Chapter 4
Privileged Scaffold Modification of Beta Fluors: Development of Bioactive and Fluorescent Pyridine-Triazole-Coumarin Hybrids through Sequential Click-Multicomponent Reactions

4.1. Introduction

Among the numerous heterocycles, pyridine scaffolds attract considerable attention in medicinal chemistry due to their widespread occurrence in natural molecules with therapeutic properties\(^1\). Consequently, several synthetic drugs have been developed for example, isoniazid (anti-tuberculosis), sulphapyridine (anti-bacterial), A3 adenosine receptor antagonist (anti-inflammatory, anti asthmatic), amirone (cardiotonic) etc\(^2\). Drugs based on 3, 5-dicyano pyridine derivatives are excellent inhibitors of adenosine receptors responsible for hypoxia, cancer, Parkinson disease, cardiovascular disease, asthma, and epilepsy\(^3\). Examples of drug molecules with broad spectrum of applications are presented in Fig 4.1\(^4\). Due the presence of too many polar surface functional groups, the molecules presented in Fig 4.1 are also not free from the drawbacks such as poor protease stability and less circulating plasma half-life.
A recent approach to overcome these drawbacks is the developments of hybrid structures contain two or more structural entities with different biological functions. Such hybrid structures have high degree of selectivity towards multiple targets due to the presence of multiple chromophores in one molecule. In accordance with this concept, Zhang et al reported the synthesis of a coumarin-piperidine diad spaced with an alkane-ether moiety useful for the treatment of schizophrenia. Based on the excellent biological properties we obtained for the coumarin scaffolds reported in chapters 2 and 3, we envisaged that, drug properties and applications of 3, 5-dicyano pyridines derivatives similar to those molecules shown in figure 4.1 can be increased manifold by peptidomimetic randomization of the pyridine core to the Beta Fluors reported in chapter 2 through a linker triazole via copper (I) catalyzed [3+2] azide-alkyne cycloaddition (CuAAC) as shown in figure 4.2.
Figure 4.2. The general structure of the pyridine-triazole-coumarin peptidomimetic hybrid 4.4 and its evolution from prion replication inhibitor (a) and a potential drug candidate for schizophrenia (b) through triazole modification of Beta Fluor (c).

4.2. Results and Discussions

4.2.1. Synthesis of alkynes and azides

We have started our studies with the synthesis of pyridine alkynes 4.1 by following a simple three-component reaction of an aldehyde having a propargyl group, malononitrile and thiophenol as shown in Scheme 4.1. The alkyne functionalization of the aldehydes was done by treating different hydroxyl aldehydes with propargyl bromide in presence of a base catalyst. The propargylated aldehydes were then refluxed with malononitrile and thiophenol in ethanol in the presence of catalytic amount of triethyl amine to afford the pyridine alkynes 4.1 in 92-94% yield.
The reaction proceeds via the formation of Knoevenagel adduct 4.2c with first molecule of malononitrile (4.2a) and aldehyde (4.2b). Thiophenol (4.2d) and the second molecule of malononitrile adds to this adduct to form the pyridine 4.1.

We then moved on to the synthesis of coumarin azides. The compound 4.3a was obtained from a two-step process comprising of an initial Mannich type four-component reaction followed by azide substitution. The four component reaction between 3-acetyl coumarin, bromopropionitrile, an aromatic aldehyde and acetyl chloride afforded the bromo derivative 4.2a. The bromine in 4.2a was then replaced with an azide moiety by treating them with sodium azide under basic condition to afford 4.3a in good to excellent yield (table 4.1). Among the six coumarin azides, those with electron donating substituents (4.3a
and **4.3c** formed in higher yields compared to that of electron withdrawing substituents.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Bromo derivative</th>
<th>Azide</th>
<th>Yield (%)</th>
<th>( \text{Abs}<em>{\text{max}}/\text{Em}</em>{\text{max}} ) nm</th>
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<td>1</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td>94%</td>
<td>312/472 nm</td>
</tr>
<tr>
<td>2</td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
<td>75%</td>
<td>300/460 nm</td>
</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td>91%</td>
<td>336/470 nm</td>
</tr>
<tr>
<td>4</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td>81%</td>
<td>333/457 nm</td>
</tr>
<tr>
<td>5</td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td>79%</td>
<td>311/461 nm</td>
</tr>
<tr>
<td>6</td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
<td>83%</td>
<td>301/451 nm</td>
</tr>
</tbody>
</table>

**4.2.2. Fragment assembly via Click chemistry**

The azide and alkyne fragments were then assembled by following the CuAAC reaction as shown in table **4.2**. In a typical experiment, an equimolar mixture of pyridine alkyne **4.1a** and coumarin azide **4.3a** were dissolved in minimum amount of DMSO. To this, a solvent mixture of t-BuOH and H₂O (4:2) containing 0.2
equivalent of CuSO₄ and 0.4 equivalent of sodium ascorbate were added and stirred for 12 h at room temperature to complete the cycloaddition. The mixture was then diluted with ice-cold water to obtain the peptidomimetic 4.4a in 92% yield. CuAAC reactions with other alkynes and azides under the same condition afforded peptidomimetics 4.4a-l in 80-99% yield as listed in table 4.2.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Yield(%)</th>
<th>Abs&lt;sub&gt;max&lt;/sub&gt; nm</th>
<th>Em&lt;sub&gt;max&lt;/sub&gt; nm</th>
<th>Stokes shift nm</th>
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<tr>
<td>1</td>
<td><img src="image" alt="4.4a" /></td>
<td>92%</td>
<td>300</td>
<td>404</td>
<td>104</td>
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<tr>
<td>2</td>
<td><img src="image" alt="4.4b" /></td>
<td>87%</td>
<td>290</td>
<td>409</td>
<td>119</td>
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<tr>
<td>3</td>
<td><img src="image" alt="4.4c" /></td>
<td>80%</td>
<td>293</td>
<td>404</td>
<td>111</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="4.4d" /></td>
<td>96%</td>
<td>292</td>
<td>400</td>
<td>108</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="4.4e" /></td>
<td>88%</td>
<td>294</td>
<td>402</td>
<td>108</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="4.4f" /></td>
<td>87%</td>
<td>290</td>
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Table 4.2 Continued

