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Synthesis, Spectral Characterization and α-Chymotrypsin Activity of 7-*O*-Substituted Derivatives of 7-Hydroxy-4-methyl-1-benzopyran-2-one

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In this work, a series of *O*-alkyl/arenyl/acyl substituted derivatives of 7-hydorxy-4-methyl-1-benzenpyran-2-one (**3a-l**) was synthesized. The parent compound 7-hydroxy-4-methyl-1-benzenpyran-2-one **(1)** was geared up by the coupling of resorcinol **(a)** with ethylacetoacetate **(b)** in the presence of conc. sulphuric acid. Further, *O*-substituted derivatives of parent compound were prepared by treating with different electrophiles (**2a-l**) using sodium hydride as base and DMF as a solvent. The structure of these synthesized compounds were characterized by IR, EI-MS and ^{1}H NMR. These derivatives were also screened against α-chymotrypsin enzyme to check their enzyme inhibition activity. All the compounds displayed α-chymotrypsin activity to varying degree.

Keywords: 7-Hydroxy-4-methyl-1-benzopyran-2-one, α-Chymotrypsin enzyme, ^{1}H NMR, EI-MS.

INTRODUCTION

The coumarin present in nature is a significant member of benzopyrones[1]. Coumarin was naturally extracted from tonka beans[2] and it is an important constituent of cinnamon and dates[3-5]. Coumarin has the properties of anticoagulant, insecticide, antibacterial and pharmacological functions[6]. Natural and synthesized coumarin is one of the main human metabolite and in human diet, it acts as dietary antioxidant. It also reduces the blood glucose level in human body[7]. Organic and medicinal chemists have considerable attraction for coumarin and its derivatives because of its antiinflammatory/anti-oxidant activities[8]. In bakery and beverage industry, it is used as flavoring agents and enhanced the flavors of different bakery items[9]. Efforts have been made for the extraction of naturally occurring coumarin from numerous plants and artificial synthesis of coumarin compounds, so that it can be further utilized as the potential drug in medicinal field[10].

Among the enzymes involved in extracellular matrix degradation, certain serine proteases (elastase, collagenase, cathepsin G and chymotrypsin) are able to solubilize fibrous proteins such as elastin and collagen[11]. Potent inhibitors have the potential to be developed as new therapeutic agents. In vertebrates, serine protease inhibitors have been studied for many years and they are known to be involved in phagocytosis, coagulation, complement activation, fibrinolysis and blood pressure regulation. In the last decade, it became obvious that in invertebrates, serine proteases and their inhibitors are also involved in parallel physiological processes, for example the blood clotting cascade in Limulus[12] and the innate immune response[13]. Therefore, the discovery of potent and safe inhibitors has been a very important area of pharmaceutical research. Coumarin and its derivatives have attracted organic chemists because of its remarkable role in biological activities.

The present work is a successful effort to synthesize such compounds exhibiting diverse and improved pharmacological potential. We have synthesized the *O*-alkyl/arenyl/acyl substituted derivatives of 7-hydorxy-4-methyl-1-benzenpyran-2-one with an objective to search new contenders of drug having significant enhanced activity and could be helpful in controlling many degenerative diseases.

EXPERIMENTAL

All melting points are in degree centigrade and were recorded on Griffin and George apparatus by using open capillary tube. Thin layer chromatography technique was performed on pre-coated silica gel G-25-UV$_{254}$ plates using mixture of *n*-hexane and ethyl acetate as solvents in different ratios for each reaction to check the purity of synthesized compound by giving single spot of product. UV light of 254 nm wavelength and ceric sulphate reagent were used for this detection. IR technique was performed on instrument Jasco-

320-A spectrophotometer (wave number in cm^{-1}) using KBr pellets. Numbers of protons calculated by nuclear magnetic resonance spectra also helped in identification of structures and were recorded on a Bruker spectrometer operating at 300 MHz in CD_3OD solution. Chemical shifts are quoted in ppm. Mass spectra (EIMS) were given on JMS-HX-110 spectrometer. Resorcinol, ethylacetoacetate and the other electrophiles (methyl iodide, ethyl iodide, isopropyl iodide, allyl iodide, 2-phenylethyliodide, 2-chlorobenzylchloride, 4-bromobenzylbromide, 4-flurobenzyl chloride and 4-chlorobenzyl chloride) were purchased from Sigma Aldrich and Alfa Aesar through local suppliers. All the other employed solvents were of analytical grade.

