub>-3), 4.18 (br.s, 2H, CH<sub>2</sub>-2''), 2.43 (s, 3H, CH<sub>3</sub>-7'), 2.29 (s, 3H, CH<sub>3</sub>-4'''). Anal. Calc. for C<sub>24</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S (452.14): C, 63.70; H, 5.35; N, 6.19. Found: C, 63.81; H, 5.46; N, 6.23.

*2-(2,3-Dihydro-1,4-benzodioxin-6-yl[(4-methylphenyl)sulfonyl]amino)-N-(2,3-dimethylphenyl)-acetamide (7g)*

Of white amorphous powder; yield 80%; mp 84-85 °C; molecular formula C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S; molecular weight 466 gmol<sup>-1</sup>; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3261 (N-H stretching), 3053 (C-H stretching of aromatic ring), 2940 (-CH<sub>2</sub> stretching), 1718 (C=O stretching), 1647 (C=C stretching of aromatic ring), 1389 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.25 (s, 1H, NHCO), 7.64 (br.t,

$J = 7.8$  Hz, H-5'''), 7.50 (d,  $J = 8.4$  Hz, 2H, H-2' & H-6'), 7.28 (d,  $J = 8.0$  Hz, 2H, H-3' & H-5'), 6.98-6.96 (m, 2H, H-4''' & H-6'''), 6.76 (d,  $J = 8.4$  Hz, 1H, H-8), 6.67 (d,  $J = 2.4$  Hz, 1H, H-5), 6.53 (dd,  $J = 2.4, 8.4$  Hz, 1H, H-7), 4.22-4.21 (m, 6H, CH<sub>2</sub>-2, CH<sub>2</sub>-3 & CH<sub>2</sub>-2''), 2.43 (s, 3H, CH<sub>3</sub>-7'), 2.26 (s, 3H, CH<sub>3</sub>-3'''), 2.24 (s, 3H, CH<sub>3</sub>-2'''). Anal. Calc. for C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S (466.16): C, 64.36; H, 5.62; N, 6.00. Found: C, 64.44; H, 5.71; N, 6.19.

*2-(2,3-Dihydro-1,4-benzodioxin-6-yl[(4-methylphenyl)sulfonyl]amino)-N-(2,4-dimethylphenyl)-acetamide (7h)*

Light pink amorphous powder; yield 80%; m.p. 150-151 °C; molecular formula C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S; molecular weight 466 gmol<sup>-1</sup>; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3265 (N-H stretching), 3055 (C-H stretching of aromatic ring), 2937 (-CH<sub>2</sub> stretching), 1721 (C=O stretching), 1649 (C=C stretching of aromatic ring), 1383 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.27 (s, 1H, NHCO), 7.48 (d,  $J = 8.4$  Hz, 2H, H-2' & H-6'), 7.26 (d,  $J = 8.4$  Hz, 2H, H-3' & H-5'), 6.78 (d,  $J = 8.4$  Hz, 1H, H-8), 6.73 (s, 1H, H-3'''), 6.69 (d,  $J = 2.4$  Hz, 1H, H-5), 6.58 (d, 2H, H-5''' & H-6'''), 6.55 (dd,  $J = 2.4, 8.4$  Hz, 1H, H-7), 4.22-4.21 (m, 6H, CH<sub>2</sub>-2, CH<sub>2</sub>-3 & CH<sub>2</sub>-2''), 2.43 (s, 3H, CH<sub>3</sub>-7'), 2.29 (s, 3H, CH<sub>3</sub>-4'''), 2.23 (s, 3H, CH<sub>3</sub>-2'''). Anal. Calc. for C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S (466.16): C, 64.36; H, 5.62; N, 6.00. Found: C, 64.49; H, 5.68; N, 6.23.

*2-(2,3-Dihydro-1,4-benzodioxin-6-yl[(4-methylphenyl)sulfonyl]amino)-N-(2,5-dimethylphenyl)-acetamide (7i)*

Light purple amorphous powder; yield 80%; m.p. 115-116 °C; molecular formula C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S; molecular weight 466 gmol<sup>-1</sup>; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3255 (N-H stretching), 3057 (C-H stretching of aromatic ring), 2937 (-CH<sub>2</sub> stretching), 1721 (C=O stretching), 1650 (C=C stretching of aromatic ring), 1391 (-SO<sub>2</sub> stretching); <sup>1</sup>H NMR: (ppm) 8.31 (s, 1H, NHCO), 7.51 (d,  $J = 8.4$  Hz, 2H, H-2' & H-6'), 7.28 (d,  $J = 8.4$  Hz, 2H, H-3' & H-5'), 6.76 (d,  $J = 8.4$  Hz, 1H, H-8), 6.68 (d,  $J = 2.4$  Hz, 1H, H-5), 6.54 (dd,  $J = 2.4, 8.4$  Hz, 1H, H-7), 4.23-4.22 (m, 6H, CH<sub>2</sub>-2, CH<sub>2</sub>-3 & CH<sub>2</sub>-2''), 2.43 (s, 3H, CH<sub>3</sub>-7'), 2.28 (s, 3H, CH<sub>3</sub>-5'''), 2.16 (s, 3H, CH<sub>3</sub>-2'''). Anal. Calc. for C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S (466.16): C, 64.36; H, 5.62; N, 6.00. Found: C, 64.47; H, 5.77; N, 6.22.

