Synthesis and preliminary in vitro activity of mono- and bis-1H-1,2,3-triazole-tethered \( \beta \)-lactam–isatin conjugates against the human protozoal pathogen \( Trichomonas vaginalis \)

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Abstract In this study, we describe the synthesis of mono- and bis-1H-1,2,3-triazole-tethered \( \beta \)-lactam–isatin conjugates using copper-catalysed azide-alkyne cycloadDITION reaction between mono- and di-propargylated azetidin-2-ones and \( \text{N} \)-alkylazido isatins. The synthesized conjugates were evaluated for their preliminary in vitro analysis against \( Trichomonas vaginalis \) at 50 \( \mu \)M. The efficacy of synthesized hybrids was observed to depend on the substituent at \( N \)-1 position of \( \beta \)-lactam ring, as well as the presence of single/double 1H-1,2,3-triazole linker. Among the synthesized conjugates, the presence of a \( p \)-tolyl substituent at \( N \)-1 of \( \beta \)-lactam ring was preferred for good activity profiles while the increase in spacer length did not influence the efficacy of the compounds. Compounds with high levels of potency were further analysed to determine their IC\textsubscript{50} values, as well as cytotoxicity profiles against mammalian cells. The most active compound in the synthesized conjugates displayed an IC\textsubscript{50} value of 10.49 \( \mu \)M against cultured G3 strain of \( T. vaginalis \) and was non-toxic to cultured mammalian HeLa cells at the same concentration.

Keywords \( \beta \)-lactam · Isatin · \textit{Trichomonas vaginalis} · Cytotoxicity · Structure–activity relationship

Introduction

With approximately 248 million new cases occurring worldwide annually (WHO, Geneva, 2012), human trichomoniasis is a far more prevalent sexually transmitted disease (STD) than either chlamydia (caused by \textit{Chlamydia trachomatis}) or gonorrhoea (caused by \textit{Neisseria gonorrhoeae}) (Soper, 2004). The causative organism for the disease in humans, \textit{Trichomonas vaginalis}, is primarily acquired through transmission of trophozoites by direct sexual contact, although neonatal infection has also been reported (McLaren et al., 1983). 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 (Wright et al., 2010; Upcroft et al., 2009). Further, it is now entrenched that trichomoniasis-infected patients are more susceptible towards human immunodeficiency virus (HIV) as it appeared as a cofactor in HIV transmission and acquisition (Sorvillo et al., 2001; van der Pol et al., 2008). The significant increase in the vulnerability to HIV with trichomoniasis (McClelland et al., 2007; Guenthner et al., 2005) has increased the importance of this disease dramatically. As evident, the identification and development of novel scaffolds with toxicity against \textit{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 (Upcroft et al., 2006).
Isatin is a privileged scaffold with well tolerance in humans and its analogues demonstrate a diverse range of biological and pharmacological properties such as anti-HIV (Bal et al., 2005), anti-viral (Quennelle et al., 2006), anti-cancer (Vine and Locke, 2007; Kopka et al., 2006), anti-fungal (Raj et al., 2003), anticonvulsants (Verma et al., 2004), anti-Parkinson’s disease therapeutic (Igoshova et al., 2005), β-lactamase inhibitors (Casey et al., 1993; Hadfield et al., 2002) and effective SARS coronavirus 3CL protease inhibitor (Chen et al., 2005). The enthralling applications of isatins in organic synthesis, its biological properties, as well as its occurrence in natural products such as spirotryprostatins, horsfiline, gelsemine, gelseverine, rhynchophylline, elacomine, etc. have generated tremendous interest among synthetic organic and medicinal chemists (Fensome et al., 2008; Kumari et al., 2011; Ding et al., 2005; Vintonyak et al., 2010; Rottmann et al., 2010). Particular examples of 2-oxoindole derivatives are SU-5416 (semaxanib) and SU-11248 (Sunitinib) that were reported to have tyrosine kinase inhibitory and anti-angiogenic properties (Ma et al., 2003; Prenen et al., 2006).