Procedure for the synthesis of 7-hydroxy-4-methyl-1-benzopyran-2-one (1): Resorcinol (4.4 g; **a**) and ethylacetoacetate (5 mL; **b**) was taken in iodine flask. Reaction mixture was shaken well until reaction mass become clear by keeping the flask on water bath and temperature was maintained at 40 ºC. Then, cooled concentrated sulphuric acid (15 mL) was added to the iodine flask and the reaction mixture was placed for overnight. Next day, the chilled water was added in the solution with continuous stirring. The reaction mixture was reserved at room temperature for 0.5 h; dark yellow solid was filtered and washed with distilled water to afford 7-hydroxy-4-methyl-1-benzopyran-2-one **(1)** on drying.

General procedure for the synthesis of *O*-substituted derivatives 2a-i: Compound (**1**) (2.80 mmol, 0.5 g) was taken in round bottom flask and *N*,*N*-dimethyl formamide (around 5 mL) and sodium hydride (2.80 mmol; 0.01g,) were added in it at room temperature. The reaction mixture was stirred for 15 min and then the electrophiles (2.80 mmol; **2a-i**) were added into the mixture. The reaction mass was further stirred and monitoring through TLC. After completion of the reaction, the reaction mixture was quenched with cold water (150 mL). The received solid was filtered, washed with water and dried to yield the corresponding *O*-substituted derivatives of 7-hydroxy-4-methyl-1-benzopyran-2-one (**3a-i**).

General procedure for the synthesis of compounds 3j-l: Compound **1** (2.8 mmol; 0.5 g) and 20 mL of 5 % sodium hydroxide solution was taken in the round bottom flask. The mixture was stirred till solution become clear and then the corresponding electrophiles (2.80 mmol; **2j-l**) were added into the reaction mixture with constant stirring at room temperature until precipitates were formed. Precipitates were filtered, washed with distilled water and dried to yield the corresponding derivatives (**3j-l**).

Characterization of the synthesized compounds

7-Hydroxy-4-methyl-1-benzopyran-2-one (1): Dark yellow amorphous powder; yield 92 %; m.p. 188-190 ºC; m.f.: $C_{10}H_8O_3$; m.w. 176; IR (KBr, ν_{max}, cm^{-1}): 3550 (O-H stretching), 3418 (C-H aromatic stretching), 1717 (C=O stretching), 1629 (C=C stretching of aromatic ring); ^{1}H NMR (300 MHz, CD_3OD δ/ppm): 7.58 (d, *J* = 8.7 Hz, 1H, H-5), 6.82 (dd, *J* = 8.7, 2.4 Hz, 1H, H-6), 6.70 (d, *J* =2.4 Hz,1H, H-8), 6.09 (s, 1H, H-3), 2.41 (d, *J* = 0.9 Hz, 3H, CH_3-4) . EI-MS: *m/z* 176 $[M]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

7-Methoxy-4-methyl-1-benzopyran-2-one (3a): White amorphous powder; yield 58 %; m.p. 88-90 ºC; m.f.: $C_{11}H_{10}O_3$; m.w. 190; IR (KBr, ν_{max}, cm^{-1}): 3535 (O-H stretching), 3401 (C-H aromatic stretching), 1729 (C=O stretching), 1616 (C=C stretching of aromatic ring). ^{1}H NMR (300 MHz, CD_3OD δ/ppm): 7.60 (d, *J* = 8.4 Hz, 1H, H-5), 6.80 (dd, *J* = 8.7, 2.5 Hz, 1H, H-6), 6.69 (d, *J* =2.5 Hz, 1H, H-8), 6.09 (s, 1H, H-3), 3.78 (s, 3H, OCH_3-1), 2.41 (s, 3H, CH_3-4). EI-MS: *m/z* 190 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

