4.1 Introduction

Parasitic infections represent a major health threat in underdeveloped countries, and have a deep impact on public health. Malaria has been a major source of parasitic infections since antiquity, yet today it remains responsible for the deaths of more than one million people every year.\(^1\) Similarly, infections caused by *Trypanosoma cruzi, Leishmania mexicana, Giardia lamblia, Entamoeba histolytica* and *Trichomonas vaginalis* have worldwide distribution, especially in under-developed countries where tropical climates prevail in combination with poor sanitation and hygiene.\(^2-4\) *T. vaginalis*, the causative organism of trichomoniasis is a mucosal pathogen which affects the human urogenital tract, producing malodorous vaginal discharge, vulval irritation, inflammation and punctate cervical microhemorrhages.\(^5\) World Health Organization (WHO) estimates that approximately 248 million new cases of trichomoniasis occur annually;\(^6\) of these, 18.5 million come from Latin America\(^7\) and more than 125,000 new cases are reported from Mexico.\(^8\) In men, it is commonly asymptomatic and may cause urethritis, prostatitis, cystitis, epididymitis and infertility. However, in women this infection results in vulvovaginitis and urethritis with vaginal discharge, irritation, dysuria and abdominal pain along with yellow-green, itchy, frothy and foul-smelling vaginal secretion.\(^9\) Metronidazole (MTZ), the current and only FDA-approved treatment for this disease, has been used for more than 40 years. However, there are ample reports on the development of resistant isolates to MTZ which in certain cases have shown to be tackled with prolonged therapy and higher dosage.\(^10,11\) Further, it is now well established that trichomoniasis infected patients are more susceptible towards human immunodeficiency virus (HIV) as it appeared as a cofactor in HIV transmission and acquisition.\(^12,13\) The significant increase in the vulnerability to HIV with trichomoniasis has increased the importance of this disease dramatically.\(^14,15\) As evident, the identification and development of novel scaffolds with toxicity against *T. vaginalis* and minimal cytotoxicity against human cells, is a challenging task and provides a strong impetus for re-engineering and re-positioning of previously characterized drug families.\(^16\)

The present chapter involves the synthesis of mono- as well as bis-1\(H\)-1,2,3-triazole tethered bifunctional hybrids of C-5 subsituted isatins with \(N\)-1 substituted \(\beta\)-
lactams and their *in vitro* evaluation against *T. vaginalis*. Compounds with high levels of potency were further analyzed to determine their IC$_{50}$ values as well as cytotoxicity profiles against mammalian cells (HeLa Cells), the results being described in sub-chapters 4.2 and 4.3.

**4.2 Synthesis of 1H-1,2,3-Triazole linked β-Lactam-Isatin Bifunctional Hybrids and Preliminary Analysis of *in vitro* Activity against the Protozoal Parasite *Trichomonas vaginalis***

**4.2.1 Results and Discussion:**

**Synthetic chemistry**

The desired bi-functional hybrids were synthesized by using Cu(I)-promoted click chemistry between *N*-substituted *β*-lactams and 5-substituted isatins containing the azide and terminal alkyne, respectively. The precursor, 5-substituted isatins were prepared according to reported procedures$^{17}$ while *N*-propargylation was done using propargyl bromide in the presence of NaH as a base and DMF as solvent (Scheme 1).

![Scheme 1](image)

Scheme 1. Reagents and conditions: (a) Propargyl bromide, NaH, DMF, rt, 3 h

The desired *N*-substituted 3-azido-*β*-lactams 4 were prepared by Staudinger reaction of appropriately functionalized 1-azadienes 3 with azido-ketene generated *in situ* from azido-acetic acid in the presence of *p*-toluenesulphonylchloride and triethylamine (Scheme 2).$^{18}$

![Scheme 2](image)

Scheme 2. Reagents and conditions: (a) *p*-toluene sulphonyl chloride, Et$_3$N, dry DCM, rt

The targeted diastereoselective, bi-functional hybrids were synthesized by room temperature stirring of variedly substituted 3-azido-2-azetidinones and propargylated...
isatins in EtOH:H_{2}O (70:30) mixture for 7 h in the presence of CuSO_{4}·5H_{2}O and sodium ascorbate (Scheme 3). After completion of reaction, as evidenced by TLC, the products were purified after usual work up and re-crystallization. The structures to desired hybrids were assigned on the basis of spectral and analytical evidences. The cis-stereochemistry assigned was on the basis of coupling constant $J=5.7$ Hz between H\(^3\) and H\(^4\).

Scheme 3. Reagent and conditions: (i) CuSO\(_4\)·5H\(_2\)O, Sodium ascorbate, EtOH:H\(_2\)O, rt, 7 h

The structure to the conjugates 5 were assigned on the basis of spectral data and analytical evidence. For example, compound 5e showed a molecular ion peak [M+H]\(^+\) at 496.2266. The \(^1\)H NMR spectrum (Fig. 1) displayed a singlet at $\delta$ 2.35 corresponding to methyl protons, characteristic peak at $\delta$ 4.97 corresponding to methylene protons, and a singlet at $\delta$ 7.74 corresponding to the triazole ring proton, along with the characteristic isatin as well as $\beta$-lactam ring protons. The appearance of the requisite number of carbons in the \(^{13}\)C NMR spectrum along with the three characteristic peaks at $\delta$ 157.2, 159.6, and 182.3 assigned corresponding to the isatin and $\beta$-lactam ring carbonyls further corroborated the assigned structure.
**In vitro activity against Trichomonas vaginalis**

The synthesized 1H-1,2,3-triazole-tethered β-lactam-isatin conjugates were evaluated for their inhibitory influence on the axenic *in vitro* growth of *T. vaginalis* strain G3 cultured in TYM Diamond’s media for 24 h at 37 °C. **Table 1** lists the data obtained from the initial percent inhibition screens. As evident from **Table 1**, the synthesized compounds show a concentration dependent inhibition of the parasite with average % age inhibition increasing with the increase in concentration from 10 to 100 μM. On comparing the effect of substituents at N-1 of the β-lactam on the % age growth inhibition at 10 μM, the compounds with N-aryl substituents (5i-5v) in general showed better activity profiles than N-alkyl substituents (5a-5h). The presence of substituent at C-4 position of N-aryl ring of β-lactam further influenced the activity profile with the compound 5m (R = H) showing 100% growth inhibition at 10 μM while the introduction of both electron donating (-CH₃) and electron withdrawing substituent (F, Cl) decreased the % age growth inhibition. A similar preference for hydrogen substituent at C-5 position of isatin has
been observed in most of the conjugates. However, the % age growth inhibition at 10 μM seem to depend profoundly on the presence of phenyl ring at N-1 position as evident by better activity profile shown by series 5m-5p among the test compound, 5q being an exception. The compound 5m, with an optimum combination of substituent (H) at N-aryl of β-lactam as well as C-5 of isatin proved to be the most potent among the synthesized conjugates exhibiting 100% growth inhibition at 10 μM. The increase in concentration from 10 μM to 100 μM greatly improved the activity with 18 of the 22 test compounds exhibiting 100% growth inhibition irrespective of the substituent present at N-1 of β-lactam or at C-5 position of isatin as depicted in Table 1 and graphically represented in Fig. 2.

Table 1. Inhibitory activity of compound library against G3 strain of *T. vaginalis* tested at 10 μM and 100 μM.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R¹</th>
<th>Average % Inhibition at 10 μM</th>
<th>Average % Inhibition at 100 μM</th>
<th>clog Pa</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>H</td>
<td>C₆H₅</td>
<td>26.80</td>
<td>100.00</td>
<td>4.02</td>
</tr>
<tr>
<td>5b</td>
<td>F</td>
<td>C₆H₅</td>
<td>0.00</td>
<td>100.00</td>
<td>4.17</td>
</tr>
<tr>
<td>5c</td>
<td>Cl</td>
<td>C₆H₅</td>
<td>7.30</td>
<td>100.00</td>
<td>4.74</td>
</tr>
<tr>
<td>5d</td>
<td>Br</td>
<td>C₆H₅</td>
<td>4.90</td>
<td>100.00</td>
<td>4.89</td>
</tr>
<tr>
<td>5e</td>
<td>CH₃</td>
<td>C₆H₅</td>
<td>9.80</td>
<td>95.65</td>
<td>4.34</td>
</tr>
<tr>
<td>5f</td>
<td>H</td>
<td>C₇H₇</td>
<td>7.80</td>
<td>100.00</td>
<td>3.58</td>
</tr>
<tr>
<td>5g</td>
<td>F</td>
<td>C₇H₇</td>
<td>0.00</td>
<td>19.57</td>
<td>3.90</td>
</tr>
<tr>
<td>5h</td>
<td>Cl</td>
<td>C₇H₇</td>
<td>7.30</td>
<td>67.39</td>
<td>4.47</td>
</tr>
<tr>
<td>5i</td>
<td>H</td>
<td>p-C₆H₆-F</td>
<td>38.50</td>
<td>100.00</td>
<td>4.17</td>
</tr>
<tr>
<td>5j</td>
<td>F</td>
<td>p-C₆H₆-F</td>
<td>19.50</td>
<td>100.00</td>
<td>4.50</td>
</tr>
<tr>
<td>5k</td>
<td>Cl</td>
<td>p-C₆H₆-F</td>
<td>14.60</td>
<td>100.00</td>
<td>5.07</td>
</tr>
<tr>
<td>5l</td>
<td>Br</td>
<td>p-C₆H₆-F</td>
<td>0.00</td>
<td>100.00</td>
<td>5.22</td>
</tr>
<tr>
<td>5m</td>
<td>H</td>
<td>C₆H₅</td>
<td>100.00</td>
<td>100.00</td>
<td>3.89</td>
</tr>
<tr>
<td>5n</td>
<td>F</td>
<td>C₆H₅</td>
<td>58.50</td>
<td>100.00</td>
<td>4.21</td>
</tr>
<tr>
<td>5o</td>
<td>Cl</td>
<td>C₆H₅</td>
<td>61.00</td>
<td>100.00</td>
<td>4.78</td>
</tr>
<tr>
<td>5p</td>
<td>Br</td>
<td>C₆H₅</td>
<td>83.00</td>
<td>100.00</td>
<td>4.93</td>
</tr>
<tr>
<td>5q</td>
<td>CH₃</td>
<td>C₆H₅</td>
<td>0.00</td>
<td>82.61</td>
<td>4.39</td>
</tr>
<tr>
<td>5r</td>
<td>H</td>
<td>p-C₆H₆-Cl</td>
<td>80.50</td>
<td>100.00</td>
<td>4.74</td>
</tr>
<tr>
<td>5s</td>
<td>Cl</td>
<td>p-C₆H₆-Cl</td>
<td>53.70</td>
<td>100.00</td>
<td>5.64</td>
</tr>
<tr>
<td>5t</td>
<td>H</td>
<td>p-C₆H₆-CH₃</td>
<td>4.90</td>
<td>100.00</td>
<td>4.39</td>
</tr>
<tr>
<td>5u</td>
<td>F</td>
<td>p-C₆H₆-CH₃</td>
<td>58.50</td>
<td>100.00</td>
<td>4.71</td>
</tr>
<tr>
<td>5v</td>
<td>Cl</td>
<td>p-C₆H₆-CH₃</td>
<td>48.80</td>
<td>100.00</td>
<td>5.28</td>
</tr>
</tbody>
</table>

*a*Calculated using Chem Draw Ultra 10.0
The active compounds from the preliminary inhibition data were chosen in order to determine their $IC_{50}$ which is the minimum concentration required for 50% growth inhibition and the results are tabulated in Table 2. As evident from Table 2, although the synthesized scaffolds are not as active as that of standard drug metronidazole, most of the compounds showed potent activity against the G3 strain of *T. vaginalis*. The compound 5n having phenyl-substituent on $N$-l of $\beta$-lactam ring and fluoro-substituent at C-5 position of isatin ring proved to be the most active scaffold among the library of compounds with an $IC_{50}$ of 7.06 $\mu$M. Further, 5i has shown no visual morphological effect on cultured CHO-K1 cells making it a good candidate for determining its $IC_{50}$ value, as shown in Table 2.