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<th>Entry</th>
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<th>Yield(%)</th>
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<th>$\lambda_{\text{Em}}$&lt;sub&gt;max&lt;/sub&gt; nm</th>
<th>Stokes shift nm</th>
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<tr>
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<td>44g</td>
<td>99%</td>
<td>290</td>
<td>481</td>
<td>191</td>
</tr>
<tr>
<td>8</td>
<td>44h</td>
<td>82%</td>
<td>296</td>
<td>472</td>
<td>176</td>
</tr>
<tr>
<td>9</td>
<td>44i</td>
<td>86%</td>
<td>296</td>
<td>490</td>
<td>184</td>
</tr>
<tr>
<td>10</td>
<td>44j</td>
<td>83%</td>
<td>298</td>
<td>474</td>
<td>176</td>
</tr>
<tr>
<td>11</td>
<td>44k</td>
<td>89%</td>
<td>296</td>
<td>470</td>
<td>174</td>
</tr>
<tr>
<td>12</td>
<td>44l</td>
<td>81%</td>
<td>296</td>
<td>478</td>
<td>182</td>
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</table>

4.2.3. Photophysical studies

The absorption and emission spectra of the alkynes, azides and the click products were measured in DMSO at neutral pH. The alkyne,
4.1a showed an emission maximum of 419 nm. Where as 4.1b, (which is structurally an electron releasing methoxy group substituted version of 4.1a) showed an emission maximum at 466 nm and thus a red shift of 47 nm compared to that of 4.1b and showed a Stokes shift of 155 nm. Similarly, the azides showed the absorption maxima in the range of 300-336 nm and emission maxima in the range 451-472 nm with high Stokes shift values ranging from 124-160 nm. These values are higher than the Beta Fluors which are the structural precursors of the coumarin azides. (Fig. 4.3(c)). For example, in the case of azide, the highest Stoke shift value obtained for compound 4.3a is 160 nm whereas the structural analogue Beta Fluor 2.1f has only 127 nm.

**Figure 4.3.** (a) and (b) represents the normalized absorption and emission spectra of the alkyne 4.1b (Stokes shift value 155 nm) and azide 4.3a (Stokes shift value 160 nm). Picture (c) represents the combined plot of Beta Fluor 2.1f and coumarin azide 4.3a in DMSO at neutral pH depicting the increase of Stokes shift value upon derivatization.

The peptidomimetic 4.4a, which is a combination of 4.1a + 4.3a, showed a blue shift in emission and an emission maximum was obtained at 404 nm, which is 68 nm less than that of its azide fragment 4.3a. However, similar studies with peptidomimetic 4.4g (4.1b+4.3a) showed a red shift in emission and afforded an emission maximum at
481 nm which is 9 nm red shifted than that of 4.3a with an enlarged Stokes shift of 191 nm as shown in Fig 4.4. Here, we have used the electron donating OCH$_3$ at the pyridine for the alkyne. This trend was followed in all the remaining peptidomimetic products and their emission maxima were obtained in the range of 472-481 nm with Stokes shift between 174-191 nm. In general, the alkynes, azides and the click peptidomimetics with strong electron donating substituents such as -OCH$_3$ as structural component were showed longer wavelength emission with high Stokes shift values.

Figure 4.4. The normalized absorption and emission spectra of the alkyne 4.1b (Stokes shift value 155 nm), azide 4.3a (Stokes shift value 160 nm) and the click product 4.4g in DMSO at neutral pH.

4.2.4. Computational study

The determination of HOMO-LUMO band gap is important to explain the observed difference in fluorescence properties of the two categories of molecule. For which, we attempted to calculate the band gap values of 4.4a & 4.4g through B3LYP/6-31G method basis set using the Gaussian 09 program$^7$. The HOMO-LUMO band gap as well
as the energy minimized structure of $4.4g$ is shown in Figs. 4.5 and 4.6. From the computational calculations, we obtained a band gap of 2.94 & 2.92 eV respectively for $4.4a$ & $4.4g$. The methoxy substituted molecule ($4.4g$) showed a low band gap of 2.92 eV as compared to that of $4.4a$. The low band gap observed could be due to the presence of an electron donating -OCH$_3$ group in the pyridine scaffold which is significantly decreasing the electron-hole gap and increasing the overlap of the frontier orbitals resulting in a red shift in the emission maxima as observed in photophysical studies.

**Figure 4.5.** The Frontier molecular energy level and band gap of $4.4a$ and $4.4g$. The lower band gap of $4.4g$ is responsible for the higher emission maxima

**Figure 4.6.** The energy minimized structure of $4.4g$
4.3. Biological property evaluation

4.3.1. Primary evaluation of drug-likeness

As done in the previous cases, here also we have calculated the drug property descriptors of all the molecules using the online service, www.molinspiration.com and were tabulated in table 4.3. As shown in table 4.3, the starting pyridine alkynes and the Beta Fluor azides are within the limit of the rule of 5 whereas the click products 4.4a-l are deviating from the rule of five with logP values in between 4.12–6.07, and molecular weight in-between 712–890. Kihlberg et al. recently reported that, extended rule of 5 (eRo5) molecules with MW between 500–700 Da and other properties slightly outside the Ro5 limits and, beyond rule of 5 (bRo5) molecules with MW>500 and one or more properties deviating from eRo5 space are better candidates for modulating difficult and emerging target classes.

4.3.2. The in vitro anti cancer study against MCF-7 Cell lines.

4.3.2. (A): Cell culture and maintenance

A representative compound 4.4g was used for the cytotoxicity evaluation. Human breast cancer MCF-7 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 L-glutamine and Earle’s BSS adjusted to contain 1.5 g/l Na bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM of Na pyruvate in a humidified atmosphere containing 5% CO₂ at 37°C.
Table 4.3. The Drug-likeness parameters of pyridine-triazole-coumarin peptido-mimetics

