Chapter 2

Synthesis of Coumarin Based Blue Emitting Fluorescent Probes with Anticancer activity- New Entries to Fluorophore Library

2.1. Introduction

As explained in the previous chapter, coumarins are a group of compounds with a wide variety of biological properties. In addition to biological applications, fluorescence labeling\(^1\) is another important application of coumarin molecules. Fluorescent labeling of biomolecules is an emerging field in bioanalytical chemistry that enables researchers to detect specific components of complex biological assemblies with sharp sensitivity and selectivity. Identification of biomolecular systems like amino acids, peptides, proteins, DNA etc. can be done by using sensitive fluorescence detectors like organic fluorophores through the in vivo or in vitro interaction\(^2\). The interaction of probes with the targets causes changes in the spectroscopic properties of the probes and these changes can be used for decoding the information about the targets. Among the various fluorogenic probes based on fluorochromes such as coumarin, rhodamine, cyanine, naphthalene, quinoline, squaraine, xanthene, etc,\(^3\) the coumarin based molecules are more prominent and the commercial versions of such fluorophores include Alexa Fluor® dyes, AMCA, (Fig. 2.1) and DyLight Fluors\(^4\). All these dyes are derivatives of aminomethylcoumarin carboxylic acids and most of them are substituted with electron attracting and electron repelling groups at
their 3 and or 7 positions. 7-diethylaminocoumarin-3-carbonyl group used for the labeling of Human Amyloid β-(1–40) protein which is believed to be responsible for Alzheimer’s disease, 3-azido-7-hydroxy coumarin used for imaging proteins, glycoconjugates, DNA etc are some of the interesting examples of such coumarin probes.

In all these examples, coumarin moiety is the key structural unit enabling the probe properties and are usually made by linking a signaling coumarin (which undergoes spectroscopic changes during interaction with target) with a labeling moiety (which enables reaction with the target) with or without the aid of a spacer by following multi-step synthetic protocols.\(^3,7\)

![Figure 2.1](image.png)

**Figure 2.1.** Representative examples of commercially available blue emitting fluorescent dyes

The cost of these fluorescent dyes can be significantly reduced through the replacement of such resource intensive multistep protocols with multicomponent coupling reactions\(^8\) coupled with other green methodologies. This chapter presents the study on the synthesis of a new series of blue light emitting fluorescent probes based on the fusion of amide moiety to the coumarin core via a four component reaction.
2.2. Results and discussions

2.2.1. Synthesis of coumarin based amidoketones

We started our work with the synthesis of N-substituted coumarin amido ketones 2.1 and its representative synthesis is given in Scheme 2.1.

\[
\begin{align*}
\text{O} & \quad \text{Cl} \\
\text{O} & \quad \text{O} \\
\text{C} & \quad \text{H}_3 \\
\text{P} & \quad \text{h} \\
\text{B} & \quad \text{(O)} \\
\text{r} & \quad \text{t} \\
4 \text{hr} \\
\text{H} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\text{R} & \quad 1 \\
\text{CN} & \quad \text{Cl} \\
\text{O} & \quad \text{R} & \quad 1 \\
\end{align*}
\]

**Scheme 2.1.** The general synthetic scheme for the generation of coumarin amido ketones.

N-substituted β-amino carbonyl compounds are usually prepared by following an alternative Mannich type reaction between a pair of enolizable and non enolizable carbonyl compounds in presence of an acid chloride and a nitrile source. Researches from our group as well as from other laboratories have shown that this reaction can be catalyzed in many ways and as a result, a good collection of catalysts are now available in chemist’s showcases for promoting this reaction. This list includes cyanuric chloride, Iodine, BiCl\textsubscript{3}, metal bisulphates, CeCl\textsubscript{3}.7H\textsubscript{2}O, Iron (III) chloride, Mont K10, selectfluor\textsuperscript{TM}, copper(II) phthalocyanines, and copper salts\textsuperscript{9}. Many of these catalysts suffer serious drawback of poor stereoselectivity and some of them require the use in stoichiometric amounts.

Apart from a collection of catalysts, in the context of developing reactions that use catalysts based on inexpensive and non-toxic materials, boronic acids are attracting increased attention of both
Boronic acids were employed for catalyzing reactions (BAC) such as direct activation of alcohols and carboxylic acids, esterifications and amidations, imine hydrolysis, epoxide opening, Biginelli reaction, cycloadditions, aldol condensation, Friedel Crafts alkylations, Nazarov cyclizations, etc. However, its use in catalyzing reactions for the synthesis of advanced functional molecules, stereoselective multicomponent reactions etc. are rare. We reasoned that boronic acids can effectively catalyze the alternative Mannich type reaction for the synthesis of N-substituted β- amino carbonyl compounds.