**Table 2.** $IC_{50}$ determination of active compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$IC_{50}$ ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5e</td>
<td>44.51</td>
</tr>
<tr>
<td>5f</td>
<td>8.68</td>
</tr>
<tr>
<td>5i</td>
<td>7.69</td>
</tr>
<tr>
<td>5n</td>
<td>7.06</td>
</tr>
<tr>
<td>5o</td>
<td>9.82</td>
</tr>
<tr>
<td>5p</td>
<td>9.34</td>
</tr>
<tr>
<td>5r</td>
<td>8.27</td>
</tr>
<tr>
<td>5s</td>
<td>9.94</td>
</tr>
<tr>
<td>5t</td>
<td>27.46</td>
</tr>
<tr>
<td>5u</td>
<td>22.09</td>
</tr>
<tr>
<td>5v</td>
<td>10.05</td>
</tr>
<tr>
<td>Metronidazole$^a$</td>
<td>0.72</td>
</tr>
</tbody>
</table>

$^a$ Current FDA approved treatment for *T. vaginalis* infections
Dose-response curves: The dose-response curve for the potent scaffolds 5e, 5f, 5i, 5n, 5o, 5p, 5r, 5s, 5t, 5u and 5v are elucidated in Fig. 3.
In conclusion, the present sub-chapter describes the synthesis of novel 1H-1,2,3-triazole-tethered β-lactam-isatin conjugates utilizing Cu(I)-mediated azide-alkyne cycloaddition reactions, and their *in vitro* activity against *T. vaginalis* at 10 and 100 μM. The preliminary growth inhibition data at 10 μM showed the dependence of activity on substituents at N-1 of β-lactam and C-5 of isatin ring. The increase in concentration to 100 μM revealed 18 of the test compounds exhibiting 100% growth inhibition irrespective of the substituents present with most potent compound 5n having an IC$_{50}$ of 7.06 μM. The cytotoxic evaluation studies of the synthesized chimeras on CHO-K1 cells showed 5i to be non-cytotoxic with an IC$_{50}$ value of 7.69 μM making it an ideal starting point for the synthesis of new pharmacological templates against *T. vaginalis*.

**Fig. 3** Dose-response curves for synthesized potent compounds 5e, 5f, 5i, 5n, 5o, 5p, 5r, 5s, 5t, 5u and 5v
4.3 Synthesis and Preliminary *in vitro* Activity of Mono- and Bis-1*H*-1,2,3-Triazole-Tethered β-Lactam-Isatin Conjugates against the Human Protozoal Pathogen *Trichomonas vaginalis*

4.3.1 Result and Discussions

**Synthetic Chemistry**

The mono- and di-propargylated precursors 7 and 8 were prepared *via* our recently reported protocol involving the treatment of 3-amino-2-azetidinone 6, with 1.1 and 2.1 mmol of propargyl bromide, respectively. The treatment with 1.1 mmol of propargyl bromide led to a mixture of 7 and 8 in the ratio of 75:25, as evidenced by the 1H NMR analysis of the crude reaction mixture while the use of 2.1 mmol of propargyl bromide resulted in the isolation of exclusive dipropargylated product 8. The observed coupling constant $J=5.4$ Hz between H$^3$ and H$^4$ confirmed the *cis*-stereochemistry of the products (Scheme 4).

![Scheme 4](image)

*Scheme 4.* Reagents and conditions: (a) K$_2$CO$_3$ (1.2 mmol), propargyl bromide (1.1 mmol), DMF, rt, 6 h; (b) K$_2$CO$_3$ (2.2 mmol), propargyl bromide (2.1 mmol), DMF, rt, 6 h

*N*-alkyl azido isatin 9, another precursor required for the synthesis of target scaffolds were prepared by an initial base-assisted *N*-alkylation of isatin with dibromoalkane followed by subsequent treatment with sodium azide in DMF at 60 °C (Scheme 5).
Scheme 5. Reagents and conditions: (a) NaH, Dibromoalkane, DMF, 60 °C, 12 h; (b) NaN₃, DMF, 60 °C, 2-3 h

The synthesized precursors 7 and 8 were then utilized in the synthesis of desired mono- and bis-1H-1,2,3-triazole-tethered β-lactam-isatin conjugates. Thus, the reaction of 7 with 9 (1 mmol) in the presence of copper sulphate and sodium ascorbate in ethanol:water (90:10) mixture led to the isolation of 10 (Scheme 6), while the reaction of 8 with 9 (2 mmol) under similar conditions led to the formation of 11 in good to excellent yields (Scheme 7).

Scheme 6. Reagents and conditions: (a) 9, CuSO₄.5H₂O, Sodium ascorbate, EtOH:H₂O, rt, 8 h

Scheme 7. Reagents and conditions: (a) 9, CuSO₄.5H₂O, Sodium ascorbate, EtOH:H₂O, rt, 8 h
The structures to the hybrids 10 and 11 were assigned on the basis of spectral data and analytical evidence. Compound 11d, for example, showed a molecular ion peak [M]$^+$ 814.8892 along with the characteristic peaks in $^1$H and $^{13}$C NMR spectra. The $^1$H NMR spectrum (Fig. 4) exhibited the presence of a singlet at $\delta$ 2.26 corresponding to methyl protons along with characteristic peaks at $\delta$ 2.32, 3.72, 4.05 and 4.27 corresponding to methylene protons and a singlet at $\delta$ 7.82 corresponding to triazole ring proton. The presence of a requisite number of carbons in $^{13}$C NMR spectrum along with two characteristic peaks at $\delta$ 164.8 and 182.9 assigned to isatin ring carbonyls further corroborated the assigned structure.

![Fig. 4 $^1$H NMR spectrum of 11d](image)

**In vitro activity against Trichomonas vaginalis:**

The synthesized mono- and bis-$^1$H-1,2,3-triazole-tethered $\beta$-lactam-isatin conjugates were evaluated for their inhibitory influence on the axenic *in vitro* growth of *T. vaginalis*
strain G3 cultured in TYM Diamond’s media for 24 h at 37 °C. Table 3 lists the data obtained from the initial percentage inhibition screens at 50 µM. As evident from Table 3, the activity profiles of test compounds showed dependence on the substituent at N-1 of β-lactam ring and the presence of single/double 1H-1,2,3-triazole linker. The increase in spacer length from n = 1 to n = 2 does not have any considerable effect on the efficacy of test compounds.

**Table 3. Biological evaluation of the compound library against T. vaginalis at 50 µM**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>n</th>
<th>Yield (%)</th>
<th>Average % Inhibition at 50 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>p-C₆H₄-CH₃</td>
<td>1</td>
<td>74</td>
<td>91.52 ± 2.78</td>
</tr>
<tr>
<td>10b</td>
<td>C₆H₁₁</td>
<td>1</td>
<td>65</td>
<td>43.72 ± 4.18</td>
</tr>
<tr>
<td>10c</td>
<td>p-C₆H₄-F</td>
<td>1</td>
<td>78</td>
<td>12.93 ± 1.93</td>
</tr>
<tr>
<td>10d</td>
<td>p-C₆H₄-CH₃</td>
<td>2</td>
<td>72</td>
<td>70.63 ± 3.80</td>
</tr>
<tr>
<td>10e</td>
<td>C₆H₁₁</td>
<td>2</td>
<td>74</td>
<td>36.90 ± 7.12</td>
</tr>
<tr>
<td>10f</td>
<td>p-C₆H₄-F</td>
<td>2</td>
<td>81</td>
<td>42.86 ± 5.23</td>
</tr>
<tr>
<td>11a</td>
<td>p-C₆H₄-CH₃</td>
<td>1</td>
<td>75</td>
<td>ND</td>
</tr>
<tr>
<td>11b</td>
<td>C₆H₁₁</td>
<td>1</td>
<td>75</td>
<td>44.23 ± 8.50</td>
</tr>
<tr>
<td>11c</td>
<td>p-C₆H₄-F</td>
<td>1</td>
<td>66</td>
<td>52.66 ± 1.47</td>
</tr>
<tr>
<td>11d</td>
<td>p-C₆H₄-CH₃</td>
<td>2</td>
<td>70</td>
<td>46.35 ± 1.16</td>
</tr>
<tr>
<td>11e</td>
<td>C₆H₁₁</td>
<td>2</td>
<td>78</td>
<td>57.61 ± 3.30</td>
</tr>
<tr>
<td>11f</td>
<td>p-C₆H₄-F</td>
<td>2</td>
<td>69</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND: Not determined

The most potent of the test compounds viz. 10a and 10d have been selected from % age inhibition data for determining their IC₅₀ values, which is defined as the minimum concentration required for 50% growth inhibition. These compounds have exhibited an IC₅₀ values of 10.49 (10a) and 25.60 µM (10d), respectively as shown in Table 4, while their dose-response curves are depicted in Fig. 5.