7-Ethoxy-4-methyl-1-benzopyran-2-one (3b): Buff amorphous powder; yield 76 %; m.p. 98-100 ºC; m.f.: $C_{12}H_{12}O_3$; m.w. 204; IR (KBr, ν_{max}, cm^{-1}): 3539 (O-H stretching), 3407 (C-H aromatic stretching), 1727 (C=O stretching), 1614 (C=C stretching of aromatic ring). ^{1}H NMR (300 MHz, CD_3OD δ/ppm): 7.68 (d, *J* = 8.7 Hz, 1H, H-5), 6.95 (dd, *J* = 8.7, 2.4 Hz, 1H, H-6), 6.87 (d, *J* =2.4 Hz, 1H, H-8), 6.15 (s, 1H, H-3), 4.11 (q, *J* = 6.9 Hz, 2H, CH_2-1), 2.43 (s, 3H, CH_3-4), 1.42 (t, J = 6.9 Hz, 3H, CH_3-2). EI-MS: *m/z* 204 $[M]^+$, 189 $[M\text{-}CH_3]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

7-Isopropoxy-4-methyl-1-benzopyran-2-one (3c): Off-white amorphous powder; yield 78 %; m.p: 88-90 ºC; m.f.: $C_{13}H_{14}O_3$; m.w. 218; IR (KBr, ν_{max}, cm^{-1}): 3542 (O-H stretching), 3406 (C-H aromatic stretching), 1728 cm^{-1} (C=O stretching), 1613 (C=C stretching of aromatic ring). ^{1}H-NMR (300 MHz, CD_3OD δ/ppm): 7.67 (d, *J* = 8.7 Hz, 1H, H-5), 6.92 (dd, *J* = 8.7, 2.4 Hz, 1H, H-6), 6.86 (d, *J* = 2.4 Hz, 1H, H-8), 6.13 (s, 1H , H-3), 4.72 (m, 1H, H-1), 2.43 (s, 3H, CH_3-4), 1.34 (d, *J* = 6.0 Hz, 6H, H-2 & 3). EI-MS: *m/z* 218$[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

7-Allyoxy-4-methyl-1-benzopyran-2-one (3d): Creamy white amorphous powder; yield 78 %; m.p. 154-157 ºC; m.f.: $C_{13}H_{12}O_3$; m.w. 216; IR (KBr, ν_{max}, cm^{-1}): 3503 (O-H stretching), 3410 (C-H aromatic stretching), 1729 (C=O stretching), 1617 (C=C stretching of aromatic ring). ^{1}H NMR (300 MHz, CD_3OD δ/ppm): 7.66 (d, *J* = 9.0 Hz, 1H, H-5), 6.96 (dd, *J* = 9.0, 2.4, 1H, H-6), 6.90 (d, *J* =2.4 Hz, 1H, H-8), 6.15 (s, 1H, H-3), 6.11-6.02 (m, 1H, H-2), 5.44 (dd, *J* =1.5, 17.1 Hz, H_b-3), 5.27 (dd , *J* = 1.5, 10.5 Hz, H_a-3), 4.66 (d, *J* = 5.4 Hz, H-1), 2.43 (s, 3H, CH_3-4). EI-MS: *m/z* 216$[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

7-(2-Phenylethoxy)-4-methyl-1-benzopyran-2-one (3e): Buff amorphous powder; yield 64 %; m.p. 96-98 ºC; m.f.: $C_{18}H_{16}O_3$; m.w. 280; IR (KBr, ν_{max}, cm^{-1}): 3510 (O-H stretching), 3418 (C-H aromatic stretching), 1723 (C=O stretching), 1613 (C=C stretching of aromatic ring). ^{1}H NMR (300 MHz, CD_3OD δ/ppm): 7.64 (d, *J* = 8.7 Hz, 1H, H-5), 7.32-7.17 (m, 5H, H-2 to 6'), 6.94 (dd, *J* = 9.0 Hz, 2.4, 1H, H-6), 6.88 (d, *J* =2.4 Hz, 1H, H-8), 6.14 (s, 1H, H-3), 4.28 (t, *J* = 6.9 Hz, 2H, H-8'), 3.12 (t, *J* = 6.6 Hz, 2H, H-7'), 2.42 (s, 3H, CH_3-4), EIMS**:** *m/z* 280 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 91 $[C_7H_7]^+$, 51 $[C_4H_3]^+$.