<table>
<thead>
<tr>
<th>Compound</th>
<th>milogP</th>
<th>tPSA</th>
<th>MW</th>
<th>nON</th>
<th>nOHNH</th>
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<tr>
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<td>5.45</td>
<td>212.06</td>
<td>802.87</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>4.4b</td>
<td>5.39</td>
<td>202.83</td>
<td>830.88</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>4.4c</td>
<td>4.53</td>
<td>238.37</td>
<td>860.91</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>4.4d</td>
<td>6.07</td>
<td>202.83</td>
<td>807.29</td>
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<td>4.4e</td>
<td>5.35</td>
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<td>817.84</td>
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<tr>
<td>4.4f</td>
<td>4.94</td>
<td>229.14</td>
<td>712.85</td>
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<td>3</td>
</tr>
<tr>
<td>4.4g</td>
<td>5.04</td>
<td>221.30</td>
<td>832.90</td>
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<td>3</td>
</tr>
<tr>
<td>4.4h</td>
<td>4.98</td>
<td>212.06</td>
<td>860.91</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>4.4i</td>
<td>4.12</td>
<td>247.6</td>
<td>890.93</td>
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<td>3</td>
</tr>
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<td>837.32</td>
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<td>3</td>
</tr>
<tr>
<td>4.4k</td>
<td>4.94</td>
<td>257.89</td>
<td>847.87</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>4.4l</td>
<td>4.53</td>
<td>238.37</td>
<td>802.87</td>
<td>15</td>
<td>3</td>
</tr>
</tbody>
</table>

4.3.2. (B): In vitro cytotoxicity of synthesized 4.4g

Cell viability was determined by MTT assay. MCF-7 cells were seeded in 96-well plates at a concentration of 1.0x10^4 cells/well and incubated overnight at 37°C in a 5% CO$_2$ humidified environment. Then the cells were treated with different concentrations of the sample 4.4g like 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100µM/mL (dissolved with RPMI medium 1640), respectively. Controls were cultivated under the same conditions without addition of 4.4g. 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 coloured 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 molecule showed excellent cytotoxicity against MCF-7 cell lines with an IC50 value 40 µM/mL.

\[ \text{Figure 4.7. MTT assay results confirming the in vitro cytotoxicity effect of 4.4g against the MCF-7 cells. The detected IC50 concentration was 40 µM/mL.} \]

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

MCF-7 cells were treated with 4.4g at its IC50 concentration (40 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 1mg/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 filed and fluorescence microscopic images are shown in
Fig. 4.8. As shown in this the strong bluish fluorescence and cellular uptake observed in the imaging studies with 4.4g reveals that these molecules have high potency against breast cancer cell lines (MCF-7).

![Image of microscopy images](image)

**Figure 4.8.** Bright field inverted light microscopy images (a) (cc), (b) (IC 25) and (c) (IC 50) and fluorescence microscopy images (d) (CC), (e) (IC 25) and (f) (IC 50) of 4.4g treated MCF-7 cells.

### 4.4. Structure elucidation by spectroscopy

#### 4.4.1. Structure elucidation of 2-amino-6-(phenylthio)-4-(4-(prop-2-yn-1-yloxy)phenyl)pyridine-3,5-dicarbonitrile 4.1a

![Structural diagram of 4.1a](image)
For the general discussion, the compound 4.1a is taken as the representative for the propargylated pyridine derivative. The molecule is numbered as shown in Figure 4.9. The FT-IR spectrum of the compound 4.1a gives major absorptions at 3278, 3227, 3067, 2924, 2228, 2211, 2117, 1641 cm\(^{-1}\) (Fig. 4.10). The band at 3278 cm\(^{-1}\) is due to the \(-\text{NH}\) stretching vibration of the amino group at position C12. The presence of propargylated group is confirmed by the bands at 3227 and 2117 cm\(^{-1}\). The peak due to the \(\text{C=CH}\) stretching vibration appeared at 3227 cm\(^{-1}\) and the \(\text{C=C}\) stretching vibration occurs at 2117 cm\(^{-1}\). The stretching vibration of the two cyano groups occurs at 2211, 2117 cm\(^{-1}\).

![Figure 4.10. FT-IR spectrum of the compound 4.1a](image)

The initial information obtained from the FT-IR spectrum about the formation of the product is further confirmed by the \(^1\text{H}\) NMR spectrum (Fig. 4.11). The \(-\text{NH}_2\) proton of the amino group is observed as a two proton singlet at \(\delta\) 5.47. The \(\text{CH}_2\) proton at position 3 is
observed as a singlet at $\delta$ 4.76 and the CH proton (alkyne CH) at position 1 is observed as another singlet at $\delta$ 3.35. The nine aromatic protons were observed as two sets of multiplets at $\delta$ 7.56-7.52 and 7.47-7.45.

![Figure 4.1](image.png)

**Figure 4.11.** $^1$HNMR spectrum of the compound4.1a

The functional group absorptions observed in the FT-IR spectrum and $^1$HNMR spectrum are in well agreement with the information obtained from its $^{13}$CNMR spectrum. The down field peak at $\delta$ 184 is due to the carbon at position 13. The amino carbonyl peak (C12) is observed at $\delta$ 166. The values at 159 and 158 are attributed to the carbons at position C10 and C4 respectively. The signals at $\delta$ 134, 129, 127, 126 and 115 are due to the aromatic carbons. The nitrile carbon peaks (C14 &C15) appears at $\delta$ 113 & 112 respectively. The other three up field resonances i.e., at $\delta$ 93 & 87 attributed to C11 and C14 carbons. The propargyl groups absorbs at $\delta$ 784, 78 and 56 corresponding to C1, C2 and C3 carbon atoms.
The structure of the compound is further confirmed by its mass spectral analysis. The molecular ion peak at 383.0966 obtained in MS further confirms the structure of the compound 4.1a (Fig. 4.13).
4.4.2. Structure elucidation of 3-azido-N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide 4.3a