**Table 4. IC₅₀ determination of active compounds against T. vaginalis**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G3 strain</td>
</tr>
<tr>
<td>10a</td>
<td>10.49 ± 1.05</td>
</tr>
<tr>
<td>10d</td>
<td>25.60 ± 1.08</td>
</tr>
<tr>
<td>Metronidazole&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72</td>
</tr>
</tbody>
</table>

<sup>a</sup> Current FDA approved treatment for T. vaginalis infections
The most potent compound 10a was then further evaluated for their cytotoxicity against HeLa cells. Test compound 10a consistently showed 80% and 90% viability, compared with untreated and DMSO-treated cells. The same cells were tested with bleomycin (54-82% viability) and doxorubicin (62-85% viability) at the same concentration as positive controls for toxicity. We also carried out these assays on three independent days with multiple trials in each experiment. Interestingly, compound 10a had consistently comparable toxicities to untreated and DMSO-treated HeLa cells when tested at 10 μM.

![Fig. 5 Dose-response curves for 10a and 10d](image)

In conclusion, the present sub-chapter describes the synthesis of mono- and bis-1H-1,2,3-triazole tethered β-lactam-isatin conjugates along with their preliminary *in vitro* evaluation against *T. vaginalis* at 50 μM. The synthesized scaffolds have shown a preference for a p-tolyl substituent at N-1 of β-lactam ring for good activity with the most potent and non-cytotoxic compounds 10a and 10d exhibiting an IC₅₀ of 10.49 and 25.60 μM, respectively.
4.4 Conclusion

Twenty-two different triazoles were prepared to examine the anti-*Trichomonas vaginalis* structure-activity relationships (SAR) within the β-lactam-isatin-triazole conjugate family. *In vitro* activity against *T. vaginalis* was determined at 10 and 100 μM for each compound, with eighteen of the synthesized hybrids showing 100% growth inhibition at 100 μM. The compound 5i, with no cytotoxicity on cultured CHO-K1 cells, is considered a good compound for further analysis.

The above approach was further extended towards the synthesis of mono- and bis-1H-1,2,3-triazole-tethered β-lactam-isatin conjugates using copper-catalyzed azide-alkyne cycloaddition reaction between mono- and di-propargylated azetidin-2-ones and N-alkylazido isatins. The efficacy of the synthesized hybrids against *T. vaginalis* was observed to depend on the substituent at N-1 position of the β-lactam ring as well as the presence of single/double 1H-1,2,3-triazole linker. The most active compound of the synthesized conjugates displayed an IC$_{50}$ value of 10.49 μM against cultured G3 strain of *T. vaginalis* and was non-toxic to cultured mammalian HeLa cells at the same concentration.
4.5 Experimental Section

Pharmacology

In vitro protozoal parasite susceptibility assay and CHO cell cytotoxic studies:

The protozoal parasites were cultured for 24 h at 37 °C. To perform the initial susceptibility screens on *T. vaginalis*, compounds were suspended in DMSO to obtain concentrations of 100 μM; 5 μl aliquots of these suspensions were diluted in 5 ml of TYM Diamond’s media to obtain a final concentration of 100 μM. After 24 h, cells were counted using a hemacytometer. Cell counts were normalized to the DMSO controls, in order to allow direct comparison and averaging of the various trials. These data sets were then transformed using Prism Software by Graphpad, by taking the log of the drug concentrations for the trials, and inputting this transform into a log(inhibitor) vs. response – Variable slope regression option. Within this nonlinear regression, constraints were set to force the maximum value (top) to 1 and the minimum value (bottom) to 0. The slope was left variable and then determined through which regression was performed. The sample size consists of 4 independent trials carried out on 4 different days (to account for possible variation in parasite culture). The assays were performed in 15 ml culture tubes, with both WT and DMSO control tubes to normalize for the effects of the solvent and *in vitro* conditions. The IC₅₀ value for active compounds were determined by running assays of increasing drug concentrations, 5 μM to 40 μM, and performing a regression analysis using Prism software, from GraphPad. For screening of compounds 5a-5v on mammalian cells, 100 μM concentrations were added to cultures of CHO-K1 cells. After 24h, cells were visualized under light microscopy to detect observable changes in morphology.

Cytotoxic evaluation of the most potent compound, 10a in the library, on cultured mammalian cells:

The HeLa cells were maintained in Dulbecco's Modified Eagle Medium that contained 1% penicillin/streptomycin and 10% fetal bovine serum in a humified 5% CO₂ atmosphere at 37 °C. Doxorubicin, bleomycin, and compound 10a (the most potent compound) were added to the medium of cells 24 h after culture. A trypan blue assay was used 24 h after drug treatment to calculate cell viability. This was done in three
separate trials to ensure that cytotoxicity results were consistent. The accuracy of our cytotoxic assay was further validated by using etoposide as a positive control which exhibited an IC\textsubscript{50} value of 0.61 \textmu M comparable to its reported value.

**Chemistry**

Melting points were determined by open capillary using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. \textsuperscript{1}H NMR spectra were recorded in deuterochloroform and DMSO-d\textsubscript{6} with Jeol 300 (300 MHz) spectrometers using TMS as internal standard. Chemical shift values are expressed as parts per million downfield from TMS and \textit{J} values are in Hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. \textsuperscript{13}C NMR spectra were recorded on Jeol 300 (75 MHz) spectrometers in deuterochloroform and DMSO-d\textsubscript{6} using TMS as internal standard. High resolution mass spectra were recorded on Bruker-micrOTOF-Q II spectrometer. Column chromatography was performed on a silica gel (60-120 mesh).

**General Procedure for the preparation of \(\beta\)-lactam-isatin hybrids 5a-5v:**

To a stirred solution of appropriate acetylenic isatins 2a-2d (1 mmol) in ethanol:water (70:30) mixture and azide 4a-4f (1 mmol) was added copper sulphate (0.05 mmol) and sodium ascorbate (0.13 mmol). The reaction mixture was allowed to stir at room temperature for 8h and the progress was monitored using TLC. After the completion of reaction, water (25 ml) was added and the reaction mixture was extracted twice with dichloromethane (2×30 ml). The combined organic layers were dried over anhydrous sodium sulphate, and concentrated under reduced pressure to yield a crude product which was purified via recrystallization using CHCl\textsubscript{3}:MeOH (80:20) mixture.

**5a.** 1-[1-(1-Cyclohexyl-2-oxo-4-styryl-azetidin-3-yl)-1\textit{H}-[1,2,3]triazol-4-ylmethyl]-1\textit{H}-indole-2,3-dione:

Brick red crystalline solid; m.p. 200-202 °C. IR (KBr) \textit{v}\textsubscript{max}: 1737, 1732, 1616 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300MHz, CDCl\textsubscript{3}): \textit{\delta} 1.08-2.04 (m, 10H, cyclohexyl-H), 3.45-3.60 (m, 1H, cyclohexyl-H), 4.72 (dd, \textit{J}=5.1, 8.7 Hz, 1H, H\textsubscript{3}), 4.98 (s, 2H, -CH\textsubscript{2}-), 5.66 (dd, \textit{J}=8.7, 15.6 Hz, 1H, H\textsubscript{2}), 5.90 (d, \textit{J}=5.1 Hz, 1H, H\textsubscript{1}), 6.60 (d, \textit{J}=15.6 Hz, 1H, H\textsubscript{1}), 7.03-7.26 (m,
7H, ArH), 7.39-7.44 (m, 1H, ArH), 7.53 (d, J=7.2 Hz, 1H, ArH), 7.76 (s, 1H, triazole-H), 
$^{13}$C NMR (75MHz, CDCl$_3$+DMSO-d$_6$): δ ppm = δ 25.0, 30.5, 31.6, 35.3, 53.1, 59.2, 67.1, 111.2, 117.4, 121.5, 123.4, 123.9, 125.2, 126.5, 128.7, 135.0, 137.1, 138.5, 141.8, 150.0, 157.8, 160.4, 182.8. HRMS Calculated for C$_{28}$H$_{27}$N$_5$O$_3$ [M+H]$^+$ 482.2114 found 482.2112; Anal. Calcd (%) for: C, 68.84; H, 5.65; N, 14.54, found: C, 68.72; H, 5.72; N, 10.62.

5b. 1-[1-(1-Cyclohexyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-5-fluoro-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 210-212 °C. IR (KBr) ν$_{max}$: 1742, 1730, 1626 cm$^{-1}$; $^1$H NMR (300MHz, CDCl$_3$): δ 1.23-2.01 (m, 10H, cyclohexyl-H), 3.46-3.61 (m, 1H, cyclohexyl-H), 4.72 (dd, J=5.1, 8.4 Hz, 1H, H$^3$), 4.97 (s, 2H, -CH$_2$-), 5.66 (dd, J=8.4, 15.6 Hz, 1H, H$^2$), 5.91 (d, J=5.1 Hz, 1H, H$^4$), 6.58 (d, J=15.6 Hz, 1H, H$^4$), 7.05-7.26 (m, 8H, ArH), 7.76 (s, 1H, triazole-H), $^{13}$C NMR (75MHz, CDCl$_3$+DMSO-d$_6$): δ ppm = 23.7, 29.3, 30.3, 34.2, 51.6, 58.0, 65.8, 111.2, 111.3, 117.0, 121.3, 123.1, 125.3, 127.3, 127.5, 134.1, 135.5, 140.2, 144.9, 151.2, 155.0, 157.1, 159.6, 182.2. HRMS Calculated for C$_{28}$H$_{26}$FN$_5$O$_3$ [M+H]$^+$ 500.2020 found 500.2017; Anal. Calcd (%) for: C, 67.32; H, 5.25; N, 14.02, found: C, 67.41; H, 5.33; N, 13.96.