*2-(2,3-Dihydro-1,4-benzodioxin-6-yl[(4-methylphenyl)sulfonyl]amino)-N-(2,6-dimethylphenyl)-acetamide (7j)*

Amorphous powder; yield 80%; m.p. 86-87 °C; molecular formula C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S; molecular weight 466 gmol<sup>-1</sup>; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3253 (N-H stretching), 3055 (C-H stretching of aromatic ring), 2939 (-CH<sub>2</sub> stretching), 1719 (C=O stretching), 1647 (C=C stretching of aromatic ring), 1388 (-SO<sub>2</sub> stretching); <sup>1</sup>H NMR: (ppm) 7.93 (s,

1H, NHCO), 7.50 (d,  $J = 8.5$  Hz, 2H, H-2' & H-6'), 7.28 (d,  $J = 8.0$  Hz, 2H, H-3' & H-5'), 7.08 (br.t,  $J = 7.5$  Hz, 1H, H-4'''), 6.78 (d,  $J = 8.5$  Hz, 1H, H-8), 6.70 (d,  $J = 2.5$  Hz, 1H, H-5), 6.57 (dd,  $J = 2.5, 8.5$  Hz, 1H, H-7), 4.27 (br.s, 2H, CH<sub>2</sub>-2''), 4.25-4.22 (m, 4H, CH<sub>2</sub>-2 & CH<sub>2</sub>-3), 2.43 (s, 3H, CH<sub>3</sub>-7'), 2.09 (s, 6H, CH<sub>3</sub>-2''' & CH<sub>3</sub>-6'''). Anal. Calc. for C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S (466.16): C, 64.36; H, 5.62; N, 6.00. Found: C, 64.46; H, 5.75; N, 6.24.

*2-{2,3-Dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl) sulfonyl]amino}-N-(3,4-dimethylphenyl)-acetamide (7k).*

Light pink amorphous powder; yield 80%; mp 159-160 °C; molecular formula C<sub>25</sub>H<sub>26</sub>O<sub>5</sub>N<sub>2</sub>S; molecular weight 466 g mol<sup>-1</sup>; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3257 (N-H stretching), 3050 (C-H stretching of aromatic ring), 2937 (-CH<sub>2</sub> stretching), 1713 (C=O stretching), 1645 (C=C stretching of aromatic ring), 1389 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.23 (s, 1H, NHCO), 7.51 (d,  $J = 8.0$  Hz, 2H, H-2' & H-6'), 7.28 (d,  $J = 8.4$  Hz, 2H, H-3' & H-5'), 7.24 (merged in the signal of CDCl<sub>3</sub>, 2H, H-2''' & H-6'''), 7.05 (br.d,  $J = 8.0$  Hz, 1H, H-5'''), 6.75 (d,  $J = 8.8$  Hz, 1H, H-8), 6.65 (d,  $J = 2.4$  Hz, 1H, H-5), 6.53 (dd,  $J = 2.8, 8.4$  Hz, 1H, H-7), 4.23-4.19 (m, 4H, CH<sub>2</sub>-2, CH<sub>2</sub>-3), 4.18 (br.s, 2H, CH<sub>2</sub>-2''), 2.43 (s, 3H, CH<sub>3</sub>-7'), 2.23 (s, 3H, CH<sub>3</sub>-3'''), 2.20 (s, 3H, CH<sub>3</sub>-4'''). Anal. Calc. for C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S (466.16): C, 64.36; H, 5.62; N, 6.00. Found: C, 64.39; H, 5.76; N, 6.12.