Azetidin-2-one (β-lactam) is the crucial structural unit present widely in the β-lactam class of antibiotics (Palomo et al., 1999; Palomo et al., 2004). Following the discovery of penicillin, an array of naturally occurring β-lactam antibiotics has been introduced as chemotherapeutics of incomparable effectiveness for the treatment of bacterial infections. Current interest in this family is focused on the synthesis and modification of the β-lactam ring to obtain compounds with diverse pharmacological potential such as tumour necrosis factor-alpha (TNF-alpha) converting enzyme (TACE) inhibitors (Rao et al., 2007), anti-cancer (O’Boyle et al., 2013; Singh et al., 2011b), anti-coccidial (Liang et al., 2008), cardiovascular (Takai et al., 2004), anti-viral (D’hooghe et al., 2012), mutagenic (Gutierrez et al., 2013), anti-fungal (O’Driscoll et al., 2008) and anti-malarial activities (Jarrahpour et al., 2012). Besides its eminence as a heterocyclic system with numerous biological potential, β-lactam have also been employed as synthetic precursor for the synthesis of a wide variety of heterocyclic scaffolds (Singh, 2003; Alcaide et al., 2007; D’hooghe et al., 2010; Singh et al., 2011a).

Recently, pharmacophore hybridization has been appeared as an attractive paradigm for medicinal chemists. The main incentives for using this strategy relates to the marked improvement in therapeutic potential, potency, mode of action and pharmacokinetics (Meunier, 2008; Muregi and Ishih, 2010; Morphy and Rankovic, 2006).

Continuing with our efforts in the synthesis of novel molecular conjugates with biological potential (Raj et al., 2013c; Raj et al., 2013a; Nisha et al., 2013; Kumar et al., 2013; Kumar et al., 2012), we recently discussed the synthesis of 1H-1,2,3-triazole-tethered β-lactam–isatin conjugates and their in vitro evaluation against T. vaginalis (Raj et al., 2013b). Most of the synthesized compounds exhibited 100 % growth inhibition at 100 μM with the most potent and non-cytotoxic compound (Fig. 1) displayed an IC₅₀ value of 7.69 μM.

The present communication is an extension of the above approach comprising of the synthesis of mono- and bis-1H-1,2,3-triazole-tethered bifunctional hybrids of isatin with N-1 substituted β-lactams (Fig. 2) and their preliminary in vitro evaluation studies against T. vaginalis. The rationale behind the use of 1H-1,2,3-triazole linker is its active participation in hydrogen bonding, dipole–dipole interaction and stability against hydrolysis and oxidative/reductive conditions (Kolb et al., 2001; Kolb and Sharpless, 2003; Wang et al., 2005; Bock et al. 2006).

**Experimental section**

Melting points were determined by open capillary using a Veego precision digital melting point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 spectrophotometer. 1H NMR spectra were recorded on a Jeol 300 (300 MHz) spectrometer using TMS as an 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 and br broad peak. 13C NMR spectra were recorded on Jeol 300 (75 MHz) spectrometer in deuterchloroform and dimethylsulphoxide-d₆ with a Bruker D-8001 spectrophotometer. 1H NMR spectra were recorded on Jeol 300 (300 MHz) spectrometer using TMS as an 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 and br broad peak. 13C NMR spectra were recorded on Jeol 300 (75 MHz) spectrometer in deuterchloroform and dimethylsulphoxide using TMS as an 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 method for the preparation of β-lactam–isatin conjugates 6 and 7**

To a stirred solution of azide 5 (1 mmol for 2 and 2 mmol for 3) in ethanol–water (10:1) was added in succession appropriate acetylenic lactam 2 or 3 (1 mmol), copper...
sulphate (0.055 mmol for 2 and 0.1 mmol for 3) and sodium ascorbate (0.13 mmol for 2 and 0.26 for 3) 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.

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 (6a)

Brick red colour, yield 74%; IR (KBr) νmax: 1733, 1612 cm⁻¹; mp 214–215 °C; ¹H NMR (CDCl₃, 300 MHz): 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, H₁); 4.82 (dd, J = 5.1, 8.1 Hz, 1H, H²); 6.16 (dd, J = 8.1, 15.9 Hz, 1H, H³); 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 (CDCl₃, 75 MHz): δ ppm = 21.1 (CH₃), 37.6 (C-13), 46.5 (C-9), 48.3 (C-10), 61.6 (C-15), 71.8 (C-14), 110.1 (C-4), 117.2 (C-16), 117.7 (C-2), 123.6 (C-24), 124.0 (C-19), 124.5 (C-17), 125.3 (C-21), 126.7 (C-20), 128.1 (C-6), 128.7 (C-1), 129.1 (C-25), 133.9 (C-26), 134.5 (C-3), 135.1 (C-11), 135.8 (C-18), 138.6 (C-23), 144.1 (C-5), 151.5 (C-12), 158.1 (C-8), 164.4 (C-22), 182.2 (C-7). HRMS calculated for C₃₁H₂₈N₆O₃ [M]⁺ 524.2536 found 524.2530; Anal. Calcd. (%) for: C, 68.68; H, 6.15; N, 16.02, found: C, 68.61; H, 6.24; N, 16.10.

