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Preliminary communication

Synthesis of 1*H*-1,2,3-triazole linked β-lactam–isatin bi-functional hybrids and preliminary analysis of *in vitro* activity against the protozoal parasite *Trichomonas vaginalis*

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ABSTRACT

Twenty-two different triazoles were prepared to examine the anti-*Trichomonas vaginalis* structure–activity relationships (SAR) within the β-lactam–isatin–triazole conjugate family. The compounds were synthesized by copper-catalyzed 'click chemistry.' *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.

1. Introduction

Parasitic infections represent a major health threat in under-developed 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 such as *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 and inflammation, and punctate cervical microhemorrhages [5]. It is now well established that *trichomoniasis*, significantly increase the host's vulnerability to contract HIV [6,7] and controlling it could significantly reduce the transmission of new HIV infections. Although the introduction of metronidazole heralded a new era for the treatment of parasitic infections [8], the recent revelations of its toxic effects, including genotoxicity, gastric mucus irritation [9,10], and the development of clinical resistance [11] demonstrate the need for the development of new and efficient scaffolds against *trichomoniasis*.

The isatin (1*H*-indole-2,3-dione) moiety is a versatile scaffold with wide possibilities for chemical modification, and is responsible for a broad spectrum of biological properties in many synthetically versatile molecules [12]. In recent years, Schiff and Mannich bases of 1*H*-indole-2,3-diones were reported to exhibit chemotherapeutic properties including antiviral [13], antitubercular [14], antifungal, and antibacterial activities [15]. Investigation of the SARs in 1*H*-indole-2,3-dione derivatives revealed that 5-halogenation [14,15], N-alkylation [16], *N*-Mannich base [15], and 3-thiosemicarbazone formation were effective in triggering a marked rise in activity against various bacteria, fungi, and viruses. Moreover, cyclization of 1*H*-indole-2,3-diones to 4-thiazoline [14], 4-thiazolidinone [17], and pyridazinoindole [16] was efficient in increasing anti-microbial activity. Previous studies have also reported the inhibitory activity of isatin–β-thiosemicarbazones and isatin derivatives on HIV replication [18], which has been extended towards the synthesis of diverse *N*-Mannich base derivatives of 5-substituted isatins with potential non-nucleoside reverse transcriptase inhibitor (NNRTI) activity.

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β-Lactam nucleus is an important heterocyclic core with pronounced biological activities and present in most widely used antibiotics such as penicillins [19], cephalosporins [20], carbapenems and monobactams [21,22]. In recent years, synthesis and modification of the β-lactam ring to obtain compounds with diverse pharmacological activities such as cholesterol absorption inhibitors, human cytomegalovirus protease inhibitors, thrombin inhibitors, anti-hyperglycaemic, anti-tumour, anti-HIV, anti-inflammatory, analgesic activities and serine-dependent enzyme inhibitors has generated a renewed interest for this class of antimicrobial agents [23].

In continuation of our pursuit of the synthesis of biologically potent novel functional entities [24], we present herein the synthesis of 1*H*-1,2,3-triazole tethered bi-functional hybrids of C-5 substituted isatins with *N*-1 substituted β-lactams (Fig. 1) and their *in vitro* evaluation against *T. vaginalis*. The introduction of 1*H*-1,2,3-triazole as linker is based on its ability to confer favourable properties *viz.* moderate dipole character, hydrogen bonding capability, rigidity and stability under *in vivo* conditions on the synthesized hybrids [25].

2. Results and discussion

2.1. Synthetic chemistry

The desired bi-functional hybrids were synthesized using click chemistry approach between *N*-substituted β-lactams and 5-substituted isatins containing the azide and terminal alkyne respectively, in the presence of Cu(I) catalyst. The 5-substituted isatins were prepared according to reported procedures and *N*-propargylation was done using propargyl bromide in the presence of NaH as a base, and DMF as solvent (Schemes 1–3). 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*-toluene sulphonylchloride and triethylamine [26].

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_2O

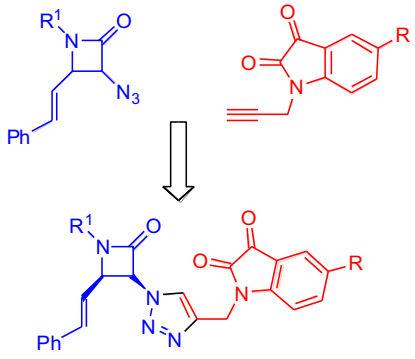
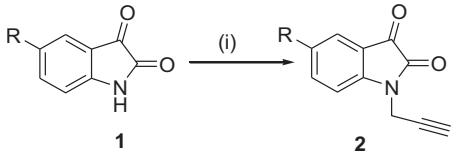
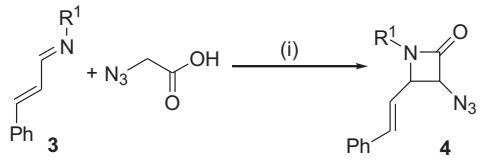


Fig. 1. General structure of lead compound and target hybrid compounds.