For the Spectroscopic identification of molecules, compound 4.2a is taken as the representative from azido ketone library. The molecule is numbered as shown in Figure 4.14. The FT-IR spectrum of the compound 4.2a (Fig. 4.15) gives major absorptions at 3423, 2102, 1725, 1658, 1606 and 1563 cm\(^{-1}\). The -NH stretching vibration band of the acetamido group occurs at 3423 cm\(^{-1}\). The band at 2102 cm\(^{-1}\) is the characteristic peak of the azide linked to the carbon at position 1. The peak at 1725 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 1658 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 1563 cm\(^{-1}\).
In the $^1$HNMR spectrum, the singlet obtained at $\delta = 8.52$ is due to the coumarin CH at position 14. Singlet obtained at $\delta = 8.19$ corresponds to the amido bond (NH). Triplet observed between $\delta$ 8.57-8.52 is attributed to the NH proton of the acetamido group. Other downfield resonances, a doublet at $\delta$ 7.93-7.92, triplet at $\delta$ 7.74-7.71 and a multiplet at $\delta$ 7.52-7.42 corresponds to the aromatic protons at the coumarin part. The remaining aromatic protons on the aldehydic portion appears a two sets of two proton doublets at $\delta$ 7.07-7.05 and $\delta$ 6.49-6.48. Due to the vicinal couplings, the CH proton at position 4 is obtained as a triplet between $\delta$ 5.08-4.94. The CH$_2$ protons at position 11 are obtained as a doublet of doublet at $\delta$ 3.62-3.57 and 3.26-3.21 with approximately equal coupling constants. The remaining two up field signals at $\delta$ 2.72-2.70 and 1.98-1.96 are due to the protons at position C2 and C1 respectively.
In the $^{13}$C NMR spectrum, the ketone carbonyl carbon C12 and the amide carbonyl C3 are observed at $\delta$ 201 and 172 respectively (Figure 4.17). The lactone carbonyl peak at C21 is observed as a downfield peak at 160. The signals at $\delta$ 156, 153, 140, 134, 131, 130, 128, 125, 121, 120, 116 and 112 are due to the aromatic carbons. The peak at $\delta$ 56 is due to the methyl carbon at position 22. The other three up field resonances i.e., at $\delta$ 51, 47, 46 and 332 are attributed to C4, C11, C1 and C2 respectively.
The structure is further confirmed by the mass spectral analysis. The M+ peak is observed at m/z 420 (Fig. 4.18).
4.4.3 Structure elucidation of 3-(4-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide 4.4a

![Figure 4.19]

For the structure elucidation of the click product, compound 4.4a is taken as a representative and is numbered as in Figure 4.19.

The FT-IR spectrum of the compound 4.4a gave major absorptions at 3467, 3352, 3292, 3220, 2212, 1724, 1627, 1607, 1548 and 1509 cm\(^{-1}\) (Fig. 4.20). The band at 3292 cm\(^{-1}\) is due to the -NH stretching vibration of the acetamido group. The amide I band, ie., band due to the C=O stretching vibration occurs at 1627 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 1548 cm\(^{-1}\). The absorption at 1724 cm\(^{-1}\) is due to the C=O stretching vibration of the coumarin part. The stretching vibration of the two cyano groups occurs at 2212 cm\(^{-1}\).
In the $^1$HNMR spectrum, the coumarin CH proton at position 29 is observed as a singlet at $\delta = 8.41$. The peaks from 7.84 to 7.17 correspond to the 17 aromatic protons. Singlet obtained at $\delta = 7.26$ corresponds to the triazole proton. The peak at $\delta 5.62$ corresponds to the two amino protons at position 1. The proton at C18 appears as a singlet at $\delta 5.49$. Due to the vicinal couplings, the CH proton at position 25 is obtained as a triplet between $\delta 5.18-5.13$. The CH$_2$ protons at position 26 are obtained as a doublet of doublet at $\delta 3.49-3.43$ and $3.29-3.23$ with approximately equal coupling constants. The three protons of the methyl group appear as a singlet at $\delta 3.85$. The remaining two up field signals at $\delta 4.15-4.13$ and $2.33-2.31$ are due to the protons at position C22 and C23 respectively.
In the $^{13}$C NMR spectrum (Fig. 4.22) the ketone carbonyl carbon at C$_{29}$, amide carbonyl at C$_{24}$ and the lactone carbonyl at C$_{35}$ are observed at their characteristic regions, at $\delta$ 202.61, 187.56 and 172.08 respectively. The amino carbonyl peak (C1) is observed at $\delta$ 162.73. The carbons at position C3 and C17 causes the values at 162, and 154 respectively. The carbons of the benzene rings give the peaks at $\delta$ 148, 147, 146, 144, 134, 132, 130, 130, 127, 124, 123, 121, 118, 116, and 115. The nitrile carbon peaks (C12 & C13) appears at $\delta$ 115, 114 respectively. The peak at 71.44 is due to the C$_{18}$ carbon atom. The remaining up field signals at $\delta$ 92, 88, 55, 50, 46, 45 and 31 are credited to the carbons C4, C2, C43, C25, C26, C21 and C22 respectively.
The structure of the compound is further confirmed by its mass spectral analysis. The (M+1) peak is observed at m/z 803 (Fig. 4.23).

**Figure 4.22.** $^{13}$CNMR spectrum of the compound 4.4a

**Figure 4.23.** Mass spectrum of the compound 4.4a
4.5. Conclusion

In short, through the exploitation of an MCR-Click strategy, we have synthesized a series of coumarin-triazole-pyridine peptidomimetics. Two new pyridine alkynes and six coumarin azides were synthesized via MCR methodology. The alkynes and azides showed good Stokes shift values like 155 nm and 160 nm respectively. The coumarin-triazole-pyridine conjugates 4.4a-l obtained through the click reactions are promising especially for further explorations towards the development of inhibitors for undruggable targets. The photophysical properties obtained are also pointing to the potential of these molecules for developing efficient bio imaging agents.

4.6. Experimental

4.6.1. Typical experimental procedure for the synthesis of 2-amino-6-(phenylthio)-4-(4-(prop-2-yn-1-yloxy)phenyl)pyridine-3,5-dicarbonitrile 4.1a: To a solution of propargylated aldehyde (1.5 mmol) and malononitrile (3.1 mmol), Et₃N was added in drop wise at room temperature. The resulting mixture was heated to 50°C and refluxed after adding a desired thiol (1.6 mmol) for 2.5-3 h and then cool to room temperature. The precipitate formed at this stage (here, a structurally similar analogue can be added to facilitate crystallization if no precipitation occurs) was collected by filtration and the filtrate was concentrated under reduced pressure. To the dark residue obtained was mixed with methanol (2 mL) to facilitate the crystallization of an additional 5-10% of the product. The solids were combined and recrystallized from acetonitrile (3 mL) or methanol (3 mL) to yield a
corresponding pure pyridine.