7-(2-Chlorobenzyloxy)-4-methyl-1-benzopyran-2-one (3f): Creamy white amorphous powder; yield 70 %; m.p. 135-137 ºC; m.f.: $C_{17}H_{13}O_3Cl$; m.w. 300; IR (KBr, ν_{max}, cm^{-1}): 3515 (O-H stretching), 3416 (C-H aromatic stretching), 1719 (C=O stretching), 1611 (C=C stretching of aromatic ring). ^{1}H NMR (300 MHz, CD_3OD δ/ppm): 7.72 (d, *J* = 8.7 Hz, 1H, H-5), 7.58-7.32 (m, 4H, H-3' to 6'), 7.05 (dd, *J* = 8.7, 2.7 Hz, 1H, H-6), 6.98 (d, *J* =2.4 Hz, 1H, H-8), 6.17 (s, 1H, H-3), 5.27 (s,

2H, H-7'), 2.44 (s, 3H, CH_3-4), EIMS: *m/z* 300 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 125 $[C_7H_6Cl]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

7-(4-Bromobenzyloxy)-4-methyl-1-benzopyran-2-one (3g): Light yellow amorphous powder; yield 72 %; m.p. 128-132 ºC; m.f.: $C_{17}H_{13}O_3Br$; m.w. 345; IR (KBr, ν_{max}, cm^{-1}): 3523 (O-H stretching), 3412 (C-H aromatic stretching), 1725 (C=O stretching), 1616 (C=C stretching of aromatic ring). ^1H NMR (300 MHz, CD_3OD δ/ppm): 7.70 (d, *J* = 9.0 Hz, 1H, H-5), 7.55 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.37 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.03 (dd, *J* = 8.7 2.4 Hz, 1H, H-6), 6.97 (d, *J* =2.4 Hz, 1H, H-8), 6.16 (s, 1H, H-3), 5.15 (s, 2H, H-7'), 2.44 (s, 3H, CH_3-4), EI-MS: *m/z* 345 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 170 $[C_7H_6Br]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

7-(4-Flurobenzyloxy)-4-methyl-1-benzopyran-2-one (3h): Creamy white amorphous powder; yield 85 %; m.p. 110-112 ºC; m.f.: $C_{17}H_{13}O_3F$; m.w. 284; IR (KBr, ν_{max}, cm^{-1}): 3511 (O-H stretching), 3417 (C-H aromatic stretching), 1721 (C=O stretching), 1617 (C=C stretching of aromatic ring). ^1H NMR (300 MHz, CD_3OD δ/ppm): 7.67 (d, *J* = 8.7 Hz, 1H, H-5), 7.65 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.13 (d, *J* = 8.4 Hz, 2H, H-2' and H-6'), 7.01 (dd, *J* = 8.7, 2.7 Hz, 1H, H-6), 6.95 (d, *J* = 2.4 Hz, 1H, H-8), 6.13 (d, *J* = 0.9 Hz, 1H, H-3), 5.17 (s, 2H, H-7'), 2.42 (s, 3H, CH_3-4), EI-MS: *m/z* 284 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 109 $[C_7H_6F]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

