Synthesis, characterization and enzyme inhibition study of O-substituted derivatives of chlorinated coumarin

Aziz-ur-Rehman1,*, Schreen Magsi1, Muhammad Athar Abbasi1, Shahid Rasool1, Abdul Malik2, Ghulam Hussain1*, Muhammad Ashrafi3 and Noreen Khalid4

1Department of Chemistry, Government College University, Lahore, Pakistan
2HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan
3Department of Biochemistry & Biotechnology; 4Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan

Abstract: Coumarins have much importance in dyes, drugs, perfumes and pesticides. In the demonstrated research work, a benignant series of chlorinated coumarins was synthesized and screened against different enzymes. First, 6-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one (3) was geared up by the reaction of 4-chlororesorcinol (1) and ethyl acetoacetate (2) in the presence of concentrated H2SO4. Second, various O-substituted derivatives of chlorinated coumarins, 5a-j, were set up by pairing different alkyl/aralkyl halides, 4a-j, with 3 in the presence of NaH in DMF as solvent. The structures of all the synthesized compounds were clarified through spectral analysis using EI-MS, IR and 1H-NMR. The different enzymes used for the evaluation of bioactivity of all the synthesized compounds were acetyl cholinesterase (AChE), butyryl cholinesterase (BChE) and lipoxygenase (LOX). The most proficient activity was shown against both cholinesterase enzymes.

Keywords: Ethyl acetoactate, 4-chlororesorcinol, chlorinated coumarin, enzyme inhibition.

INTRODUCTION

Coumarin is naturally occurring heterocyclic compound, first isolated from tonka bean in 1820 (Smyth et al., 2009). It is also present in different plants like vanilla grass woodruff, licorice, strawberries, sweet clover etc. (Kinza et al., 2010). General structure for coumarin is

Antibacterial activities of coumarin were first accounted in 1945 (Smyth et al., 2009). By the ancient Egypt literature, it acts as therapeutic agent and widely used as medicine in aboriginal culture (Dekic et al., 2010). Coumarin also acts as anticoagulant agent and its derivatives are reported to be used as anti HIV agents (Dekic et al., 2010 and Sinhamahapatra et al., 2011). Coumarin is ministrant in curing cancer and presents a vital role in anti-inflammatory and antioxidant activities (Sinha mahapatra et al., 2011 and Upadhyay et al., 2008). There are thousands of derivatives of coumarin which have different uses in biological and agrochemical fields (Dekic et al., 2010 and Sinhamahapatra et al., 2011). Cigarettes, alcoholic beverages and laser dyes contain coumarin. These molecules are used as food additive and pesticide (Smyth et al., 2009). Coumarin gives specific features to different perfumes and cosmetics and also used to remove the unpleasant smell of rubber, sprays, paints and objects made up of plastic material (Lake, 1999).

Acetyl cholinesterase (AChE, EC 3.1.1.7) and Butyryl cholinesterase (BChE, EC 3.1.1.8) constitute a group of enzymes including serine hydrolases. The particularities for substrates and inhibitors regarding these enzymes are because of the divergences in residues of amino acid of active sites possessed by AChE and BChE. Both of the enzymes are creditworthy for the terminus of acetylcholine at cholinergic synapses and are key components of cholinergic brain synapses and neuromuscular junctions (Tougu et al., 2001).

Butyryl cholinesterase inhibition is an efficient one for the ailment of AD and associated dementedness. Much eminent quantities of BChE are detected in Alzheimer’s plaques instead of plaques related to normal age non-demented brains. Blood circulation is enriched by BChE and is present in animals and plants (Ahmad et al., 2005; Cyglar et al., 1993; Ellman et al., 1961; Tougu et al., 2001 and Ye et al., 2002).

