Chapter 05

Studies on

Phenyl Ester of

Coumarin 4-

Acetic acid
5.1 Introduction

Coumarins are both naturally occurring as well as synthetic derivatives are having widespread applications as HIV protease inhibitors, anticoagulant, spasmylytic and bacteriostatic agents. However, the most widely reported activities for Coumarin derivatives are their anti-inflammatory and anti-cancer activities. Coumarin derivatives with anticancer activities include aromatase inhibitors, carbonic anhydrase inhibitors, and steroid sulfatase inhibitors. Other Coumarin derivatives based anticancer compounds include the naturally occurring GUT-70 from C. brasiliense, 7-Isopentenyloxy coumarin from H. lanatum, 5-Oxygenated-6,7-methylenedioxy coumarins from P. polystachyum as well as the synthetic Coumarin derivatives such as 7-Hydroxycoumarin, 6-Nitro-7-hydroxycoumarin, Coumarin-3-(Naryl)-Sulfonamides, and 3-Bromophenyl-6-acetoxymethyl-2-oxo-2H-benzopyran-3-carboxylate. Some of the medicinal compounds containing Coumarin nucleus are Warfarin (vitamin K antagonists), Ensaculin (antidementia agent), Umbelliferone or 7- Hydroxycoumarin (find use in sunscreen creams and lotions), Psoralen (for psoriasis, eczema and vitiligo).

Coumarins scavenge reactive oxygen species and suppress inflammation, oedema and pain. Linkage of various heterocycles at C-4 position in 2-Arylaminothiazoles has resulted in good pharmacological activity. Number of 2,4-disubstituted Thiazoles, Imidazolythiazoles and Pyrazolythiazoles have been recognized as potent anti-inflammatory and analgesic agents. Synthesis of many 3-substituted biheterocyclic coumarins with Thiazoles and fused Thiazoles possessing antimicrobial and anti-inflammatory agents has been reported.

It is observed that ester linked with amino group possesses good anti-inflammatory activity and analgesic activity. Coumarin possesses good anti-inflammatory and analgesic activity. So, it was decided to carry out amino group linked by ester linkage in Coumarin nucleus. There was no much structural modification at 7th position of Coumarin ring. Hence it was decided to modify the structural nucleus Coumarin at 7th position by substituting with different amines linked by ester.
Thus in the present investigation it was contemplated to synthesize a series of Coumarin derivatives by substituting biologically active amino alkanone group with a view to increase their biologically activity.

5.2 Synthesis aspect

Simion A. M. et al\textsuperscript{11} have reported various o-acylated compounds by using different phenol and various alkanoyl chlorides using phase transfer catalyst.

\[
\text{OH} - \text{ClO} \xrightarrow{\text{Pd(OAc)}_4} \text{O}\text{C}\text{Ph}
\]

Khalfina I. A. and Vlasov V. M. et al\textsuperscript{12} have prepared phenyl ester and studied its physical property. They have prepared it by enthalpy-entropy co-relation in reaction of 2,4-Dinitro phenyl benzoate and phenols.

\[
\text{NO}_2\text{O}\text{C}\text{Ph} + \text{OH} \xrightarrow{\text{Pd(OAc)}_2} \text{O}\text{C}\text{Ph}
\]

Ueda Tsuyoshi et al\textsuperscript{13}, have prepared Phenyl acetate palladium catalyst by carbonylation of Aryl, Alkenyl and Allyl Halide with phenyl formate.

\[
\text{I} + \text{O-CHO} \xrightarrow{\text{Pd(OAc)}_4} \text{O}\text{C}\text{Ph}
\]

\[
\text{Br} + \text{O-CHO} \xrightarrow{\text{Pd(OAc)2}} \text{O}\text{Ph}
\]
Yang Hung-Ming et al\textsuperscript{14} have synthesized phenyl benzoate by solid-supported catalyst, green chemistry approach, divinylbenzene-crosslinked styrene-(chloromethyl)- styrene copolymer-supported Bu3N used as phase transfer catalyst.

Ghazanfari Dadkhoda et al\textsuperscript{15} have prepared by using the Benzophenone as starting material and oxidised it chemo selectively by using solid supported per-acid (Beyer-Villigar Oxidation).