7-(4-Chlorobenzyloxy)-4-methyl-1-benzopyran-2-one (3i): White amorphous powder; yield 60 %; m.p. 178-180 ºC; m.f.: $C_{17}H_{13}O_3Cl$; m.w. 300; IR (KBr, ν_{max}, cm^{-1}): 3519 (O-H stretching), 3423 (C-H aromatic stretching), 1721 (C=O stretching), 1619 (C=C stretching of aromatic ring). ^1H-NMR (300 MHz, CD_3OD δ/ppm): 7.67 (d, *J* = 9.0 Hz, 1H, H-5), 7.43 (d, *J* = 8.4 Hz, 2H, H-2' and H-6'), 7.37 (d, *J* = 8.4 Hz, 2H, H-3' and H-5'), 7.03 (dd, *J* = 8.7, 2.7 Hz, 2.7, 1H, H-6), 6.97 (d, *J* = 2.4 Hz, 1H, H-8), 6.15 (s, 1H, H-3), 5.17 (s, 2H, H-7'), 2.43 (s, 3H, CH_3-4), EIMS: *m/z* 300 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 125 $[C_7H_6Cl]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$.

4-Methyl-2-oxo-1-benzopyran-7-yl-acetate (3j): White amorphous powder; yield 88 %; m.p. 128-130 ºC; m.f.: $C_{12}H_{10}O_4$; m.w. 218; IR (KBr, ν_{max}, cm^{-1}): 3534 (O-H stretching), 3412 (C-H aromatic stretching), 1715, 1729 (C=O stretching), 1627 (C=C stretching of aromatic ring). ^1H NMR (300 MHz, CD_3OD δ/ppm): 7.69 (d, *J* = 8.4 Hz, 1H, H-5), 7.11 (dd, *J* = 8.4 Hz, 2.1, 1H, H-6), 6.32 (d, *J* =1.2 Hz, 1H, H-8), 6.1 (s, 1H, H-3), 2.4 (s, 3H, CH_3-4), 2.3 (s, 3H, CH_3COO). EIMS: *m/z* 218 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 51 $[C_4H_3]^+$, 43 $[C_2H_3O]^+$.

4-Methyl-2-oxo-1-benzopyran-7-yl-benzoate (3k): Creamy white amorphous powder; yield 78 %; m.p. 136-138 ºC; m.f.: $C_{17}H_{12}O_4$; m.w. 280; IR (KBr, ν_{max}, cm^{-1}): 3531 (O-H stretching), 3410 (C-H aromatic stretching), 1718, 1734 cm^{-1} (C=O stretching), 1623 (C=C stretching of aromatic ring). ^1H NMR (300 MHz, CD_3OD δ/ppm): 8.20-7.84 (m, 5H, H-2' to H-6'), 7.80 (d, *J* = 9.0 Hz, 1H, H-5), 7.30 (dd, *J* = 8.7, 2.1 Hz, 1H, H-6), 6.90 (d, *J* =2.4 Hz, 1H, H-8), 6.30 (s, 1H, H-3), 2.50 (s, 3H, CH_3-4), EIMS: *m/z* 280 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 105 $[C_7H_5O]^+$, 51 $[C_4H_3]^+$.