4.6.2. **Typical experimental procedure for the synthesis of 3-azido-N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide 4.3a**: A mixture of 4-methoxybenzaldehyde (134 mg, 1 mmol), 3-acetylcoumarin (188 mg, 1 mmol), and 3-bromopropionitrile (133 mg, 1 mmol) in acetonitrile (8 ml) were stirred in the presence of catalytic amount of BF$_3$.Et$_2$O at room temperature for 4 h. After completion of the reaction as indicated by TLC, the reaction mixture was poured into ice cold water and extracted with CH$_2$Cl$_2$ (15 ml). Evaporation of the solvent followed by purification on silica gel (100–200 mesh) using ethyl acetate/hexane mixture (3:1) afforded the 3-bromo-N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide 4.2a. The resulted bromide (426 mg, 1 mmol), K$_2$CO$_3$ (414 mg, 3 mmol), NaN$_3$ (65 mg, 1 mmol) were dissolved in dimethylacetamide and stirred for 6–8 h. After completion, the reaction mixture was poured into ice cold water and the precipitate was filtered, dried under vacuum to afford the azide 4.3a.

4.6.3. **Typical experimental procedure for the synthesis of 3-(4-((2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide 4.4a**: An equimolar amount 2-amino-6-(phenylthio)-4-(4-(prop-2-yn-1-yloxy)phenyl)pyridine-3,5-dicarbonitrile 4.1a (47.75 mg, 0.12 mmol) and the coumarin azide 4.3a (52.5 mg, 0.12 mol) are dissolved in minimum amount of DMSO. To this, 2 ml of t-BuOH, 1 ml of water, CuSO$_4$.5H$_2$O (200 mg) and
sodium ascorbate (150 mg) are added and stirred in room temperature for 12 h. and then poured in to cold water. The precipitated click product was filtered, washed with water and dried under vacuum to afford 4.4a in pure form (180 mg, 92%).

4.6.4. Spectral data of compounds

2-amino-4-(3-methoxy-4-(prop-2-yn-1-yl)oxy)phenyl)-6-(phenylthio)pyridine-3,5-dicarbonitrile (4.1b) $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 3.75(1H, s), 3.95(3H, s), 4.84 (2H, s), 5.47 (2H, s), 7.19-7.17 (d, 2H, J= 7 Hz), 7.09 (1H, s), 7.49-7.46 (3H, m), 7.56-7.55 (2H, m); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 184.5, 166.0, 159.7, 158.1, 148.6, 134.7, 129.6, 127.2, 126.7, 121.2, 115.5, 113.2, 112.6, 93.4, 87.0, 78.9, 55.9; FT-IR (KBr) $\nu_{\text{max}}$: 3435, 3333, 3278, 3227, 3067, 2924, 2228, 2211, 1641, 1603, 1581, 1549, 1533, 1490, 1464, 1451, 1439, 1417, 1384, 1316, 1298, 1286, 1255, 1222, 1166, 1154, 1115, 1067, 1049, 1019, 942, 923, 888, 853 cm$^{-1}$; HRMS: m/z. calcd: 413.1072, found: 413.1065.

3-bromo-N-(3-oxo-3-(2-oxo-2H-chromen-3-yl)-1-phenylpropyl)propanamide (4.2b): $^1$H NMR (500 MHz, DMSO-$d_6$) : $\delta$ 8.59(1H, s), 8.17(1H, s), 7.94-7.93(1H, d, J=7.5 Hz), 7.78-7.74(1H, t), 7.53-7.48 (4H, m), 7.30-7.28 (1H, t), 6.80-6.78 (2H, d), 5.18-5.14 (1H, t), 3.89-3.84 (1H, dd, J= 8 Hz, J= 6 Hz), 3.45-3.40 (1H, dd, J= 8 Hz, J= 20 Hz), 3.19-3.18 (2H, t), 2.36-2.31(2H, t); $^{13}$CNMR (100MHz, DMSO-$d_6$): 199.7, 175.3, 159.9, 153.6, 143.6, 139.0, 134.9, 129.7, 128.9, 125.4, 123.8, 121.8, 116.1, 113.2, 50.1, 47.5, 38.2, 29.4; FT-IR (KBr) $\nu_{\text{max}}$: 3423, 3292, 2923, 2852, 1725, 1676, 1608, 1560, 1527,
1455, 1349, 1225, 1174, 1108, 1024, 759 cm⁻¹

4-(1-(3-bromopropanamido)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)-2-methoxyphenyl acetate (4.2c) ¹H NMR (500 MHz, DMSO-ᵈᶠ): δ 8.29 (1H, s), 8.08 (1H, d, J = 8 Hz), 7.82-7.80 (1H, d, J = 8 Hz), 7.59-7.58 (1H, t), 7.50-7.49 (1H, t), 7.16-7.14 (2H, d, J = 8 Hz), 6.89-6.88 (2H, d, J = 7.5 Hz, 2H), 5.13-5.11 (1H, t), 3.97 (3H, s), 3.80-3.76 (1H, dd, J = 8 Hz, J = 6 Hz) 3.49-3.45 (1H, dd, J = 8 Hz, J = 20 Hz), 3.27-3.26 (2H, t), 2.21-2.20 (2H, t), 2.18 (3H, s); ¹³CNMR (100 MHz, DMSO-ᵈᶠ): 199.5, 172.7, 168.0, 159.0, 155.7, 150.8, 142.7, 139.0, 134.9, 129.9, 128.9, 125.0, 122.7, 120.8, 119.2, 114.2, 110.2, 106.2, 56.1, 52.6, 49.6, 47.6, 37.6, 29.9; FT-IR (KBr) νₘₐₓ: 3429, 2923, 2853, 1760, 1724, 1677, 1607, 1560, 1509, 1489, 1455, 1422, 1370, 1269, 1199, 1123, 1083, 1031, 914, 862 cm⁻¹