Pasha et. al\textsuperscript{16} have reported green and efficient method for preparation of phenyl ester by using the TiO\textsubscript{2} as catalyst in reaction of phenol and acid chloride.

Petersen, Tue B. et al\textsuperscript{17} have given the metal free efficient method for the preparation of the aryl ester by using carboxylic acid derivatives and Diaryliodonium salts.

Wu Xiao-Feng et al\textsuperscript{18} reported the methods for preparation of various phenyl ester using phenol and Bromobenzine and Carbondioxide in the presence of Pd/Di-1-adamentyl-n-butylphosphine catalyst.
Zarchi, Mohammad Ali Karimi et al\textsuperscript{19} have used solid supported Benzoyl chloride and phenol to prepare benzoate ester.

Yang Tianle et al\textsuperscript{20} and French Jarrod B. et al\textsuperscript{21} have prepared phenyl ester of Pyridine-2-carboxylic acid chloride.

Romanelli, Gustavo P. et al\textsuperscript{22} have synthesised various benzoate by using reusable hetereo poly acid catalyst and simple mild acylation method.

Liu Jianhua et al\textsuperscript{23} have reported the synthesis of alkoxy carbonylation using recyclable phosphine free catalyst for sonogashira coupling reaction of Iodobenzene and phenol.
Poisson Thomas et al\textsuperscript{24} have prepared benzoate by using o-Silyphenol derivative and Benzoylfluoride and DMAP catalyst.

Zhang Lingli et al\textsuperscript{25} have used Boronic acid and Copper triflate for synthesis of the phenyl ester with Benzoic acid.

Rosa, Joao N. et al\textsuperscript{26} have reported that in the above method Benzaldehyde also can be used to get desire product.

Wiles Charlotte et al\textsuperscript{27} have synthesised phenolic ester in micro-level by using borosilicate micro-reactor with electro-osmotic flow gives higher yield.
Tanasa Fulga et al\textsuperscript{28} have prepared phenyl benzoate by using Diphenyl carbonate and Benzoic acid and DMNP as a catalyst.

Mohammadpoor-Baltork I. et al\textsuperscript{29} proposed that the Bismuth (III) salt as catalyst for the synthesis of ester by phenol and Benzoic anhydride.

5.3 Aim of current work

The present work aims at preparation of Phenolic ester derivatives of 7-Hydroxy Coumarin-4-Acetic Acid with different substituted phenols, to find out optimum reaction conditions and further study of its properties. By exploring the literature as mention above, found that there was no any work done related to Phenolic ester derivatives of 7-HydroxyCoumarin-4-Acetic Acid.

5.4 Reaction scheme

5.4.1 Step-I: Synthesis of 7-Hydroxy coumarin-4-acetic acid
5.4.2 Step-II Synthesis of Phenyl ester of Coumarin-4-acetic acid

5.4.3 Plausible reaction mechanism

![Reaction Mechanism Diagram]

5.5 Experimental

5.5.1 Materials and methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 using Direct Injection Probe technique and Turbo spray model using chemical ionisation technique. $^1$H NMR was determined in CDCl$_3$/DMSO solution on a Bruker Ac 400 MHz spectrometer.
5.5.2 **General Method of Preparation of Coumarin-4-Acetic Acid**

A mixture of Citric acid anhydrate (0.1mol) and 28 ml of concentrated Sulphuric acid was stirred at room temperature for sixty minutes, and then slowly heated to 70 °C on oil bath. After half an hour at this temperature, with stirring throughout, the evolution of carbon monoxide had slackened and the clean yellow colored solution was rapidly cooled to 0 °C. To this stirred solution, was added phenol (0.08 mol) and 11.2 ml of concentrated Sulphuric acid, each in three equal portions, at a rate that the temperature does not exceed 10°C. The resulting reaction mixture was stored at 0 °C for sixteen hours, poured onto ice and the resulting precipitates were filtered off and washed thoroughly with water. It was then treated with 10% Sodium bicarbonate solution and then filtered. The filtrate, on acidification gave Coumarin-4-acetic acid. The residue after bicarbonate treatment is 4-Methylcoumarin. The purity of the compound is checked by TLC (Methanol: Chloroform = 1:9). Several substituted Coumarin-4-acetic acids were similarly prepared by using different phenols.  