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 (6c)

Brick red colour, yield 78%; IR (KBr) νmax: 1733, 1612 cm⁻¹; mp 203–204 °C; ¹H NMR (300 MHz CDCl₃): δ 3.92 (s, 2H, –CH₂–); 4.16–4.19 (m, 2H, –CH₂–); 4.26 (d, J = 5.1 Hz, 1H, H¹); 4.47–4.64 (m, 2H, –CH₂–); 7.47 (dd, J = 5.4, 8.1 Hz, 1H, H¹); 7.53–7.85 (m, 9H, ArH); 7.90 (s, 1H, triazole-H); 13C NMR (CDCl₃, 75 MHz): δ ppm = 21.1 (CH₃), 37.6 (C-13), 46.5 (C-9), 48.3 (C-10), 61.6 (C-15), 71.8 (C-14), 110.1 (C-4), 117.2 (C-16), 117.7 (C-2), 123.6 (C-24), 124.0 (C-19), 124.5 (C-17), 125.3 (C-21), 126.7 (C-20), 128.1 (C-6), 128.7 (C-1), 129.1 (C-25), 133.9 (C-26), 134.5 (C-3), 135.1 (C-11), 135.8 (C-18), 138.6 (C-23), 144.1 (C-5), 151.5 (C-12), 158.1 (C-8), 164.4 (C-22), 182.2 (C-7). HRMS calculated for C₃₉H₂₈F₂N₆O₃ [M]⁺ 552.2223 found 552.2230; Anal. Calcd. (%) for: C, 69.91; H, 5.30; N, 15.78, found: C, 69.99; H, 5.38; N, 15.73.
**Brick red colour, yield 72 %; IR (KBr) **\( \nu_{\text{max}} \): 1732, 1617 cm\(^{-1}\); mp 197–198 °C; \(^1\)H NMR (300 MHz CDCl\(_3\)): \( \delta \) ppm: 2.24 (3H, -CH\(_3\)); 2.32 (dd, \( J=6.6, 12.9 \) Hz, 2H, -CH\(_2\)); 3.75 (a pair of doublet, \( J=15.0 \) Hz, 2H, -CH\(_2\)); 4.10–4.21 (m, 2H, -CH\(_2\)); 4.59 (dd, \( J=5.1, 11.1 \) Hz, 2H, -CH\(_2\)); 4.68 (d, \( J=5.1 \) Hz, 1H, H\(^1\)); 4.84 (dd, \( J=5.1, 8.4 \) Hz, 1H, H\(^2\)); 6.30 (dd, \( J=8.4, 15.9 \) Hz, 1H, H\(^3\)); 6.54 (d, \( J=8.1 \) Hz, 1H, ArH); 6.73 (d, \( J=15.9 \) Hz, 1H, H\(^1\)); 6.97 (d, \( J=8.1 \) Hz, 1H, ArH); 7.09–7.45 (m, 11H, ArH); 7.76 (s, 1H, triazole-H); \(^1\)C NMR (CDCl\(_3\), 75 MHz): \( \delta \) ppm: 21.3 (CH\(_3\)); 28.0 (C-10), 38.1 (C-14), 45.2 (C-9), 47.4 (C-11), 61.2 (C-16), 71.9 (C-15), 108.9 (C-4), 111.7 (C-17), 117.6 (C-2), 124.1 (C-25), 124.6 (C-20), 124.9 (C-18), 125.3 (C-22), 126.7 (C-21), 128.2 (C-6), 128.6 (C-1), 129.5 (C-26), 134.1 (C-27), 135.4 (C-3), 135.6 (C-12), 135.9 (C-19), 138.4 (C-24), 144.2 (C-5), 150.2 (C-13), 158.1 (C-8), 164.6 (C-23), 182.1 (C-7). HRMS calculated for C\(_{23}\)H\(_{26}\)N\(_6\)O\(_3\) [M\(^+\)] = 546.2379 found 546.2372; Anal. Calcld. (%) for: C: 70.31, H: 5.53; N, 15.37, found: C: 70.37; H: 5.59; N, 15.29.