*2-{2,3-Dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl) sulfonyl]amino}-N-(3,5-dimethylphenyl)-acetamide (7l).*

Light pink amorphous powder; yield 80%; mp 130-131 °C; molecular formula C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S; molecular weight 466 g mol<sup>-1</sup>; IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3260 (N-H stretching), 3058 (C-H stretching of aromatic ring), 2935 (-CH<sub>2</sub> stretching), 1715 (C=O stretching), 1648 (C=C stretching of aromatic ring), 1390 (-SO<sub>2</sub> stretching); <sup>1</sup>H-NMR: (ppm) 8.24 (s, 1H, NHCO), 7.51 (d,  $J = 8.0$  Hz, 2H, H-2' & H-6'), 7.28 (d,  $J = 8.0$  Hz, 2H, H-3' & H-5'), 7.12 (br.s, 2H, H-2''' & H-6'''), 6.76-6.74 (m, 2H, H-8, H-4'''), 6.66 (d,  $J = 2.4$  Hz, 1H, H-5), 6.53 (dd,  $J = 2.4, 8.4$  Hz, 1H, H-7), 4.22-4.21 (m, 4H, CH<sub>2</sub>-2 & CH<sub>2</sub>-3), 4.17 (br.s, 2H, CH<sub>2</sub>-2''), 2.43 (s, 3H, CH<sub>3</sub>-7'), 2.28 (s, 6H, CH<sub>3</sub>-3''' & CH<sub>3</sub>-5'''). Anal. Calc. for C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S (466.16): C, 64.36; H, 5.62; N, 6.00. Found: C, 64.41; H, 5.73; N, 6.14.

## Enzyme inhibition assays

### $\alpha$ -Glucosidase assay

The enzyme inhibition activity against  $\alpha$ -glucosidase was performed according to a reported method (Chapdelaine, Trembley, Dube, 1978). The enzyme assay was optimized for the given time at temperature to

maintain the linearity of the enzyme reaction wherein the concentration of the substrate was not limiting (data not shown). The reaction mix consisted of phosphate buffer (50 mM, pH 6.8, 70  $\mu$ L), test compound (0.5 mM, 10  $\mu$ L) and 10  $\mu$ L (0.057 U) of enzyme, (Sigma Inc., Cat No. G5003, Type-I from *Saccharomyces cerevisiae*) to make a total volume of 100  $\mu$ L, which was mixed well and pre-incubated for 10 min at 37 °C and pre-read at 400 nm. Next, 10  $\mu$ L of 0.5 mM substrate, *p*-nitrophenyl-*D*-glucopyranoside, was added to start the reaction, and incubation continued for another 30 min, followed by reading at 400 nm with a microplate reader (Epoch, BioTek, USA). The change in absorbance was used as an index for the measurement of percentage inhibition. Acarbose was used as the positive control. All the experiments were performed in triplicates. Inhibition (%) was calculated by the following equation:

$$\% \text{age Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA) was used to calculate IC<sub>50</sub> values of the compounds. Active compounds were serially diluted to suitable concentrations of 0.25, 0.125, 0.0625, 0.03125, 0.0156 mM and their percentage inhibition was determined, and the data were used for the calculations of IC<sub>50</sub> values.

### Acetylcholinesterase assay

The enzyme inhibition assay against AChE was performed according to a reported method (Ellman *et al.*, 1961). The reaction mixture was prepared with 50 mM Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 7.7, 60  $\mu$ L), test compound (0.5 mM, 10  $\mu$ L well<sup>-1</sup>), followed by the addition 10  $\mu$ L of 0.005 U *Electrophorus electricus* AChE (Sigma Inc.) to make a total volume of 100  $\mu$ L. The contents were mixed, pre-incubated for 10 minutes at 37°C, and pre-read at 405 nm. Next, 10  $\mu$ L of 0.5 mM the substrate acetylthiocholine iodide and 10  $\mu$ L of 0.5 mM DTNB were added and reaction was started. The reaction contents were incubated for 15 minutes at 37°C and absorbance was measured at 405 nm. These experiments were carried out in triplicate. Eserine (0.5 mM well<sup>-1</sup>) was employed as a positive control. The percentage inhibition and IC<sub>50</sub> values were calculated by the same method as described for  $\alpha$ -glucosidase assay.

## Molecular docking

To predict the bioactive conformations, various ligands were docked into the binding pockets of the

enzymes by using the default parameters of MOE-Dock program.

### Ligand preparation

The three-dimensional (3D) structures of the synthesized compounds were made by using ChemDraw Ultra 12.0 (Cambridge Soft, 2001) and saved in MDL Mol file format which were then opened in Molecular Operating Environment (MOE 2009-2010). The energies of the compounds were minimized by using the default parameter of MOE energy minimization algorithm (gradients: 0.05, force field: MMFF94X). A database was created in which all the compounds were saved in the mdb file format for the next step of docking.

### Receptor protein preparation

The 3D structures of receptor protein molecules of yeast  $\alpha$ -glucosidase (PDB Code: 3NO4) and AChE (PDB Code: 1GQR) were retrieved from Protein Data Bank (Tan *et al.*, 2010; Bar-on *et al.*, 2002). All water molecules were released from the receptor proteins and 3D protonation was carried out by using Protonate 3D Option (Tan *et al.*, 2010; Bar-on *et al.*, 2002). Protein molecules were energy minimized by using the default parameters of MOE 2009-10 energy minimization algorithm (gradient: 0.05, Force Field: MMFF94X). By using default parameters of MOE-Dock Program, all compounds were docked into binding pockets of the above proteins. The re-docking procedure was also used to increase the validity of docking protocol (Boström, Greenwood, Gottfries, 2003).