3-bromo-N-(1-(4-nitrophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide (4.2e) ¹H NMR (500 MHz, DMSO-ᵈᶠ): δ 8.60 (1H, s), 8.39-8.38 (2H, d, J = 7.5 Hz), 8.08 (1H, s), 7.81-7.79 (1H, d, J = 6.5 Hz), 7.59-7.54 (2H, m), 7.43-7.42 (2H, d, J = 7 Hz), 6.94-6.92 (2H, d, 1 J = 8.5 Hz), 5.14-5.12 (1H, t), 3.69-3.66 (1H, dd, J = 8 Hz, J = 16 Hz) 3.39-3.35 (1H, dd, J = 8 Hz, J = 20 Hz), 3.18-3.13 (2H, t), 2.19-2.18 (2H, t); ¹³CNMR (100 MHz, DMSO-ᵈᶠ): 200.5, 172.2, 159.0, 153.9, 149.3, 145.0, 135.2, 130.2, 128.9, 126.5, 124.7, 122.1, 118.9, 116.9, 113.2, 50.7, 46.5, 38.6, 29.0; FT-IR (KBr) νₘₐₓ: 3423, 2923, 2853, 1719, 1647, 1607, 1560, 1519, 1489, 1455, 1346, 1226, 1176, 1110, 1083, 1028, 856, 759 cm⁻¹
3-azido-N-(3-oxo-3-(2-oxo-2H-chromen-3-yl)-1-phenylpropyl)propanamide (4.3b) \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) : \(\delta\) 8.58 (1H, s), 8.40 (1H, s), 7.94-7.93 (2H, d, J = 8 Hz), 7.77-7.74 (2H, t), 7.52-7.48 (2H, m), 7.30-7.28 (1H, t), 6.79-6.78 (2H, d, J = 8 Hz), 5.15-5.14 (1H, t), 3.60-3.55 (1H, dd, J = 8 Hz, J = 16 Hz) 3.18-3.14 (1H, dd, J = 8 Hz and J = 20 Hz), 2.13-2.12 (2H, t), 1.31-1.30 (2H, t) ; \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): 199.7, 175.3, 159.8, 153.6, 143.7, 139.7, 134.9, 129.7, 128.9, 125.7, 123.7, 121.8, 116.2, 113.2, 51.1, 47.6, 45.9, 34.2; FT-IR (KBr) \(\nu\) max: 3415, 2923, 2852, 2109, 1730, 1654, 1607, 1560, 1508, 1455, 1422, 1370, 1271, 1202, 1122, 1030, 910 cm\(^{-1}\)

4-(1-(3-azidopropanamido)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)-2-methoxyphenyl acetate (4.3c) \(^1\)H NMR(500 MHz, DMSO-\(d_6\)): \(\delta\) 8.57 (1H, s), 8.39 (1H, s), 7.94-7.92 (1H, d, J = 8 Hz), 7.76-7.73 (1H, t), 7.59-7.49 (2H, m), 6.97-6.96 (1H, d, J = 7 Hz), 5.14-4.95 (1H, t), 3.73 (3H, s), 3.63-3.58 (1H, dd, J = 8 Hz, J = 6 Hz), 3.38-3.34 (1H, dd, J = 8 Hz, J = 20 Hz, 1H), 2.46-2.44 (2H, t), 2.04 (3H, s), 1.68-1.66 (2H, t) ; \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): 200.5, 173.0, 169.0, 160.8, 156.6, 149.6, 139.7, 137.0, 134.9, 129.7, 128.9, 125.7, 123.7, 121.8, 119.2, 114.20, 110.0, 106.2, 56.1, 52.5, 48.6, 47.6, 35.6, 29.2; FT-IR (KBr) \(\nu\) max: 3429, 2923, 2853, 1760, 1724, 1677, 1607, 1560, 1509, 1489, 1455, 1422, 1370, 1269, 1199, 1123, 1083, 1031, 914, 862 cm\(^{-1}\)

3-azido-N-(1-(4-nitrophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide (4.3e) \(^1\)H NMR(500 MHz, DMSO-\(d_6\)) : \(\delta\) 8.577 (1H, s), 8.22-8.21 (2H, d, J = 7 Hz), 7.93 (1H, s), 7.53-7.52 (1H, d), 7.29-7.52 (2H, t), 7.09-7.02 (1H, m), 6.59-6.58 (2H, d), 5.17-5.13
(2H, t), 3.60-3.55 (1H, dd, J = 8 Hz, J = 16 Hz) 2.95-2.92 (1H, dd, J = 8 Hz, J=20 Hz), 2.13-2.12 (t, 2H), 1.31-1.30 (2H, t) ;

\(^{13}\)CNMR(100MHz, DMSO-\(d_6\)) : 200.5, 177.2, 160.6, 155.9, 147.0, 140.7, 135.3, 130.2, 128.9, 126.5, 124.7, 122.1, 118.9, 116.9, 112.2, 50.7, 4, 45.0, 35.6; FT-IR (KBr) \(\nu_{\text{max}}\): 3405, 2923, 2852, 2109, 1720, 1657, 1606, 1562, 1518, 1489, 1455, 1400, 1346, 1215, 1109, 1015, 856, 756 cm\(^{-1}\)

3-(4-(4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(3-oxo-3-(2-oxo-2H-chromen-3-yl)-1-phenylpropyl)propanamide (4.4b)

\(^1\)HNMR(500MHz, DMSO-\(d_6\)) : \(\delta\) 8.57 (1H, s), 8.13 (1H, s), 7.99-94 (4H, m), 7.68 (1H, s), 7.65-7.55 (6H, m), 7.39 (1H, s), 7.36-7.28 (6H, m), 5.41 (2H, s), 5.21 (2H, s), 5.10-5.08 (1H, t, 1H), 4.50-4.49 (2H, t), 3.92 (3H, s), 3.43-3.38 (1H, dd, J = 8 Hz, J = 16 Hz), 3.07-3.02 (1H, dd, J = 8 Hz, J = 16 Hz), 2.33-2.19 (2H, t); FT-IR (KBr) \(\nu_{\text{max}}\): 3457, 3347, 3255, 3220, 3080, 2955, 2923, 2853, 2216, 1729, 1628, 1608, 1547, 1509, 1455, 1425, 1371, 1321, 1254, 1219, 1175, 1142, 1117, 1020, 918, 859, 822 cm\(^{-1}\)

4-(1-(3-(4-(4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propanamido)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)-2-methoxyphenyl acetate (4.4c) :

\(^{13}\)C NMR (100MHz, DMSO-\(d_6\)) : 202.5, 188.0, 177.9, 174.8, 169.6, 163.9, 159.8, 156.0, 151.6, 146.8, 142.7, 139.8, 134.2, 131.2, 131.6, 130.6, 129.2, 126.9, 121.9, 118.14, 112.8, 109.8, 104.2, 92.7, 86.0, 71.0, 56.4, 54.2, 51.2, 45.9, 44.2, 34.5, 23.2; FT-IR (KBr) \(\nu_{\text{max}}\) :3469, 3352, 3293, 2923, 2852, 2360, 2212, 1628, 1608, 1579, 1546, 1508, 1474, 1455, 1420, 1384, 1349, 1311, 1292, 1263, 1249, 1233, 1185,
119, 1022, 824, 754 cm$^{-1}$ HRMS: m/z. Calcd: 861.2455, Found: 861.2350.