**I-(3-[4-[(2-Oxo-4-styryl-1-p-tolyazetidin-3-ylamino)-methyl]-[1,2,3]triazol-1-yl-propyl]-1H-indole-2,3-dione (6d)**

Brick red colour, yield 81 %; IR (KBr) \( \nu_{\text{max}} \): 1730, 1616 cm\(^{-1}\); mp 206–207 °C; \(^1\)H NMR (300 MHz CDCl\(_3\)): \( \delta \) ppm: 2.35 (d, \( J=6.6, 13.2 \) Hz, 2H, -CH\(_2\)); 3.84 (s, 2H, -CH\(_2\)); 4.10–4.21 (m, 2H, -CH\(_2\)); 4.25 (d, \( J=5.1 \) Hz, 1H, H\(^1\)); 4.66–4.63 (m, 2H, -CH\(_2\)); 4.73 (dd, \( J=5.1, 8.1 \) Hz, 1H, H\(^2\)); 6.24 (dd, \( J=8.1, 15.9 \) Hz, 1H, H\(^3\)); 6.58 (d, \( J=8.1 \) Hz, 1H, ArH); 6.69 (d, \( J=15.9 \) Hz, 1H, H\(^1\)); 6.94–7.44 (m, 12H, ArH); 7.66 (s, 1H, triazole-H); \(^1\)C NMR (CDCl\(_3\), 75 MHz): \( \delta \) ppm: 27.6 (C-10), 37.2 (C-14), 46.6 (C-9), 47.8 (C-11), 61.6 (C-16), 72.1 (C-15), 110.0 (C-4), 117.0 (C-17), 117.7 (C-2), 123.5 (C-26), 124.2 (C-25), 124.7 (C-20), 125.4 (C-18), 126.8 (C-22), 128.0 (C-21), 128.8 (C-6), 129.1 (C-1), 134.5 (C-3), 135.1 (C-12), 135.7 (C-19), 138.8 (C-24), 144.2 (C-5), 148.8 (C-27), 151.4 (C-13), 158.3 (C-8), 164.7 (C-23), 182.3 (C-7). HRMS calculated for C\(_{31}\)H\(_{34}\)N\(_6\)O\(_3\) [M\(^+\)] = 550.2129 found 550.2122; Anal. Calcld. (%) for: C: 76.63, H: 4.94; N, 15.26, found: C: 67.70, H: 4.88; N, 15.31.

**3-{3-[4-[(1-Cyclohexyl-2-oxo-4-styryl-azetidin-3-ylamino)-methyl]-[1,2,3]triazol-1-yl-propyl]-1H-indole-2,3-dione (6e)**

Brick red colour, yield 75 %; IR (KBr) \( \nu_{\text{max}} \): 1730, 1621 cm\(^{-1}\); mp >320 °C; \(^1\)H NMR (300 MHz CDCl\(_3\)): \( \delta \) ppm: 2.27 (3H, -CH\(_3\)); 3.73 (a pair of doublet, \( J=15.0 \) Hz, 4H, 2\times-CH\(_2\)); 4.10–4.21 (m, 5H, H\(^1\), 2\times-CH\(_2\)); 4.56 (dd, \( J=5.1, 10.8 \) Hz, 4H, 2\times-CH\(_2\)); 4.82 (dd, \( J=5.1, 8.1 \) Hz, 1H, H\(^2\)); 6.29 (dd, \( J=8.4, 15.9 \) Hz, 1H, H\(^3\)); 6.56 (d, \( J=7.8 \) Hz, 2H, ArH); 6.69 (d, \( J=16.2 \) Hz, 1H, H\(^1\)); 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); \(^1\)C NMR (CDCl\(_3\), 75 MHz): \( \delta \) ppm: 20.8 (CH\(_3\)); 40.6 (C-13), 45.3 (C-9), 47.5 (C-10), 61.3 (C15), 71.5 (C14), 109.4 (C-4), 117.0 (C-16), 117.4 (C-2), 124.0 (C-24), 124.7 (C-19), 124.8 (C-17), 125.5 (C-21), 126.6 (C-20), 128.3 (C-6), 128.7 (C-1), 129.5 (C-25), 133.9 (C-26), 135.1 (C-3), 135.3 (C-11), 135.9 (C-18), 138.5 (C-23), 144.3 (C-5), 149.9 (C-12), 158.4 (C-8), 164.7 (C-22), 182.5 (C-7). HRMS calculated for C\(_{33}\)H\(_{38}\)N\(_8\)O\(_3\) [M\(^+\)] = 786.3027 found 786.3034; Anal. Calcld. (%) for: C: 76.16, H: 4.87; N, 17.80, found: C: 76.07, H: 4.90; N, 17.87.
3-{Bis-[1-(3-{2,3-dioxo-1H-indol-1-yl}-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-amino}-1-cyclohexyl-4-styryl-azetidine-2-one (7b)