### Statistical analysis

All the experiments were carried out in triplicate. Statistical analysis was performed using Microsoft Excel 2010 and the results are provided as mean  $\pm$  SEM.

## RESULTS AND DISCUSSION

### Chemistry

The designed 2-{2,3-dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl)sulfonyl]amino}-*N*-(un/substituted-phenyl)acetamides (**7a-l**) were synthesized according to scheme 1 and Table I. The procedures and conditions of the reactions are discussed in the experimental section. For the synthesis of the target derivatives, the first step of the reaction was carried between *N*-2,3-dihydrobenzo[1,4]-

dioxin-6-amine (**1**) and 4-methylbenzenesulfonyl chloride (**2**) in aqueous alkaline medium. The reaction mixture was stirred for 4-5 hours at room temperature yielding the parent *N*-(2,3-dihydrobenzo[1,4]-dioxin-6-yl)-4-methylbenzenesulfonamide (**3**). The parent compound was obtained as a light brown amorphous powder in good yield by the acidification of the reaction mixture with concentrated HCl to adjust pH 2-3. In a parallel reaction, different amines (4a-l, Table I) were reacted, one by one, with bromoacetyl bromide (**5**) to obtain respective electrophiles, 2-bromo-*N*-(un/substituted-phenyl)acetamides (**6a-l**). In the final step, parent, **3**, was coupled with different electrophiles (**6a-l**), in polar aprotic solvent, i.e. DMF using LiH as a base to afford the targeted *N*-2-{2,3-dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl)sulfonyl]amino}-*N*-(un/substituted-phenyl)acetamides (**7a-l**). The structures of these molecules were deduced by their IR and <sup>1</sup>H-NMR spectral data, and CHN analysis also supported the assignment.

The structural analysis of one of the compounds is discussed hereby in detail as a representative molecule. The molecule, **7f**, was obtained as an off-white amorphous powder with 88% yield, having a melting point of 134-135 °C. The molecular formula (C<sub>24</sub>H<sub>24</sub>O<sub>5</sub>N<sub>2</sub>S) of this compound was predicted by counting the number of protons in its <sup>1</sup>H-NMR spectrum, and it was also supported by its CHN analysis data. Various functional groups in this molecule were determined by its IR data. The absorption band at 3258 cm<sup>-1</sup> was peculiar for N-H stretching (amide). The other bands were observed at 3047 (C-H stretching of aromatic ring), 2936 (-CH<sub>2</sub> stretching), 1711 (C=O stretching), 1643 (C=C stretching of aromatic ring), and 1385 (-SO<sub>2</sub> stretching). In its <sup>1</sup>H NMR spectrum, a singlet in highly deshielded region at  $\delta$  8.29 accounted for an acetamidic proton (-NHCO). The 4-methylbenzenesulfonyl moiety in this molecule was characterized by an A<sub>2</sub>B<sub>2</sub> spin system in the aromatic region, represented by two *ortho*-coupled doublets at  $\delta$  7.51 (2H, H-2' & H-6') and  $\delta$  7.36 (2H, H-3' & H-5') along with a methyl singlet in the aliphatic region at  $\delta$  2.43. Similarly, another A<sub>2</sub>B<sub>2</sub> spin system was observed for the aromatic protons of 4-methylphenyl moiety substituted on nitrogen atom. This moiety was characterized by two *ortho*-coupled doublets at  $\delta$  7.28 (2H, H-2''' & H-6'''), and  $\delta$  7.11 (2H, H-3''' & H-5''') along with a methyl signal at  $\delta$  2.29 as singlet. An AMX spin system for a 6-amino-benzodioxane moiety in the molecule, was corroborated by an *ortho*-coupled doublet at  $\delta$  6.75 (1H, H-8, *J* = 8.5 Hz), a *meta*-coupled doublet at  $\delta$  6.66 (1H, H-5, *J* = 2.5 Hz), and a reciprocal doublet of doublet at  $\delta$  6.53 (1H, H-7, *J* = 2.5, 8.5 Hz). A multiplet at  $\delta$  4.22-4.19 with integration of four

protons was deduced for two symmetrical methylene groups (CH<sub>2</sub>-2 & CH<sub>2</sub>-3) in 1,4-benzodioxane moiety. A very close lying broad singlet at 4.18 was assignable to a methylene group (2H, CH<sub>2</sub>-2'') of an acetamido unit attached by its carbon atom with the sulfonamidic nitrogen atom in the molecule. Thus, on the basis of the above cumulative spectral evidence, the structure of **7f** was designated 2-{2,3-dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl)sulfonyl]amino}-N-(4-methylphenyl)acetamide. Similarly, the structures of all other synthetic derivatives were characterized, and their spectral data is given in experimental section.