Reagents and conditions: (i) Propargyl bromide, NaH, DMF, rt, 3 h.

Scheme 1. Synthesis of *N*-propargylated isatins.



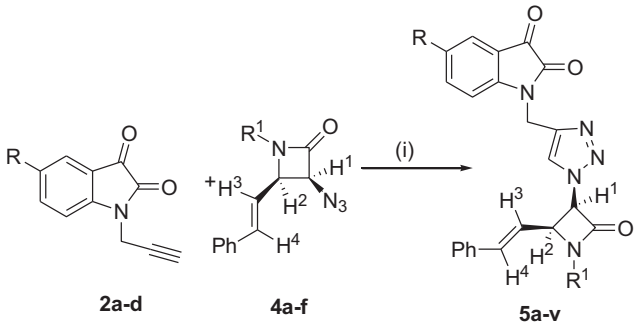
Reagents and conditions: (i) *p*-toluene sulphonyl chloride, Et_3N, Dry Dichloromethane, rt.

Scheme 2. Synthesis of 3-azido-2-azetidinones.

(7:3) mixture in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate for 7 h. 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^1 and H^2.

2.2. In vitro protozoal parasite susceptibility assay and mammalian cell cytotoxic studies

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. The protozoal parasites were cultured for 24 h at 37 °C. 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. After 24 h, cells were counted using a hemacytometer. The IC_{50} value for compound **5i** was determined by running assays of increasing drug concentrations, 5 μM–40 μM, and performing a regression analysis using Prism software, from GraphPad. To screen compounds on mammalian cells, 100 μM concentrations were added to cultures of CHO-K1 cells. After 24 h, cells were visualized under light microscopy to detect observable changes in morphology.



Reagent and conditions: (i) $CuSO_4 .5H_2O$ (0.05 mmol), Sodium ascorbate (0.13 mmol), EtOH/H_2O, rt, 7h.

Scheme 3. Diastereoselective synthesis of 1*H*-1,2,3-triazole-tethered β-lactam–isatin conjugates.

2.3. In vitro activity against T. vaginalis

The synthesized 1*H*-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 at 10 and 100 μM. 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**–**v**) in general showed better activity profiles than with *N*-alkyl substituents (**5a**–**h**). 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 (−CH_3) 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**–**p** among the test compound; **5q** being an exception. The compound **5h**, 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. 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*-1 of β-lactam ring and flouro-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 μM. All the compounds except **5i** exerted cytotoxic effects on CHO-k1 cells as observed by direct visualization of cellular morphology. **5i** with an IC_{50} of 7.69 μM and no visual morphological effect on cultured CHO-K1 cells is considered as a good candidate for further evaluations. The dose–response curve for the potent scaffolds **5i** and **5n** are elucidated in Fig. 3 while the dose–response curves for the scaffolds **5e**, **5f**, **5i**, **5n**, **5o**, **5p**, **5r**, **5s**, **5t**, **5u** and **5v** are provided in the Supplementary material.

In conclusion, the present manuscript describes the synthesis of novel 1*H*-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*.

3. Experimental section

Melting points were determined by open capillary using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. ^{1}H NMR spectra were recorded in deuterochloroform and DMSO-d_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 *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. ^{13}C NMR spectra were recorded on Jeol 300 (75 MHz) spectrometers in deuterochloroform and DMSO-d_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).

3.1. Procedure for the preparation of β-lactam–isatin hybrids

To a stirred solution of appropriate acetylenic isatins **2a**–**d** (1 mmol) in ethanol:water (7:3) mixture and azide **4a**–**f** (1 mmol) were added copper sulphate (0.05 mmol) and sodium ascorbate (0.13 mmol). The reaction mixture was allowed to stir at room temperature for 8 h 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* re-crystallization using 8:2 (chloroform:methanol) mixture.