Brick red colour, yield 75%; IR (KBr) \( \nu_{\text{max}} \): 1739, 1614 cm\(^{-1}\); mp >230 °C; \(^1\)H NMR (300 MHz CDCl\(_3\)): \( \delta \) 1.27–1.98 (m, 10H, cyclohexyl), 3.47–3.62 (m, 1H, cyclohexyl), 3.71 (a pair of doublet, \( J = 15.0 \) Hz, 4H, 2\text{–}CH\(_2\)); 4.11–4.22 (m, 4H, 2\text{–}CH\(_2\)); 4.27 (d, \( J = 5.1 \) Hz, 1H, H\(^3\)); 4.58 (dd, \( J = 5.4, 11.1 \) Hz, 4H, 2\text{–}CH\(_2\)); 4.84 (dd, \( J = 5.1, 8.4 \) Hz, 1H, H\(^3\)); 6.31 (dd, \( J = 8.4, 15.9 \) Hz, 1H, H\(^4\)); 6.68 (d, \( J = 15.9 \) Hz, 1H, H\(^1\)); 7.03–7.46 (m, 13H, ArH); 7.75 (s, 2H, triazole-H); \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): \( \delta \) ppm = 20.8 (CH\(_3\)), 27.5 (C-10), 37.3 (C-14), 46.0 (C-9), 47.3 (C-11), 61.8 (C-16), 72.5 (C-15), 110.1 (C-4), 117.1 (C-17), 117.6 (C-2), 123.9 (C-25), 124.2 (C-20), 124.8 (C-18), 125.5 (C-22), 126.5 (C-21), 128.2 (C-6), 128.7 (C-1), 129.5 (C-26), 133.8 (C-27), 134.8 (C-3), 135.3 (C-12), 135.9 (C-19), 135.8 (C-24), 144.3 (C-5), 150.2 (C-13), 158.3 (C-8), 164.8 (C-23), 182.9 (C-7). HRMS calculated for C\(_{43}\)H\(_{42}\)N\(_{10}\)O\(_{5}\) \([\text{M}]^+\): 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.

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-azetidine-2-one (7e)

Brick red colour, yield 66%; IR (KBr) \( \nu_{\text{max}} \): 1739, 1618 cm\(^{-1}\); mp >230 °C; \(^1\)H NMR (300 MHz CDCl\(_3\)): \( \delta \) 3.98 (s, 4H, –CH\(_2\)); 4.10–4.21 (m, 5H, H\(^3\) + 2\text{–}CH\(_2\)); 4.39 (d, \( J = 5.1 \) Hz, 1H, H\(^4\)); 4.56 (dd, \( J = 5.1, 10.8 \) Hz, 4H, 2\text{–}CH\(_2\)); 4.92 (dd, \( J = 5.1, 8.4 \) Hz, 1H, H\(^3\)); 6.32 (dd, \( J = 8.4, 15.6 \) Hz, 1H, H\(^4\)); 6.50 (d, \( J = 7.8 \) Hz, 2H, ArH); 6.71 (d, \( J = 16.2 \) Hz, 1H, H\(^2\)); 6.99–7.49 (m, 15H, ArH); 7.59 (s, 2H, triazole-H); \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): \( \delta \) ppm = 41.2 (C-13), 45.8 (C-9), 47.6 (C-10), 61.1 (C-15), 72.3 (C-14), 110.2 (C-4), 117.1 (C-16), 117.5 (C-2), 124.1 (C-25), 124.6 (C-24), 124.8 (C-19), 125.7 (C-17), 126.8 (C-21), 128.1 (C-20), 128.6 (C-6), 129.4 (C-1), 135.2 (C-3), 135.6 (C-11), 136.0 (C-18), 138.4 (C-23), 144.1 (C-5), 149.0 (C-26), 150.4 (C-12), 159.2 (C-8), 164.7 (C-22), 181.9 (C-7). HRMS calculated for C\(_{43}\)H\(_{42}\)N\(_{10}\)O\(_{5}\) \([\text{M}]^+\): 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.