5.5.3 **General Method of Preparation of various derivatives of phenyl ester Coumarin-4-Acetic Acid**

In the R.B.F. 0.0045 mole Coumarin-4-acetic acid was dissolved in 20 ml of dry THF and cooled to 0 °C and then 0.009 mole of SOCl₂ was dropwise added by maintaining temperature. The reaction mixture was stirred at room temp. For 20-25 minute & concentrate it by evaporated solvent. Add 0.004 mol phenol solution in pyridine to the concentrated acid chloride solution at 0 °C temp. & stirr for 30 minutes. Reaction mass was poured on to the crushed ice and decomposed with dil HCl to obtained solid mass. Product was filterd and wash with aq. NaHCO₃ solution to remove unreacted acid, washed with water and finally with the Ethyl Acetate to give pure product.
5.5.4 Physical Data

Physical data of coumarin-4-acetic acids

The physical data and \( R_f \) value of various Coumarin-4-acetic acids were recorded in the Table No. 1.

![Chemical structure of Coumarin-4-acetic acid](image)

**Table 1**

<table>
<thead>
<tr>
<th>Codes</th>
<th>Substitution</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Melting point ( (\degree C) )</th>
<th>( R_f ) value</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSP-500</td>
<td>-OH</td>
<td>C_{11}H_{8}O_{5}</td>
<td>220</td>
<td>202-204(a)</td>
<td>0.43</td>
<td>58</td>
</tr>
</tbody>
</table>

TLC solvent system: Methanol: Chloroform = 1:9 Ref.: (a= Reported m. p.: 204-206 \( \degree C \)) (1)

5.6 Physical data of phenolic ester of coumarin-4-acetic acid

The physical data and \( R_f \) value of various Phenolic ester of Coumarin-4-acetic acid were recorded in the Table No. 2.

![Chemical structure of Phenolic ester of coumarin-4-acetic acid](image)
Table 2

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Code No.</th>
<th>Substitution R</th>
<th>Molecular Formula</th>
<th>Molecular Weight gm/mole</th>
<th>Found MP (°C)</th>
<th>Rf Value %</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KSP-501</td>
<td>-H</td>
<td>C_{17}H_{12}O_3</td>
<td>296</td>
<td>184-186</td>
<td>0.64</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>KSP-502</td>
<td>4-OCH_3</td>
<td>C_{18}H_{14}O_6</td>
<td>326</td>
<td>202-204</td>
<td>0.63</td>
<td>58</td>
</tr>
<tr>
<td>3</td>
<td>KSP-503</td>
<td>2-CH_3</td>
<td>C_{18}H_{14}O_3</td>
<td>310</td>
<td>192-194</td>
<td>0.67</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>KSP-504</td>
<td>3-CH_3</td>
<td>C_{18}H_{14}O_3</td>
<td>310</td>
<td>192-194</td>
<td>0.62</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>KSP-505</td>
<td>4-CH_3</td>
<td>C_{19}H_{14}O_3</td>
<td>310</td>
<td>178-180</td>
<td>0.64</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>KSP-506</td>
<td>3,5-di-CH_3</td>
<td>C_{19}H_{16}O_5</td>
<td>324</td>
<td>184-186</td>
<td>0.60</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td>KSP-507</td>
<td>3-OH</td>
<td>C_{19}H_{14}O_6</td>
<td>312</td>
<td>216-218</td>
<td>0.43</td>
<td>58</td>
</tr>
<tr>
<td>8</td>
<td>KSP-508</td>
<td>2-OH</td>
<td>C_{19}H_{14}O_6</td>
<td>312</td>
<td>218-220</td>
<td>0.63</td>
<td>59</td>
</tr>
<tr>
<td>9</td>
<td>KSP-509</td>
<td>2-Br</td>
<td>C_{17}H_{11}O_3Br</td>
<td>375</td>
<td>186-188</td>
<td>0.62</td>
<td>52</td>
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<tr>
<td>10</td>
<td>KSP-510</td>
<td>4-Br</td>
<td>C_{17}H_{11}O_3Br</td>
<td>375</td>
<td>172-174</td>
<td>0.58</td>
<td>47</td>
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<tr>
<td>11</td>
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<td>2-Cl</td>
<td>C_{17}H_{11}O_3Cl</td>
<td>330.5</td>
<td>198-200</td>
<td>0.52</td>
<td>58</td>
</tr>
<tr>
<td>12</td>
<td>KSP-512</td>
<td>4-Cl</td>
<td>C_{17}H_{11}O_3Cl</td>
<td>330.5</td>
<td>174-176</td>
<td>0.55</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
<td>KSP-513</td>
<td>2-NO_2</td>
<td>C_{17}H_{11}NO_2</td>
<td>341</td>
<td>172-174</td>
<td>0.43</td>
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<tr>
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<td>KSP-514</td>
<td>3-NO_2</td>
<td>C_{17}H_{11}NO_2</td>
<td>341</td>
<td>178-180</td>
<td>0.47</td>
<td>53</td>
</tr>
</tbody>
</table>