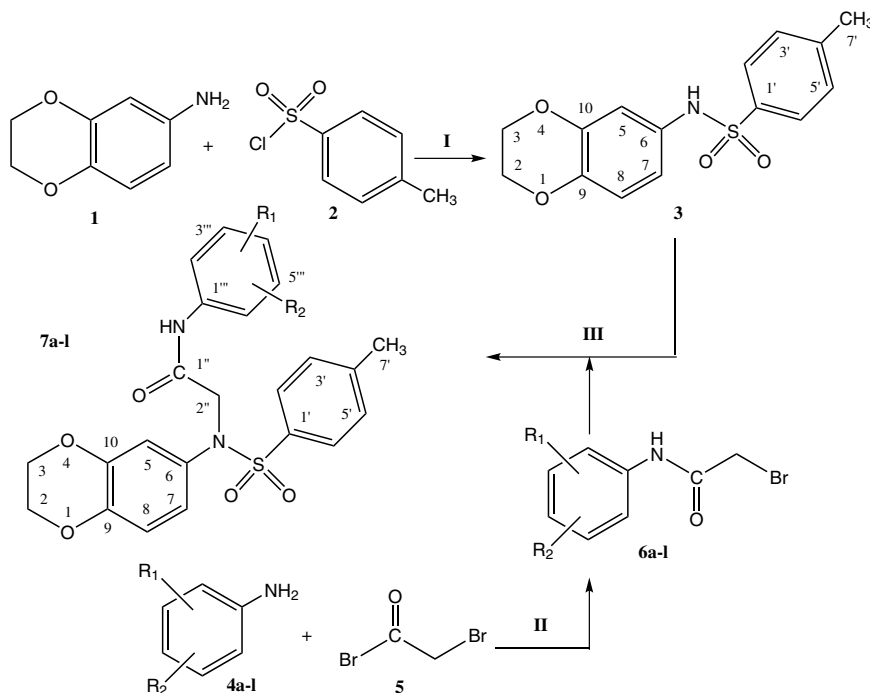
### Enzyme inhibition, molecular docking (*in silico*) and SAR study

In search for new suitable therapeutic agents for the control of type 2 Diabetes mellitus and for the treatment of Alzheimer's disease, these synthesized molecules, **7a-l**, were screened against  $\alpha$ -glucosidase and AChE, respectively. In general, these compounds exhibited moderate to high inhibitory potential against  $\alpha$ -glucosidase and weak inhibition against AChE, which was evident from their IC<sub>50</sub> values as compared to the standard

acarbose and serine, respectively (Table II).

Compound **7h** showed potent inhibition against  $\alpha$ -glucosidase with an IC<sub>50</sub> of 34.21  $\pm$  0.12  $\mu$ M, which was better than acarbose, which had an IC<sub>50</sub> of 37.38  $\pm$  0.12  $\mu$ M. The enhanced inhibitory potential of this molecule against this enzyme might be attributed to the incorporation of a 2,4-dimethylphenyl moiety in this molecule. Similarly, molecules **7g** and **7k**, with 2,3-dimethylphenyl and 3,4-dimethylphenyl moieties, respectively, also showed excellent activity. The IC<sub>50</sub> values of these molecules were 47.23  $\pm$  0.14  $\mu$ M and 52.45  $\pm$  0.14  $\mu$ M, respectively. All molecules showed weak inhibitory profiles against AChE. Molecule **7g** exhibited an IC<sub>50</sub> of 213.47  $\pm$  0.14  $\mu$ M which was the lowest among the studied molecules. Its possibly weak inhibitory potential may be due to the amalgamation of 2,3-dimethylphenyl in the core sulfonamide bearing a benzodioxane entity.

The *in silico* molecular docking data of these molecules was also in agreement with their *in vitro* enzyme inhibition data. Molecular docking was performed with selected residues of the active pockets of the enzymes. It is shown (Figure 1; 2D & 3D) that compound **7h** was deeply bound in the binding pocket of  $\alpha$ -glucosidase by making two strong interactions. Asp420 created polar interaction



**SCHEME 1** - Outline for the synthesis of 2-{2,3-dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl)sulfonyl]amino}-N-(un/substituted-phenyl)acetamides (**7a-l**). Reagents & Conditions: (1) 2,3-dihydrobenzo[1,4]dioxin-6-amine (**1**)/aq. Na<sub>2</sub>CO<sub>3</sub> soln./pH 9-10/4-methylbenzenesulfonyl chloride (**2**)/stirring at RT for 3-4 hours. (2) Un/substituted anilines (**4a-l**)/aq. Na<sub>2</sub>CO<sub>3</sub> soln./pH 9-10/stirring/bromoacetyl bromide (**5**)/vigorous manual shaking. (3) *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide(**3**)/DMF/LiH/stirring for half an hour/addition of un/substituted electrophiles, **4a-l**, followed by stirring for 4-5 hours at room temperature.