3-{Bis-[1-(3-{2,3-dioxo-1H-indol-1-yl}-propyl)-1H-[1,2,3]triazol-4-ylmethyl]-amino}-4-styryl-1-p-toly-azetidine-2-one (7f)

Brick red colour, yield 69%; IR (KBr) \( \nu_{\text{max}} \): 1734, 1613 cm\(^{-1}\); mp >230 °C; \(^1\)H NMR (300 MHz CDCl\(_3\)): \( \delta \) 2.34 (dd, \( J = 6.0, 12.6 \) Hz, 4H, 2\text{–}CH\(_2\)); 3.88 (s, 4H, –CH\(_2\)); 4.07–4.18 (m, 4H, 2\text{–}CH\(_2\)); 4.33 (d, \( J = 5.1 \) Hz, 1H, H\(^3\)); 4.56 (m, 4H, 2\text{–}CH\(_2\)); 4.90 (dd, \( J = 5.1, 8.4 \) Hz, 1H, H\(^4\)); 6.34 (dd, \( J = 8.4, 15.6 \) Hz, 1H, H\(^3\)); 6.51 (d, \( J = 8.1 \) Hz, 2H, ArH); 6.68 (d, \( J = 15.6 \) Hz, 1H, H\(^1\)); 7.04–7.46 (m, 15H, ArH); 7.62 (s, 2H, triazole-H); \(^{13}\)C
NMR (CDCl₃, 75 MHz): δ ppm = 27.4 (C-10), 41.5 (C-14), 45.9 (C-9), 47.1 (C-11), 62.4 (C-16), 72.0 (C-15), 110.3 (C-4), 117.2 (C-17), 117.6 (C-2), 124.2 (C-26), 124.6 (C-25), 124.8 (C-20), 125.5 (C-18), 126.7 (C-22), 128.0 (C-21), 128.5 (C-6), 129.5 (C-1), 135.1 (C-3), 135.4 (C-12), 136.3 (C-19), 138.1 (C-24), 144.5 (C-5), 148.4 (C-27), 149.8 (C-13), 159.1 (C-8), 164.4 (C-23), 182.3 (C-7).

HRMS calculated for C₄₅H₃₉FN₁₀O₅ [M]⁺ 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.

Biological evaluation

In vitro protozoal parasite susceptibility assay

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) versus response—variable slope regression option. Within this non-linear 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 four 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, 5–40 μM, and performing a regression analysis using Prism software, from GraphPad.

Cytotoxic evaluation of 6a, the most potent compound 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% foetal bovine serum in a humidified 5% CO₂ atmosphere at 37 °C. Doxorubicin, bleomycin, and 6a (the most potent compound in the library) 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 IC₅₀ value of 0.61 μM comparable to its reported value (Travelli *et al.*, 2011).
Result and discussions

Chemistry

The mono- and di-propargylated precursors 2 and 3 were prepared via our recently reported protocol involving the treatment of 3-amino-2-azetidinone 1 (Singh et al., 2011c), 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 2 and 3 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 di-propargylated product 3. The observed coupling constant \( J = 5.4 \text{ Hz} \) between \( H^3 \) and \( H^4 \) confirmed the cis-stereochemistry of the products (Scheme 1).

N-alkyl azido isatin 5, another precursor required for the synthesis of target scaffolds, was prepared by an initial base-assisted N-alkylation of isatin with dibromoalkane followed by subsequent reaction with sodium azide in DMF at 60 °C (Scheme 2) (Singh et al., 2012).

The synthesized precursors 2 and 3 were utilized in the synthesis of desired mono- and bis-1H-1,2,3-triazole-tethered \( \beta \)-lactam–isatin conjugates. Thus, the reaction of 2 with 5 (1 mmol) in the presence of copper sulphate and sodium ascorbate in ethanol–water (10:1) mixture led to the isolation of 6 (Scheme 3), while the reaction of 3 with 5 (2 mmol) under similar conditions led to the formation of 7 in good to excellent yields (Scheme 4).