3.1.1. 1-[1-(1-Cyclohexyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3] triazol-4-ylmethyl]-1H-indole-2,3-dione (**5a**)

Brick red crystalline solid, mp 200–202 °C, ^{1}H NMR: δ 1.08–2.04 (m, 10H, cyclohexyl), 3.45–3.60 (m, 1H, cyclohexyl), 4.72 (dd, *J* = 5.1, 8.7 Hz, 1H, H^2), 4.98 (s, 2H, −CH_2), 5.66 (dd, *J* = 8.7, 15.6 Hz, 1H, H^3), 5.90 (d, *J* = 5.1 Hz, 1H^1), 6.60 (d, *J* = 15.6 Hz, 1H, H^4), 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 ring), ^{13}C NMR: 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 $C_{28}H_{27}N_5O_3$ $[M + H]^+$ 482.2114 found 482.2112.

3.1.2. 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 (**5b**)

Brick red crystalline solid, mp 210–212 °C, ^{1}H NMR: δ 1.23–2.01 (m, 10H, cyclohexyl), 3.46–3.61 (m, 1H, cyclohexyl), 4.72 (dd, *J* = 5.1, 8.4 Hz, 1H, H^2), 4.97 (s, 2H, −CH_2), 5.66 (dd, *J* = 8.4, 15.6 Hz, 1H, H^3), 5.91 (d, *J* = 5.1 Hz, 1H, H^1), 6.58 (d, *J* = 15.6 Hz, 1H, H^4), 7.05–7.26 (m, 8H, ArH), 7.76 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{28}H_{26}FN_5O_3$ $[M + H]^+$ 500.2020 found 500.2017.

3.1.3. 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 (**5c**)

Brick red crystalline solid, mp 219–220 °C, ^{1}H NMR: δ 1.23–2.31 (m, 10H, cyclohexyl), 3.45–3.60 (m, 1H, cyclohexyl), 4.71 (dd, *J* = 5.1, 8.7 Hz, 1H, H^2), 4.96 (s, 2H, −CH_2), 5.65 (dd, *J* = 8.7, 15.6 Hz, 1H, H^3), 5.89 (d, *J* = 5.1 Hz, 1H, H^1), 6.59 (d, *J* = 15.6 Hz, 1H, H^4), 7.00–7.34 (m, 8H, ArH), 7.72 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{28}H_{26}ClN_5O_3$ $[M + H]^+$ 516.1724 found 516.1727.