As shown in Scheme 2.1, compound 2.1 is prepared by condensing 3-acetyl coumarin with an aromatic aldehyde, acetyl chloride, and acetonitrile in the presence of 20 mol % of phenyl boronic acid at room temperature. Optimization reactions for the synthesis of 2.1a were carried out for finding out the amount of catalyst and temperature requirements. In the present work, we used two different boronic acid derivatives; 1A and 1B for catalyst screening to synthesize coumarin β-amidoketone scaffolds. For optimizing the reaction conditions, we have carried out a reaction between benzaldehyde and 3-acetyl coumarin using phenyl boronic acid 1A. Optimizations were carried out in terms of the amount of catalyst and temperature while keeping acetonitrile as solvent in all the cases. Taking into account of our previous experiences with Mont K10 and Selectfluor™, we decided to carry out the screening experiments at room temperature and at the boiling temperature of acetonitrile.
The efficiency of phenyl boronic acid (1A) and 2, 6-difluoro phenyl boronic acid (1B) for catalyzing this reaction at room temperature and at the boiling point of acetonitrile were studied. As given in Table 1, the room temperature reactions with 20 mol\% of both the catalysts afforded maximum amount of products and the performance of both the catalysts were found to be almost equal. Since 1A is cheaper than 1B, we used 1A for further studies.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Loading (mol%)</th>
<th>T°C</th>
<th>Time</th>
<th>Yield (%)</th>
</tr>
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<tr>
<td>1</td>
<td>1A</td>
<td>5</td>
<td>rt</td>
<td>4h</td>
<td>76</td>
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<td>2</td>
<td>1A</td>
<td>10</td>
<td>rt</td>
<td>4h</td>
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<tr>
<td>3</td>
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<td>15</td>
<td>rt</td>
<td>4h</td>
<td>79</td>
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<td>1B</td>
<td>20</td>
<td>70</td>
<td>4h</td>
<td>83</td>
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</tbody>
</table>

Following this protocol, we have synthesized 10 coumarin acetamides with various substitution patterns at the alkyl amide part (Table 2.2) and an aqueous work-up of the reaction mixture was sufficient to afford the products in near quantitative amount with high purity. The products with an electron withdrawing group at the acetamide phenyl
ring gave better yield, compared to the products with electron donating groups at the same phenyl. A marginal decrease in yield was observed for 2.1e which was formed in 52% yield. In this case, we have isolated a side product from the reaction mixture (α,β-unsaturated ketone, 26%) formed via an aldol type reaction.

2.2.2. Mechanism

Phenylboronic acid here acts as a catalyst for the formation of amidoketone skeleton. The suggested mechanism of the reaction is shown in Scheme 2.2.

Scheme 2.2. Proposed catalytic cycle for the formation of coumarin amido ketone using phenyl boronic acid catalyst

The reaction is initiated by the co-ordination of the carbonyl oxygen of the coumarin moiety with the metal atom of the catalyst. Phenylboronic acid acts as a Lewis acid and thus activates the enol 2.2c formation. The addition of aldehyde moiety 2.2d followed by acid
chloride to this complex resulted in the carbon-carbon bond formation to produce a β-acyloxy ketone derivative 2.2e. The acyloxy group in 2.2e is then displaced by the more nucleophilic nitrogen of the nitrile to produce a stable cation intermediate 2.2f. Addition of water leads to the formation of the coumarin amido ketone derivative 2.1.

2.2.3 Photophysical studies of coumarin amido ketones

We then moved on to study the photophysical properties of the newly synthesized molecules. The fluorophoric properties of all the compounds were studied by measuring the absorption and emission spectra in dichloromethane from 0 to 10 pH. As a representative example, the normalized absorption and emission spectra of 2.1f recorded in dichloromethane at neutral pH is given in Figure 2.2. Compound 2.1f showed absorption maximum centered at 304 nm and an emission maximum centered at 431 nm with a high Stokes shift value (the distance between the excitation maxima and emission maxima) 127 nm.
Table 2.2. List of Beta Fluors synthesized