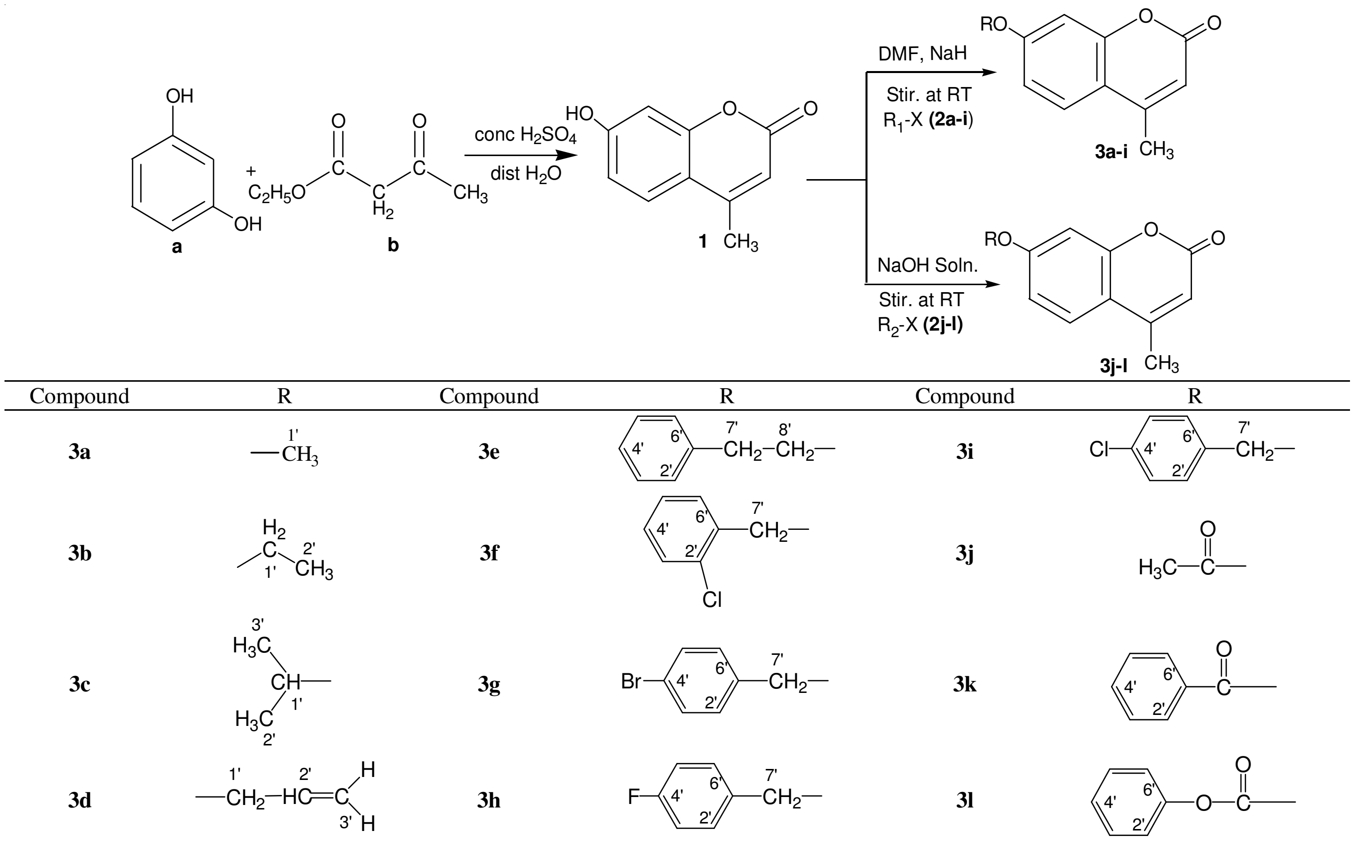
O-(4-Methyl-2-oxo-1-benzopyran-7-yl)-O-phenylcarbonate (3l): Oily liquid; yield 54 %; m.f.: $C_{17}H_{12}O_5$; m.w. 296; IR (KBr, ν_{max}, cm^{-1}): 3529 (O-H stretching), 3409 cm (C-H aromatic stretching), 1706, 1727 (C=O stretching), 1621 (C=C stretching of aromatic ring). ^1H NMR (300 MHz, CD_3OD δ/ppm): 7.40 (d, *J* = 8.7 Hz, 1H, H-5), 7.17 (d, *J* = 2.1 Hz, 1H, H-8), 6.70 (dd, *J* = 8.7, 2.1 Hz, 1H, H-6), 6.75-6.81 (m, 5H, H-2' to 6'), 6.00 (s, 1H, H-3), 2.30 (s, 3H, CH_3-4). EIMS: *m/z* 296 $[M]^+$, 175 $[C_{10}H_7O_3]^+$, 159 $[C_{10}H_7O_2]^+$, 144 $[C_9H_4O_2]^+$, 108 $[C_6H_4O_2]^+$, 93 $[C_6H_5O]^+$, 51 $[C_4H_3]^+$.