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

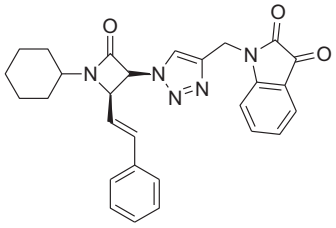
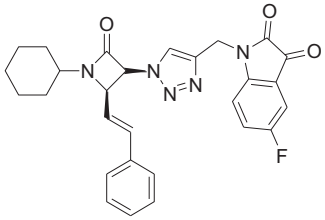
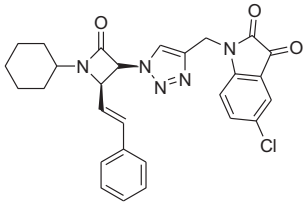
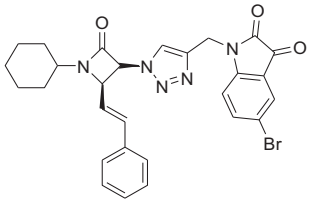
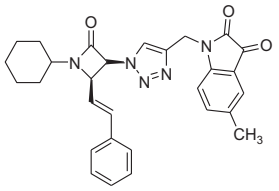
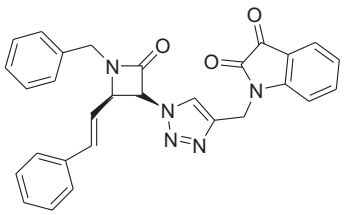
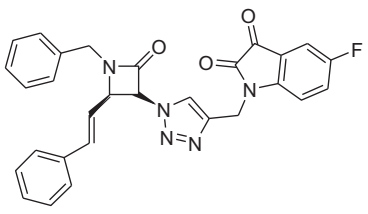
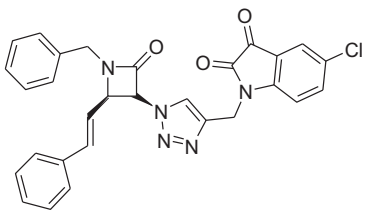
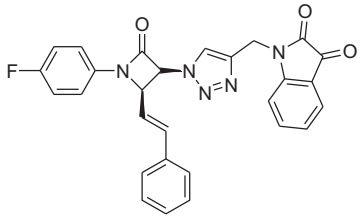
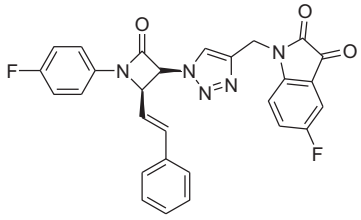
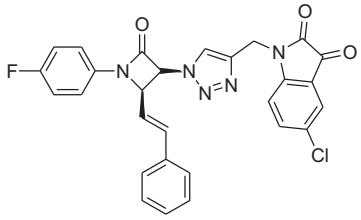
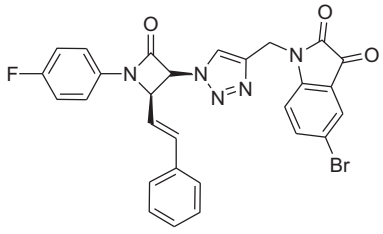
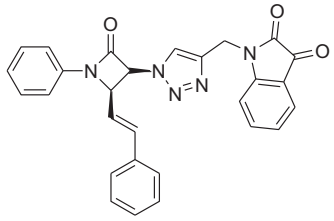
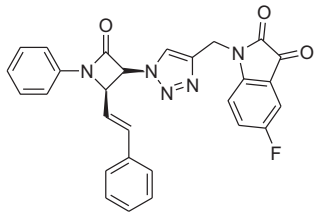
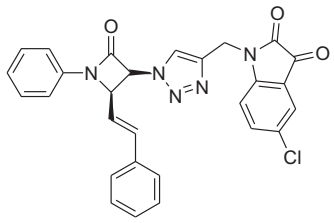
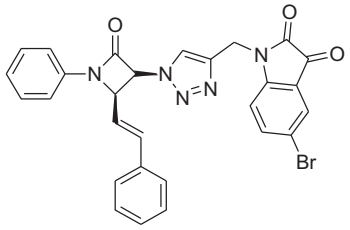
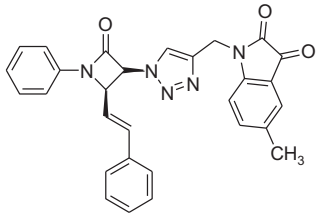
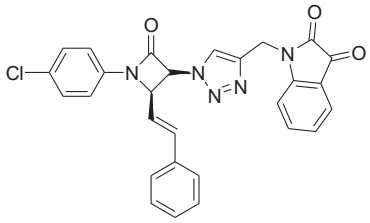
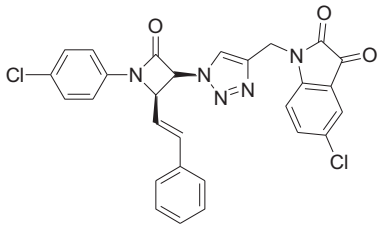
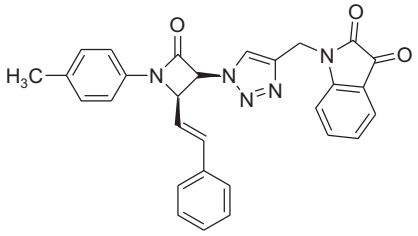
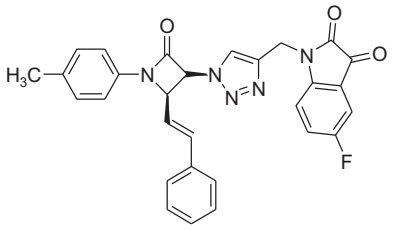
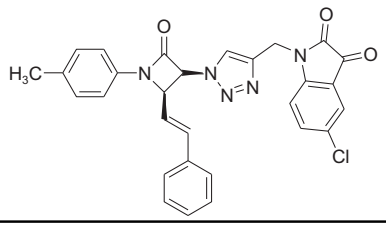
Code	Structure	Average % inhibition 10 μM	Average % inhibition at at 100 μM	C log P [27] [a]
5a		26.80	100.00	4.02
5b		0.00	100.00	4.17
5c		7.30	100.00	4.74
5d		4.90	100.00	4.89
5e		9.80	95.65	4.34
5f		7.80	100.00	3.58
5g		0.00	19.57	3.90
5h		7.30	67.39	4.47

Table 1 (*continued*)

Code	Structure	Average % inhibition 10 μM	Average % inhibition at at 100 μM	C log P [27] [a]
5i		38.50	100.00	4.17
5j		19.50	100.00	4.50
5k		14.60	100.00	5.07
5l		0.00	100.00	5.22
5m		100.00	100.00	3.89
5n		58.50	100.00	4.21
5o		61.00	100.00	4.78

(*continued on next page*)

Table 1 (*continued*)

Code	Structure	Average % inhibition 10 μM	Average % inhibition at at 100 μM	C log P [27] [a]
5p		83.00	100.00	4.93
5q		0.00	82.61	4.39
5r		80.50	100.00	4.74
5s		53.70	100.00	5.64
5t		4.90	100.00	4.39
5u		58.50	100.00	4.71
5v		48.80	100.00	5.28

[a] Calculated using Chem Draw Ultra 10.0.

