Synthetic N-(Alkyl/Aralkyl)-N-(2,3-Dihydro-1,4-Benzodioxin-6-Yl)-4-Methylbenzenesulfonamides as Acetyl cholinesterase Inhibitors

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Introduction

The basic sulfonamide group SO2NH is found in numerous biological active compounds including antiviral, anticancer, anti-thyroid, antimicrobial, anti-inflammatory and antibiotics drugs along with inhibitors of carbonic anhydrase [1]. Because of their less cost, less toxicity and astonishing activity, these are extensively used as antibacterial agents [2-6]. Furthermore, sulfonamides are also employed as, anti-leprotic, diuretics, antitumor agents, tuberculostatics and oral hypoglycemic drugs [7-8]. Aliphatic sulfonamide derivatives act as antifungal agents [9]. Sulfonamide based antibiotics are utilized as veterinary medicines to treat infections in livestock herds [10]. Compounds bearing benzodioxane ring exhibits different biological activities such as anti-oxidant [11], anti-hepatotoxic and anti-inflammatory [12,13]. Aryl sulfonamides having benzodioxane moiety have been recognized as inhibitors of ExoU [14]. Because of their non-interaction to defence mechanism of host and broad spectrum activity some effective derivatives of sulfonamides are widely used to treat gastrointestinal and urinary tract infections [15]. Some sulfonamides were also found to be potent carbonic anhydrase, COX-2 and caspase inhibitors [16-18].

Acetyl cholinesterase (AChE, EC 3.1.1.7) belongs to serine hydrolases class of enzymes. This enzyme system is accountable for the termination of acetylcholine at cholinergic synapses. The main function of AChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses [19]. The inhibitors of this enzyme are the targets for the treatment of Alzheimer’s disease [20]. Biological literature review on sulfonamides displayed that little modification in the structure of sulfonamides can cause remarkable changes in biological activity. These outcomes stimulated us to focus on synthesis of variety of N-alkyl/aralkyl-N-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-4-methylbenzenesulfonamides. Recent research work was a successful attempt to synthesize new therapeutic agents for the inhibition of cholinesterase enzyme.
Experimental Measurements and materials

All of the essential chemicals/solvents were of analytical grade and procured from authorized suppliers, Merck and Alfa Aeser branded. The pre-coated silica gel G-25-UV254 plates were applied for TLC to monitor the completion of reactions using various percentages of n-hexane and ethyl acetate as mobile phase. Open capillary tubes were used in Gallon kamp melting point apparatus to record the melting points. Developed TLC visualized under 254nm UV lamp and UV inactive substances were identified with the spray of ceric sulfate solution. Infrared spectra were noted in KBr pellet on a Jasco-320-A spectrophotometer. 1H-NMR spectra were recorded by Bruker spectrometer in CDCl3 operating at 400MHz at 25 °C. The chemicals shifts (δ) were observed in ppm and coupling constants (J) were noted in Hertz (Hz). The abbreviations used in 1HNMR spectral analysis were; s=singlet, d=doublet, dd=doublet of doublet, t=triplet, br=t broad triplet, q=quartet, quint=quintet, sex=sexet, sep=septet, m=multiplet. Mass spectra (EIMS) were taken on Finngen Mass Spectrometer.

Synthesis of N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (3)

2,3-Dihydro-1,4-benzodioxin-6-amine (1.22mL;0.01mol;1) and 4-methylbenzenesulfonyl chloride (0.90g; 0.01mol; 2) were poured into a 250ml round bottom flask having 30ml of distilled water. The pH of the suspension was maintained at 9.0 by introducing 10% Na2CO3 solution at room temperature. The content of reaction was stirred for 2-3 hours and progress of the reaction was examined by TLC time to time till single spot confirm the completion of reaction. The product was obtained by the slow addition of concentrated HCl at pH 2-3 as brown coloured precipitates. These were filtered, washed with distilled water and air-dried to afford pure N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (3); Yield: 82%, IR (KBr, cm−1): v max: 3284 (N-H stretching, 3H), 1598 (C=O stretching of aromatic ring), 1410 (SO2 stretching), 1140 (C-O-C stretching of ether); EI-MS (m/z): 305 (M+ C17H18NO4S), 340 (C15H14ClNO4S), 304 (C15H14NO4S), 155 (C8H7O2)+, 107 (C6H5)+, 51 (C4H3)+.