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>% Yield</th>
<th>$\lambda_{\text{Abs}}$ (nm)</th>
<th>$\lambda_{\text{Em}}$ (nm)</th>
<th>Stokes shift (nm)</th>
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</thead>
<tbody>
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<td>1</td>
<td><img src="image1" alt="Compound 1" /></td>
<td>94%</td>
<td>345</td>
<td>436</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Compound 2" /></td>
<td>99%</td>
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<td>428</td>
<td>125</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Compound 3" /></td>
<td>96%</td>
<td>305</td>
<td>386</td>
<td>81</td>
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<tr>
<td>4</td>
<td><img src="image4" alt="Compound 4" /></td>
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<tr>
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<td><img src="image5" alt="Compound 5" /></td>
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<td>346</td>
<td>426</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6" alt="Compound 6" /></td>
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<td>431</td>
<td>127</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7" alt="Compound 7" /></td>
<td>72%</td>
<td>304</td>
<td>422</td>
<td>118</td>
</tr>
<tr>
<td>8</td>
<td><img src="image8" alt="Compound 8" /></td>
<td>94%</td>
<td>346</td>
<td>436</td>
<td>90</td>
</tr>
<tr>
<td>9</td>
<td><img src="image9" alt="Compound 9" /></td>
<td>91%</td>
<td>303</td>
<td>382</td>
<td>79</td>
</tr>
<tr>
<td>10</td>
<td><img src="image10" alt="Compound 10" /></td>
<td>98%</td>
<td>304</td>
<td>393</td>
<td>89</td>
</tr>
<tr>
<td>11</td>
<td><img src="image11" alt="Compound 11" /></td>
<td>55%</td>
<td>348</td>
<td>422</td>
<td>74</td>
</tr>
</tbody>
</table>
These values were found to be stable to the changes in pH from 0 to 10. Fluorophores with high Stokes shift values are highly useful for bio-imaging applications, because, when using such compounds, there is no possibility of overlapping the excitation wavelengths with the emission wavelengths and therefore it is easy to detect the fluorescence emission from biological targets without background errors\(^{20}\). All the compounds \(2.1a-k\) gave fluorescence emission at the blue emitting region with high Stokes shift values and remains intact to changes in pH. The substituent effects on the absorption/emission properties of \(2.1a-k\) did not follow any pattern. The molecules \(2.1b, 2.1c, 2.1f, 2.1g, 2.1i\) and \(2.1j\) showed absorption maxima at 303–305 nm region and \(2.1a, 2.1d, 2.1e, 2.1h\) and \(2.1k\) showed the same at 345–350 regions. In the emission part, the molecules \(2.1a, 2.1b, 2.1d, 2.1e–h,\) and \(2.1k\) showed emission maxima at 422–436 region and \(2.1c, 2.1i,\) and \(2.1j\) showed the same at 382–393 regions.

\[\text{Figure 2.2.} \text{ The normalized absorption and emission spectra of blue emitting ‘Beta Fluor’ 2.1f in dichloromethane at neutral pH with large Stokes shift value (127nm)}\]
The absorption and emission spectra of our compounds **2.1a–k** were then compared with the same of commercially available coumarin based fluorescent probe Alexa Fluor 350. The fluorescence spectra for the commercial molecules were obtained from web sources and the comparisons revealed that, the fluorescence properties of **2.1a–k** are comparable with Alexa Fluor 350. Since the alkyl amide part of **2.1a–k** are β-amido ketones and are considered as β-amino acid derivatives, we decided to name our molecules as ‘Beta Fluors’.

### 2.3. Biological Study

#### 2.3.1. Primary evaluation of drug-likeness

The drug-likeness of the molecules were reported based on Lipinski’s rule\(^2^1\) and mainly depends on molecular size, lipophilicity as well as polarity (topological polar surface area; tPSA)\(^2^2\). Drug like molecules usually have log P values between -0.4 and 5.6 and a molecular weight of <500. An orally bioavailable drug will have tPSA between 75 and 160 Å\(^2\). The drug property descriptors of our molecules were calculated using molinspiration calculation service. The logP values of these compounds were obtained in the range from 0.92 to 2.53 with tPSA between 76 and 122 Å\(^2\). Similarly all the molecules have a molecular weight <500 indicating that these molecules are well within the limits of Lipinski’s rule of five suitable for further explorations on biological activities.
Table 2.3. Drug-Likeness parameters of Beta Fluors