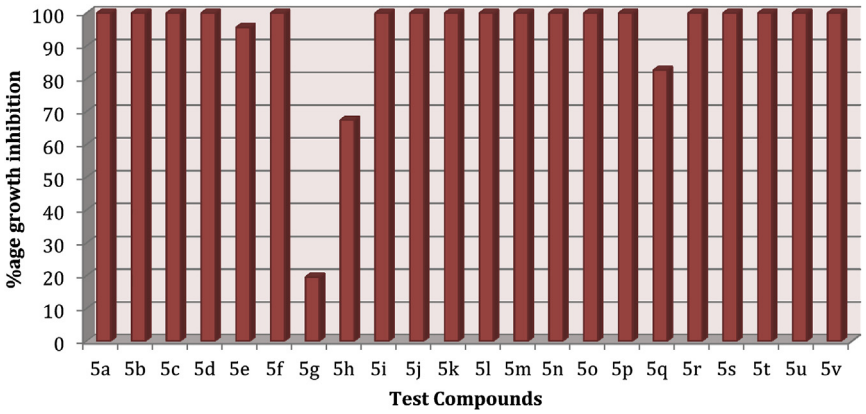


Fig. 2. Graphical representation of inhibitory activities of test compounds at 100 μM.

*3.1.4. 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 (**5d**)*

Brick red crystalline solid, mp 201–203 °C, ^{1}H NMR: δ 1.27–1.98 (m, 10H, cyclohexyl), 3.47–3.62 (m, 1H, cyclohexyl), 4.72 (dd, J = 5.1, 8.4 Hz, 1H, H^2), 4.94 (q, J = 15.6 Hz, 2H, −CH$_2$), 5.65 (dd, J = 8.4, 15.9 Hz, 1H, H^3), 5.89 (d, J = 5.1 Hz, 1H, H^1), 6.57 (d, J = 15.9 Hz, 1H, H^4), 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 ring), ^{13}C NMR: δ 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 $C_{28}H_{26}BrN_5O_3$ [M + H]$^+$ 560.1219 found 560.1217.

*3.1.5. 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 (**5e**)*

Brick red crystalline solid, mp 207–208 °C, ^{1}H NMR: δ 1.27–1.97 (m, 10H, cyclohexyl), 2.35 (s, 3H, −CH$_3$), 3.46–3.61 (m, 1H, cyclohexyl), 4.72 (dd, J = 5.1, 8.4 Hz, 1H, H^2), 4.97 (q, J = 15.6 Hz, 2H, −CH$_2$), 5.65 (dd, J = 8.4, 15.9 Hz, 1H, H^3), 5.90 (d, J = 5.1 Hz, 1H, H^1), 6.57 (d, J = 15.9 Hz, 1H, H^4), 7.03–7.26 (m, 6H, ArH), 7.31–7.34 (m, 1H, ArH), 7.46 (d, J = 2.1 Hz, 1H, ArH), 7.74 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{29}H_{29}BrN_5O_3$ [M + H]$^+$ 496.2270 found 496.2266.

*3.1.6. 1-[1-(1-Benzyl-2-oxo-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione (**5f**)*

Brick red crystalline solid, mp 214–215 °C, ^{1}H NMR: δ 4.19 (d, J = 15.0 Hz, 1H, −CH$_2$ benzyl), 4.54 (dd, J = 5.1, 8.4 Hz, 1H, H^2), 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^3), 5.95 (d, J = 5.1 Hz, 1H, H^1), 6.47 (d, J = 15.9 Hz, 1H, H^4), 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: δ 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 $C_{29}H_{23}N_5O_3$ [M + H]$^+$ 490.1811 found 490.1815.

Table 2
IC$_{50}$ determination of active compounds.

Compound	IC$_{50}$ (μM) (G3)
5e	44.51
5f	8.68
5i	7.69
5n	7.06
5o	9.82
5p	9.34
5r	8.27
5s	9.94
5t	27.46
5u	22.09
5v	10.05
Metronidazole[a]	0.72

[a] Current FDA approved treatment for *T. vaginalis* infections.

*3.1.7. 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 (**5g**)*

Brick red crystalline solid, mp 215–217 °C, ^{1}H NMR: δ 4.17 (d, J = 14.9 Hz, 1H, −CH$_2$ benzyl), 4.53 (dd, J = 5.1, 8.4 Hz, 1H, H^2), 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^3), 5.93 (d, J = 5.1 Hz, 1H, H^1), 6.43 (d, J = 15.9 Hz, 1H, H^4), 6.95–7.35 (m, 13H, ArH), 7.71 (s, 1H, triazole ring), ^{13}C NMR: δ 19.1, 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 $C_{29}H_{22}FN_5O_3$ [M + H]$^+$ 508.1707 found 508.1705.