**TABLE I** - Different substituents (-R<sub>1</sub> & -R<sub>2</sub>) in scheme 1

Compd.	-R <sub>1</sub>	-R <sub>2</sub>	Compd.	-R <sub>1</sub>	-R <sub>2</sub>
<b>4a, 6a, 7a</b>	-H	-H	<b>4g, 6g, 7g</b>	2-CH <sub>3</sub>	3-CH <sub>3</sub>
<b>4b, 6b, 7b</b>	2-OCH <sub>3</sub>	-H	<b>4h, 6h, 7h</b>	2-CH <sub>3</sub>	4-CH <sub>3</sub>
<b>4c, 6c, 7c</b>	4-OC <sub>2</sub> H <sub>5</sub>	-H	<b>4i, 6i, 7i</b>	2-CH <sub>3</sub>	5-CH <sub>3</sub>
<b>4d, 6d, 7d</b>	2-CH <sub>3</sub>	-H	<b>4j, 6j, 7j</b>	2-CH <sub>3</sub>	6-CH <sub>3</sub>
<b>4e, 6e, 7e</b>	3-CH <sub>3</sub>	-H	<b>4k, 6k, 7k</b>	3-CH <sub>3</sub>	4-CH <sub>3</sub>
<b>4f, 6f, 7f</b>	4-CH <sub>3</sub>	-H	<b>4l, 6l, 7l</b>	3-CH <sub>3</sub>	5-CH <sub>3</sub>

with the amino proton of the ligand giving a bond length of 1.97 Å while Lys422 made an acidic interaction with sulfonyl oxygen showing a bond distance of 2.30 Å. Similarly, molecule **7g** also made four interactions. Arg440 showed a couple of strong acidic interactions with both the sulfonyl oxygen with a bond length of 2.02 and 2.69 Å. Lys422 also made two arene-cation interactions with bond distances of 3.25 and 4.31 Å as shown in 2-D and 3-D (Figure 2). Likewise, **7k** showed two acidic interactions between Arg404 and both with the sulfonyl oxygen with bond distances of 1.85 Å and 2.36 Å (Figure 3; 2D & 3D).

The molecular docking of **7g** into the active pocket of AChE indicated two polar interactions, i.e., one between

Tyr121 and sulfonyl oxygen (bond length: 1.89 Å) while the second with carbonyl oxygen (bond length: 2.64 Å) as depicted (**Figure 4**; 2D & 3D). The strength of the bond was clear from the bond distances of ligand and amino acid residues of protein, i.e., the shorter the bond distance, the stronger the interaction, and vice versa.

## CONCLUSIONS

The targeted 2- {2,3-dihydro-1,4-benzodioxin-6-yl}[(4-methylphenyl)sulfonyl]amino}-N-(un/substituted-phenyl)acetamides (**7a-l**) were synthesized in good yields. Some of the molecules exhibited promising inhibition

**TABLE II** - Percent inhibition at 0.5 mM and IC<sub>50</sub> values for α-Glucosidase and AChE.

Compound	α-Glucosidase		AChE	
	Inhibition (%)	IC <sub>50</sub> (μM)	Inhibition (%)	IC <sub>50</sub> (μM)
<b>7a</b>	91.21 ± 0.18	127.32 ± 0.12	17.32 ± 0.14	-
<b>7b</b>	76.31 ± 0.23	245.12 ± 0.16	-	-
<b>7c</b>	87.54 ± 0.17	132.21 ± 0.11	-	-
<b>7d</b>	82.51 ± 0.24	252.12 ± 0.17	28.56 ± 0.13	-
<b>7e</b>	91.21 ± 0.21	74.49 ± 0.11	72.52 ± 0.18	374.63 ± 0.16
<b>7f</b>	86.42 ± 0.22	136.34 ± 0.14	86.58 ± 0.19	316.42 ± 0.12
<b>7g</b>	94.57 ± 0.21	47.23 ± 0.14	79.24 ± 0.19	213.47 ± 0.14
<b>7h</b>	95.78 ± 0.19	34.21 ± 0.12	51.26 ± 0.16	482.53 ± 0.11
<b>7i</b>	91.21 ± 0.18	85.41 ± 0.12	52.65 ± 0.15	471.25 ± 0.13
<b>7j</b>	93.76 ± 0.23	92.12 ± 0.15	57.43 ± 0.18	429.72 ± 0.12
<b>7k</b>	94.23 ± 0.17	52.45 ± 0.14	81.62 ± 0.19	352.26 ± 0.16
<b>7l</b>	87.74 ± 0.29	239.31 ± 0.18	82.53 ± 0.17	342.54 ± 0.14
<b>Acarbose</b>	<b>92.23 ± 0.16</b>	<b>37.38 ± 0.12</b>	-	-
<b>Eserine</b>	-	-	<b>91.27 ± 1.17</b>	<b>0.04 ± 0.0001</b>