5.7 Spectral discussion

5.7.1 Mass spectra

The mass spectrum of compounds was recorded by GCMS-QP2010 spectrometer (EI method) and Turbo spray using chemical ionisation technique. The molecular ion peak (M^+ and M^-) were obtained by positive chemical ionization mass spectrometry. The molecular ion peak and the base peak in all compounds were clearly obtained in mass spectrom study. The molecular ion peak (M^+) values are in good agreement with molecular formula of all the compounds synthesized.
5.7.2 IR spectra

Infra Red spectra of 2-(2-Oxo-2H-chromen-4-yl)-acetic acids were taken on SHIMADZU FT-IR-8400 Spectrometer using KBr Pellet method. The characteristic carbonyl group in Coumarin moiety is observed at 1710-1680 cm\(^{-1}\). The carboxylic acid group in 2-(2-Oxo-2H-chromen-4-yl) acetic acids was observed in the range of 1844-1794 cm\(^{-1}\). The Coumarin moiety showed the ring skeleton vibrations at 1645-1600, 1590-1550, 1550-1520, 1495-1470 cm\(^{-1}\). The peak of methylene band was observed at 1458-1396 cm\(^{-1}\). The ether (C-O-C) was observed at 1275-1235 cm\(^{-1}\) and 1085-1025 cm\(^{-1}\). Mono, ortho as well as meta substitution at C\(_6\), C\(_7\) as well as C\(_8\) of coumarin ring showed the absorbance at 890-830 cm\(^{-1}\), 780-760 cm\(^{-1}\), 760-720 cm\(^{-1}\).

5.7.3 \(^1\)HNMR spectra

\(^1\)HNMR spectra of 2-(7-Hydroxy-2-oxo-2H-chromen-4-yl)-acetic acid was recorded on Bruker Avance 300 MHz FT-NMR Spectrometer using TMS (Tetramethyl Silane) as an internal standard and DMSO-d\(_6\) as a solvent. In the NMR spectra of 2-(7-Hydroxy-2-oxo-2H-chromen-4-yl)acetic acid, various proton values like methylene (-CH\(_2\)), methyl (-CH\(_3\)), aromatic protons (Ar-H) were observed. The values for methyl (-CH\(_3\)) protons are seen between 2.3-2.6 δ ppm and for methylene (-CH\(_2\)) protons at 3.5-4 δ ppm. The aromatic proton (Ar-H) shows doublets or multiplets between 6.4-7.8 δ ppm.

5.8 Analytical Data

7-Hydroxycoumarin-4-Acetic acid (KSP-500)

\[
\text{HO} \quad \text{O} \quad \text{O} \quad \text{O} \\
\text{H} \quad \text{O} \quad \text{O} \quad \text{OH} \\
\]