Lipoxygenase (LOX, EC 1.13.11.12) relates to dioxygenases bearing iron but without haem and possessed by animals and plants. LOX shows a vital role in metabolism of arachidonic acid, in genesis of many biologically active lipids presenting role in inflammation, in thrombosis & tumor angiogenesis, in establishment of fresh vessels relating capillary from already present and in corroborations of many physiologic actions along with the maturation in pathological situations. These are probably targets in drug designing and also of inhibitors regarding the ailment of great disorders (Abbasi et al., 2005; Alitonou et al., 2006; Byrum et al., 1997; Clapp et al.,

*Corresponding author: e-mail: azizryk@yahoo.com
This research work is a productive endeavor to inaugurate pharmacologically significant ether derivatives of coumarin having antimicrobial and antioxidant potential (Shi and Zhou, 2011; Chimenti et al., 2004 and Zhang et al., 2011). In continuation of our last work of O-substituted derivatives (Aziz-ur-Rehman et al., 2012), the synthesis of the chlorinated coumarin and its derivatives was performed with an aim to prepare new competitors of drug having prominent activity for the remedy of numerous diseases.

**MATERIALS AND METHODS**

**General**
4-Chlororesorcinol, ethyl acetocetate, alkyl/aralkyl halides (Merck and Alfa Aeser) and other solvents of analytical grade were purchased through local suppliers and applied without further purification. Purity of the synthesized compounds was evaluated by thin layer chromatography (TLC) using a mixture of EtOAc and n-C₆H₁₄ as solvent system. TLC plates (purchased from local supplier) were visualized via UV at 254 nm and also by spraying ceric sulfate solution. The I.R. spectra were put down in potassium bromide pellet method on a Jasco-320-A spectrophotometer with wave number in cm⁻¹. Melting points of all the synthesized compounds were recorded on a Griffin-George melting point apparatus by open capillary tube. ¹H-NMR and ¹³C-NMR spectra were recorded in CDCl₃ on a Bruker spectrometer operating at 300 and 125 MHz respectively. The chemical shift values are reported in ppm (δ) units taking TMS as reference, and the coupling constants (J) are in Hz. Mass spectra (EI-MS) were recorded on a JMS-HX-110 spectrometer.

**Synthesis of 6-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one (3)**
4-Chlororesorcinol (0.020 moles, 1) was thoroughly dissolved in ethyl acetocetate (0.020 moles, 2) in a 500 mL iodine flask. 10 mL of concentrated H₂SO₄ was added drop wise with continuous shaking at 10 °C and allowed to stand for 12-14 hours. The precipitates of product were formed after the addition of ice cold water or crushed ice to stand for 12-14 hours. The precipitates of product were formed after the addition of ice cold water or crushed ice to stand for 12-14 hours. The precipitates of product were collected by filtration, washed with cold distilled water and finally dried to obtain the synthesized products, 5a-j. Further recrystallization of the synthesized products was not performed.

**BIOLOGICAL ACTIVITY**

**Cholinesterase assays**
The AChE and BChE inhibition activity were carried out almost by the reported method (Ellman et al., 1961). Reaction mixture of volume 100×10⁻⁶ L containing 60×10⁻⁶ L Na₂HPO₄ buffer (50×10⁻³ M & pH 7.7), 10×10⁻⁶ L test compound (0.5×10⁻³ M well⁻¹) and 10×10⁻⁶ L (0.005 unit well⁻¹ AChE or 0.5 unit well⁻¹ BChE) enzyme was prepared. The pre-incubation of contents was performed for 10 min at 37 °C after mixing and pre-reading at 405 nm. The reaction was set up by 10×10⁻⁶ L (0.5×10⁻³ M well⁻¹) substrate (acetylthiocholine iodide for AChE or butyrylthiocholine chloride for BChE) and 10×10⁻⁶ L DTNB (0.5×10⁻³ M well⁻¹). After incubation for 15 min at 37 °C, the measurement of absorbance was performed at 405 nm using 96-well plate reader (Synergy HT, Biotek, USA). All the experiments were executed in three-folds. As a positive control and reference standard, eserine (0.005 M well⁻¹) was employed. The %age inhibition was computed through the given equation.

\[
\text{Inhibition(\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100
\]

Where Control=Total enzyme activity without inhibitor
Test =Activity in the presence of synthesized compound
IC₅₀ values were accounted utilizing EZ-Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA).
IC₅₀ values were calculated from the graph by a serial dilution of compounds to different concentrations. The IC₅₀ values were expressed as average of three values obtained via three different observations.