<table>
<thead>
<tr>
<th>Entry</th>
<th>milogP</th>
<th>tPSA</th>
<th>MW</th>
<th>nON</th>
<th>nOHNH</th>
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<tbody>
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<td>76.38</td>
<td>335.36</td>
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</tr>
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<td>2.1b</td>
<td>2.19</td>
<td>76.38</td>
<td>369.80</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>2.1c</td>
<td>2.24</td>
<td>76.38</td>
<td>369.80</td>
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</tr>
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<td>2.1d</td>
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<td>2.1g</td>
<td>0.90</td>
<td>105.84</td>
<td>381.38</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>2.1h</td>
<td>2.53</td>
<td>76.38</td>
<td>428.80</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>2.1i</td>
<td>1.50</td>
<td>122.20</td>
<td>380.36</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>2.1j</td>
<td>2.37</td>
<td>76.38</td>
<td>414.25</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>2.1k</td>
<td>2.32</td>
<td>76.38</td>
<td>414.25</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

2.3.2. The in vitro anti cancer activity evaluation

Based on the promising drug-likeness parameters obtained from computational studies, we decided to study the cytotoxicity of our molecules. Out of various disease classes, cancer is a major global problem due to the uncontrolled growth of abnormal cells. Among various cancerous varieties, Breast cancer is the most common form of cancer present in women and is the second leading cause of death after lung cancer. According to literature reports, one in every eight women develops metastatic breast cancer in her lifetime. Therefore, there is a tremendous unmet need for more safe, potent and selective anticancer drugs. Since coumarin family is well known for its very rare nephrotoxicity, hepatotoxicity, cardiotoxicity, dermal toxicity and other biological effects, we decided to screen our molecules against human breast cancer cell line MCF-7
2.3.2. (A) **Cell culture and maintenance**

Human breast cancer MCF-7 (purchased from National Centre for Cell Science, Pune, India) cells were maintained in RPMI medium 1640 supplemented with 10% fetal bovine serum as well as 100 µg/mL streptomycin, 100 U/mL penicillin, 2 mM-glutamine and Earle’s BSS adjusted to contain 1.5 g/l Na bicarbonate, 0.1 mM nonessential amino acids, and 1.0 mM of Na pyruate in a humidified atmosphere containing 5% CO₂ at 37°C.

2.3.2. (B) **In vitro cytotoxicity of synthesized 2.1f**

Cell viability was determined by MTT assay. MCF-7 cells were seeded in 96-well plates at a concentration of 1.0x10⁴ cells/well and incubated overnight at 37°C in a 5% CO₂ humidified environment. Then the cells were treated with different concentrations of the sample 2.1f like 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µM/mL (dissolved with RPMI medium 1640), respectively. Controls were cultivated under the same conditions without addition of 2.1f. The treated cells were incubated for 48 h for cytotoxicity analysis. The cells were then subjected for MTT assay. The stock concentration (5 mg/mL) of MTT-(3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) was prepared and 100 µL of MTT was added in each wells and incubated for 4 h. Purple color formazan crystals were observed and these crystals were dissolved with 100 µL of dimethyl sulphoxide (DMSO), and read at 620 nm in a multi well ELISA plate reader (Thermo, Multiskan). The dose dependent cytotoxicity was observed in the case of Beta Fluor (2.1f) treated MCF-7 cells. Fifty percentage of cell death, which determines the inhibitory concentration (IC50) value of 2.1f against MCF-7 cells holds at 8 µM/mL in 48 h.
(Fig. 2.3). Which indicates that these molecules exhibit increased bioavailability and have tremendous anticancer potential.

2.3.2. (C) DAPI (4, 6-diamidino-2-phenylindole dihydrochloride) staining

MCF-7 cells were treated with 2.1f at its IC50 concentration (8 µM/mL) for 48 h, and then fixed with methanol:acetic acid (3:1, v/v) prior to washing with PBS. The washed cells were then stained with 1 mg/mL DAPI (4, 6-diamidino-2-phenylindole, dihydrochloride) for 20 min in the dark atmosphere. Stained images were recorded with fluorescent microscope with appropriate excitation filter. The bright field and fluorescence microscopic images are shown in Fig. 2.4. As shown in this the strong bluish fluorescence and cellular uptake observed in the imaging studies with 2.1f reveals that these molecules have high potency against breast cancer cell lines (MCF-7).

![Figure 2.3](image)

**Figure.** 2.3. MTT assay results confirming the in vitro cytotoxicity effect of 2.1f against the MCF-7 cells. The detected IC50 concentration was 8 µM/mL.
Figure 2.4. Bright field inverted light microscopy images (a, b, c) and the DAPI nuclear staining (d, e, f) of control cells and 2.1f treated cells. The DAPI images exhibit condensed form of nuclear materials in apoptotic cells.