***in vitro* α-Chymotrypsin assay:** The chymotrypsin inhibitory activity of the compounds was performed by the method of Cannell *et al.*[14]. Chymotrypsin (9 units/mL of 50 mM *Tris*-HCl buffer pH 7.6; Sigma Chemical Co. USA) was pre-incubated with the compounds for 20 min at 258 ºC. 100 mL of substrate solution (1 mg of N-succinyl-phenylalanine-*p*-nitroanilide/mL of 50 mM *Tris*-HCl buffer pH 7.6) was added to start the enzyme reaction. The absorbance of released *p*-nitroaniline was continuously monitored at 410 nm until a significant colour change was achieved. The final DMSO concentration in the reaction mixture was 7 %. The percentage (%) inhibition was calculated as follows (ES)/E x 100, where E is the activity of the enzyme without test compound and S is the activity of enzyme in the presence of the test compound. The concentrations of test compounds that inhibited the hydrolysis of substrate upto 50 % (IC_{50}) were determined by monitoring the effect of various concentrations of these compounds in the assays on the inhibition values. The IC_{50} value was then designed using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

Statistical analysis: All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± sem.

RESULTS AND DISCUSSION

In the undertaken research, heterocyclic *O*-substituted derivatives of coumarin were synthesized. The parent compound 7-hydroxy-4-methyl-1-benzenpyran-2-one (**1**), was prepared by the coupling of resorcinol (**a**) with ethylacetoacetate (**b**) in the presence of sulphuric acid. Further, the reaction of **1** with different electrophiles (**2a-l**) yielded a series of *O*-substituted derivative of 7-hydroxy-4-methyl-1-benzenpyran-2-one (**3a-l**) as represented in **Scheme-I**. Synthesis of all *O*-substituted derivatives **3a-i** was performed in DMF (*N,N*-dimethyl-formamide) and sodium hydride (NaH) which act as a base. Complete conversion was achieved within 40-120 min by stirring. The products were isolated by adding cold water in the reaction mixture and filtering off the precipitated solid. In some cases, compound was taken out through solvent extraction method by chloroform/ ethyl acetate. The structure of all the prepared compounds were characterized by ^1H NMR, IR and mass spectral data as elaborated in experimental section. Parent compound **(1)** was synthesized as dark yellow powder. The molecular formula $C_{10}H_8O_3$ was established by molecular ion peak observed at *m/z* 296 in EI-MS and by counting the number of protons in its ^1H-NMR spectrum. The IR spectrum showed absorption peaks at 3550, 3418, 1717 and 1629 cm^{-1} which were assigned to O-H (stretching of hydroxyl group), C-H (aromatic stretching), C=O (stretching of carbonyl bond) and



Scheme-1: Outline for the synthesis of *O*-substituted derivatives of 7-hydroxy-4-methyl-1-benzopyarn-2-one

C=C (stretching of aromatic ring), respectively. In the ^{1}H-NMR spectrum, the signals appeared at δ 7.58 (d, *J* = 8.7, 1H, H-5), 6.82 (dd, *J* = 8.7, 2.4 Hz, 1H, H-6), 6.70 (d, *J* =2.4 Hz, 1H, H-8) and 6.09 (d, *J* = 0.9, 1H, H-3). In the aliphatic region of the spectrum, signal resonated at δ 2.41 (d, *J* = 0.9, 3H, CH_3-4) indicated the presence of one methyl group present in the molecule. On the basis of mentioned cumulative evidences, the structure of compound **1** was assigned 7-hydroxy-4-methyl-1-benzenpyran-2-one. Mass fragmentation pattern of one of the compound, 7-(4-chlorobenzyloxy)-4-methyl-1-benzopyran-2-one (**3i**) is clearly elaborated in Fig. 1. Similarly, the structures of other 7-*O*-alkyl/arenyl/acyl derivatives were characterized by IR, ^{1}H NMR and EI-MS data.