**Lipoxygenase assay**
Lipoxygenase (LOX) activity was attempted almost by the mentioned procedure (Baylac et al., 2003; Bertaccini et al., 1982; Clapp et al., 1985 and Kemal et al., 1987). Total 200×10⁻⁶ L volume containing 150×10⁻⁶ L Na₃PO₄ buffer (100 mM and pH 8.0), 10×10⁻⁶ L test compound (0.5 mM well⁻¹) and 15×10⁻⁶ L (600 units well⁻¹) enzyme was developed. This mixture was passed through pre-reading at 234 nm and then pre-incubation for 0.17 hours at 25°C. 25×10⁻⁶L substrate solution was applied as reaction initiator. The absorbance change was detected after 0.1 hours at 234 nm using 96-well plate reader (Synergy HT, Biotek, USA). All the experiments were
executed in three-folds. As a positive control and reference standard, baicalein (0.005 M well⁻¹) was applied. The %age inhibition was computed by the same method as described above for cholinesterase assays. IC₅₀ values were calculated employing the same procedure as mentioned for acetyl cholinesterase and butyryl cholinesterase enzyme.

STATISTICAL ANALYSIS

All the measurements were accomplished in three-folds and the analysis was executed by Microsoft Excel 2010. The outcomes are offered as mean ± sem.

SPECTRAL CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

6-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one (3)
Light brown amorphous solid; Yield: 78%; M. P. 262-264 °C; Mol. formula: C₁₀H₇ClO₃; Mol. weight: 210 gmol⁻¹; IR (KBr): max (cm⁻¹): 3410 (stretching of O-H), 3056 (C-H aromatic stretching), 1720 (stretching of α, β-unsaturated C=O), 1625 (C=C stretching of aromatic ring); ¹H-NMR (300 MHz, CDCl₃, ppm): δ 7.54 (s, 1H, H-5), 6.98 (s, 1H, H-8), 6.17 (s, 1H, H-3), 2.37 (s, 3H, CH₃-11); ¹³C-NMR (125 MHz, CDCl₃, ppm): δ 160.5 (C-2), 157.7 (C-7), 153.4 (C-4), 151.5 (C-9), 125.4 (C-5), 118.7 (C-6), 113.6 (C-10), 112.7 (C-3), 100.3 (C-8), 18.5 (C-11); EIMS (m/z): 212 [M+2]⁺, 210 [M]⁺, 193 [M-OH]⁺, 182 [M-CO]⁺, 175 [M-Cl]⁺, 166 [M-CO₂]⁺, 143 [M-CH₃ClO]⁺, 134 [M-C₂HClO]⁺.

6-Chloro-7-ethoxy-4-methyl-2H-chromen-2-one (5a)
Buff amorphous solid; Yield: 75%; M. P. 150-152 °C; Mol. formula: C₁₂H₁₁ClO₃; Mol. weight: 238 gmol⁻¹; IR (KBr): max (cm⁻¹): 3054 (C-H aromatic stretching), 1726 (stretching of α, β-unsaturated C=O), 1628 (C=C stretching of aromatic ring); ¹H-NMR (300 MHz, CDCl₃, ppm): δ 7.55 (s, 1H, H-5), 6.82 (s, 1H, H-8), 6.15 (s, 1H, H-3), 4.14 (q, J = 6.9 Hz, 2H, H-1'), 2.37 (s, 3H, CH₃-11), 1.25 (t, J = 7.2 Hz, 3H, CH₃-2'); EIMS (m/z): 240 [M+2]⁺, 238 [M]⁺, 210 [M-C₂H₄]⁺, 203 [M-Cl]⁺, 194 [M-CO₂]⁺, 193 [M-OC₂H₅]⁺, 143 [M-C₃H₈ClO]⁺, 134 [M-C₄H₅ClO]⁺.