3-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(1-(4-chlorophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl) propyl) propanamide (4.4d): FT-IR (KBr) $\nu_{max}$: 3351, 3292, 3223, 2922, 2852, 2212, 1725, 1628, 1608, 1578, 1548.56, 1508, 1455, 1234, 1263, 1292, 1022, 1185 cm$^{-1}$ HRMS: m/z. calcd: 807.1905, Found: 807.1896.

4-((1-(3-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)propanamido)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)-2-methoxyphenyl acetate(4.4e) FT-IR (KBr) $\nu_{max}$: 3468, 3352, 3293, 3227, 2923, 2852, 2212, 1718, 1628, 1608, 1578, 1548, 1508, 1474, 1454, 1421, 1383, 1346, 1311, 1292.07, 1263, 1249 1233, 1185, 1022, 824 cm$^{-1}$

4-(1-(3-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propanamido)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)phenyl acetate (4.4f) : $^1$H NMR (500MHz, DMSO-$d_6$): $\delta$ 8.39 (1H, s), 8.24 (1H, s), 8.08 (1H, s), 7.92-7.91 (1H, d), 7.79-7.77 (3H, m), 7.71-7.69 (3H, t), 7.59-7.51 (4H, m), 6.89-6.82 (5H, m), 5.47 (2H, s), 5.29 (2H, s), 5.13-5.11 (1H, t), 4.31-4.30 (2H, t), 3.83-3.79 (1H, dd, J= 8 Hz, J=16 Hz) 3.60-3.56 (1H, dd, J=8 Hz, J=20 Hz), 2.99-2.93 (2H, t), 2.11 (3H, s); FT-IR (KBr) $\nu_{max}$; 3469, 3352, 3293, 3222, 2922, 2852, 2212, 1725, 1629, 1609, 1578, 1549, 1508, 1473, 1455, 1376, 1311, 1292, 1264, 1249, 1234, 1185, 1120, 1022, 825 cm$^{-1}$
3-(4-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(1-(4-methoxyphenyl)-3-oxo-3-(2-oxo-2H-chromen-3yl)propyl)propanamide (4.4g). $^1$H NMR (500 MHz, DMSO- $d_6$): $\delta$ 8.40 (1H, s), 8.08 (1H, s), 7.62 (1H, s), 7.55-7.54 (3H, t), 7.52-7.45 (4H, m), 7.38-7.33 (4H, m), 7.26-7.15(4H, m), 7.05 (1H, s) 5.57 (2H, s), 5.46 (2H, s), 5.29-5.27 (1H, t), 4.09-4.07 (2H, t), 3.79 (6H, s), 3.29-3.25 (1H, dd, J=8 Hz, J=16 Hz) 3.16-3.11 (1H, dd, J=8 Hz, J=20 Hz), 2.62-2.60 (2H, t); $^{13}$C NMR (100 MHz, DMSO- $d_6$): 203.6, 187.0, 170.8, 167.1, 161.1, 159.5, 158.0, 154.3, 150.3, 145.8, 139.5, 138.1, 133.2, 131.9, 130.1, 129.3, 128.6, 127.4, 124.0, 123.1, 122.0, 114.9, 114.0, 113.5, 112.8, 112.3, 111.7, 89.0, 83.4, 74.1, 59.4, 55.0, 50.7, 46.2, 44.2, 32.1; FT-IR (KBr) $\nu_{max}$: 3523, 3458, 33472, 3254, 3077, 2922, 2853, 2215, 1624, 1607, 1546, 1509, 1468, 1455, 1441, 1424, 1371, 1322, 1305, 1253, 1219, 1175, 1142, 1021, 919, 875, 861 cm$^{-1}$ ESIMS m/z 855 (M+Na) Exact mass : 832.2428.

4-(1-(3-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)propanamido)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)-2-methoxyphenyl acetate (4.4i). $^1$H NMR (500 MHz, DMSO- $d_6$): $\delta$ 8.50 (1H, s), 8.03 (1H, s), 7.79 (1H, s), 7.54-7.49 (6H, m), 7.33-7.26 (4H, m), 7.16-7.14(4H, d), 7.06-7.04(2H, d), 5.55 (2H, s), 5.35 (2H, s), 5.19-5.17 (1H, t), 4.09-4.06 (2H, t), 3.96 (6H, s), 3.49-3.27 (1H, dd, J=8 Hz, J=16 Hz) 3.19-3.10(1H, dd, J=8 Hz, J=20 Hz), 2.51-2.49 (2H, t); 2.35 (3H, s); FT-IR (KBr) $\nu_{max}$: 3525, 3458, 33472, 3254, 3077, 2922, 2853, 2215, 1624,
3-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(1-(4-chlorophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide (4.4j) $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.60 (1H, s), 7.99 (1H, s), 7.71 (1H, s), 7.59-7.51 (1H, d), 7.46-7.44 (6H, d), 7.39 (1H, s), 7.36-7.34 (2H, d), 7.26-7.19 (4H, m), 7.12 (1H, s), 6.97 (1H, s), 5.52 (2H, s), 5.29 (2H, s), 5.14-5.10 (1H, t), 4.176-4.16 (2H, t), 3.81 (3H, s), 3.30-3.25 (1H, dd, J=8 Hz, J=16 Hz) 3.19-3.14 (1H, dd, J=8 Hz, J=20 Hz), 2.40-2.33(2H, t); FT-IR (KBr) $\nu_{\text{max}}$: 3459, 3346, 3254, 3016, 2923, 2853, 2215, 1724, 1625, 1607, 1546, 1509, 1455, 1425, 1424, 1253, 1142, 1019,755 cm$^{-1}$; ESIMS m/z 859 (M+Na) Exact mass : 836.1932.