5c. 5-Chloro-1-[1-(1-cyclohexyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 219-220 °C. IR (KBr) ν$_{max}$: 1745, 1735, 1613 cm$^{-1}$; $^1$H NMR (300MHz, CDCl$_3$): δ 1.23-2.31 (m, 10H, cyclohexyl-H), 3.45-3.60 (m, 1H, cyclohexyl-H), 4.71 (dd, J=5.1, 8.7 Hz, 1H, H$^3$), 4.96 (s, 2H, -CH$_2$-), 5.65 (dd, J=8.7, 15.6 Hz, 1H, H$^2$), 5.89 (d, J=5.1 Hz, 1H, H$^4$), 6.59 (d, J=15.6 Hz, 1H, H$^4$), 7.00-7.34 (m, 8H, ArH), 7.72 (s, 1H, triazole-H), $^{13}$C NMR (75MHz, CDCl$_3$+DMSO-d$_6$): δ ppm = 25.5, 30.8, 31.5, 35.2, 53.3, 59.5, 67.8, 111.3, 117.5, 121.7, 123.2, 123.8, 125.5, 126.4, 128.4, 135.6, 137.2, 138.6, 141.4, 150.9, 156.6, 157.3, 160.2, 182.6. HRMS Calculated for C$_{28}$H$_{26}$ClN$_5$O$_3$ [M+H]$^+$ 516.1724 found 516.1727; Anal. Calcd (%) for: C, 65.18; H, 5.08; N, 13.57, found: C, 65.10; H, 5.17; N, 13.65.
5d. 5-Bromo-1-[1-(1-cyclohexyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 201-203 °C. IR (KBr) \( \nu_{\text{max}} \): 1741, 1736, 1630 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)): \( \delta \) 1.27-1.98 (m, 10H, cyclohexyl-H), 3.47-3.62 (m, 1H, cyclohexyl-H), 4.72 (dd, \( J = 5.1, 8.4 \) Hz, 1H, H\(^3\)), 4.94 (q, \( J = 15.6 \) Hz, 2H, -CH\(_2\)-), 5.65 (dd, \( J = 8.4, 15.9 \) Hz, 1H, H\(^2\)), 5.89 (d, \( J = 5.1 \) Hz, 1H, H\(^4\)), 6.57 (d, \( J = 15.9 \) Hz, 1H, H\(^1\)), 7.04-7.26 (m, 6H, ArH), 7.46 (t, \( J = 1.8, 9.6 \) Hz, 1H, ArH), 7.60 (d, \( J = 2.4 \) Hz, 1H, ArH), 7.75 (s, 1H, triazole-H), \(^{13}\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)): δ ppm = 25.5, 30.8, 31.2, 35.4, 53.4, 59.8, 67.2, 111.1, 117.9, 121.6, 123.3, 123.6, 125.6, 126.2, 128.8, 135.2, 137.4, 138.1, 141.9, 150.2, 156.9, 157.9, 160.5, 182.9. HRMS Calculated for C\(_{28}\)H\(_{26}\)BrN\(_5\)O\(_3\)\([M+H]^+\) 560.1219 found 560.1217; Anal. Calcd (%) for: C, 60.01; H, 4.68; N, 12.50, found: C, 60.12; H, 4.74; N, 10.58.

5e. 1-[1-(1-Cyclohexyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 207-208 °C. IR (KBr) \( \nu_{\text{max}} \): 1736, 1733, 1619 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)): \( \delta \) 1.27-1.97 (m, 10H, cyclohexyl-H), 2.35 (s, 3H, -CH\(_3\)), 3.46-3.61 (m, 1H, cyclohexyl-H), 4.72 (dd, \( J = 5.1, 8.4 \) Hz, 1H, H\(^3\)), 4.97 (q, \( J = 15.6 \) Hz, 2H, -CH\(_2\)-), 5.65 (dd, \( J = 8.4, 15.9 \) Hz, 1H, H\(^2\)), 5.90 (d, \( J = 5.1 \) Hz, 1H, H\(^4\)), 6.57 (d, \( J = 15.9 \) Hz, 1H, H\(^1\)), 7.03-7.26 (m, 6H, ArH), 7.31-7.34 (m, 1H, ArH), 7.46 (t, \( J = 1.8, 9.6 \) Hz, 1H, ArH), 7.60 (d, \( J = 2.4 \) Hz, 1H, ArH), 7.74 (s, 1H, triazole-H), \(^{13}\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)): δ ppm = 19.4, 23.7, 29.3, 30.3, 34.1, 51.6, 58.1, 65.8, 109.7, 116.3, 121.3, 122.9, 124.0, 125.4, 127.3, 127.4, 134.1, 135.6, 137.5, 140.5, 150.2, 156.6, 157.2, 159.6, 182.3. HRMS Calculated for C\(_{29}\)H\(_{28}\)BrN\(_5\)O\(_3\)\([M+H]^+\) 496.2270 found 496.2266; Anal. Calcd (%) for: C, 70.28; H, 5.90; N, 14.13, found: C, 70.19; H, 5.83; N, 14.24.

5f. 1-[1-(1-Benzyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 214-215 °C. IR (KBr) \( \nu_{\text{max}} \): 1747, 1740, 1611 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)): \( \delta \) 4.19 (d, \( J = 15.0 \) Hz, 1H, -CH\(_2\)- benzyl), 4.54 (dd, \( J = 5.1, 8.4 \) Hz, 1H, H\(^3\)), 4.79 (d, \( J = 15.0 \) Hz, 1H, -CH\(_2\)- benzyl), 4.98 (a pair of doublet, \( J = 15.9 \) Hz, 2H, -CH\(_2\)-), 5.55 (dd, \( J = 8.4, 15.9 \) Hz, 1H, H\(^2\)), 5.95 (d, \( J = 5.1 \) Hz, 1H, H\(^4\)), 6.47 (d, \( J = 15.9 \) Hz, 1H, H\(^1\)), 7.01-7.45 (m, 13H, ArH), 7.53 (d, \( J = 6.9 \) Hz, 1H, ArH), 7.74 (s, 1H,
triazole-H), $^{13}$C NMR (75MHz, CDCl$_3$+DMSO-d$_6$): δ ppm = 35.2, 45.3, 59.6, 67.9, 111.1, 117.4, 119.6, 123.5, 123.9, 125.2, 126.5, 128.2, 128.6, 128.7, 129.0, 134.3, 134.8, 137.9, 138.4, 141.8, 149.9, 157.8, 160.9, 182.7. HRMS Calculated for C$_{29}$H$_{23}$N$_5$O$_3$ [M+H]$^+$ 490.1811 found 490.1815; Anal. Calcd (%) for: C, 71.15; H, 4.74; N, 14.31, found: C, 71.21; H, 4.86; N, 14.26.

5g. 1-[1-(1-Benzyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-5-fluoro-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 215-217 °C. IR (KBr) $\nu_{\text{max}}$: 1739, 1736, 1625 cm$^{-1}$; $^1$H NMR (300MHz, CDCl$_3$): δ 4.17 (d, $J$=14.9 Hz, 1H, -CH$_2$ benzyl), 4.53 (dd, $J$=5.1, 8.4 Hz, 1H, H$^3$), 4.77 (d, $J$=14.9 Hz, 1H, -CH$_2$ benzyl), 4.95 (q, $J$=15.6 Hz, 2H, -CH$_2$-), 5.53 (dd, $J$=8.4, 15.9 Hz, 1H, H$^2$), 5.93 (d, $J$=5.1 Hz, 1H, H$^4$), 6.43 (d, $J$=15.9 Hz, 1H, H$^1$), 6.95-7.35 (m, 13H, ArH), 7.71 (s, 1H, triazole-H), $^{13}$C NMR (75MHz, CDCl$_3$+DMSO-d$_6$): δ ppm = 9.1, 37.7, 58.9, 67.7, 109.3, 114.4, 114.7, 117.4, 117.5, 119.3, 123.0, 123.6, 125.1, 127.1, 127.2, 133.4, 133.6, 136.4, 137.1, 140.5, 146.4, 156.2, 158.5, 160.0, 182.4. HRMS Calculated for C$_{29}$H$_{22}$FN$_5$O$_3$ [M+H]$^+$ 508.1707 found 508.1705; Anal. Calcd (%) for: C, 68.63; H, 4.37; N, 13.80, found: C, 68.73; H, 4.26; N, 13.93.

5h. 1-[1-(1-Benzyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-5-chloro-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 210-211 °C. IR (KBr) $\nu_{\text{max}}$: 1743, 1738, 1634 cm$^{-1}$; $^1$H NMR (300MHz, CDCl$_3$): δ 4.17 (d, $J$=15.0 Hz, 1H, -CH$_2$ benzyl), 4.53 (dd, $J$=5.1, 8.4 Hz, 1H, H$^3$), 4.78 (d, $J$=15.0 Hz, 1H, -CH$_2$ benzyl), 4.97 (q, $J$=15.6 Hz, 2H, -CH$_2$-), 5.53 (dd, $J$=8.4, 15.9 Hz, 1H, H$^2$), 5.92 (d, $J$=5.1 Hz, 1H, H$^4$), 6.46 (d, $J$=15.9 Hz, 1H, H$^1$), 7.00-7.53 (m, 13H, ArH), 7.73 (s, 1H, triazole-H), $^{13}$C NMR (75MHz, CDCl$_3$+DMSO-d$_6$): δ ppm = 35.1, 45.2, 59.4, 67.8, 111.4, 117.3, 119.6, 123.8, 123.7, 125.6, 126.2, 128.5, 128.8, 128.9, 129.1, 134.2, 134.9, 137.8, 138.5, 141.9, 149.5, 156.4, 157.3, 160.2, 182.5. HRMS Calculated for C$_{29}$H$_{22}$ClN$_5$O$_3$ [M+H]$^+$ 524.1411 found 524.1414; Anal. Calcd (%) for: C, 66.48; H, 4.23; N, 13.37, found: C, 66.40; H, 4.32; N, 13.43.
5i. 1-{\(1-(4\text{-fluoro-phenyl})-2\text{-oxo-4-styryl-azetidin-3-yl}\)}-{\(1H\text{-}[1,2,3]\text{triazol-4-ylmethyl}\)}-{\(1H\text{-indole-2,3-dione}\)}:

Brick red crystalline solid; m.p. 230-231 °C. IR (KBr) \( \nu_{\text{max}} \): 1746, 1727, 1631 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)): \( \delta \) 5.01 (a pair of doublet, \( J=15.6 \) Hz, 2H, -CH\(_2\)-), 5.15 (dd, \( J=5.4, 7.2 \) Hz, 1H, H\(^3\)), 5.82 (dd, \( J=7.2, 15.9 \) Hz, 1H, H\(^2\)), 6.16 (d, \( J=5.4 \) Hz, 1H, H\(^4\)), 6.37 (d, \( J=15.9 \) Hz, 1H, H\(^1\)), 7.00-7.51 (m, 13H, ArH), 7.80 (s, 1H, triazole-H), \(^1\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)): \( \delta \) ppm = 34.2, 46.1, 64.1, 111.8, 116.9, 117.7, 120.6, 123.3, 123.6, 125.9, 126.5, 128.1, 128.8, 129.7, 134.3, 134.8, 137.2, 138.7, 140.1, 149.6, 157.3, 160.8, 182.5. HRMS Calculated for C\(_{28}\)H\(_{20}\)FN\(_5\)O\(_3\) [M+H]\(^+\) 494.1550 found 494.1552; Anal. Calcd (%) for: C, 68.15; H, 4.08; N, 14.19, found: C, 68.06; H, 4.20; N, 14.11.