*3.1.8. 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 (**5h**)*

Brick red crystalline solid, mp 210–211 °C, ^{1}H NMR: δ 4.17 (d, J = 15.0 Hz, 1H, −CH$_2$ benzyl), 4.53 (dd, J = 5.1, 8.4 Hz, 1H, H^2), 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^3), 5.92 (d, J = 5.1 Hz, 1H, H^1), 6.46 (d, J = 15.9 Hz, 1H, H^4), 7.00–7.53 (m, 13H, ArH), 7.73 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{29}H_{22}ClN_5O_3$ [M + H]$^+$ 524.1411 found 524.1414.

*3.1.9. 1-{1-[1-(4-Fluoro-phenyl)-2-oxo-4-styryl-azetidin-3-yl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**5i**)*

Brick red crystalline solid, mp 230–231 °C, ^{1}H NMR: δ 5.01 (a pair of doublet, J = 15.6 Hz, 2H, −CH$_2$), 5.15 (dd, J = 5.4, 7.2 Hz, 1H, H^2), 5.82 (dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.16 (d, J = 5.4 Hz, 1H, H^1), 6.37 (d, J = 15.9 Hz, 1H, H^4), 7.00–7.51 (m, 13H, ArH), 7.80 (s, 1H, triazole ring), ^{13}C NMR: δ 34.2, 46.1, 67.4, 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, 141.0, 149.6, 157.3, 160.8, 182.5, HRMS Calculated for $C_{28}H_{20}FN_5O_3$ [M + H]$^+$ 494.1550 found 494.1552.

*3.1.10. 5-Fluoro-1-{1-[1-(4-fluoro-phenyl)-2-oxo-4-styryl-azetidin-3-yl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**5j**)*

Brick red crystalline solid, mp 218–219 °C, ^{1}H NMR: δ 5.03 (q, J = 15.6 Hz, 2H, −CH$_2$), 5.17 (t, J = 5.4, 7.2 Hz, 1H, H^2), 5.81

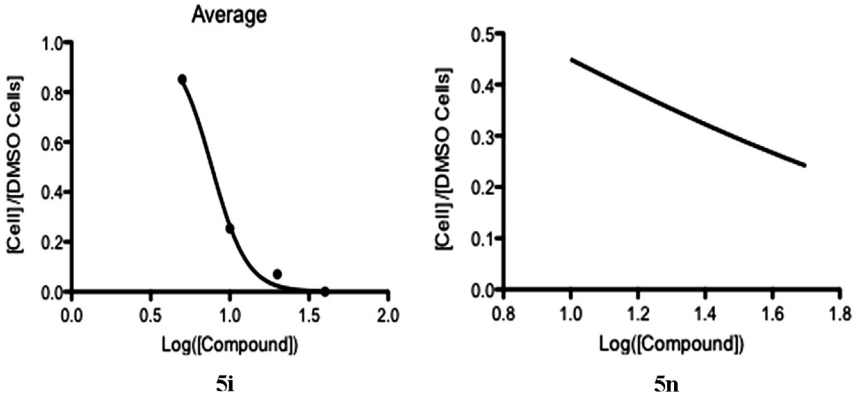


Fig. 3. Dose–response curve for **5i** and **5n**.

(dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.18 (d, J = 5.4 Hz, 1H, H^1), 6.39 (d, J = 15.9 Hz, 1H, H^4), 6.98–7.26 (m, 10H, ArH), 7.45–7.49 (m, 2H, ArH), 7.81 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{28}H_{19}F_2N_5O_3$ $[M + H]^+$ 512.1456 found 512.1459.

*3.1.11. 5-Chloro-1-{1-[1-(4-fluoro-phenyl)-2-oxo-4-styryl-azetidin-3-yl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**5k**)*

Brick red crystalline solid, mp 203–205 °C, ^{1}H NMR: δ 5.01 (q, J = 15.9 Hz, 2H, $-CH_2$), 5.18 (t, J = 5.4, 7.2 Hz, 1H, H^2), 5.80 (dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.19 (d, J = 5.4 Hz, 1H, H^1), 6.37 (d, J = 15.9 Hz, 1H, H^4), 6.99–7.26 (m, 10H, ArH), 7.30–7.48 (m, 2H, ArH), 7.82 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{28}H_{19}ClFN_5O_3$ $[M + H]^+$ 528.1160 found 528.1158.