$3$-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-N-(1-(4-nitrophenyl)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)propanamide (4.4k) $^{13}$C NMR (100 MHz, DMSO-$d_6$): 201.8, 188.0,170.8, 166.1, 161.1, 159.6, 158.1, 154.5, 150.3, 145.8, 139.3, 198.1, 133.2, 131.2, 130, 129.4, 128.6, 128.3, 127.3, 124.0, 123.1, 122.02, 114.40, 114.0, 113.5, 112.8, 112.0, 117.7, 89.0, 83.4, 74.0, 58.4, 56.0, 50.7, 46.2, 44.3, 32.1; FT-IR (KBr) $\nu_{\text{max}}$: 3459, 3417, 3348, 3254, 3077, 2961, 2923, 2853, 1673, 1624, 1546, 1510, 1469, 1455, 1425, 1404, 1371, 1347, 1321, 1253, 1218, 1142, 1109, 1020, 858, 822, 809, 781 cm$^{-1}$
4-(1-(3-(4-((4-(2-amino-3,5-dicyano-6-(phenylthio)pyridin-4-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)propanamido)-3-oxo-3-(2-oxo-2H-chromen-3-yl)propyl)phenyl acetate (4.4l) 

$^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 8.40 (1H, s), 8.24 (1H, s), 8.01 (1H, s), 7.99-7.91 (1H, d), 7.79-7.77 (3H, m), 7.70-7.69 (3H, t), 7.59-7.50 (4H, m), 6.89-6.81 (5H, m), 5.46(2H, s, 2H), 5.29 (2H, s), 5.13-5.11(1H, t), 4.31-4.30 (2H, t), 3.95 (3H, s), 3.83-3.78 (1H, dd, J=8 Hz, J=16 Hz, 1H) 3.60-3.56(1H, dd, J=8 Hz, J=20 Hz), 2.99-2.93(2H, t) 2.19(3H, s); 

$^{13}$C NMR (100 MHz, DMSO-$d_6$): 201.2, 188.6, 178.9, 174.8, 169.0, 163.6, 159.5, 156.0, 151.6, 147.8, 142.7, 139.8, 134.1, 131.2, 131.5, 130.6, 129.2, 126.9, 121.9, 118.1, 112.8, 103.9, 104.2, 92.7, 86.1, 72.0, 56.2, 54.2, 51.7, 45.9, 44.2, 33.6, 23.2; FT-IR (KBr) $\nu_{\text{max}}$: 3459, 3346, 3254, 3221, 3075, 2960, 2923, 2852, 2215, 1725, 1625, 1546, 1509, 1469, 1455, 1425, 1371, 1322, 1253, 1219, 1191, 1142, 1020, 971, 916, 861, 822, 809 cm$^{-1}$; HRMS: m/z. Calcd: 861.2455; Found: 861.2494
References


Supplementary material

Copies of $^1$H NMR, $^{13}$C NMR, FT-IR and Mass spectra of selected compounds.
Computational Results

Figure 3.24: IR spectrum of compound 3.4g obtained from DFT

Optimized geometry Energy: -2983.8834HF

Dipole moment: 11Debye

**Thermo chemical parameters**

Temperature: 298K and Pressure: 1atm

Zeropoint vibrational energy: 1874867.8 J/mol

Enthalpy ΔH: -2983.1172 HF

Entropy ΔS: 318.215 cal/mol

Free energy ΔG: -2983.2684

Heat capacity $C_V$: 193.809
$^1$HNMR spectrum of the compound 4.1b

$^{13}$CNMR spectrum of the compound 4.1b
FT-IR spectrum of the compound 4.1b

Mass spectrum of the compound 4.1b
$^1$HNMR spectrum of the compound 4.2a

\[ \text{Compound Structure} \]

$^{13}$CNMR spectrum of the compound 4.2a
FT-IR spectrum of the compound 4.2a

Mass spectrum of the compound 4.2a
$^1$HNMR spectrum of the compound 4.2b

$^{13}$CNMR spectrum of the compound 4.2b
FT-IR spectrum of the compound $4.2b$

Mass spectrum of the compound $4.2b$
$^1$HNMR spectrum of the compound 4.2c

FT-IR spectrum of the compound 4.2c
$^{13}$CNMR spectrum of the compound 4.2c

$^1$HNMR spectrum of the compound 4.2c
\textsuperscript{13}CNMR spectrum of the compound \textit{4.2e}
Mass spectrum of the compound 4.2e

\[ \text{Mass spectrum of the compound 4.2e} \]

\[ \text{\textsuperscript{1}HNMR spectrum of the compound 4.3a} \]
$^{13}$CNMR spectrum of the compound 4.3a

FT-IR spectrum of the compound 4.3a
Mass spectrum of the compound 4.3a

1^{1}HNMR spectrum of the compound 4.3b
$^{13}\text{CNMR spectrum of the compound 4.3b}$

$^{13}\text{CNMR spectrum of the compound 4.3b}$

$\text{FT-IR spectrum of the compound 4.3b}$
Mass spectrum of the compound 4.3b

13CNMR spectrum of the compound 4.3c
FT-IR spectrum of the compound 4.3c

$^{1}$HNMR spectrum of the compound 4.3c
$^{13}$CNMR spectrum of the compound 4.3e

Mass spectrum of the compound 4.3e
FT-IR spectrum of the compound 4.3e

$^1$HNMR spectrum of the compound 4.4b
FT-IR spectrum of the compound 4.4b

\[ \text{CNMR spectrum of the compound 4.4c} \]
FT-IR spectrum of the compound 4.4c

Mass spectrum of the compound 4.4c
FT-IR spectrum of the compound 4.4d

Mass spectrum of the compound 4.4d
$^1$HNMR spectrum of the compound 4.4f

FT-IR spectrum of the compound 4.4f
$^{1}$HNMR spectrum of the compound 4.4g

$^{13}$CNMR spectrum of the compound 4.4g
FT-IR spectrum of the compound 4.4g

Mass spectrum of the compound 4.4g
$^1$HNMR spectrum of the compound 4.4i

FT-IR spectrum of the compound 4.4i
$^1$HNMR spectrum of the compound 4.4j

FT-IR spectrum of the compound 4.4j
Mass spectrum of the compound **4.4j**

![Mass spectrum of compound 4.4j](image)

$^{13}$C NMR spectrum of the compound **4.4k**

![$^{13}$C NMR spectrum of compound 4.4k](image)
FT-IR spectrum of the compound 4.4k

\(^1\)HNMR spectrum of the compound 4.4l
\[ ^{13}\text{CNMR spectrum of the compound 4.4l} \]

\[ \text{FT-IR spectrum of the compound 4.4l} \]
Mass spectrum of the compound 4.41