All the compounds were screened against α-chymotrypsin enzyme and data is given in Table-1. All compounds showed varying degree of enzyme inhibition. It is clearly evident that the compounds 4-methyl-2-oxo-1-benzopyran-7-yl benzoate **(3k)** and 7-(4-chlorobezyloxy)-4-methyl-1-benzopyran-2-one **(3i)** were found to be good inhibitors against *a*-chymotrypsin enzyme having IC_{50} value 58.11 ± 0.22 and 115.11 ± 0.05 μmol/L, relative to Chymostatin a reference standard with IC_{50} value of 8.24 ± 0.11 μmoles/L, respectively. This indicates that nature of the substituent has great effect on inhibitory activity of these derivatives. These compounds can further be exploited and their derivatives could be synthesized to get closer to IC_{50} values of the standard, chymostatin. In this way, the compounds could be potential target in the drug discovery and drug development program. Compounds **(3e, 3f)** were found to be the least inhibitor having IC_{50} value > 400. Similarly, the compounds **3a, 3g, 3j** and **3l** were remained inactive against α-chymotrpsin enzyme.

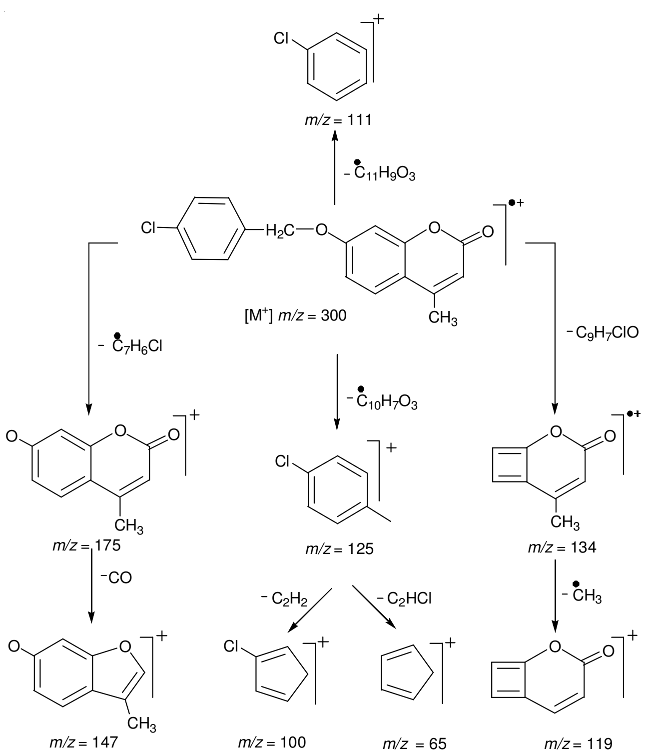


Fig. 1. Mass fragmentation pattern of 7-(4-chlorobezyloxy)-4-methyl-1-benzopyran-2-one (**3i**)

Conclusion

The projected structures of the synthesized compounds are well supported by spectroscopic data. The synthesized compounds showed varying degree of chymostatin activity.

TABLE-1
BIOACTIVITY STUDIES OF *O*-SUBSTITUTED DERIVATIVES OF 7-HYDROXY-4-METHYL-1-BENZOPYARN-2-ONE

S. No.	α-Chymotrypsin		
	Conc. (mM)	Inhibition (%)	IC_{50} (µmol)
3a	0.5	30.33 ± 0.11	–
3b	0.5	65.33 ± 0.11	253.6 ± 0.01
3c	0.5	72.22 ± 0.14	239.3 ± 0.15
3d	0.5	64.81 ± 0.12	229.7 ± 0.10
3e	0.5	52.45 ± 0.15	>400
3f	0.5	50.68 ± 0.11	>400
3g	0.5	48.91 ± 0.16	–
3h	0.5	59.24 ± 0.19	229.3 ± 0.11
3i	0.5	65.08 ± 0.61	115.11 ± 0.05
3j	0.5	30.43 ± 0.17	–
3k	0.5	92.93 ± 0.15	58.11 ± 0.22
3l	0.5	41.58 ± 0.13	–
Control	Chymostatin	93.50 ± 0.91	8.24 ± 0.11

Note: 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).

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