2.4. Structure elucidation by spectroscopy

2.4.1. Structure identification of $\text{N}-(3\text{-oxo-3-(2-oxo-2H-chromen-3-yl)-1-phenylpropyl})$acetamide 2.1a.

For the spectroscopic identification of molecules, compound 2.1a is taken as the representative from coumarin amido ketone library. The molecule is numbered as shown in figure 2.5. The FT-IR spectrum
of the compound 2.1a (Fig. 2.6) gives major absorptions at 3318, 1747, 1681, 1648, 1610, and 1560 cm⁻¹. The band at 3318 cm⁻¹ is due to the NH stretching vibration of the acetamido group. The peak at 1747 cm⁻¹ is due the lactone part at position C21. The amide I band, i.e., the band due to the C=O stretching vibration of (C2) occurs at 1648 cm⁻¹ and the amide II band which arises from the interaction between the N-H bending and the C-N stretching of the C-N-H group is obtained at 1560 cm⁻¹. The absorption at 1681 cm⁻¹ is due to the C=O stretching vibration of the ketone part (C12).

**Figure 2.6.** FT-IR spectrum of the compound 2.1a

The initial information obtained from FT-IR spectrum for the formation of coumarin acetamido ketone was further confirmed by analyzing the ¹H NMR spectrum (Fig. 2.7.). In the ¹H NMR spectrum, a strong singlet obtained at δ = 8.65 is due to the coumarin CH at position 14. The formation of amide bond is confirmed via the doublet peak obtained for the NH proton at δ = 8.35-8.33. The 9 aromatic protons appear from δ = 7.97-7.20. The doublet at δ =7.97-7.95 is due to the proton at carbon 16 on the benzene ring on coumarin part. At the same part, there is a proton at C18 which appears as a triplet with δ
=7.78-7.74. The remaining two protons in that part appeared as a multiplet between \( \delta = 7.49-7.41 \). Another multiplet at \( \delta = 7.37-7.30 \) is attributed to the presence four aromatic protons on benzene ring. The remaining single proton at C8 is a triplet due to the two adjacent protons and observed at \( \delta = 7.25-7.20 \). The CH proton at position 4 is observed as a multiplet at \( \delta = 5.42-5.36 \). The two protons on C11 are in different chemical environments due to the presence of the adjacent chiral carbon C4. In the \(^1\)H NMR spectrum, these two proton signals are observed between \( \delta = 3.59-3.53 \) and between \( \delta = 3.45-3.38 \) respectively. Both these signals observed as doublet of doublets with approximately equal coupling constants (16 and 20 Hz) and the splitting of signals is occurred due to the spin-spin couplings of the protons of the same carbon and of the adjacent carbon C4. Finally, the three methyl protons at position C1 are observed as a singlet at \( \delta = 1.79 \).

![Figure 2.7. \(^1\)H NMR spectrum of the compound 2.1a](image-url)
In the $^{13}$C NMR spectrum, the ketone carbonyl carbon C12 and the amide carbonyl C2 are observed at δ 195 and 168 respectively (Fig. 2.8). The lactone carbonyl peak at C21 is observed as a downfield peak at 158. The signals at δ 155, 147, 143, 135, 131, 128, 125, 124, 118 and 116 are due to the aromatic carbons. The other three up field resonances i.e., at δ 48.9, 48.7 and 23 are attributed to C11, C4 and C1 respectively.

![Figure 2.8. $^{13}$C spectrum of the compound 2.1a](image)

**2.5. Conclusion**

In conclusion, in this chapter we have discussed the development of a new series of coumarin based blue emitting fluorescent probes through a low-cost and green synthetic method using a boronic acid catalyzed multicomponent coupling strategy. These fluorescent probes are having comparable performance with
commercial molecules of the same category. All the molecules showed emission in the blue region and are featured with the presence of enough number of diversity points useful for substitution with electron withdrawing or donating groups for fine tuning the emission properties. In vitro biological evaluation of the representative molecule was also done and the molecules showed excellent cytotoxicity against human breast cancer cell line MCF-7 indicating the potential of this molecule for further development as potential anticancer agents.