6-Chloro-7-(propan-2-yloxy)-4-methyl-2H-chromen-2-one (5b)
Peach amorphous solid; Yield: 58%; M. P. 118-120 °C; Mol. formula: C₁₃H₁₃ClO₃; Mol. weight: 252 gmol⁻¹; IR

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>-R</th>
<th>Compd. No.</th>
<th>-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>-CH₂-CH₃</td>
<td>5f</td>
<td>H₂C</td>
</tr>
<tr>
<td>5b</td>
<td>-CH₂CH₃</td>
<td>5g</td>
<td>H₂C</td>
</tr>
<tr>
<td>5c</td>
<td>H₂C</td>
<td>5h</td>
<td>H₂C</td>
</tr>
<tr>
<td>5d</td>
<td>H₂C</td>
<td>5i</td>
<td>H₂C</td>
</tr>
<tr>
<td>5e</td>
<td>H₂C</td>
<td>5j</td>
<td>H₂C</td>
</tr>
</tbody>
</table>

Scheme 1: Outline for the synthesis of various derivatives of chlorinated coumarin (3)
6-Chloro-7-(2-propen-1-yloxy)-4-methyl-2H-chromen-2-one (5e)

Milky white amorphous solid; Yield: 67%; M. P. 116-118°C; Mol. formula: C12H11ClO3; Mol. weight: 250 gmol⁻¹; IR (KBr): max (cm⁻¹): 3054 (C-H aromatic stretching), 1723 (stretching of α, β-unsaturated C=O), 1621 (C=O -unsaturated C=O), 1596 (C=C stretching of aromatic ring); 1H-NMR (300 MHz, CDCl₃, ppm): δ 7.56 (s, 1H, H-5), 6.84 (s, 1H, H-8), 6.16 (s, 1H, H-3), 5.98-6.09 (m, 1H, H-2%), 5.47 (dd, J = 15.9, 1.2 Hz, 1H, H-3%), 5.36 (dd, J = 9.3, 1.2 Hz, 1H, H-2%), 4.66 (d, J = 6.9 Hz, 2H, H-1%), 2.37 (s, 3H, CH₃-11); EIMS (m/z): 252 [M⁺²]+, 250 [M⁺]+, 215 [M⁺-CO]+, 209 [M-C₃H₇]+, 206 [M+CO₂]+, 193 [M-OC₃H₇]+, 143 [M-C₃H₆ClO]+, 134 [M-C₃H₅ClO]+.

6-Chloro-7-((ethoxycabonyl)methoxy)-4-methyl-2H-chromen-2-one (5d)

White amorphous solid; Yield: 54%; M. P. 184-186°C; Mol. formula: C₁₃H₁₂ClO₃; Mol. weight: 296 gmol⁻¹; IR (KBr): max (cm⁻¹): 3054 (C-H aromatic stretching), 1727 (stretching of α, β-unsaturated C=O), 1626 (C=O -unsaturated C=O), 1607 (C=C stretching of aromatic ring); 1H-NMR (300 MHz, CDCl₃, ppm): δ 7.59 (s, 1H, H-5), 6.71 (s, 1H, H-8), 6.18 (s, 1H, H-3), 4.74 (s, 2H, H-1'), 4.27 (q, J = 7.2 Hz, 2H, H-4'), 2.37 (s, 3H, CH₃-11), 1.30 (t, J = 6.9 Hz, 3H, CH₃-7'); EIMS (m/z): 298 [M⁺²]+, 296 [M⁺], 268 [M-CO]+, 267 [M-C₃H₇]+, 261 [M-Cl]+, 252 [M+CO₂]+, 209 [M-C₃H₆ClO]+, 193 [M-OC₃H₇]+, 143 [M-C₃H₆ClO]+, 134 [M-C₃H₅ClO]+.