5j. 5-Fluoro-1-{\(1-(4\text{-fluoro-phenyl})-2\text{-oxo-4-styryl-azetidin-3-yl}\)}-{\(1H\text{-indole-2,3-dione}\)}:

Brick red crystalline solid; m.p. 218-219 °C. IR (KBr) \( \nu_{\text{max}} \): 1741, 1737, 1628 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)): \( \delta \) 5.03 (q, \( J=15.6 \) Hz, 2H, -CH\(_2\)-), 5.17 (t, \( J=5.4, 7.2 \) Hz, 1H, H\(^3\)), 5.81 (dd, \( J=7.2, 15.9 \) Hz, 1H, H\(^2\)), 6.18 (d, \( J=5.4 \) Hz, 1H, H\(^4\)), 6.39 (d, \( J=15.9 \) Hz, 1H, H\(^1\)), 6.98-7.26 (m, 10H, ArH), 7.45-7.49 (m, 2H, ArH), 7.81 (s, 1H, triazole-H), \(^1\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)): \( \delta \) ppm = 34.5, 46.3, 67.1, 111.6, 116.1, 117.6, 120.2, 123.5, 123.8, 125.3, 126.7, 128.2, 128.6, 129.4, 134.6, 134.9, 137.1, 138.6, 141.4, 149.8, 157.0, 158.2, 160.7, 182.9. HRMS Calculated for C\(_{28}\)H\(_{19}\)F\(_2\)N\(_5\)O\(_3\) [M+H]\(^+\) 512.1456 found 512.1459; Anal. Calcd (%) for: C, 65.75; H, 3.74; N, 13.69, found: C, 65.68; H, 3.66; N, 13.79.

5k. 5-Chloro-1-{\(1-(4\text{-fluoro-phenyl})-2\text{-oxo-4-styryl-azetidin-3-yl}\)}-{\(1H\text{-indole-2,3-dione}\)}:

Brick red crystalline solid; m.p. 203-205 °C. IR (KBr) \( \nu_{\text{max}} \): 1744, 1736, 1622 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)): \( \delta \) 5.01 (q, \( J=15.9 \) Hz, 2H, -CH\(_2\)-), 5.18 (t, \( J=5.4, 7.2 \) Hz, 1H, H\(^3\)), 5.81 (dd, \( J=7.2, 15.9 \) Hz, 1H, H\(^2\)), 6.19 (d, \( J=5.4 \) Hz, 1H, H\(^4\)), 6.37 (d, \( J=15.9 \) Hz, 1H, H\(^1\)), 6.99-7.26 (m, 10H, ArH), 7.30-7.48 (m, 2H, ArH), 7.82 (s, 1H, triazole-H), \(^1\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)): \( \delta \) ppm = 34.2, 46.1, 67.6, 111.5, 116.0, 117.5, 120.1, 123.6, 123.4, 125.8, 126.9, 128.3, 128.9, 129.1, 134.2, 134.6, 137.7, 138.5, 141.1, 149.4, 156.2, 157.5, 160.3, 182.1. HRMS Calculated for C\(_{28}\)H\(_{19}\)ClFN\(_5\)O\(_3\) [M+H]\(^+\) 528.1160
found 528.1158; Anal. Calcd (%) for: C, 63.70; H, 3.63; N, 13.27, found: C, 63.81; H, 3.58; N, 13.40.

5l. 5-Bromo-1-\{1-(4-fluoro-phenyl)-2-oxo-4-styryl-azetidin-3-yl\}-1H-[1,2,3]triazol-4-ylmethyl\}-1H-indole-2,3-dione:
Brick red crystalline solid; m.p. 227-228 °C. IR (KBr) \( \nu_{\text{max}} \): 1738, 1729, 1620 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)) : \( \delta \) 4.94 (q, \( J=15.6 \) Hz, 2H, -CH\(_2\)-), 5.17 (t, \( J=5.1 \) Hz, 1H, H\(^3\)), 5.79 (dd, \( J=7.2 \), 15.9 Hz, 1H, H\(^2\)), 6.18 (d, \( J=5.1 \) Hz, 1H, H\(^6\)), 6.62 (d, \( J=15.9 \) Hz, 1H, H\(^1\)), 7.00-7.26 (m, 10H, ArH), 7.33-7.51 (m, 2H, ArH), 7.80 (s, 1H, triazole-H), \(^1^3\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)): \( \delta \) ppm = 34.5, 46.5, 67.6, 111.9, 116.3, 117.4, 120.3, 123.3, 123.8, 125.8, 126.7, 128.2, 128.7, 129.5, 134.5, 134.6, 137.4, 138.8, 141.3, 149.8, 155.4, 157.8, 160.8, 182.9. HRMS Calculated for C\(_{28}\)H\(_{19}\)BrFN\(_5\)O\(_3\) [M+H]+ 572.0655 found 572.0651; Anal. Calcd (%) for: C, 58.75; H, 3.35; N, 12.24, found: C, 58.84; H, 3.46; N, 12.32.

5m. 1-[1-(2-Oxo-1-phenyl-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl\}-1H-indole-2,3-dione:
Brick red crystalline solid; m.p. 210-211 °C. IR (KBr) \( \nu_{\text{max}} \): 1748, 1731, 1610 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)) : \( \delta \) 4.96 (a pair of doublet, \( J=15.6 \) Hz, 2H, -CH\(_2\)-), 5.19 (dd, \( J=5.1 \), 7.2Hz, 1H, H\(^3\)), 5.81 (dd, \( J=7.2 \), 15.9 Hz, 1H, H\(^2\)), 6.17 (d, \( J=5.1 \) Hz, 1H, H\(^4\)), 6.67 (d, \( J=15.9 \) Hz, 1H, H\(^1\)), 7.02-7.57 (m, 14H, ArH), 7.82 (s, 1H, triazole-H), \(^1^3\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)): \( \delta \) ppm = 35.1, 45.4, 67.6, 111.0, 117.3, 119.4, 123.6, 123.8, 125.2, 126.3, 128.2, 128.6, 129.2, 134.5, 134.8, 137.4, 138.8, 141.9, 149.3, 157.7, 160.2, 181.8. HRMS Calculated for C\(_{28}\)H\(_{21}\)N\(_5\)O\(_3\) [M+H]+ 476.1644 found 476.1642; Anal. Calcd (%) for: C, 70.73; H, 4.45; N, 14.73, found: C, 70.80; H, 4.34; N, 14.82.

5n. 5-Fluoro-1-\{1-(2-oxo-1-phenyl-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl\}-1H-indole-2,3-dione:
Brick red crystalline solid; m.p. 209-210 °C. IR (KBr) \( \nu_{\text{max}} \): 1743, 1737, 1628 cm\(^{-1}\); \(^1\)H NMR (300MHz, CDCl\(_3\)) : \( \delta \) 4.97 (q, \( J=15.6 \) Hz, 2H, -CH\(_2\)-), 5.20 (dd, \( J=5.1 \), 7.2Hz, 1H, H\(^3\)), 5.82 (dd, \( J=7.2 \), 15.9 Hz, 1H, H\(^2\)), 6.18 (d, \( J=5.1 \) Hz, 1H, H\(^6\)), 6.66 (d, \( J=15.9 \) Hz, 1H, H\(^1\)), 7.00-7.55 (m, 13H, ArH), 7.80 (s, 1H, triazole-H), \(^1^3\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)): \( \delta \) ppm = 35.0, 45.2, 67.7, 111.5, 117.6, 119.8, 123.4, 123.8, 125.8, 126.3, 128.1, 128.6, 128.7, 129.4, 134.6, 134.9, 137.6, 138.5, 141.5, 149.2, 156.4, 157.9, 199
5o. 5-Chloro-1-[1-(2-oxo-1-phenyl-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 216-217 °C. IR (KBr) ν\text{max} (cm\textsuperscript{-1}): 1737, 1731, 1636; \textsuperscript{1}H NMR (300MHz, CDCl\textsubscript{3}): δ 4.95 (q, J=15.6 Hz, 2H, -CH\textsubscript{2}-), 5.20 (dd, J=5.1, 7.2Hz, 1H, H\textsuperscript{3}), 5.80 (dd, J=7.2, 15.9 Hz, 1H, H\textsuperscript{2}), 6.15 (d, J=5.1 Hz, 1H, H\textsuperscript{4}), 6.68 (d, J=15.9 Hz, 1H, H\textsuperscript{1}), 7.01-7.56 (m, 13H, ArH), 7.81 (s, 1H, triazole-H); \textsuperscript{13}C NMR (75MHz, CDCl\textsubscript{3}+DMSO-d\textsubscript{6}): δ ppm = 35.0, 45.8, 67.4, 111.2, 117.9, 119.5, 123.8, 123.9, 125.1, 126.6, 128.4, 128.7, 128.8, 129.2, 134.4, 134.7, 137.9, 138.5, 141.2, 149.1, 156.1, 157.2, 160.3, 182.0. HRMS Calculated for C\textsubscript{28}H\textsubscript{20}ClN\textsubscript{5}O\textsubscript{3} [M+H]\textsuperscript{+} 510.1255 found 510.1251; Anal. Calcd (%) for: C, 65.95; H, 3.95; N, 13.73, found: C, 65.88; H, 4.19; N, 13.66.

5p. 5-Bromo-1-[1-(2-oxo-1-phenyl-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 234-235 °C. IR (KBr) ν\text{max} (cm\textsuperscript{-1}): 1744, 1728, 1623; \textsuperscript{1}H NMR (300MHz, CDCl\textsubscript{3}): δ 4.98 (s, 2H, -CH\textsubscript{2}-), 5.25 (dd, J=5.4, 8.1 Hz, 1H, H\textsuperscript{3}), 5.93 (dd, J=8.1, 15.9 Hz, 1H, H\textsuperscript{2}), 6.36 (d, J=5.4 Hz, 1H, H\textsuperscript{4}), 6.78 (d, J=15.9 Hz, 1H, H\textsuperscript{1}), 6.85 (d, J=8.4 Hz, 1H, ArH), 7.06-7.70 (m, 12H, ArH), 8.17 (s, 1H, triazole-H); \textsuperscript{13}C NMR (75MHz, CDCl\textsubscript{3}+DMSO-d\textsubscript{6}): δ ppm = 35.2, 45.2, 67.5, 111.3, 117.6, 119.4, 123.3, 123.5, 125.8, 126.3, 128.1, 128.5, 128.7, 129.4, 134.4, 134.6, 137.3, 138.6, 141.5, 149.4, 156.8, 157.5, 160.7, 182.1. HRMS Calculated for C\textsubscript{28}H\textsubscript{20}BrN\textsubscript{5}O\textsubscript{3} [M+H]\textsuperscript{+} 554.0750 found 554.0753; Anal. Calcd (%) for: C, 60.66; H, 3.64; N, 12.63, found: C, 60.73; H, 3.73; N, 12.66.