2.6. Experimental

2.6.1. Materials and methods

All solvents and reagents were of reagent grade quality from Aldrich Chemical Company, Fluka, or Merck and used without any further purification. Reactions were monitored by thin-layer chromatography (TLC) using plates prepared with Merck silica gel G by irradiation with UV light and/or treatment with iodine. Fourier transform infrared (FT-IR) spectra were recorded on a Jasco FTIR-4100 spectrometer. $^1$H and $^{13}$C NMR spectra were determined in CDCl$_3$ and DMSO with a Bruker amx 500 MHz spectrometer. The chemical shifts (δ) are given relative to tetramethyl silane (TMS) and the coupling constants (J) are reported in hertz (Hz). Absorption spectra of the compounds were recorded on JASCO V 550 UV/Vis spectrophotometer and Fluorescence measurements were carried out on Perkin Elmer LS 55 spectrophotometer.
2.6.2. General procedure for the one-pot synthesis of N-(3-oxo-3-(2-oxo-2H-chromen-3-yl)-1-phenylpropyl)acetamide (2.1a). A 100 mL round-bottomed flask was charged with anhydrous acetonitrile (10 mL), benzaldehyde (0.212 g, 2 mmol), 3-acetyl coumarin (376 mg, 2 mmol), acetyl chloride (4 mL), and phenyl boronic acid (20 mol %) under constant stirring at room temperature. The reaction was monitored by TLC and was found to complete after 4 h. The mixture was then poured into distilled water and kept for 1 h. The precipitated colourless solid was collected on a filter, washed with distilled water (3x 25 mL), and dried under vacuum. The vacuum-dried solid was then washed with anhydrous diethyl ether (2x15 mL) and air dried to obtain the product 2.1a in pure form (630 mg, 94%). $^1$H NMR(400MHz, DMSO-d6): δ 1.79 (3H, s); 3.45-3.38 (1H, dd, J=8 Hz, J=20 Hz), 3.59-3.53 (1H, dd, J=8 Hz, J=16 Hz), 5.42-5.36 (1H, m), 7.25-7.20 (1H, t), 7.37-7.30 (4H, m) 7.49-7.41(2H, m), 7.78–7.74(1H,t), 7.97-7.95(1H, d, J=8Hz), 8.35-8.33 (2H, d, J=8 Hz), 8.65 (1H s); $^{13}$CNMR(100MHz, DMSO-d6): δ 195, 168, 158, 155, 147, 143, 135, 131, 128, 125, 124, 118, 116, 48, 23; FT-IR (KBr) $\nu_{\text{max}}$: 3318, 2953, 2851, 1747,1681,1648,1610, 1560, 1493, 1448, 1369,1174, 982, 831,764, 543 cm$^{-1}$.

2.6.3. Spectral data of compounds

N-(1-(2-chlorophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl) propyl) acetamide (2.1b) $^1$H NMR (400 MHz, DMSO-d6): δ 8.69 (1H, s), 8.34-8.32 (1H, d, J= 8 Hz), 7.98-7.96 (1H, d, J=8 Hz), 7.79-7.75 (1H, m), 7.49-7.43 (4H, m), 7.37-7.26 (2H, m), 5.63-5.58 (1H, m), 3.61-3.54 (1H, dd, J = 12 Hz, J=20 Hz), 3.49-3.44 (1H, dd, J= 8 Hz, J=16
Hz), 1.81 (3H, s); $^{13}$C NMR (100 MHz, DMSO-d6): δ 194.3, 168.2, 161.5, 158.1, 155.5, 147.6, 140.3, 135.2, 131.1, 129.9, 129.5, 128.6, 127.5, 125.6, 124.2, 118.1, 116.6, 47.3, 46.1, 22.2; FT-IR (KBr) ν$_{max}$: 3292, 2922, 2853, 1730, 1685, 1649, 1608, 1561, 1509, 1487, 1454, 1373, 1298, 1256, 1175, 1046, 758, cm$^{-1}$;

**N-(1-(4-chlorophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-y1)propyl)acetamide (2.1c)** $^1$H NMR (400 MHz, DMSO-d6): δ 8.65(1H, s), 8.39-8.37 (1H, d, J= 8 Hz,), 7.97-7.95 (1H, d, J=8 Hz), 7.81-7.69 (1H, t), 7.49-7.41(2H, m), 7.37(1H, s), 5.39-5.33(1H, m), 3.58-3.52 (1H, dd, J=4 Hz, and J= 6 Hz), 3.47-3.40 (1H, dd, J=8Hz, J=20 Hz), 1.80 (3H, s); $^{13}$C NMR(100 MHz, DMSO-d6): δ 194.2, 168.3, 158.5, 155.4, 147.6, 142.1, 135.8, 131.7, 131.6, 128.1, 128.4, 125.1, 114.6, 118.6, 116.4, 48.5, 48.8, 23.6 ; FT-IR (KBr) ν$_{max}$: 3310, 2922, 2852, 1746,1681,1647,1611, 1560, 1541, 1492, 1448, 1370, 1295, 1175, 1090, 1014, 984, 822, 765, 594, 535cm$^{-1}$