General procedure for the synthesis of N-substituted derivatives 5a-n

Compound 3 (0.1g; 0.3mmol) solubilised in 10ml of N,N-dimethyl formamide (DMF) aprotic solvent in 100ml round bottom flask. Lithium hydride (0.004g) was mixed in the reaction mixture to activate the reaction followed by stirring for 2-3 hours at room temperature. Then various alkyl/arylalkyl halides (4a-n) were introduced and stirring was continued for further 3-4 hours. Completion of reaction was assured by TLC displaying single spot. Then reaction content was quenched with ice cold distilled water along with vigorous shaking to get the precipitates of N-alkyl/arylalkyl-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfon-amides (5a-n) which were left for some time undisturbed and collected by the filtration or solvent extraction (using CHCl3) depending upon the nature of the derived compound.

Spectral analysis

The spectral analysis of 5b, 5g, 5h, 5j and 5l has already been reported by our group [21] while that of other compounds is given hereby.

N-(2-Chloroethyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5a): Off white powder; Yield: 95 %; m.p: 142 °C; Molecular formula: C17H18ClNO4S; Molecular weight: 347g mol-1; IR (KBr cm-1): v max: 2989 (C-H stretching of aromatic ring), 1379 (-SO2 stretching), 1250 (C-O-C stretching of ether); EI-MS (m/z): 347 (M+ C17H18ClNO4S), 319 (C16H17ClNO4S), 237 (C12H11ClNO2), 213 (C12H11NO2), 155 (C8H7O2)+, 107 (C6H5)+, 51 (C4H3)+.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-N-(2-lodoethyl)-4-methylbenzenesulfonamide (5c): Greyish white solid; Yield: 95%; m.p: 132 °C; Molecular formula: C17H18INO4S; Molecular weight: 459g mol-1; IR (KBr cm-1): v max: 2989 (C-H stretching of aromatic ring), 1660 (C=C stretching of aromatic ring), 1379 (-SO2 stretching), 1148 (C-O-C stretching of ether); 1H-NMR (CDCl3 400MHz, δ in ppm): 9.70 (d, J=8.5Hz, 2H, H-2' & H-6'), 7.42 (d, J=8.5Hz, 2H, H-3' & H-5'). 6.50 (d, J=5.8Hz, 1H, H-8), 6.30 (d, J=2.5Hz, 1H, H-5), 4.28 (br.s, 4H, CH2 & CH2), 3.60 (t, J=6.5Hz, 2H, CH2-2'), 3.46 (t, J=7.4Hz, 2H, CH2-1'), 2.34 (s, 3H, CH3-7'), 1.60-1.56 (m, 2H, CH2-3), 3.10 (s, 3H, CH3-2 & CH3-3), 2.36 (s, 3H, CH3-7'); EI-MS (m/z): 371 (M+ C17H18INO4S), 340 (C15H14INO4S), 304 (C15H14NO4S), 237 (C12H11NO2), 213 (C12H11INO2), 155 (C8H7O2), 135 (C17H18O), 107(C8H7O), 91 (C7H5), 81 (C6H5); 1H NMR (CDCl3, 400MHz, δ in ppm): δ 7.46 (d, J=8.2Hz, 2H, H-2' & H-6'), 7.42 (d, J=8.5Hz, 2H, H-3' & H-5'), 6.50 (d, J=2.5Hz, 1H, H-8), 6.32 (d, J=2.5Hz, 1H, H-5), 4.26 (brs, 4H, CH2 & CH2), 3.62 (t, J=6.8Hz, 2H, CH2-2'), 3.36 (t, J=6.8Hz, 2H, CH2-2'), 2.36 (s, 3H, CH3-7'); EI-MS (m/z): 459 [M+ C17H18I04S], 431 (C15H12INO4S), 395 (C15H12INO2), 368(C12H11INO4S), 326 (C12H11INO2), 304 (C15H14NO4S), 155 (C8H7O2), 135 (C14H0), 107 (C8H7O), 