6-Chloro-7-((4-bromophenyl)methoxy)-4-methyl-2H-chromen-2-one (5f)

Light tea pink amorphous solid; Yield: 57%; M. P. 204-206°C; Mol. formula: C₁₇H₁₆Cl₂O₃; Mol. weight: 335 gmol⁻¹; IR (KBr): max (cm⁻¹): 3052 (C-H aromatic stretching), 1722 (stretching of α, β-unsaturated C=O), 1624 (C=O stretching of aromatic ring), 703 (stretching of C-Cl); 1H-NMR (300 MHz, CDCl₃, ppm): δ 7.59 (s, 1H, H-5), 7.57 (br.s, 1H, H-3), 7.40 (t, J = 6.9 Hz, 1H, H-5'), 7.33 (br.s, 1H, H-6), 7.29 (dd, J = 6.9, 1.5 Hz, 1H, H-4'), 6.90 (s, 1H, H-8), 6.17 (s, 1H, H-3), 5.28 (s, 2H, H-7), 2.38 (s, 3H, CH₃-11); EIMS (m/z): 339 [M⁺-CO]+, 337 [M⁺-2]+, 335 [M⁺], 307 [M⁺-CO₂]+, 300 [M⁺-Cl], 291 [M⁺-CO₂], 210 [M-C₃H₇Cl], 193 [M-OC₃H₇Cl], 143 [M-C₃H₆ClO]+, 134 [M-C₃H₅ClO]+, 111 [M-C₃H₇Cl], 99 [M-C₄H₇Cl], 65 [C₆H₅Cl], 51 [C₄H₇].

6-Chloro-7-((3-chlorophenyl)methoxy)-4-methyl-2H-chromen-2-one (5g)

Brown sticky solid; Yield: 58%; M. P. 224-226°C; Mol. formula: C₁₃H₁₁Cl₂O₃; Mol. weight: 335 gmol⁻¹; IR (KBr): max (cm⁻¹): 3060 (C-H aromatic stretching), 1730 (stretching of α, β-unsaturated C=O), 1631 (C=O stretching of aromatic ring); 1H-NMR (300 MHz, CDCl₃, ppm): δ 7.59 (s, 1H, H-5), 7.35-7.45 (m, 2H, H-4', H-5'), 7.33 (br.s, 1H, H-6), 7.31 (br.s, 1H, H-2'), 6.85 (s, 1H, H-8), 6.16 (s, 1H, H-3), 5.17 (s, 2H, H-7), 2.37 (s, 3H, CH₃-11); EIMS (m/z): 339 [M⁺+4]+, 337 [M⁺+2]+, 335 [M⁺], 307 [M⁺-CO₂]+, 300 [M⁺-Cl], 291 [M⁺-CO₂], 210 [M-C₃H₇Cl], 193 [M-OC₃H₇Cl], 143 [M-C₃H₆ClO]+, 134 [M-C₃H₅ClO]+, 111 [M-C₃H₇Cl], 99 [M-C₄H₇Cl], 65 [C₆H₅Cl], 51 [C₄H₇].

6-Chloro-7-((4-flourophenyl)methoxy)-4-methyl-2H-chromen-2-one (5h)

Light tea pink amorphous solid; Yield: 53%; M. P. 174-176°C; Mol. formula: C₁₇H₁₄Cl₂O₃; Mol. weight: 318 gmol⁻¹; IR (KBr): max (cm⁻¹): 3062 (C-H aromatic stretching), 1731 (stretching of α, β-unsaturated C=O), 1621 (C=O stretching of aromatic ring), 1035 (stretching of C-F); 1H-NMR (300 MHz, CDCl₃, ppm): δ 7.58 (s, 1H, H-5), 7.43 (d, J = 8.7 Hz, 2H, H-3', H-5'), 7.08 (d, J = 8.7 Hz, 2H, H-2', H-6'), 6.87 (s, 1H, H-8), 6.16 (s, 1H, H-3), 5.15 (s, 2H, H-7), 2.37 (s, 3H, CH₃-11); EIMS (m/z): 320 [M⁺+2]+, 318 [M⁺], 290 [M⁺-CO₂], 283 [M⁺-Cl], 274 [M⁺-CO₂], 210 [M-C₃H₇F], 193 [M-OC₃H₇F], 143 [M-C₃H₆ClO], 134 [M-C₃H₅ClO], 95 [C₆H₄F], 83 [C₃H₇F], 65 [C₆H₅Cl], 51 [C₄H₇].