**N-(1-(2-fluorophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-y1)propyl)acetamide (2.1d)** $^1$H NMR (400 MHz, DMSO-d6): δ 8.66 (1H, s), 8.34-8.32 (1H, d, J=8 Hz), 7.97-7.95 (1H, d, J=8 Hz), 7.77-7.76 (3H, t), 7.49-7.40 (3H, m), 7.32-7.27 (1H, m), 7.19-7.12 (1H, m), 5.63-5.58 (1H, m), 3.61-3.54 (1H, dd, J = 8 Hz and J = 20 Hz), 3.49-3.17 (1H,dd, J = 8Hz and J = 20 Hz), 1.80 (3H, s); $^{13}$C NMR (100 MHz, DMSO-d6): δ 194, 168, 161, 158, 155, 147, 135, 131, 130, 130, 129, 129, 128, 128, 125, 124,124, 118, 16, 115, 47, 43, 22; FT-IR (KBr)ν$_{max}$: 3282, 3074, 2924, 2851, 1731, 1684, 1649, 1608, 1561, 1492, 1455, 1170, 980, 757cm-1.
4-(1-acetamido-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)phenyl acetate (2.1e) $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 8.66 (1H, s), 8.36-8.34 (1H, d, J=8 Hz), 7.97-7.95 (1H, d, J=8 Hz), 7.78-7.74 (1H, m), 7.49-7.37 (4H, m), 7.08-7.06 (2H, m), 5.42-5.37 (1H, m), 3.61-3.55 (1H, dd, J=8 Hz and J=20 Hz, 1H), 3.47-3.40 (1H, dd, J=12 Hz, J=20 Hz), 2.26 (3H, s), 1.80 (3H, s); $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 194.5, 169.9, 168.7, 158.2, 155.6, 149.4, 147.7, 140.7, 135.8, 131.4, 128.6, 125.6, 124.2, 122.3, 118.7, 116.8, 48.9, 48.7, 23.7, 21.6; FT-IR (KBr) $\nu_{max}$: 3315, 2923, 2852, 1747, 1680, 1644, 1612, 1560, 1509, 1448, 1369, 1218, 1176, 1019, 765, 541 cm$^{-1}$

N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)acetamide (2.1f) $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 8.62 (1H, s), 8.28-8.26 (1H, d, J=8 Hz), 7.97-7.94 (1H, d, J=8 Hz), 7.78-7.72 (1H, m), 7.50-7.41 (2H, m), 7.27-7.25 (2H, m), 6.88-6.86 (2H, m), 5.32-5.38 (1H, m), 3.72 (3H, s), 3.58-3.52 (1H, dd, J=8 Hz, J=16 Hz), 3.40-3.34 (1H, dd, J=8 Hz, J=16 Hz), 1.77 (3H, s); $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 195.3, 168.5, 161.4, 158.8, 155.9, 147.1, 135.2, 131.5, 128.6, 125.8, 124.1, 118.3, 116.8, 116.6, 114.8, 55.4, 48.1, 40.6, 23.9; FT-IR (KBr) $\nu_{max}$: 3313.64, 2928, 2856, 1746, 1681, 1644, 1609, 1560, 1514, 1448, 1370, 1290, 1251, 1174, 1030, 982, 831, 764, 543 cm$^{-1}$

N-(1-(4-hydroxy-3-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)acetamide (2.1g) $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 8.66 (1H, s), 8.35-8.33 (1H, d, J=8 Hz), 7.98-7.95 (1H, m), 7.79-7.74 (1H, m), 7.49-7.41 (2H, m), 7.12 (1H, s) 7.02-7.00 (1H, d, J=8 Hz), 6.93-6.90 (1H, m), 5.42-5.36 (1H, m), 4.10-4.06 (1H, s), 3.77 (3H, s),