Yield: 48%; MP 202-204 ºC; MS: \(m/z\) M\(^+\) 220 IR (cm\(^{-1}\)): 3502 (-O-H stretching of -COOH), 3142 (-C-H stretching of aromatic ring), 2960 (-C-H asymmetrical
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stretching of -CH\textsubscript{2} group), 1712, 1666, (>C=O stretching of coumarin), 1604, 1523 and 1386 (-C=C stretching of aromatic ring), 1323 (-C-H asymmetrical deformation of -CH\textsubscript{2} group), 1260 (-C-H symmetrical deformation of -CH\textsubscript{2} group), 1211, 1146 (-C-O-C stretching); Anal. Calcd. C\textsubscript{11}H\textsubscript{8}O\textsubscript{5}: C, 60.00; H, 3.66; O, 36.33; Found: C, 59.86; H, 3.45; O, 36.13%;

5.8.1 Phenyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate (KSP-501)

![Chemical structure](image1)

Yield: 58%; MP 202-204 °C; MS: m/z 296, IR (cm\textsuperscript{-1}): 3112 (-O-H stretching), 3054 (-C-H stretching of aromatic ring), 2824 (-C-H asymmetrical stretching of -CH\textsubscript{2} group), 1412, (>C=O stretching of coumarin), 1605, 1329 (-C=C stretching of aromatic ring), 1326 (-C-H asymmetrical deformation of Alkyl group), 1210 (-C-H symmetrical deformation of -CH\textsubscript{2} group), 1130 (-C-O-C stretching); Anal. Calcd. C\textsubscript{17}H\textsubscript{12}O\textsubscript{5}: C, 68.92; H, 4.08; O, 27.00; Found: C, 68.75; H, 4.01; O, 26.46%.

5.8.2 4-Methoxyphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-502)

![Chemical structure](image2)

Yield: 58%; MP 202-204 °C; MS: m/z M\textsuperscript{+}+1 327, M\textsuperscript{+}-1 325, IR (cm\textsuperscript{-1}): 3102 (-O-H stretching), 3064 (-C-H stretching of aromatic ring), 2808 (-C-H asymmetrical stretching of -CH\textsubscript{2} group), 1730, (>C=O stretching of coumarin), 1612, 1390 (-C=C stretching of aromatic ring), 1319 (-C-H asymmetrical deformation of Alkyl group), 1219 (-C-H symmetrical deformation of -CH\textsubscript{2} group), 1136 (-C-O-C stretching);
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$^1$HNMR: (DMSO-$d_6$) $\delta$ ppm: 3.13 (s, 2H), 4.16 (s, 3H), 6.50 (s, 1H), 6.89 (m, 4H), 7.03 (d, 2H), 7.66 (d, 1H), 10.97 (s, 1H(-OH)), Anal. Calcd. C$_{18}$H$_{14}$O$_6$: C, 66.26; H, 4.32; O, 29.42; Found: CC, 66.16; H, 4.22; O, 29.32%.

5.8.3 o-Tolyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate
(KSP-503)

![Structure of o-Tolyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate](image1)

Yield: 58%; MP 200-201 ºC; MS: $m/z$ 310, IR (cm$^{-1}$): 3100 (-O-H stretching), 3064 (-C-H stretching of aromatic ring), 2801 (-C-H asymmetrical stretching of -CH$_2$ group), 2871 (-C-H asymmetrical stretching of -CH$_3$ group), 1720, (>C=O stretching of coumarin), 1612, 1370 (-C=C stretching of aromatic ring), 1319 (-C-H asymmetrical deformation of Alkyl group), 1219 (-C-H symmetrical deformation of -CH$_2$ group), 1136 (-C-O-C stretching); Anal. Calcd. C$_{18}$H$_{14}$O$_5$: C, 69.67; H, 4.55; O, 25.78; Found: C, 69.47; H, 4.25; O, 25.55%.

5.8.4 m-Tolyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate
(KSP-504)

![Structure of m-Tolyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate](image2)

Yield: 52%; MP 200-205 ºC; MS: $m/z$ 310, IR (cm$^{-1}$): 3111 (-O-H stretching), 3061 (-C-H stretching of aromatic ring), 2821 (-C-H asymmetrical stretching of -CH$_2$ group), 2854 (-C-H asymmetrical stretching of -CH$_3$ group), 1715, (>C=O stretching of coumarin), 1602, 1351 (-C=C stretching of aromatic ring), 1329 (-C-H asymmetrical deformation of Alkyl group), 1222 (-C-H symmetrical deformation of -CH$_2$ group),
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1136 (-C-O-C stretching); Anal. Calcd. C_{18}H_{14}O_{5}: C, 69.67; H, 4.55; O, 25.78; Found: C, 69.43; H, 4.21; O, 25.50%.