5q. 5-Methyl-1-[1-(2-oxo-1-phenyl-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 223-224 °C. IR (KBr) ν\text{max} (cm\textsuperscript{-1}): 1736, 1730, 1640; \textsuperscript{1}H NMR (300MHz, CDCl\textsubscript{3}): δ 2.34(s, 3H, -CH\textsubscript{3}), 4.97 (q, J=15.6 Hz, 2H, -CH\textsubscript{2}-), 5.20 (dd, J=5.1, 7.2Hz, 1H, H\textsuperscript{3}), 5.82 (dd, J=7.2, 15.9 Hz, 1H, H\textsuperscript{2}), 6.18 (d, J=5.1 Hz, 1H, H\textsuperscript{4}), 6.68 (d, J=15.9 Hz, 1H, H\textsuperscript{1}), 7.00-7.55 (m, 13H, ArH), 7.80 (s, 1H, triazole-H); \textsuperscript{13}C NMR (75MHz, CDCl\textsubscript{3}+DMSO-d\textsubscript{6}): δ ppm = 22.4, 35.0, 45.3, 67.5, 111.1, 117.8, 119.7, 123.1, 123.6, 125.3, 126.8, 128.5, 128.7, 128.8, 129.9, 134.3, 134.6, 137.5, 138.8, 141.7, 149.2,
156.2, 157.4, 160.1, 182.2. HRMS Calculated for C_{29}H_{23}N_{5}O_{3} [M+H]^+ 490.1801 found 490.1804; Anal. Calcd (%) for: C, 71.15; H, 4.74; N, 14.31, found: C, 71.24; H, 4.81; N, 14.38.

5r. 1-{1-[1-(4-Chloro-phenyl)-2-oxo-4-styryl-azetidin-3-yl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione:
Brick red crystalline solid; m.p. 203-205 °C. IR (KBr) ν_{max}: 1747, 1731, 1622 cm^{-1}; ¹H NMR (300MHz, CDCl₃): δ 4.95 (a pair of doublet, J=15.9 Hz, 2H, -CH₂-), 5.17 (t, J=5.4, 7.2Hz, 1H, H⁺), 5.79 (dd, J=7.2, 16.2 Hz, 1H, H²), 6.17 (d, J=5.4 Hz, 1H, H⁴), 6.67 (d, J=16.2 Hz, 1H, H¹), 7.02-7.56 (m, 13H, ArH), 7.80 (s, 1H, triazole-H), ¹³C NMR (75MHz, CDCl₃+DMSO-d₆): δ ppm = 34.4, 46.3, 67.2, 111.4, 116.6, 117.0 121.1, 122.9, 123.7, 125.4, 126.5, 128.2, 128.5, 129.6, 134.5, 134.8, 137.4, 138.6, 141.2, 149.9, 157.2, 160.8, 182.4. HRMS Calculated for C_{28}H_{20}ClN_{5}O_{3} [M+H]^+ 510.1255 found 510.1258; Anal. Calcd (%) for: C, 65.95; H, 3.95; N, 13.73, found: C, 65.87; H, 4.07; N, 13.83.

5s. 5-Chloro-1-{1-[1-(4-chloro-phenyl)-2-oxo-4-styryl-azetidin-3-yl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione:
Brick red crystalline solid; m.p. 218-219 °C. IR (KBr) ν_{max}: 1740, 1733, 1629 cm^{-1}; ¹H NMR (300MHz, CDCl₃): δ 4.96 (q, J=15.6 Hz, 2H, -CH₂-), 5.16 (t, J=5.4, 7.2Hz, 1H, H⁺), 5.81 (dd, J=7.2, 16.2 Hz, 1H, H²), 6.17 (d, J=5.4 Hz, 1H, H⁴), 6.69 (d, J=16.2 Hz, 1H, H¹), 7.00-7.54 (m, 12H, ArH), 7.81 (s, 1H, triazole-H), ¹³C NMR (75MHz, CDCl₃+DMSO-d₆): δ ppm = 34.1, 46.3, 67.8, 111.6, 117.2, 117.8 121.0, 123.7, 125.2, 126.4, 128.0, 128.4, 129.5, 134.6, 134.8, 137.7, 138.8, 141.1, 149.7, 155.8, 157.6, 160.8, 182.0. HRMS Calculated for C_{28}H_{19}ClN_{5}O_{3} [M+H]^+ 544.0865 found 544.0867; Anal. Calcd (%) for: C, 61.78; H, 3.52; N, 13.73, found: C, 61.89; H, 3.60; N, 13.77.

5t. 1-[1-(2-Oxo-4-styryl-1-p-tolyl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:
Brick red crystalline solid; m.p. 230-231 °C. IR (KBr) ν_{max}: 1739, 1725, 1608 cm^{-1}; ¹H NMR (300MHz, CDCl₃): δ 2.31 (s, 3H, -CH₃), 4.97 (a pair of doublet, J=15.9 Hz, 2H, -CH₂-), 5.16 (dd, J=5.4, 7.2Hz, 1H, H⁺), 5.79 (dd, J=7.2, 16.2 Hz, 1H, H²), 6.16 (d, J=5.4 Hz, 1H, H⁴), 6.64 (d, J=16.2 Hz, 1H, H¹), 7.01-7.42 (m, 12H, ArH), 7.51 (dd, J=7.5 Hz, 1H, ArH), 7.80 (s, 1H, triazole-H), ¹³C NMR (75MHz, CDCl₃+DMSO-d₆): δ ppm = 23.2, 34.1, 45.8, 67.5, 111.0, 117.2, 117.4, 121.8, 123.3, 123.7, 125.3, 126.6, 128.3, 128.5,
129.6, 134.4, 134.6, 137.6, 138.5, 149.4, 157.5, 160.2, 182.4. HRMS Calculated for C_{29}H_{23}N_{5}O_{3} [M+H]^+ 490.1801 found 490.1802; Anal. Calcd (%) for: C, 71.15; H, 4.74; N, 14.31, found: C, 71.07; H, 4.63; N, 14.24.

5u. 5-Fluoro-1-[1-(2-oxo-4-styryl-1-p-tolyl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 224-225 °C. IR (KBr) \( \nu_{\text{max}} \): 1737, 1731, 1631 cm\(^{-1} \); \(^1\)H NMR (300MHz, CDCl\(_3\)) \( \delta \) 2.35 (s, 3H, \(-\text{CH}_3\)), 4.95 (q, \( J=15.6 \) Hz, 2H, \(-\text{CH}_2-\)), 5.15 (dd, \( J=5.4, 7.2 \) Hz, 1H, \( H^3 \)), 5.79 (dd, \( J=7.2, 16.2 \) Hz, 1H, \( H^2 \)), 6.17 (d, \( J=5.4 \) Hz, 1H, \( H^4 \)), 6.65 (d, \( J=16.2 \) Hz, 1H, \( H^1 \)), 7.00-7.41 (m, 11H, ArH), 7.50 (dd, \( J=7.5 \) Hz, 1H, ArH), 7.77 (s, 1H, triazole-H), \(^{13}\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)) \( \delta \) ppm = 23.5, 34.7, 45.9, 67.1, 111.4, 117.5, 117.8, 121.5, 123.4, 123.7, 125.5, 126.7, 128.1, 128.7, 129.4, 134.5, 134.7, 137.8, 138.4, 141.6, 149.6, 157.2, 159.0, 160.6, 182.0. HRMS Calculated for C_{29}H_{22}FN_{5}O_{3} [M+H]^+ 508.1707 found 508.1721; Anal. Calcd (%) for: C, 68.63; H, 4.37; N, 13.80, found: C, 68.72; H, 4.49; N, 13.73.

5v. 5-Chloro-1-[1-(2-oxo-4-styryl-1-p-tolyl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione:

Brick red crystalline solid; m.p. 212-213 °C. IR (KBr) \( \nu_{\text{max}} \): 1746, 1735, 1615 cm\(^{-1} \); \(^1\)H NMR (300MHz, CDCl\(_3\)) \( \delta \) 2.33 (s, 3H, \(-\text{CH}_3\)), 4.96 (q, \( J=15.6 \) Hz, 2H, \(-\text{CH}_2-\)), 5.16 (dd, \( J=5.4, 7.2 \) Hz, 1H, \( H^3 \)), 5.80 (dd, \( J=7.2, 15.9 \) Hz, 1H, \( H^2 \)), 6.17 (d, \( J=5.4 \) Hz, 1H, \( H^4 \)), 6.66 (d, \( J=15.9 \) Hz, 1H, \( H^1 \)), 7.02-7.43 (m, 11H, ArH), 7.51 (dd, \( J=7.5 \) Hz, 1H, ArH), 7.81 (s, 1H, triazole-H), \(^{13}\)C NMR (75MHz, CDCl\(_3\)+DMSO-d\(_6\)) \( \delta \) ppm = 23.3, 34.5, 46.0, 67.3, 111.6, 117.4, 117.7, 121.6, 123.1, 123.7, 125.2, 126.7, 128.2, 128.6, 129.4, 134.2, 134.7, 137.2, 138.9, 141.5, 149.7, 155.8, 157.6, 160.4, 182.1. HRMS Calculated for C_{29}H_{22}ClN_{5}O_{3} [M+H]^+ 524.1411 found 524.1415; Anal. Calcd (%) for: C, 66.48; H, 4.23; N, 13.37, found: C, 66.58; H, 4.16; N, 13.45.

General method for the preparation of \( \beta \)-lactam isatin conjugates 10 and 11:

To a stirred solution of azide 9 (1 mmol for 7 and 2 mmol for 8) in EtOH:H\(_2\)O (90:10) was added in succession appropriate acetylenic lactam 7 or 8 (1 mmol), copper sulphate (0.055 mmol for 7 and 0.1 mmol for 8) and sodium ascorbate (0.13 mmol for 7 and 0.26 for 8) at room temperature. On completion, as monitored by TLC, water was added to the
reaction mixture and extracted with chloroform. Combined organic layers were dried over anhydrous sodium sulphate and concentrated under reduced pressure to result in a crude product which was purified by silica gel column chromatography.