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3.60-3.55 (1H, dd, J=4 Hz, J=16 Hz), 3.46-3.39 (1H, m), 1.80 (3H, s);  
$^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 194.8, 169.4, 168.2, 158.5, 155.8, 151.1, 147.5, 142.3, 138.5, 135.6, 131.7, 125.4, 124.0, 118.8, 118.4, 116.4, 111.3, 56.8, 49.9, 48.9, 48.6, 23.5, 20.7; FT-IR (KBr)$v_{max}$: 3312, 3069, 28516, 1766, 1731, 1681, 1644, 1609, 1560, 1536, 1513, 1449, 1370, 1291., 1213, 1176, 1154, 1125, 1031, 983, 765 cm$^{-1}$

N-(1-(3-nitrophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)acetamide (2.1i) $^1$HNMR (400 MHz, DMSO-d6): $\delta$ 8.68(1H, s), 8.55-8.53(d, J= 8Hz, 1H), 8.22(1H, s), 8.12-8.09(1H, m), 7.97-7.95(1H, d, J = 8Hz), 7.84-7.75(2H, m), 7.66-7.62(3H, t), 7.49-7.41(2H, m), 5.49-5.55(1H, m), 3.61-3.55(2H, m) 1.83(3H, s);  
$^{13}$C NMR(100MHz, DMSO-d6): $\delta$ 194.2, 169.7, 158.6, 158.1, 155.9, 155.4, 148.8, 148.1, 147.5, 145.6, 135.4, 134.6, 134.1, 131.5, 130.1, 125.2, 125.1, 124.7, 124.3, 122.2, 121.9, 118.2, 116.4, 116.1, 48.5, 48.2, 23.8; FT-IR (KBr)$v_{max}$: 3302, 3085, 2924, 2852, 1746, 1698, 1643, 1612, 1560, 1550, 1446, 1348, 1173, 978, 762, 692cm$^{-1}$.

N-(1-(4-bromophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)acetamide(2.1j) $^1$HNMR (400MHz,DMSO-d6) : $\delta$ 8.65(1H, s), 8.39-8.37(1H, d, J = 8Hz), 7.97-7.95(1H, d, J=8Hz), 7.78-7.74(1H, t), 7.53-7.41(4H, m), 7.33-7.29(2H, m), 5.37-5.31(1H, m), 3.57-3.52(1H, dd, J=4 Hz, J=16 Hz) 3.46-3.40(1H, dd, J=8 Hz, J=16Hz), 1.79 (3H, s); $^{13}$C NMR (100 MHz, DMSO-d6): 197.5, 168.8, 158.7, 155.5, 147.6, 142.8, 135.1, 131.6, 131.3, 129.4, 125.5, 124.4, 120.1, 118.5, 116.9, 48.8, 48.2, 23.9.; FT-IR (KBr)$v_{max}$: 3298, 2923, 2853, 1746, 1688, 1646, 1611, 1560, 1542, 1449, 1374, 1175, 1010, 765, cm$^{-1}$
References


Supplementary Material

Copies of $^1$H NMR, $^{13}$C NMR and FT-IR spectra of selected compounds.
FT-IR spectrum of the compound 2.1a

$^1$HNMR spectrum of the compound 2.1a
\(^{13}\)C spectrum of the compound 2.1a

FT-IR spectrum of the compound 2.1b
$^1$HNMR spectrum of the compound 2.1b

$^{13}$C spectrum of the compound 2.1b
FT-IR spectrum of the compound 2.1c

\[ \text{FT-IR spectrum} \]

\[ \text{HNMR spectrum of the compound 2.1c} \]
$^{13}$C spectrum of the compound **2.1c**

FT-IR spectrum of the compound **2.1d**
$^1$HNMR spectrum of the compound 2.1d

$^{13}$C spectrum of the compound 2.1d
FT-IR spectrum of the compound 2.1e

\( ^1\text{HNMR} \) spectrum of the compound 2.1e
$^{13}$C spectrum of the compound 2.1e

FT-IR spectrum of the compound 2.1f
$^1$HNMR spectrum of the compound 2.1f

$^{13}$C spectrum of the compound 2.1f
FT-IR spectrum of the compound 2.1g

$^1$HNMR spectrum of the compound 2.1g
$^{13}$C spectrum of the compound 2.1g

FT-IR spectrum of the compound 2.1i
$^1$HNMR spectrum of the compound 2.1i

$^{13}$C spectrum of the compound 2.1i
$^1$HNMR spectrum of the compound **2.1j**

FT-IR spectrum of the compound **2.1j**
$^{13}$C spectrum of the compound 2.1j