5.8.5  *p*-Tolyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate
(KSP-505)

Yield: 48%; MP 213-215 ºC; MS: m/z 310, IR (cm⁻¹): 3101 (-O-H stretching), 3061 (-C-H stretching of aromatic ring), 2801 (-C-H asymmetrical stretching of -CH₂ group), 2804 (-C-H asymmetrical stretching of -CH₃ group), 1705, (>C=O stretching of coumarin), 1602, 1341 (-C=C stretching of aromatic ring), 1329 (-C-H asymmetrical deformation of Alkyl group), 1242 (-C-H symmetrical deformation of -CH₂ group), 1106 (-C-O-C stretching); Anal. Calcd. C_{18}H_{14}O_{5}: C, 69.67; H, 4.55; O, 25.78; Found: C, 69.40; H, 4.45; O, 25.44%.

5.8.6  2,5-Dimethylphenyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)
acetate (KSP-506)

Yield: 55%; MP 184-186 ºC; MS: m/z M⁺+1 325, M⁺-1 323, IR (cm⁻¹): 3182 (-O-H stretching), 3082 (-C-H stretching of aromatic ring), 2956 (-C-H asymmetrical stretching of alkyl group), 1714, (>C=O stretching of coumarin), 1614, 1377 (-C=C stretching of aromatic ring), 1321 (-C-H asymmetrical deformation of Alkyl group), 1266 (-C-H symmetrical deformation of -CH₂ group), 1132 (-C-O-C stretching); ¹HNMR: (DMSO-d₆) δ ppm: 2.25 (s, 6H), 3.22 (s, 2H), 6.45 (s, 1H), 6.90 (s, 3H), 7.00 (s, 2H).
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7.03 (s, 1H), 7.39 (s, 1H), 7.76 (d, 1H) 10.58 (s, 1H(-OH)), Anal. Calcd. C_{19}H_{16}O_{5}: C, 70.36; H, 4.97; O, 24.67; Found: C, 70.06; H, 4.57; O, 24.47%.

5.8.7 2-Hydroxyphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-507)

Yield: 53%; MP 210-215 °C; MS: m/z 312, IR (cm\(^{-1}\)): 3345 (-O-H stretching of free -OH)3101 (-O-H stretching), 3061 (-C-H stretching of aromatic ring), 2801 (-C-H asymmetrical stretching of -CH\(_2\) group), 1705, (>C=O stretching of coumarin), 1602, 1341 (-C=C stretching of aromatic ring), 1329 (-C-H asymmetrical deformation of Alkyl group), 1242 (-C-H symmetrical deformation of -CH\(_2\) group), 1106 (-C-O-C stretching); Anal. Calcd. C\(_{17}\)H\(_{12}\)O\(_6\): C, 65.39; H, 3.87; O, 30.74; Found: C, 65.19; H, 3.77; O, 30.43%.

5.8.8 2-Hydroxyphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-508)

Yield: 52%; MP 207-210 °C; MS: m/z 312, IR (cm\(^{-1}\)): 3340 (-O-H stretching of free -OH) 3101 (-O-H stretching), 3043 (-C-H stretching of aromatic ring), 2833 (-C-H asymmetrical stretching of -CH\(_2\) group), 1703, (>C=O stretching of coumarin), 1603, 1341 (-C=C stretching of aromatic ring), 1323 (-C-H asymmetrical deformation of Alkyl group), 1233 (-C-H symmetrical deformation of -CH\(_2\) group), 1136
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(-C-O-C stretching); Anal. Calcd. C_{17}H_{12}O_6: C, 65.39; H, 3.87; O, 30.74; Found: C, 65.05; H, 3.43; O, 30.24%.