91 (C7H5), 81 (C6H5); 1H NMR (CDCl3, 400MHz, δ in ppm): δ 7.46 (d, J=8.2Hz, 2H, H-2' & H-6'), 7.42 (d, J=8.5Hz, 2H, H-3' & H-5'), 6.50 (d, J=2.5Hz, 1H, H-8), 6.32 (d, J=2.5Hz, 1H, H-5), 4.26 (brs, 4H, CH2 & CH2), 3.62 (t, J=6.8Hz, 2H, CH2-2'), 3.36 (t, J=6.8Hz, 2H, CH2-2'), 2.36 (s, 3H, CH3-7'); EI-MS (m/z): 459 [M+ C17H18I04S], 431 (C15H12INO4S), 395 (C15H12INO2), 368(C12H11INO4S), 326 (C12H11INO2), 304 (C15H14NO4S), 155 (C8H7O2), 135 (C14H0), 107 (C8H7O), 91 (C7H5), 81 (C6H5).
N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methyl-N-(1-pentyl)benzenesulfonamide (5e): Brown powder; Yield: 85%; m.p.: 114 °C; Molecular formula: C_{29}H_{28}NO_{4}S; Molecular weight: 375g mol⁻¹; IR (KBr, cm⁻¹): ν max: 2984 (C-H stretching of aromatic ring), 1678 (C=C stretching of aromatic ring), 1376 (SO₂ stretching), 1142 (C-O-C stretching of ether); 1H-NMR (CDCl₃, 400 MHz, δ in ppm): δ 7.40 (d, J=8.4Hz, 2H, H-2' & H-6'), 7.34 (d, J=8.5Hz, 2H, H-3' & H-5'), 6.66, (d, J=7.8Hz, 1H, H-8), 6.44 (dd, J=2.7, 8.5Hz, 1H, H-7), 6.28 (d, J=2.6Hz, 1H, H-5), 4.28 (brs, 4H, CH₂-2 & CH-3), 3.20 (t, J=7.5Hz, 2H, CH₂-1'), 2.34 (s, 3H, CH₃-7'), 1.50-1.55 (m, 2H, CH₂-1'' to CH₂-4''), 1.40-1.34 (m, 6H, CH₂-2'' to CH₂-4''); EI-MS (m/z): 375 [M+; C_{29}H_{27}NO_{4}S]+, 347 [C_{18}H_{15}NO_{3}S]+, 311 [C_{16}H_{13}NO_{2}S]+, 304 [C_{15}H_{12}NO_{2}S]+, 284 [C_{14}H_{11}NO_{2}S]+, 220 [C_{13}H_{9}NO]+, 155 [C_{12}H_{7}SO₃]+, 135 [C_{12}H_{7}O₄]+, 107 [C_{11}H_{5}O₆]+, 91 [CH₃]+, 81 [C(H)O₄]+.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methyl-N-(N-(2,3-Dihydro-1,4-Benzodioxin-6-Yl)-4-methylbenzenesulfonamide (5f): Brown powder; Yield: 96%; m.p.: 124 °C; Molecular formula: C_{24}H_{23}NO_{4}S, 381 (C_{20}H_{19}NO_{4}S)+, 354 (C_{20}H_{19}NO_{2}S)+, 311 (C_{16}H_{13}NO_{2}S)+, 304 (C_{15}H_{12}NO_{2}S)+, 284 (C_{14}H_{11}NO_{2}S)+, 220 (C_{13}H_{9}NO)+, 155 (C_{12}H_{7}SO₃)+, 135 (C_{12}H_{7}O₄)+, 107 (C_{11}H_{5}O₆)+, 91 (CH₃)+, 81 (C(H)O₄)+.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methyl-N-(N-(2,3-Dihydro-1,4-Benzodioxin-6-Yl)-4-methyl-N-(4-chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5m): Brown powder; Yield: 96%; m.p.: 160 °C; Molecular formula: C_{28}H_{27}BrNO_{4}S, 385 (C_{24}H_{23}BrNO_{3}S)+, 349 (C_{22}H_{19}BrNO_{3}S)+, 322 (C_{18}H_{15}BrNO_{2}S)+, 304 (C_{15}H_{14}BrNO_{2}S)+, 155 (C_{14}H_{12}BrSO₃)+, 135 (C_{14}H_{12}O₄)+, 107 (C_{13}H_{10}O₆)+, 91 (CH₃)+, 81 (C(H)O₄)+.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methyl-N-(4-fluorobenzyl)benzenesulfonamide (5n): Light grey powder; Yield: 90%; m.p.: 122 °C; Molecular formula: C_{25}H_{25}FNO_{4}S, 349 (C_{21}H_{21}FNO_{3}S)+, 322 (C_{17}H_{17}FNO_{2}S)+, 304 (C_{15}H_{14}FNO_{2}S)+, 155 (C_{14}H_{12}FSO₃)+, 135 (C_{14}H_{12}O₄)+, 107 (C_{13}H_{10}O₆)+, 91 (CH₃)+, 81 (C(H)O₄)+.