**Note:** IC<sub>50</sub> values (concentration at which there is 50% enzyme inhibition) of compounds were calculated from the inhibition data obtained after performing assays at high dilutions of the compounds as given in the assay method, and data were analyzed using EZ-Fit enzyme kinetics software (Perrella Scientific Inc. Amherst, MA, USA). Data are given as mean of three values (mean ± SEM., n = 3).



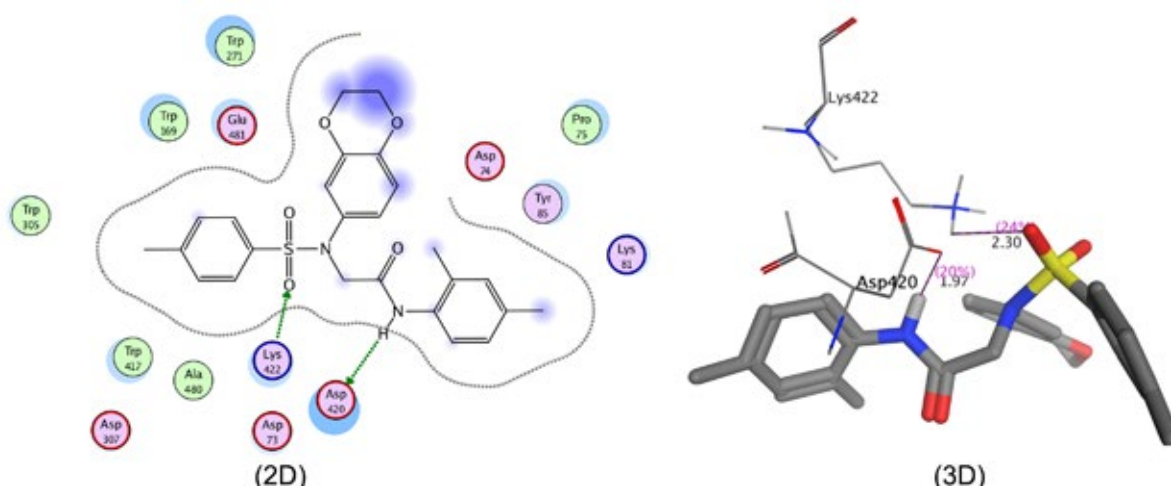


FIGURE 1 - Molecular docking of **7h** against  $\alpha$ -glucosidase.

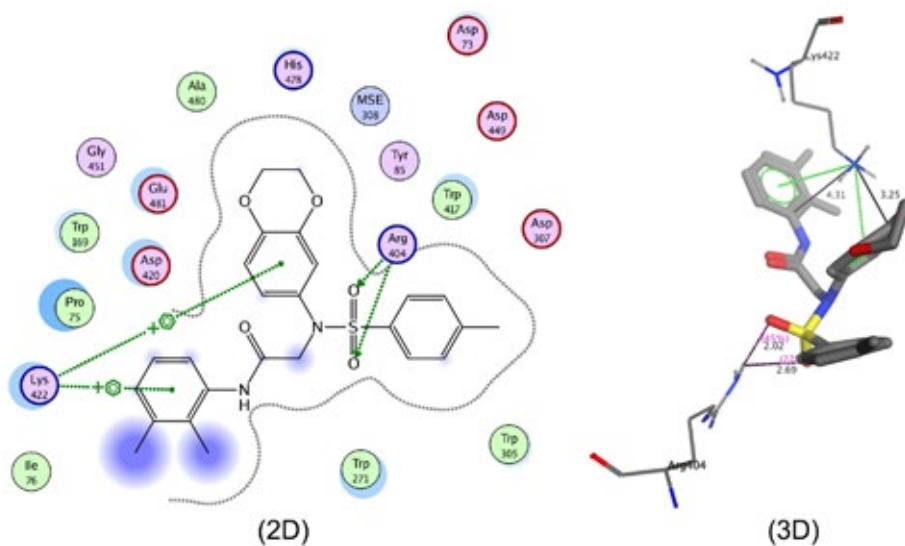


FIGURE 2 - Molecular docking of **7g** against yeast  $\alpha$ -glucosidase.