5.8.9 2-Bromophenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-509)

Yield: 46%; MP 195-200 °C; MS: m/z 375, IR (cm\(^{-1}\)): 3124 (-O-H stretching), 3075 (-C-H stretching of aromatic ring), 2812 (-C-H asymmetrical stretching of -CH\(_2\) group), 1704, (>C=O stretching of coumarin), 1611, 1342 (-C=C stretching of aromatic ring), 1323 (-C-H asymmetrical deformation of Alkyl group), 1243 (-C-H symmetrical deformation of -CH\(_2\) group), 1136 (-C-O-C stretching); 810 (-C-Br stretching); Anal. Calcd. C_{17}H_{11}BrO_5: C, 54.42; H, 2.96; Br, 21.30; O, 21.32; Found: C, 54.16; H, 2.75; Br, 21.12; O, 21.11%.

5.8.10 4-Bromophenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-510)

Yield: 58%; MP 199-200 °C; MS: m/z 375, IR (cm\(^{-1}\)): 3120 (-O-H stretching), 3070(-C-H stretching of aromatic ring), 2810 (-C-H asymmetrical stretching of -CH\(_2\) group), 1704, (>C=O stretching of coumarin), 1610, 1342 (-C=C stretching of aromatic ring), 1320 (-C-H asymmetrical deformation of Alkyl group), 1240 (-C-H symmetrical deformation of -CH\(_2\) group), 1130 (-C-O-C stretching); 810 (-C-Br stretching); Anal.
Studies on Phenyl Ester of Coumarin 4-Acetic acid

Calcd. C\textsubscript{17}H\textsubscript{11}BrO\textsubscript{5}: C, 54.42; H, 2.96; Br, 21.30; O, 21.32; Found: C, 54.06; H, 2.65; Br, 21.06; O, 21.21%.

5.8.11 2-Chlorophenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-511)

Yield: 55%; MP 199-200 °C; MS: \textit{m/z} 330, IR (cm\textsuperscript{-1}): 3133 (-O-H stretching), 3085 (-C-H stretching of aromatic ring), 2874 (-C-H asymmetrical stretching of -CH\textsubscript{2} group), 1701, (>C=O stretching of coumarin), 1610, 1324 (-C=C stretching of aromatic ring), 1315 (-C-H asymmetrical deformation of Alkyl group), 1235 (-C-H symmetrical deformation of -CH\textsubscript{2} group), 1134 (-C-O-C stretching); 845 (-C-Cl stretching); Anal. Calcd. C\textsubscript{17}H\textsubscript{11}ClO\textsubscript{5}: C, 61.74; H, 3.35; Cl, 10.72; O, 24.19; Found: C, 61.74; H, 3.23; Cl, 10.34; O, 24.01%.

5.8.12 4-Chlorophenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-512)

Yield: 57%; MP 195-200 °C; MS: \textit{m/z} 330, IR (cm\textsuperscript{-1}): 3131 (-O-H stretching), 3075 (-C-H stretching of aromatic ring), 2844 (-C-H asymmetrical stretching of -CH\textsubscript{2} group), 1707 (>C=O stretching of coumarin), 1610, 1314 (-C=C stretching of aromatic ring), 1315 (-C-H asymmetrical deformation of Alkyl group), 1215 (-C-H symmetrical deformation of -CH\textsubscript{2} group), 1114 (-C-O-C stretching); 815 (-C-Cl stretching); Anal.
Studies on Phenyl Ester of Coumarin 4-Acetic acid

Calcd. C_{17}H_{11}ClO_{5}: C, 61.74; H, 3.35; Cl, 10.72; O, 24.19; Found: C, 61.74; H, 3.03; Cl, 10.14; O, 24.11%.

5.8.13 2-Nitrophenyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-513)

![Chemical structure of 2-Nitrophenyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate]

Yield: 61%; MP 190-195 °C; MS: m/z 341, IR (cm^-1): 3116 (-O-H stretching), 3045 (-C-H stretching of aromatic ring), 2830 (-C-H asymmetrical stretching of -CH_{2} group), 1710 (>C=O stretching of coumarin), 1512 (-N-O stretching of -NO_{2}); 1612, 1311 (-C=C stretching of aromatic ring), 1312 (-C-H asymmetrical deformation of Alkyl group), 1215 (-C-H symmetrical deformation of -CH_{2} group), 1102 (-C-O-C stretching); Anal. Calcd. C_{17}H_{11}NO_{7}: C, 59.83; H, 3.25; N, 4.10; O, 32.82; Found: C, 59.80; H, 3.05; N, 4.00; O, 32.53%.