6-Chloro-7-((2-phenylethan-1-yloxy)-4-methyl-2H-chromen-2-one (5i)

Light brown amorphous solid; Yield: 55%; M. P. 112-114°C; Mol. formula: C₁₃H₁₂ClO₂; Mol. weight: 314 gmol⁻¹; IR (KBr): max (cm⁻¹): 3064 (C-H aromatic stretching), 1717 (stretching of α, β-unsaturated C=O), 1617 (C=C stretching of aromatic ring); 1H-NMR (300 MHz, CDCl₃, ppm): δ 7.54 (s, 1H, H-5), 7.25-7.33 (m, 5H, H-2' to H-6'),
Table 1: Enzyme inhibition studies of various derivatives of chlorinated coumarin

<table>
<thead>
<tr>
<th>Compound</th>
<th>AChE Inhibition (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (0.5 mM)</th>
<th>BChE Inhibition (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (0.5 mM)</th>
<th>LOX Inhibition (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (0.5 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>76.98±0.78</td>
<td>169.41±0.09</td>
<td>71.28±0.31</td>
<td>125.61±0.15</td>
<td>93.22±0.21</td>
<td>57.31±0.19</td>
</tr>
<tr>
<td>5a</td>
<td>64.41±0.11</td>
<td>211.52±0.06</td>
<td>59.28±0.13</td>
<td>231.52±0.06</td>
<td>53.39±0.33</td>
<td>&gt;400</td>
</tr>
<tr>
<td>5b</td>
<td>61.91±0.69</td>
<td>245.36±0.14</td>
<td>78.72±0.27</td>
<td>121.21±0.15</td>
<td>60.47±0.24</td>
<td>289.11±0.63</td>
</tr>
<tr>
<td>5c</td>
<td>70.99±0.35</td>
<td>152.91±0.35</td>
<td>64.74±0.11</td>
<td>201.31±0.14</td>
<td>44.67±0.15</td>
<td>127.31±0.14</td>
</tr>
<tr>
<td>5d</td>
<td>67.71±0.15</td>
<td>212.71±0.12</td>
<td>67.15±0.13</td>
<td>217.51±0.12</td>
<td>41.38±0.81</td>
<td>-</td>
</tr>
<tr>
<td>5e</td>
<td>91.88±0.62</td>
<td>59.11±0.15</td>
<td>87.36±0.33</td>
<td>63.91±0.08</td>
<td>52.03±0.31</td>
<td>&gt;400</td>
</tr>
<tr>
<td>5f</td>
<td>66.56±0.18</td>
<td>226.36±0.11</td>
<td>69.38±0.15</td>
<td>206.11±0.18</td>
<td>53.68±0.81</td>
<td>&gt;400</td>
</tr>
<tr>
<td>5g</td>
<td>65.57±0.33</td>
<td>71.25±0.04</td>
<td>90.53±0.87</td>
<td>24.51±0.04</td>
<td>41.86±0.81</td>
<td>&gt;400</td>
</tr>
<tr>
<td>5h</td>
<td>74.27±0.31</td>
<td>152.31±0.22</td>
<td>86.28±0.22</td>
<td>68.11±0.21</td>
<td>39.73±0.27</td>
<td>-</td>
</tr>
<tr>
<td>5i</td>
<td>53.38±0.11</td>
<td>&gt;400</td>
<td>68.87±0.18</td>
<td>63.91±0.17</td>
<td>44.19±0.15</td>
<td>-</td>
</tr>
<tr>
<td>5j</td>
<td>77.56±0.55</td>
<td>144.91±0.14</td>
<td>97.01±0.21</td>
<td>34.71±0.14</td>
<td>65.89±0.21</td>
<td>199.51±0.14</td>
</tr>
</tbody>
</table>

Control

<table>
<thead>
<tr>
<th>Eserine</th>
<th>Eserine</th>
<th>Baicalein</th>
</tr>
</thead>
<tbody>
<tr>
<td>91.29±1.17</td>
<td>0.04±0.001</td>
<td>82.82±1.09</td>
</tr>
</tbody>
</table>

Note: IC<sub>50</sub> values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

AChE = Acetyl cholinesterase. BChE = Butyryl cholinesterase. LOX = Lipoxygenase.