The structures to the hybrids 6 and 7 were assigned on the basis of spectral data and analytical evidence. Compound 7d, for example, showed a molecular ion peak [M]¹ 814.8892 along with the characteristic peaks in \(^1\text{H} \) and \(^13\text{C} \) NMR spectra. The \(^1\text{H} \) NMR spectrum 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

Table 1 Biological evaluation of the compound library against \( T. \) vaginalis at 50 μM

| Code | R              | n | Yield (%) | Average % inhibition at 50 μM |
|------|----------------|---|-----------|-----------------------------|
| 6a   | \( p-C_6H_5–CH_3 \) | 1 | 74        | 91.52 ± 2.78                |
| 6b   | \( C_6H_{11} \)     | 1 | 65        | 43.72 ± 4.18                |
| 6c   | \( p-C_6H_5–F \)    | 1 | 78        | 12.93 ± 1.93                |
| 6d   | \( p-C_6H_5–CH_3 \) | 2 | 72        | 70.63 ± 3.80                |
| 6e   | \( C_6H_{11} \)     | 2 | 74        | 36.90 ± 7.12                |
| 6f   | \( p-C_6H_5–F \)    | 2 | 81        | 42.86 ± 5.23                |
| 7a   | \( p-C_6H_5–CH_3 \) | 1 | 75        | ND                          |
| 7b   | \( C_6H_{11} \)     | 1 | 75        | 44.23 ± 8.50                |
| 7c   | \( p-C_6H_5–F \)    | 1 | 66        | 52.66 ± 1.47                |
| 7d   | \( p-C_6H_5–CH_3 \) | 2 | 70        | 46.35 ± 1.16                |
| 7e   | \( C_6H_{11} \)     | 2 | 78        | 57.61 ± 3.30                |
| 7f   | \( p-C_6H_5–F \)    | 2 | 69        | ND                          |

Table 2 IC50 determination of active compounds against \( T. \) vaginalis

| Compound | IC50 (μM) (G3) |
|----------|---------------|
| 6a       | 10.49 ± 1.05  |
| 6d       | 25.60 ± 1.08  |
| Metronidazolea | 0.72          |

a Current FDA-approved treatment for \( T. \) vaginalis infections

Scheme 3 Reagents and conditions i 5 (1 mmol), CuSO\(_4\)-5H\(_2\)O (0.05 mmol), sodium ascorbate (0.13 mmol), EtOH: H\(_2\)O, rt, 8 h

Scheme 4 Reagents and conditions i 5 (2 mmol), CuSO\(_4\)-5H\(_2\)O (0.1 mmol), sodium ascorbate (0.26 mmol), EtOH: H\(_2\)O, rt, 8 h
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.

**In vitro activity against T. vaginalis**

The synthesized mono- and bis-$\text{H}-1,2,3$-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 1 lists the data obtained from the initial percentage inhibition screens at 50 lM. As evident from Table 1, the activity profiles of test compounds showed dependence on the substituent at N-1 of $\beta$-lactam ring and the presence of single/double $\text{H}-1,2,3$-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.

The most potent of the test compounds viz. 6a and 6d have been selected from % age inhibition data for determining their IC$_{50}$ values, which is defined as the minimum concentration required for 50% growth inhibition. These compounds have exhibited an IC$_{50}$ values of 10.49 (6a) and 25.60 lM (6d), respectively, as shown in Table 2, while their dose–response curves are depicted in Fig. 3.

The most potent compound 6a was then further evaluated for its cytotoxicity against HeLa cells. Results of these cytotoxicity tests consistently showed between 80 and 90 % viability compared with untreated and DMSO-treated cells. The same passage of cells was also tested with bleomycin and doxorubicin (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. Compound 6a consistently showed comparable toxicities to untreated and DMSO-treated HeLa cells when tested at 10 lM.

**Conclusion**

The present communication describes the synthesis of mono- and bis-$\text{H}-1,2,3$-triazole-tethered $\beta$-lactam–isatin conjugates along with their preliminary in vitro evaluation against *T. vaginalis* at 50 lM. The synthesized scaffolds have shown a preference for a p-tolyl substituent at N-1 of $\beta$-lactam ring for good activity with the most potent and non-cytotoxic compounds 6a and 6d exhibiting an IC$_{50}$ of 10.49 and 25.60 lM, respectively. However, the exact inhibition site ($\beta$-lactam or isatin) responsible for the activity of these conjugates is still uncertain and further studies are currently underway.

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