5.8.14 3-Nitrophenyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-514)

![Chemical structure of 3-Nitrophenyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate]

Yield: 61%; MP 185-190 °C; MS: m/z 341, IR (cm^-1): 3110 (-O-H stretching), 3045 (-C-H stretching of aromatic ring), 2850 (-C-H asymmetrical stretching of -CH_{2} group), 1750, (>C=O stretching of coumarin), 1515 (-N-O stretching of -NO_{2}); 1614, 1314 (-C=C stretching of aromatic ring), 1317 (-C-H asymmetrical deformation of Alkyl group), 1216 (-C-H symmetrical deformation of -CH_{2} group), 1107 (-C-O-C stretching).
Studies on Phenyl Ester of Coumarin 4-Acetic acid

stretching); Anal. Calcd. C_{17}H_{11}NO_{7}: C, 59.83; H, 3.25; N, 4.10; O, 32.82; Found: C, 59.74; H, 3.01; N, 4.01; O, 32.43%.

5.9 Results and discussion

Various Phenolic esters of 7-Hydroxycoumarin-4-Acetic acid derivatives were prepared by reaction of different Phenols with the 7-Hydroxycoumarin-4-acetic acid. The compounds prepared in this chapter possess chromene nucleus and possesses ester linkage.

5.10 Spectra of some compounds

5.10.1 Mass spectra

Mass spectra of 4-Methoxyphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate (KSP-502)
Studies on Phenyl Ester of Coumarin 4-Acetic acid

Mass spectra of 2,5-Dimethylphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-506)

![Mass spectra](image1)

M.Wt. 324 gm/mol

5.9.2 IR spectra

IR spectra of 4-Methoxyphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-502)

![IR spectra](image2)
Studies on Phenyl Ester of Coumarin 4-Acetic acid

IR spectra of 2,5-Dimethylphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate (KSP-506)

5.9.3 $^1$HNMR spectra

$^1$HNMR spectra of 4-Methoxyphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetate (KSP-502)
Studies on Phenyl Ester of Coumarin 4-Acetic acid

$^1$HNMR spectra of 2,5-Dimethylphenyl-2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate (KSP-506)

5.10 Biological evaluation

5.10.1 Antimicrobial evaluation

All of the synthesized compounds (KSP- 501 to 514) were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method$^{30,33}$ with two Gram-positive bacteria Staphylococcus aureus MTCC-96, Streptococcus pyogenes MTCC 443, two Gram-negative bacteria Escherichia coli MTCC 442, Pseudomonas aeruginosa MTCC 441 and three fungal strains Candida albicans MTCC 227, Aspergillus Niger MTCC 282, Aspergillus clavatus MTCC 1323 taking Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin and Greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MT3CC), Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards$^{30}$.
**Minimal Inhibition Concentration [MIC]:**

The main advantage of the ‘Broth Dilution Method’ for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

- Serial dilutions were prepared in primary and secondary screening.
- The control tube containing no antibiotic is immediately subculturated (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight.
- The MIC of the control organism is read to check the accuracy of the drug concentrations.
- The lowest concentration inhibiting growth of the organism is recorded as the MIC.
- The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

**Methods used for primary and secondary screening:**

Each synthesized drug was diluted obtaining 2000 μg mL⁻¹ concentration, as a stock solution. Inoculum size for test strain was adjusted to 10⁸ cfu (colony forming unit) per milliliter by comparing the turbidity.

**Primary screen:** In primary screening 1000 μg mL⁻¹, 500 μg mL⁻¹ and 250 μg mL⁻¹ concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

**Secondary screen:** The drugs found active in primary screening were similarly diluted to obtain 200 μg mL⁻¹, 100 μg mL⁻¹, 50 μg mL⁻¹, 25 μg mL⁻¹, 12.5 μg mL⁻¹, and 6.250 μg mL⁻¹ concentrations.

**Reading Result:** The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10⁸ organism/mL.

The results obtained from antimicrobial susceptibility testing are depicted in Table 1.
### Table-1: *In vitro* Antimicrobial Screening Results for KSP-501 to 514

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5.11 References