*3.1.12. 5-Bromo-1-{1-[1-(4-fluoro-phenyl)-2-oxo-4-styryl-azetidin-3-yl]-1H-[1,2,3]triazol-4-ylmethyl}-1H-indole-2,3-dione (**5l**)*

Brick red crystalline solid, mp 227–228 °C, ^{1}H NMR: δ 4.94 (q, J = 15.6 Hz, 2H, $-CH_2$), 5.17 (t, J = 5.1, 7.2 Hz, 1H, H^2), 5.79 (dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.18 (d, J = 5.1 Hz, 1H, H^1), 6.62 (d, J = 15.9 Hz, 1H, H^4), 7.00–7.26 (m, 10H, ArH), 7.33–7.51 (m, 2H, ArH), 7.80 (s, 1H, triazole ring), ^{13}C NMR: δ 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, 137.8, 138.3, 141.3, 149.8, 155.4, 157.8, 160.8, 182.9, HRMS Calculated for $C_{28}H_{19}BrFN_5O_3$ $[M + H]^+$ 572.0655 found 572.0651.

*3.1.13. 1-[1-(2-Oxo-1-phenyl-4-styryl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione (**5m**)*

Brick red crystalline solid, mp 210–211 °C, ^{1}H NMR: δ 4.96 (a pair of doublet, J = 15.6 Hz, 2H, $-CH_2$), 5.19 (dd, J = 5.1, 7.2 Hz, 1H, H^2), 5.81 (dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.17 (d, J = 5.1 Hz, 1H, H^1), 6.67 (d, J = 15.9 Hz, 1H, H^4), 7.02–7.57 (m, 14H, ArH), 7.82 (s, 1H, triazole ring), ^{13}C NMR: δ 35.1, 45.4, 67.6, 111.0, 117.3, 119.4, 123.6, 123.8, 125.2, 126.3, 128.2, 128.6, 128.8, 129.2, 134.5, 134.8, 137.4, 138.8, 141.9, 149.3, 157.7, 160.2, 181.8, HRMS Calculated for $C_{28}H_{21}N_5O_3$ $[M + H]^+$ 476.1644 found 476.1642.

*3.1.14. 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 (**5n**)*

Brick red crystalline solid, mp 209–210 °C, ^{1}H NMR: δ 4.97 (q, J = 15.6 Hz, 2H, $-CH_2$), 5.20 (dd, J = 5.1, 7.2 Hz, 1H, H^2), 5.82 (dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.18 (d, J = 5.1 Hz, 1H, H^1), 6.66 (d, J = 15.9 Hz, 1H, H^4), 7.00–7.55 (m, 13H, ArH), 7.80 (s, 1H, triazole ring), ^{13}C NMR: δ 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, 160.0, 182.4, HRMS Calculated for $C_{28}H_{20}FN_5O_3$ $[M + H]^+$ 494.1550 found 494.1557.

*3.1.15. 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 (**5o**)*

Brick red crystalline solid, mp 216–217 °C, ^{1}H NMR: δ 4.95 (q, J = 15.6 Hz, 2H, $-CH_2$), 5.20 (dd, J = 5.1, 7.2 Hz, 1H, H^2), 5.80 (dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.15 (d, J = 5.1 Hz, 1H, H^1), 6.68 (d, J = 15.9 Hz, 1H, H^4), 7.01–7.56 (m, 13H, ArH), 7.81 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{28}H_{20}ClN_5O_3$ $[M + H]^+$ 510.1255 found 510.1251.

*3.1.16. 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 (**5p**)*

Brick red crystalline solid, mp 234–235 °C, ^{1}H NMR: δ 4.98 (s, 2H, $-CH_2$), 5.25 (dd, J = 5.4, 8.1 Hz, 1H, H^2), 5.93 (dd, J = 8.1, 15.9 Hz, 1H, H^3), 6.36 (d, J = 5.4 Hz, 1H, H^1), 6.78 (d, J = 15.9 Hz, 1H, H^4), 6.85 (d, J = 8.4 Hz, 1H, ArH), 7.06–7.70 (m, 12H, ArH), 8.17 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{28}H_{20}BrN_5O_3$ $[M + H]^+$ 554.0750 found 554.0753.

*3.1.17. 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 (**5q**)*

Brick red crystalline solid, mp 223–224 °C, ^{1}H NMR: δ 2.34(s, 3H, $-CH_3$), 4.97 (q, J = 15.6 Hz, 2H, $-CH_2$), 5.20 (dd, J = 5.1, 7.2 Hz, 1H, H^2), 5.82 (dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.18 (d, J = 5.1 Hz, 1H, H^1), 6.68 (d, J = 15.9 Hz, 1H, H^4), 7.00–7.55 (m, 13H, ArH), 7.80 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{29}H_{23}N_5O_3$ $[M + H]^+$ 490.1801 found 490.1804.