Fig. 1: <sup>1</sup>H-NMR spectrum of 6-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one (3)
Synthesis, characterization and enzyme inhibition study of O-substituted derivatives of chlorinated coumarin

276


6-Chloro-7-(3-phenylpropan-1-yloxy)-4-methyl-2H-chromen-2-one (5j)
Brown crystalline solid; Yield: 83%; M. P. 126-128 oC; Mol. formula: C$_{19}$H$_{17}$ClO$_3$; Mol. weight: 328 gmol$^{-1}$; IR (KBr): max (cm$^{-1}$): 3057 (C-H aromatic stretching), 1728 (stretching of $\alpha,\beta$-unsaturated C=O), 1629 (C=C stretching of aromatic ring); $^1$H-NMR (300 MHz, CDCl$_3$, ppm): $\delta$ 7.56 (s, 1H, H-5), 7.18-7.30 (m, 5H, H-2' to H-6'), 6.78 (s, 1H, H-8), 6.15 (s, 1H, H-3), 4.04 (t, $J = 6.3$ Hz, 2H, H-9'); EIMS (m/z): 330 [M+2]$^+$, 328 [M]$^+$, 286 [M-CO]$^+$, 279 [M-Cl]$^+$, 270 [M-CO$_2$]$^+$, 209 [M-C$_9$H$_{11}$]$^+$, 193 [M-OC$_9$H$_{11}$]$^+$, 143 [M-C$_{10}$H$_{14}$ClO]$^+$, 134 [M-C$_{11}$H$_{14}$ClO]$^+$, 105 [C$_8$H$_9$]$^+$, 91 [C$_7$H$_7$]$^+$, 77 [C$_6$H$_5$]$^+$, 65 [C$_5$H$_5$]$^+$, 51 [C$_4$H$_3$]$^+$.

Fig. 2: Mass Fragmentation pattern of 6-Chloro-7-((4-bromophenyl)methoxy)-4-methyl-2H-chromen-2-one (5e)

RESULTS

In the presented research work, a series of compounds was derived from chloro substituted coumarin. The research work comprised a two step synthesis. First, the chlorinated coumarin (3) was synthesized in a highly acidic medium by the intermixing of 4-chlororesorcinol (1) with ethyl acetoacetate (2) in the presence of concentrated sulphuric acid as catalyst in 12-14 hours. The high %age yield of product, 3, was collected by filtration after adding ice cold water and washing was processed with cold distilled water. 3 was further treated with different alkyl/aralkyl halides as electrophiles, 4a-j, to yield different substituted products, 5a-j, in the presence of NaH acting as base and aprotic polar solvent, DMF. The
products were collected as precipitates by filtration after the addition of ice cold distilled water and 10% aqueous NaOH. The sodium hydroxide was utilized to get rid of non-reacted chlorinated coumarin by getting it soluble in the form of salt. All the synthesized compounds were screened against acetyl cholinesterase (AChE), butyryl cholinesterase (BChE) and lipooxygenase (LOX) enzymes and their structures were clarified through spectral data.