N-(2-Chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methyl-N-(4-fluorobenzyl)benzenesulfonamide (5p): Light grey powder; Yield: 90%; m.p.: 122 °C; Molecular formula: C_{25}H_{25}FNO_{4}S, 349 (C_{21}H_{21}FNO_{3}S)+, 322 (C_{17}H_{17}FNO_{2}S)+, 304 (C_{15}H_{14}FNO_{2}S)+, 155 (C_{14}H_{12}FSO₃)+, 135 (C_{14}H_{12}O₄)+, 107 (C_{13}H_{10}O₆)+, 91 (CH₃)+, 81 (C(H)O₄)+.

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Acetylcholinesterase inhibition assay

The inhibition activity of acetylcholinesterase was performed by according to a reported method [22] with little modifications. Total volume of the reaction mixture was 100μL and it also contained 60μL NaHPO₄, which acts as buffer having 50mm concentration (pH 7.7). Ten μl test compound 0.5mm well-1, followed by the addition of 10μl (0.5 unit well-1) enzyme. The reaction contents was agitated well and read prior at the wavelength 405nm. Then mixture was pre-incubated at 37°C for 5min.
Results and Discussion

Chemistry

The synthesis of various derivatives, 5a-n, derived by the N-substitution of N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (3) has been outlined in Scheme 1 and Table 1. All methods and conditions for this research work are mentioned in experimental section. The synthesis was initiated by the reaction of N-2,3-dihydro-1,4-benzodioxin-6-amine (1) with 4-methylbenzenesulfonyl chloride (2) in the presence of 10% Na₂CO₃ at adjusted pH 9 under constant stirring for 2-3 hours at room temperature to yield the parent sulfonamide, N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (3). Then, N-alkylation/aralkylation of this parent compound was carried out with different alkyl/aralkyl halides (4a-n) in DMF as a polar aprotic solvent and LiH as the base to yield the target compounds, 5a-n. We have already reported 5b, 5g, 5h, 5j and 5l along with their structural characterizations [21], however, we are reporting other compounds as new molecules in this investigation. The structures of the studied molecules were deduced through IR, other compounds as new molecules in this investigation. The chemical shift values at δ 2.34 (s, 3H, CH₃-7ʹ) along with a broad singlet at δ 4.28 (br.s, 4H, CH₂-2’une, CH₂-5’,) and 0.90 (t, J=7.5Hz, 3H, CH₂-5”). So, on the basis of above collected evidences, the structure of 5f was named as N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methyl-N-(2-pentyl)benzenesulfonamide. In an analogous manner, the structures of other derivatives of the series were characterized [22].