10a. 1-(2-{4-[(2-Oxo-4-styryl-1-p-toly1-azetidin-3-ylamino)-methyl]-[1,2,3]triazol-1-yl]-ethyl)-1H-indole-2,3-dione:

Brick red colour; yield 74%; m.p. 214-215 °C. IR (KBr) ν max: 1738, 1612 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 2.23 (s, 3H, -CH₃); 3.76 (s, 2H, -CH₂-); 4.10-4.13 (m, 2H, -CH₂-); 4.40-4.57 (m, 2H, -CH₂-); 4.61 (d, J=5.1 Hz, 1H, H₄); 4.82 (dd, J=5.1, 8.1 Hz, 1H, H₃); 6.16 (dd, J=8.1, 15.9 Hz, 1H, H²); 6.48 (d, J=8.1 Hz, 1H, ArH); 6.64 (d, J=15.9 Hz, 1H, H¹); 6.90-7.42 (m, 12H, ArH); 7.58 (s, 1H, triazole-H); ¹³C NMR (75MHz, CDCl₃): δ ppm = 21.1, 37.6, 46.5, 48.3, 61.6, 71.8, 110.1, 117.2, 117.7, 123.6, 124.0, 124.5, 125.3, 126.7, 128.1, 128.7, 129.1, 133.9, 134.5, 135.1, 135.8, 138.6, 144.1, 151.5, 158.1, 164.4, 182.2. HRMS Calculated for C₃₁H₂₈N₆O₃ [M]+ 532.2223 found 532.2230; Anal. Caled (%) for: C, 69.91; H, 5.30; N, 15.78, found: C, 69.99; H, 5.38; N, 15.73.

10b. 1-(2-{4-[(1-Cyclohexyl-2-oxo-4-styryl-azetidin-3-ylamino)-methyl]-[1,2,3]triazol-1-yl]-ethyl)-1H-indole-2,3-dione:

Brick red colour; yield 65%; m.p. 219-220 °C. IR (KBr) ν max: 1737, 1616 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.26-1.97 (m, 10H, cyclohexyl-H), 3.48-3.63 (m, 1H, cyclohexyl-H), 3.70 (s, 2H, -CH₂-); 4.12-4.23 (m, 2H, -CH₂-); 4.28 (d, J=5.1 Hz, 1H, H⁴); 4.60 (dd, J=5.4, 11.4 Hz, 2H, -CH₂-); 4.83 (dd, J=5.1, 8.4 Hz, 1H, H³); 6.27 (dd, J=8.4, 15.9 Hz, 1H, H¹); 6.71 (d, J=15.9 Hz, 1H, H¹); 6.99-7.44 (m, 9H, ArH); 7.68 (s, 1H, triazole-H); ¹³C NMR (CDCl₃, 75MHz): δ ppm = 24.1, 24.8, 25.4, 30.2, 31.3, 39.4, 45.4, 47.5, 51.6, 61.7, 72.0, 110.3, 117.3, 117.8, 124.1, 124.6, 124.8, 125.5, 126.7, 128.4, 134.2, 135.1, 135.6, 144.4, 150.1, 158.4, 164.1, 182.1. HRMS Calculated for C₃₀H₃₂N₆O₃ [M]+ 524.2536 found 524.2530; Anal. Caled (%) for: C, 68.68; H, 6.15; N, 16.02, found: C, 68.61; H, 6.24; N, 16.10.
10c. 1-[2-(4-[[1-(4-Fluoro-phenyl)-2-oxo-4-styryl-azetidin-3-ylamino]-methyl]-[1,2,3]triazol-1-yl]-ethyl]-1H-indole-2,3-dione:
Brick red colour; yield 78%; m.p. 203-204 °C. IR (KBr) ν<sub>max</sub>: 1733, 1612 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 3.92 (s, 2H, -CH<sub>2</sub>-); 4.16-4.19 (m, 2H, -CH<sub>2</sub>-); 4.26 (d, J=5.1 Hz, 1H, H<sup>4</sup>); 4.47-4.64 (m, 2H, -CH<sub>2</sub>-); 4.70 (dd, J=5.1, 7.5 Hz, 1H, H<sup>3</sup>); 6.19 (dd, J=7.8, 16.2 Hz, 1H, H<sup>2</sup>); 6.51 (d, J=8.1 Hz, 1H, ArH); 6.65 (d, J=16.2 Hz, 1H, H<sup>1</sup>); 6.95-7.50 (m, 12H, ArH); 7.55 (s, 1H, triazole-H); <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>): δ ppm = 37.1, 46.4, 47.7, 61.8, 72.3, 110.2, 117.0, 117.6, 123.8, 124.1, 124.6, 125.4, 126.9, 128.1, 128.6, 129.0, 134.4, 135.2, 135.8, 138.9, 144.0, 148.7, 151.1, 158.2, 164.9, 182.1. HRMS Calculated for C<sub>30</sub>H<sub>25</sub>F<sub>6</sub>N<sub>6</sub>O<sub>3</sub> [M]<sup>+</sup> 536.1972 found 536.1979; Anal. Calcd (%) for: C, 67.15; H, 4.70; N, 15.66, found: C, 67.06; H, 4.77; N, 15.60.

10d. 1-(3-{4-[(2-Oxo-4-styryl-1-p-toly-azetidin-3-ylamino)-methyl]-[1,2,3]triazol-1-yl}-propyl)-1H-indole-2,3-dione:
Brick red colour; yield 72%; m.p. 197-198 °C. IR (KBr) ν<sub>max</sub>: 1732, 1617 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 2.24 (s, 3H, -CH<sub>3</sub>); 2.32 (dd, J=6.6, 12.9 Hz, 2H, -CH<sub>2</sub>-); 3.75 (a pair of doublet, J=15.0 Hz, 2H, -CH<sub>2</sub>-); 4.10-4.21 (m, 2H, -CH<sub>2</sub>-); 4.59 (dd, J=5.1, 11.1 Hz, 2H, -CH<sub>2</sub>-); 4.68 (d, J=5.1 Hz, 1H, H<sup>4</sup>); 4.84 (dd, J=5.1, 8.4 Hz, 1H, H<sup>3</sup>); 6.30 (dd, J=8.4, 15.9 Hz, 1H, H<sup>2</sup>); 6.54 (d, J=8.1 Hz, 1H, ArH); 6.73 (d, J=15.9 Hz, 1H, H<sup>1</sup>); 6.97 (d, J=8.1 Hz, 1H, ArH); 7.09-7.45 (m, 11H, ArH); 7.76 (s, 1H, triazole-H); <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>): δ ppm = 21.3, 28.0, 38.1, 45.2, 47.4, 61.2, 71.9, 109.8, 117.1, 117.6, 124.1, 124.6, 124.9, 125.3, 125.7, 128.2, 128.6, 129.5, 134.1, 135.4, 135.6, 135.9, 138.4, 144.2, 150.2, 158.1, 164.6, 182.1. HRMS Calculated for C<sub>32</sub>H<sub>30</sub>N<sub>6</sub>O<sub>3</sub> [M]<sup>+</sup> 546.2379 found 546.2372; Anal. Calcd (%) for: C, 70.31; H, 5.53; N, 15.37, found: C, 70.37; H, 5.59; N, 15.29.

10e. 1-(3-{4-[(1-Cyclohexyl-2-oxo-4-styryl-azetidin-3-ylamino)-methyl]-[1,2,3]triazol-1-yl}-propyl)-1H-indole-2,3-dione:
Brick red colour; yield 74%; m.p. 207-208 °C. IR (KBr) ν<sub>max</sub>: 1731, 1610 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.27-1.95 (m, 10H, cyclohexyl-H), 2.33 (dd, J=6.3, 13.2 Hz, 2H, -CH<sub>2</sub>-); 3.47-3.63 (m, 1H, cyclohexyl-H), 3.73 (s, 2H, -CH<sub>2</sub>-); 4.13-4.24 (m, 2H, -CH<sub>2</sub>-); 4.34 (d, J=5.1 Hz, 1H, H<sup>4</sup>); 4.56 (m, 2H, -CH<sub>2</sub>-); 4.87 (dd, J=5.1, 8.4 Hz, 1H, H<sup>3</sup>); 6.25 (dd, J=8.4, 15.6 Hz, 1H, H<sup>2</sup>); 6.69 (d, J=15.6 Hz, 1H, H<sup>1</sup>); 7.06-7.53 (m, 9H, ArH); 7.71
(s, 1H, triazole-H); 13C NMR (75MHz, CDCl3): δ ppm = 23.7, 24.8, 25.5, 28.2, 31.1, 31.9, 39.1, 45.6, 47.4, 51.2, 62.9, 72.4, 109.9, 117.1, 117.8, 124.1, 124.7, 124.8, 125.3, 126.2, 128.5, 133.8, 135.2, 135.8, 144.5, 150.2, 158.6, 164.2, 182.0. HRMS Calculated for C31H34N6O3 [M]+ 538.2692 found 538.2699; Anal. Calcd (%) for: C, 69.12; H, 6.36; N, 15.60, found: C, 69.19; H, 6.28; N, 15.67.

10f. 1-[3-(4-{[1-(4-Fluoro-phenyl)-2-oxo-4-styryl-azetidin-3-ylamino]-methyl}-[1,2,3]triazol-1-yl)-propyl]-1H-indole-2,3-dione:

Brick red colour; yield 81%; m.p. 206-207 °C. IR (KBr) νmax: 1730, 1616 cm⁻¹; 1H NMR (300 MHz, CDCl3): δ 2.35 (dd, J=6.6, 13.2 Hz, 2H, -CH₂-); 3.84 (s, 2H, -CH₂-); 4.10-4.21 (m, 2H, -CH₂-); 4.25 (d, J=5.1 Hz, 1H, H⁴); 4.46-4.63 (m, 2H, -CH₂-); 4.73 (dd, J=5.1, 8.1 Hz, 1H, H³); 6.24 (dd, J=8.1, 15.9 Hz, 1H, H²); 6.58 (d, J=8.1 Hz, 1H, ArH); 6.69 (d, J=15.9 Hz, 1H, H¹); 6.94-7.44 (m, 12H, ArH); 7.66 (s, 1H, triazole-H); 13C NMR (75MHz, CDCl3): δ ppm = 27.6, 37.2, 46.6, 47.8, 61.6, 72.1, 110.0, 117.0, 117.7, 123.5, 124.2, 124.7, 125.4, 126.8, 128.0, 128.8, 129.1, 134.5, 135.1, 135.7, 138.8, 144.2, 148.8, 151.4, 158.3, 164.7, 182.3. HRMS Calculated for C₃₁H₃₄FN₆O₃ [M]+ 550.2129 found 550.2122; Anal. Calcd (%) for: C, 67.63; H, 4.94; N, 15.26, found: C, 67.70; H, 4.88; N, 15.31.