*3.1.18. 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 (**5r**)*

Brick red crystalline solid, mp 203–205 °C, ^{1}H NMR: δ 4.95 (a pair of doublet, J = 15.9 Hz, 2H, $-CH_2$), 5.17 (t, J = 5.4, 7.2 Hz, 1H, H^2), 5.79 (dd, J = 7.2, 16.2 Hz, 1H, H^3), 6.17 (d, J = 5.4 Hz, 1H, H^1), 6.67 (d, J = 16.2 Hz, 1H, H^4), 7.02–7.56 (m, 13H, ArH), 7.80 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{28}H_{20}ClN_5O_3$ $[M + H]^+$ 510.1255 found 510.1258.

*3.1.19. 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 (**5s**)*

Brick red crystalline solid, mp 218–219 °C, ^{1}H NMR: δ 4.96 (q, J = 15.6 Hz, 2H, $-CH_2$), 5.16 (t, J = 5.4, 7.2 Hz, 1H, H^2), 5.81 (dd, J = 7.2, 16.2 Hz, 1H, H^3), 6.17 (d, J = 5.4 Hz, 1H, H^1), 6.69 (d, J = 16.2 Hz, 1H, H^4), 7.00–7.54 (m, 12H, ArH), 7.81 (s, 1H, triazole ring), ^{13}C NMR: δ 34.1, 46.3, 67.8, 111.6, 117.2, 117.8, 121.0, 123.7, 123.9, 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 $C_{28}H_{19}Cl_2N_5O_3$ $[M + H]^+$ 544.0865 found 544.0867.

*3.1.20. 1-[1-(2-Oxo-4-styryl-1-p-tolyl-azetidin-3-yl)-1H-[1,2,3]triazol-4-ylmethyl]-1H-indole-2,3-dione (**5t**)*

Brick red crystalline solid, mp 230–231 °C, ^{1}H NMR: δ 2.31 (s, 3H, $-CH_3$), 4.97 (a pair of doublet, J = 15.9 Hz, 2H, $-CH_2$), 5.16 (dd, J = 5.4, 7.2 Hz, 1H, H^2), 5.79 (dd, J = 7.2, 16.2 Hz, 1H, H^3), 6.16 (d, J = 5.4 Hz, 1H, H^1), 6.64 (d, J = 16.2 Hz, 1H, H^4), 7.01–7.42 (m, 12H, ArH), 7.51 (dd, J = 7.5 Hz, 1H, ArH), 7.80 (s, 1H, triazole ring), ^{13}C NMR: δ 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, 141.3, 149.4, 157.5, 160.2, 182.4, HRMS Calculated for $C_{29}H_{23}N_5O_3$ $[M + H]^+$ 490.1801 found 490.1802.

*3.1.21. 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 (**5u**)*

Brick red crystalline solid, mp 224–225 °C, ^{1}H NMR: δ 2.35 (s, 3H, $-CH_3$), 4.95 (q, J = 15.6 Hz, 2H, $-CH_2$), 5.15 (dd, J = 5.4, 7.2 Hz, 1H, H^2), 5.79 (dd, J = 7.2, 16.2 Hz, 1H, H^3), 6.17 (d, J = 5.4 Hz, 1H, H^1), 6.65 (d, J = 16.2 Hz, 1H, H^4), 7.00–7.41 (m, 11H, ArH), 7.50 (dd, J = 7.5 Hz, 1H, ArH), 7.77 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{29}H_{22}FN_5O_3$ $[M + H]^+$ 508.1707 found 508.1721.

*3.1.22. 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 (**5v**)*

Brick red crystalline solid, mp 212–213 °C, ^{1}H NMR: δ 2.33 (s, 3H, $-CH_3$), 4.96 (q, J = 15.6 Hz, 2H, $-CH_2$), 5.16 (dd, J = 5.4, 7.2 Hz, 1H, H^2), 5.80 (dd, J = 7.2, 15.9 Hz, 1H, H^3), 6.17 (d, J = 5.4 Hz, 1H, H^1), 6.66 (d, J = 15.9 Hz, 1H, H^4), 7.02–7.43 (m, 11H, ArH), 7.51 (dd, J = 7.5 Hz, 1H, ArH), 7.81 (s, 1H, triazole ring), ^{13}C NMR: δ 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 $C_{29}H_{22}ClN_5O_3$ $[M + H]^+$ 524.1411 found 524.1415.

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Appendix A. Supplementary material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.019.

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