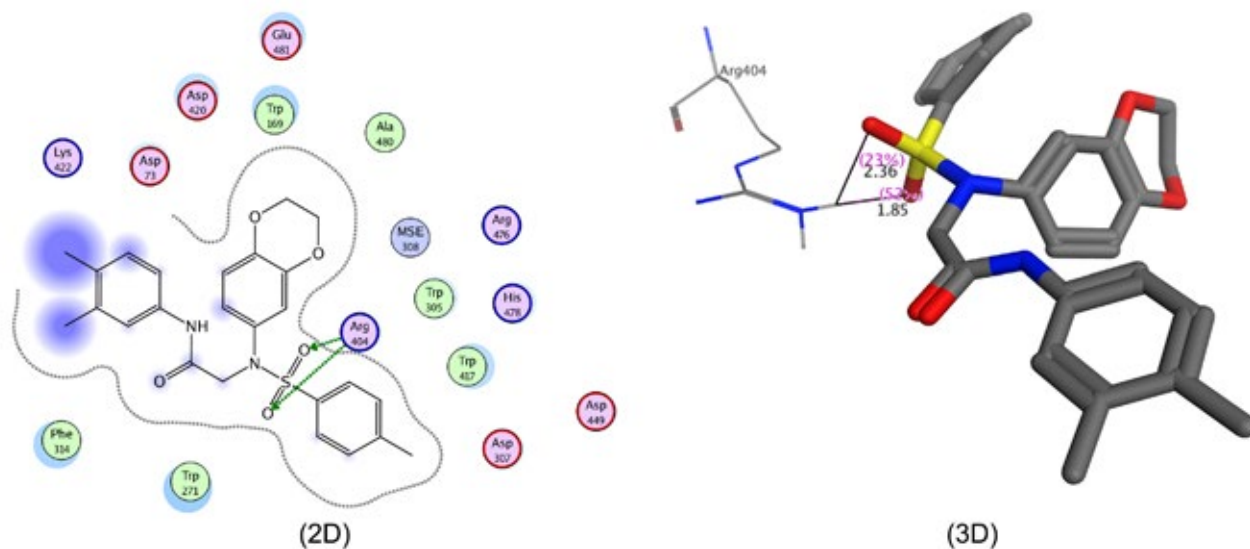
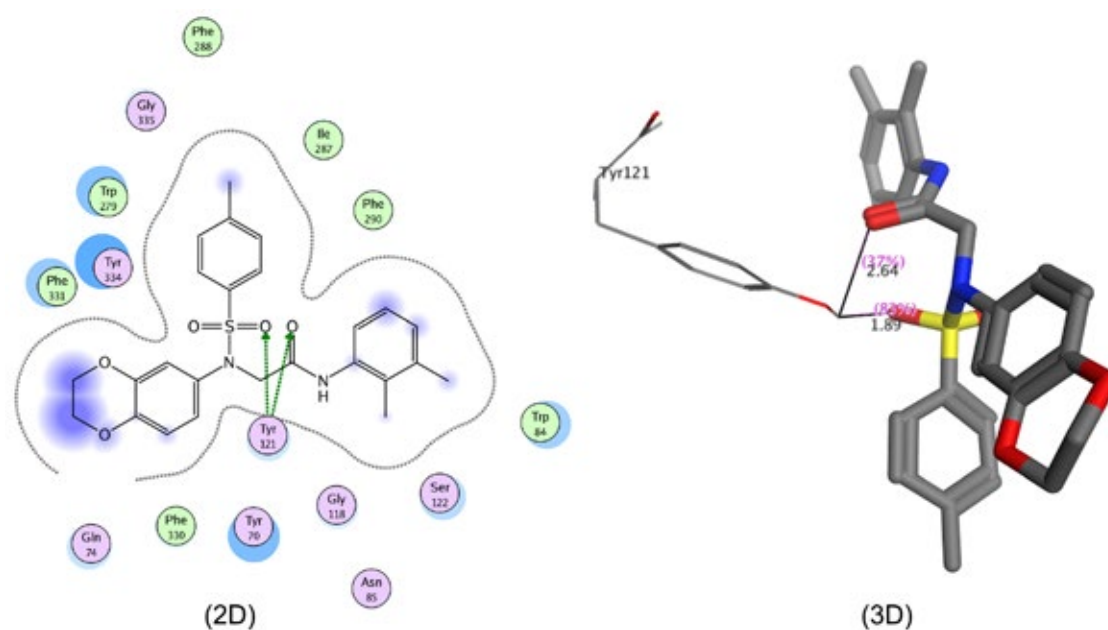


FIGURE 3 - Molecular docking of **7k** against yeast  $\alpha$ -glucosidase.



**FIGURE 4** - Molecular docking of **7g** against AChE.

against  $\alpha$ -glucosidase which was also supported by molecular docking studies. So, it was concluded that some of the molecules might be considered as suitable therapeutic agents for type 2 diabetes.

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