![Scheme 1: Outline for the synthesis of N-(Alkyl/aralkyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methyl-N-(2-pentyl)benzenesulfonamides (5a–n). Reagents & Conditions: (I) Aq. 10% Na₂CO₃ soln./pH 9-10/stirring at RT for 3hrs. (II) DMF/LiH/stirring at RT for 2-3hrs.](image)

![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-methylbenzenesulfonamides (5a-n).](image)
Acetylcholinesterase inhibition

The screening of all the derivatives 5a-n, against acetylcholinesterase (AChE) enzyme demonstrated that all the molecules of the series were active, except 5a. These molecules exhibited moderate to weak inhibitory potential and the results are tabulated in Table 2 in the form of % age inhibition and IC$_{50}$ values. Among these molecules, 5f, was found to be better inhibitor against this enzyme having IC$_{50}$ value of 71.62±0.09µM, probably due to the substitution of a branched aliphatic group i.e. 2-pentyl group. The molecule, 5n, having substitution of 4-fluorobenzyl group, also showed notable inhibitory potential with IC$_{50}$ value of 131.78±0.14µM. An extremely potent, eserine molecule was used as reference standard in this assay which has an IC$_{50}$ value of 0.85±0.001µM.

Table 2: Acetylcholinesterase inhibition study of N-alkyl/aralkyl-N-(2,3-Dihydro-[1,4]benzodioxin-6-yl)-4-methylbenzenesulfonylamides (5a-n).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Inhibition (%) at 0.5mm</th>
<th>IC$_{50}$ (µM)</th>
</tr>
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<tbody>
<tr>
<td>5a</td>
<td>44.29±0.19</td>
<td>-</td>
</tr>
<tr>
<td>5b</td>
<td>82.63±0.31</td>
<td>237.69±0.19</td>
</tr>
<tr>
<td>5c</td>
<td>91.71±0.24</td>
<td>269.54±0.15</td>
</tr>
<tr>
<td>5d</td>
<td>96.55±0.24</td>
<td>254.59±0.18</td>
</tr>
<tr>
<td>5e</td>
<td>94.65±0.26</td>
<td>318.64±0.15</td>
</tr>
<tr>
<td>5f</td>
<td>98.75±0.11</td>
<td>71.62±0.09</td>
</tr>
<tr>
<td>5g</td>
<td>94.31±0.24</td>
<td>145.32±0.13</td>
</tr>
<tr>
<td>5h</td>
<td>93.25±0.21</td>
<td>289.47±0.16</td>
</tr>
<tr>
<td>5i</td>
<td>93.55±0.19</td>
<td>148.58±0.11</td>
</tr>
<tr>
<td>5j</td>
<td>94.75±0.24</td>
<td>145.34±0.11</td>
</tr>
<tr>
<td>5k</td>
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<td>161.89±0.12</td>
</tr>
<tr>
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<td>218.64±0.14</td>
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<tr>
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<td>254.48±0.12</td>
</tr>
<tr>
<td>5n</td>
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<td>131.78±0.14</td>
</tr>
<tr>
<td>Eserine</td>
<td>82.82±1.09</td>
<td>0.85±0.0001</td>
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Conclusion

The targeted derivatives, 5a-n, were synthesized in good yields with a facile method and some of them exhibited a notable inhibitory potential against acetyl cholinesterase enzyme, therefore, these molecules might find their utility as possible therapeutic agents for the treatment of Alzheimer’s disease.

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References