DISCUSSION

Chemistry
The compound 3 was synthesized as light brown amorphous solid having yield of 78% and melting point of 262-264 °C. The mol. formula C_{10}H_{7}ClO_{3} was launched by EI-MS pointing molecular ion peak at m/z 210 and also by counting the number of protons via 1H-NMR spectral data. The IR spectrum depicted absorption bands at 3410 cm⁻¹, 3056 cm⁻¹, 1720 cm⁻¹ and 1625 cm⁻¹ because of O-H stretching of hydroxyl group, C-H aromatic stretching, stretching of α, β-unsaturated C=O and C=C stretching of aromatic ring in the molecule. The EI-MS presented two distinct peaks at m/z 182 and m/z 166 after the removal of CO and CO₂ molecules respectively. The other eminent peaks were mentioned in the spectral data. In 1H-NMR spectrum, two signals appeared at δ 7.54 (s, 1H, H-5) and 6.98 (s, 1H, H-8) in aromatic section owing to two protons of aromatic ring at fifth & eighth position and one signal at δ 6.17 (s, 1H, H-3) because of one proton of six membered ring at third position. The signal appearing at δ 2.37 (s, 3H, CH₃-11) was allotted to three protons of methyl group attached to fourth position of six membered ring. The 1H-NMR spectrum is given in fig. 1 for confirmation of coumarin synthesis. In the 13C-NMR (BB and DEPT) spectrum, the ten signals resonated for six quaternary carbons at δ 160.5 (C-2), 157.7 (C-7), 153.4 (C-4), 151.5 (C-9), 118.7 (C-6) & 113.6 (C-10), three methine carbons at δ 125.4 (C-5), 112.7 (C-3) & 100.3 (C-8) and one methyl carbon at δ 18.5 (C-11). On the basis of all these manifests, the structure of compound 3 was identified as 6-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one. The mass fragmentation pattern of 6-Chloro-7-(4-bromophenylmethoxy)-4-methyl-2H-chromen-2-one (5e) was sketched in fig. 2. Similarly, the structures of other synthesized compounds, 5a-j, were characterized by 1H-NMR, IR and EI-MS as described in spectral data section.

Enzyme inhibition activity
The screening of these synthesized compounds against AChE, BChE and LOX enzymes displayed good inhibitory potential against cholinesterase enzymes as shown by their IC₅₀ values (table 1). Inhibition study showed that all the compounds possessed prominent activity against AChE except 6-chloro-7-(2-phenylethanol-1-yloxy)-4-methyl-2H-chromen-2-one (5i). 6-Chloro-7-((4-bromophenyl)methoxy)-4-methyl-2H-chromen-2-one (5e) and 6-chloro-7-((3-chlorophenyl)methoxy)-4-methyl-2H-chromen-2-one (5g) were the most efficient AChE inhibitors having IC₅₀ values of 59.11±0.15 and 71.25±0.04 µmoles/L, respectively, with respect to eserine, a reference standard with IC₅₀ value of 0.04±0.001 µmoles/L. The efficient inhibitory results of these compounds were likely due to the presence of chlorine at para position and chlorine at meta position of aralkyl groups, respectively, attached to coumarin nucleus via oxygen.

The screening against BChE revealed that all the synthesized compounds were active but 6-chloro-7-((3-chlorophenyl)methoxy)-4-methyl-2H-chromen-2-one (5g) and 6-chloro-7-(3-phenylpropan-1-yloxy)-4-methyl-2H-chromen-2-one (5j) were found to be the most potent inhibitors having IC₅₀ values of 24.51±0.04 µmoles/L and 34.71±0.14 µmoles/L, relative to eserine, a reference standard with IC₅₀ value of 0.85±0.0001 µmoles/L. The beneficial inhibition activity of these compounds was likely due to the occurrence of meta chloro-substituted and unsubstituted aralkyl groups, respectively, attached to coumarin via oxygen. Compounds 5e, 5h and 5i also inhibited BChE activity with low IC₅₀ values. The descending order of BChE inhibition activity is as follows; 5g<5j<5e<5i<5h.

Against LOX, most of the compounds were inactive. The parent compound, 6-chloro-7-hydroxy-4-methyl-2H-chromen-2-one (3) was the most efficient having IC₅₀ value of 57.31±0.19 µmoles/L, relative to baicalein, a reference standard with IC₅₀ value of 22.4±1.3 µmoles/L. However, the derivatives of 3 could not reveal good LOX inhibition activity.

CONCLUSION
The presented series was synthesized to elaborate the biological activities of the O-substituted derivatives of chlorinated coumarin. The synthesized compounds showed relatively better results against cholinesterase enzymes rather than lipooxygenase enzyme. The most active compounds can be further evaluated for in vivo study and so might be helpful for the designing of pharmacologically important new drug candidates.

ACKNOWLEDGEMENT
The authors are much thankful to the Higher Education Commission (HEC) of Pakistan for financial assistance.

REFERENCES