11a. 3-{Bis-[1-(3-[2,3-dioxo-1H-indol-1-yl]-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-amino}-4-styryl-1-p-tolyl-azetidin-2-one:

Brick red colour; yield 75%; m.p. >230 °C. IR (KBr) νmax: 1730, 1621 cm⁻¹; 1H NMR (300 MHz, CDCl₃): δ 2.27 (s, 3H, -CH₃); 3.73 (a pair of doublet, J=15.0 Hz, 4H, 2×-CH₂-); 4.10-4.21 (m, 5H, H⁴+2×-CH₂-); 4.56 (dd, J=5.1, 10.8 Hz, 4H, 2×-CH₂-); 4.82 (dd, J=5.1, 8.1 Hz, 1H, H³); 6.29 (dd, J=8.4, 15.9 Hz, 1H, H²); 6.56 (d, J=7.8 Hz, 2H, ArH); 6.69 (d, J=16.2 Hz, 1H, H¹); 6.97 (t, J=7.8 Hz, 2H, ArH); 7.07 (d, J=8.1 Hz, 2H, ArH); 7.25-7.47 (m, 11H, ArH); 7.68 (s, 2H, triazole-H); 13C NMR (75MHz, CDCl₃): δ ppm = 20.8, 40.6, 45.3, 47.5, 61.3, 71.5, 109.4, 117.0, 117.4, 124.0, 124.7, 124.8, 125.5, 126.6, 128.3, 128.7, 129.5, 133.9, 135.1, 135.3, 135.9, 138.5, 144.3, 149.9, 158.4, 164.7, 182.5. HRMS Calculated for C₄₄H₄₃N₁₀O₅ [M]+ 786.3027 found 786.3034; Anal. Calcd (%) for: C, 67.16; H, 4.87; N, 17.80, found: C, 67.07; H, 4.90; N, 17.87.
11b. 3-{Bis-[1-(3-[2,3-dioxo-1H-indol-1-yl]-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-amino}-1-cyclohexyl-4-styryl-azetidin-2-one:

Brick red colour; yield 75%; m.p. >230 °C. IR (KBr) ν_max: 1739, 1614 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.27-1.98 (m, 10H, cyclohexyl-H), 3.47-3.62 (m, 1H, cyclohexyl-H), 3.71 (a pair of doublet, J=15.0 Hz, 4H, 2×-CH₂-); 4.11-4.22 (m, 4H, 2×-CH₂-); 4.27 (d, J=5.1 Hz, 1H, H₄); 4.58 (dd, J=5.4, 11.1 Hz, 4H, 2×-CH₂-); 4.84 (dd, J=5.1, 8.4 Hz, 1H, H₃); 6.31 (dd, J=8.4, 15.9 Hz, 1H, H²); 6.68 (d, J=15.9 Hz, 1H, H¹); 7.03-7.46 (m, 13H, ArH); 7.75 (s, 2H, triazole-H); ¹³C NMR (75MHz, CDCl₃): δ ppm = 24.4, 24.9, 25.3, 30.1, 31.4, 39.8, 45.2, 47.3, 51.5, 61.6, 71.8, 110.2, 117.1, 117.5, 124.2, 124.7, 124.9, 125.7, 126.6, 128.5, 134.0, 135.2, 135.8, 144.5, 150.2, 158.6, 164.3, 182.0. HRMS Calculated for C₄₃H₄₁N₁₀O₅ [M]+ 778.3340 found 778.3347; Anal. Calcd (%) for: C, 66.31; H, 5.44; N, 17.98, found: C, 66.37; H, 5.39; N, 17.93.

11c. 3-{Bis-[1-(3-[2,3-dioxo-1H-indol-1-yl]-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-amino}-1-(4-Fluoro-phenyl)-4-styryl-azetidin-2-one:

Brick red colour; yield 66%; m.p. >230 °C. IR (KBr) ν_max: 1732, 1618 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.98 (s, 4H, -CH₂-); 4.10-4.21 (m, 5H, H₄+2×-CH₂-); 4.39 (d, J=5.1 Hz, 1H, H¹); 4.56 (dd, J=5.1, 10.8 Hz, 4H, 2×-CH₂-); 4.92 (dd, J=5.1, 8.4 Hz, 1H, H³); 6.32 (dd, J=8.4, 15.6 Hz, 1H, H²); 6.50 (d, J=7.8 Hz, 2H, ArH); 6.71 (d, J=16.2 Hz, 1H, H¹); 6.99-7.49 (m, 15H, ArH); 7.59 (s, 2H, triazole-H); ¹³C NMR (75MHz, CDCl₃): δ ppm = 41.2, 45.8, 47.6, 61.1, 72.3, 110.2, 117.1, 117.5, 124.1, 124.6, 124.8, 125.7, 126.8, 128.1, 128.6, 129.4, 135.2, 135.6, 136.0, 138.4, 144.1, 149.0, 150.4, 159.2, 164.7, 181.9. HRMS Calculated for C₄₃H₃₅FN₁₀O₅ [M]+ 790.2776 found 7990.2770; Anal. Calcd (%) for: C, 65.31; H, 4.46; N, 17.71, found: C, 65.37; H, 4.53; N, 17.64.

11d. 3-{Bis-[1-(3-[2,3-dioxo-1H-indol-1-yl]-propyl)-1H-[1,2,3]triazol-4-ylmethyl]-amino}-4-styryl-1-p-tolyl-azetidin-2-one:

Brick red colour; yield 70%; m.p. >230 °C. IR (KBr) ν_max: 1737, 1610 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 2.26 (s, 3H, -CH₃); 2.32 (dd, J=6.6, 13.2 Hz, 4H, 2×-CH₂-); 3.70-3.75 (m, 4H, -CH₂-); 4.04-4.10 (m, 4H, 2×-CH₂-); 4.27 (t, J=6.6 Hz, 4H, 2×-CH₂-); 4.66 (d, J=5.1 Hz, 1H, H¹); 4.82 (dd, J=5.1, 8.1 Hz, 1H, H³); 6.31 (dd, J=8.4, 15.9 Hz, 1H,
H\textsuperscript{3}; 6.68 (d, J=15.9 Hz, 1H, H\textsuperscript{1}); 6.87 (d, J=8.4 Hz, 2H, ArH); 7.04-7.60 (m, 15H, ArH); 7.82 (s, 2H, triazole-H); \textsuperscript{13}C NMR (75MHz, CDCl\textsubscript{3}): δ ppm = 20.8, 27.5, 37.3, 46.0, 47.3, 61.8, 72.5, 110.1, 117.1, 117.6, 123.9, 124.2, 124.8, 125.5, 126.5, 128.2, 128.7, 129.5, 133.8, 134.8, 135.3, 135.9, 144.3, 150.2, 158.3, 164.8, 182.9. HRMS Calculated for C\textsubscript{46}H\textsubscript{42}N\textsubscript{10}O\textsubscript{5} [M]\textsuperscript{+} 814.3340 found 814.3347; Anal. Calcd (%) for: C, 67.80; H, 5.19; N, 17.19, found: C, 67.85; H, 5.24; N, 17.11.

11e. 3-{Bis-[1-(3-[2,3-dioxo-1H-indol-1-yl]-propyl)-1H-[1,2,3]triazol-4-ylmethyl]-amino}-1-cyclohexyl-4-styryl-azetidin-2-one:

Brick red colour; yield 78%; m.p. >230 °C. IR (KBr) \(\nu_{\text{max}}\): 1739, 1614 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ 1.29-1.97 (m, 10H, cyclohexyl-H), 2.34 (dd, J=6.3, 12.9 Hz, 4H, 2×-CH\textsubscript{2}-); 3.46-3.61 (m, 1H, cyclohexyl-H); 3.84 (s, 4H, -CH\textsubscript{2}-); 4.15-4.26 (m, 4H, 2×-CH\textsubscript{2}-); 4.58 (m, 4H, 2×-CH\textsubscript{2}-); 4.67 (d, J=5.1 Hz, 1H, H\textsuperscript{4}); 4.91 (dd, J=5.1, 8.7 Hz, 1H, H\textsuperscript{3}); 6.28 (dd, J=8.7, 15.6 Hz, 1H, H\textsuperscript{2}); 6.70 (d, J=15.6 Hz, 1H, H\textsuperscript{1}); 7.01-7.51 (m, 13H, ArH); 7.63 (s, 2H, triazole-H); \textsuperscript{13}C NMR (75MHz, CDCl\textsubscript{3}): δ ppm = 24.1, 24.6, 25.5, 27.9, 30.8, 31.6, 39.3, 45.1, 47.5, 51.7, 62.8, 72.1, 109.8, 117.2, 117.7, 124.0, 124.6, 124.8, 125.4, 126.3, 128.4, 133.7, 135.3, 135.9, 144.6, 150.1, 158.8, 164.1, 182.3. HRMS Calculated for C\textsubscript{45}H\textsubscript{46}N\textsubscript{10}O\textsubscript{5} [M]\textsuperscript{+} 806.3653 found 806.3645; Anal. Calcd (%) for: C, 66.98; H, 5.75; N, 17.36, found: C, 66.90; H, 5.82; N, 17.49.

11f. 3-{Bis-[1-(3-[2,3-dioxo-1H-indol-1-yl]-propyl)-1H-[1,2,3]triazol-4-ylmethyl]-amino}-1-(4-Fluoro-phenyl)-4-styryl-azetidin-2-one:

Brick red colour; yield 69%; m.p. >230 °C. IR (KBr) \(\nu_{\text{max}}\): 1734, 1613 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ 2.34 (dd, J=6.0, 12.6 Hz, 4H, 2×-CH\textsubscript{2}-); 3.88 (s, 4H, -CH\textsubscript{2}-); 4.07-4.18 (m, 4H, 2×-CH\textsubscript{2}-); 4.33 (d, J=5.1 Hz, 1H, H\textsuperscript{4}); 4.56 (m, 4H, 2×-CH\textsubscript{2}-); 4.90 (dd, J=5.1, 8.4 Hz, 1H, H\textsuperscript{3}); 6.34 (dd, J=8.4, 15.6 Hz, 1H, H\textsuperscript{2}); 6.51 (d, J=8.1 Hz, 2H, ArH); 6.68 (d, J=15.6 Hz, 1H, H\textsuperscript{1}); 7.04-7.46 (m, 15H, ArH); 7.62 (s, 2H, triazole-H); \textsuperscript{13}C NMR (75MHz, CDCl\textsubscript{3}): δ ppm = 27.4, 41.5, 45.9, 47.1, 62.4, 72.0, 110.3, 117.2, 117.6, 124.2, 124.8, 125.5, 126.7, 128.0, 128.5, 129.5, 135.1, 135.4, 136.3, 138.1, 144.5, 148.4, 149.8, 159.1, 164.4, 182.3. HRMS Calculated for C\textsubscript{45}H\textsubscript{39}FN\textsubscript{10}O\textsubscript{5} [M]\textsuperscript{+} 818.3089 found 818.3095; Anal. Calcd (%) for: C, 66.00; H, 4.80; N, 17.11, found: C, 66.07; H, 4.74; N, 17.18.
4.6 References: