Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

N-Propargylated isatin-Mannich mono- and bis-adducts: Synthesis and preliminary analysis of *in vitro* activity against *Tritrichomonas foetus*

Nisha^a, Kewal Kumar^a, Gaurav Bhargava^b, Kirkwood M. Land^c, Kai-Hsiang Chang^c, Reena Arora^d, Somdutta Sen^d, Vipan Kumar^{a,*}

^a Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, India

^b Department of Applied Sciences, Punjab Technical University, Kapurthala 144601, India

^c Department of Biological Sciences, University of the Pacific, Stockton, CA 95211, USA

^d Pharmacology Department, Sphaera Pharma Pvt. Ltd, Manesar 12205, India

ARTICLE INFO

Article history: Received 26 September 2013 Received in revised form 26 November 2013 Accepted 10 January 2014 Available online 18 January 2014

Keywords:

N-Propargylated-isatin Mannich adducts N-Propargylated-isatin-7-chloroquinoline conjugates *Tritrichomonas foetus* Cytotoxicity

1. Introduction

Bovine trichomoniasis is a veneral disease caused by the protozoal pathogen *Tritrichomonas foetus* and is considered to be one of the most important infectious causes of reproductive failure in beef cattle drastically reducing their breeding efficiency [1–4]. The infection in bulls is primarily acquired through direct sexual contact and is asymptomatic in nature affecting neither the semen quality nor the sexual behaviour although in some cases, early death of developing foetus has also been observed [5–7]. Literature rationale has also revealed that the parasite provoked cell death to bovine oviduct epithelial cells [8] and extracellular products secreted by *T. foetus* were cytotoxic to bovine sperm [9]. Although the use of artificial insemination in breeding cattle has led to the virtual eradication of this organism from the cattle population in many countries, bovine trichomonad infection still is responsible for significant economic losses in the free-ranging cattle industry

E-mail address: vipan_org@yahoo.com (V. Kumar).

ABSTRACT

Cu(I)Cl promoted synthesis of *N*-propargylated-isatin Mannich mono- and bis-adducts with an extension towards the synthesis of *N*-propargylated-isatin-7-chloroquinoline conjugates was described. The synthesized scaffolds were evaluated for their *in vitro* activity against the veterinary protozoal pathogen *Tritrichomonas foetus* and cytotoxicity against human prostate (PC-3) cancer cell line. The preliminary evaluation data revealed the enhancement in the activity profiles with the introduction of 7-chloroquinoline ring with the most active conjugates **7a**, **7c** and **7d** exhibiting an IC₅₀ of 22.2, 11.3 and 24.5 μ M respectively against *T. foetus* and minimal toxicity against human prostate (PC-3) cell lines.

around the world. In light of this current problem, the identification of new chemotherapies for this pathogen is still warranted.

Isatin is a versatile scaffold with wide possibility of chemical modification and display a diverse array of biological and pharmacological activities which include anti-HIV [10], antiviral [11], anti-tumour [12-14], antifungal [15,16], anti-angiogenic [17], anticonvulsants [18], anti-Parkinson's disease therapeutic [19], and effective SARS coronavirus 3CL protease inhibitor [20]. An extensive investigation on the synthetic versatility of isatin has disclosed many interesting aspects of organic synthesis allowing the generation of a large number of structurally diverse analogues derived from electrophilic aromatic substitution at positions C-5 and C-7 of the phenyl ring, nucleophilic additions onto the C-3 carbonyl group, chemoselective reductions, oxidations, ring-expansions, and spiroannulations [21]. However there are only few reports regarding the antineoplastic activities of N-alkyl and N-aryl isatins. Vine and coworkers have reported the apoptosis behaviour of N-alkyl/aryl isatins through introduction of various hydrophobic substituents like N-phenethyl, N-benzyl and N-2-naphthylmethyl on the parent nucleus that significantly increased their cytotoxicity towards lymphoma cells and against a wide range of human cancer cell lines, including MDA-MB-231 metastatic breast adenocarcinoma





CrossMark



^{*} Corresponding author. Tel.: +91 183 2258802 09x3320; fax: +91 183 2258819 20.

^{0223-5234/\$ –} see front matter @ 2014 Elsevier Masson SAS. All rights reserved. http://dx.doi.org/10.1016/j.ejmech.2014.01.015



Scheme 1. Cu(I)Cl promoted *N*-propargylated isatin Mannich adducts.

cells and U937 human monocyte-like histolytic lymphoma cells [22]. Recent report from our lab has shown the synthesis of *N*-alkylisatin along with their cytotoxic profiles against a panel of cancer cell lines [23]. The approach has been further extended towards the synthesis of 1*H*-1,2,3-triazole-tethered isatin conjugates with four of the synthesized scaffolds being twice as potent as 5-fluorouracil on THP-1 cell line [24].

In continuation with our interest towards the synthesis of novel molecular frameworks having biological relevance [25], we recently disclosed the synthesis of 1H-1,2,3-triazole-tethered isatin- β -lactam conjugates along with the preliminary analysis of their in vitro activity against Trichomonas vaginalis at 10 and 100 µM [26]. Eighteen of the synthesized hybrids have shown 100% growth inhibition at 100 μ M with the most potent and non-cytotoxic compound showing and IC_{50} of 7.69 μ M. The methodology was further extended towards the synthesis of β -amino alcohol based β lactam-isatin conjugates and their evaluation against T. vaginalis at $50 \,\mu\text{M}$ with the most active chimeric scaffold among the test series exhibited an IC₅₀ of 9.73 μ M along with low cytotoxicity [27]. As an extension of this work, we now describes the Cu(I)Cl-facilitated synthesis of N-propargylated-isatin Mannich adducts along with their preliminary in vitro evaluation studies against the veterinary protozoal pathogen T. foetus and cytotoxicity against human prostate (PC-3) cancer cell line.

2. Results and discussion

2.1. Synthetic chemistry

The synthetic approach devised for the synthesis of desired hybrids involved sodium hydride promoted initial propagylation of C-5 substituted isatins **1** with propargyl bromide in dry DMF to yield **2**. This upon refluxing at 110 °C in dry dioxane with secondary amines and para-formaldehyde in the presence of catalytic amount of Cu(I)Cl resulted in the isolation of desired N-propargylated-isatin Mannich adducts. Mechanistically, the reaction is thought to proceed by an initial formation of Cu-acetylide **I** which undergoes Mannich addition as shown in Scheme 1. Interestingly, the reaction employing piperazine as the secondary amine unit resulted in the isolation of piperazine with para-formaldehyde at 1st and 4th position to result in the corresponding bis-iminium ion with subsequent Mannich addition.

The developed methodology has been further extended towards the synthesis of *N*-propargylated isatin-7-chloroquinoline hybrids *via* Cu-mediated Mannich reaction of C-5 substituted *N*-propargylated isatins with 7-chloro-4-piperazin-1-yl-quinoline **6**, prepared by heating 4,7-dichloroquinoline with an excess of piperazine and triethylamine at 120 °C overnight Scheme 2. The inclusion of 7-



Scheme 2. Cu(I)Cl promoted N-propargylated isatin-quinoline Mannich adducts.

chloroquinoline nucleus in the synthesized conjugates is based on the recent disclosure by Dwivedi and co-workers on the promising activity of the quinoline based compounds against different strains of *T. vaginalis* [28].

The structure to the synthesized Mannich adducts were assigned on the basis of spectral data and analytical evidences. The compound **3d**, for example was characterized as 1-(4-Morpholine-4-yl-but-2-ynyl)-1*H*-2-indole-2,3-dione analysed for C₁₆H₁₆N₂O₃ and showed molecular ion peak at *m*/*z* 284.11 (M⁺) in its mass spectrum. The salient features of its ¹H NMR spectrum include characteristic peaks at δ 2.49(*J* = 4.4 Hz, 4H) and 3.69 (*J* = 4.4 Hz, 4H) corresponding to the morpholine group along with two singlets corresponding to two methylenes at δ 3.25 and 4.55 respectively. ¹³C NMR spectrum of compound **3d** exhibited the appearance of a characteristic peak at δ 157.0 and 182.58 assigned to the isatin ring carbonyls further corroborating the assigned structure.

2.2. In vitro evaluation against T. foetus and cytotoxicity against PC-3 cell lines

The synthesized compounds were evaluated for their antitrichomonas activity against the veterinary protozoal pathogen *T. foetus* at 50 μ M and the results are provided in Table 1. As evident from Table 1, the anti-trichomonas activity of the test compounds were found to be dependent upon the nature of substituent at C-5 position as well as the presence of nature of secondary amine. The presence of a $-CH_3$ substituent at C-5 position of isatin ring of the synthesized Mannich adducts considerably improved the activity profile as evident by compounds **3a–3p** and **5a–5d**. Further, the introduction of 7-chloro-4-piperazin-1-yl-quinoline moiety remarkably improved the *anti-trichomonas* activity with a

Table 1

| Inhibitory | activity | of | compound | library | Tritrichomonas | foetus | and | cytotoxicity |
|------------|----------|------|--------------|-----------|----------------|--------|-----|--------------|
| against hu | man pros | stat | e (PC-3) can | icer cell | line. | | | |

| Compound | R | R ¹ | Average % | Cytotox | cicity% inh | ibition |
|----------|--------|------------------|--|---------|-------------|---------|
| | | | inhibition at 50 μM (Tritrichomonas foetus) | | 2 μM | 20 µM |
| 3a | Н | Piperidine | 18.2 | 6.0 | 4.0 | 7.0 |
| 3b | Н | Pyrrolidine | 10.6 | 7.0 | 7.0 | 3.0 |
| 3c | Н | Morpholine | 9.1 | 13.4 | 11.6 | 8.7 |
| 3d | Н | N,N-Diethylamine | 22.7 | 8.0 | 4.6 | 8.0 |
| 3e | Cl | Piperidine | 3.0 | 11.1 | 10.7 | 18.7 |
| 3f | Cl | Pyrrolidine | 15.2 | 4.6 | 4.3 | 9.6 |
| 3g | Cl | Morpholine | 22.7 | 0.8 | 4.3 | 3.4 |
| 3h | Cl | N,N-Diethylamine | 42.4 | 4.8 | 5.6 6.4 | |
| 3i | F | Piperidine | 40.9 | -4.7 | $-6.0\ 5.1$ | |
| 3ј | F | Pyrrolidine | 33.3 | 5.0 | 4.3 | -2.7 |
| 3k | F | Morpholine | 56.1 | 3.8 | 0.7 | 3.2 |
| 31 | F | N,N-Diethylamine | 18.2 | 11.6 | 14.5 | 7.9 |
| 3m | CH_3 | Piperidine | 31.8 | 6.8 | 10.5 | 2.4 |
| 3n | CH_3 | Pyrrolidine | 65.2 | 1.9 | 4.7 | 6.1 |
| 30 | CH_3 | Morpholine | 57.6 | 8.0 | 7.8 | 3.5 |
| 3р | CH_3 | N,N-Diethylamine | 51.5 | 11.5 | 10.2 | 11.2 |
| 5a | Н | - | 3.0 | 6.5 | 10.3 | 45.8 |
| 5b | Cl | - | 7.6 | 24.4 | 28.9 | 24.8 |
| 5c | F | - | 9.1 | -0.6 | -3.6 | 7.4 |
| 5d | CH_3 | - | 71.2 | -1.0 | 6.7 | 7.1 |
| 7a | Н | - | 98.5 | 9.0 | 10.1 | -3.2 |
| 7b | F | | 100.0 | 2.1 | 5.0 | 3.0 |
| 7c | Cl | - | 100.0 | 6.1 | 1.2 | 1.4 |
| 7d | CH_3 | - | 59.1 | 1.3 | 8.1 | 8.2 |
| 7e | NO_2 | - | 60.6 | 0.5 | 3.2 | 4.8 |
| GDC-0941 | | | | 30.2 | 56.8 | 72.2 |

preference for halogen substituent at C-5 position of isatin ring. The most potent of the synthesized hybrids **7b** and **7c** showing 100% growth inhibition at 50 µM. The active compounds viz. 7a, 7b and 7c from the preliminary inhibition data were chosen in order to determine their IC₅₀ 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 conjugates are not as active as the standard drug metronidazole, the compounds showed potent activity against the veterinary protozoal pathogen T. foetus. The compound **7c** having a chloro-substituent at C-5 position of the isatin ring proved to be the most potent of the synthesized scaffolds with an IC₅₀ of 11.3 µM. The synthesized compounds were further evaluated for their cytotoxicity against human prostate (PC-3) cancer cell lines in order to confirm if the observed activity profiles are because of their intrinsic anti-trichomonas activity or the cytotoxicity. The cytotoxic evaluation results of the test compounds at 0.2, 2.0 and 20 µM are enlisted in Table 1. As evident, virtually all the synthesized Mannich adducts are devoid of cytotoxicity except 5a and 5f, showing that the bis-adducts were cytotoxic against PC-3 cell lines compared to the mono-adducts. The most potent anti-trichomonas scaffolds viz. 7a, 7b, 7c and 7d showed no cytotoxicity against PC-3 cell line and can be considered as a good hit.

In conclusion, the present manuscript describes the Cu(I)Cl promoted *N*-propargylated-isatin-Mannich mono- as well as bisadducts with an extension towards the synthesis of *N*-propargylated-isatin-7-chloroquinoline conjugates. The synthesized scaffolds were evaluated for their activity against veterinary protozoal pathogen *T. foetus*. The preliminary evaluation data revealed that the introduction of 7-chloroquinoline ring markedly enhanced the activity profiles with the most active compound **7c** exhibiting an IC₅₀ of 11.3 μ M. The cytotoxic studies against human prostate (PC-3) cell lines showed that the synthesized Mannich adducts are virtually devoid of any cytotoxicity and the isatin-7-chloroquinoline framework can act as an ideal starting point for the synthesis of new pharmacological templates against *T. foetus*.

3. Experimental section

Melting points were determined by open capillary using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. ¹H 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. ¹³C 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).

| Table 2 | | |
|-------------|-------------|----------|
| IC50 of the | synthesized | hybrids. |

| Compound | IC ₅₀ (μM) on protozoal pathogen Tritrichomonas foetus |
|----------------------------|--|
| 7a 7c 7d | 22.2 11.3 24.5 |
| Metronidazole ^a | 0.72 |

^a Current FDA approved treatment for *T. vaginalis* infections.

3.1. Procedure for the synthesis of C-5 substituted N-propargylated isatins ${f 2}$

To a stirred suspension of sodium hydride (1.5 mmol) in dry DMF (10 mL) was added isatin (1 mmol), resulting in the formation of purple coloured anion. The solution was stirred at room temperature till the evolution of hydrogen ceases. To this reaction mixture was added dropwise a solution of propargyl bromide (1.1 mmol) in dry DMF. The reaction mixture was heated to 60 °C with constant stirring for about 6 h. After the completion of reaction, as evident from TLC, it was quenched by dropwise addition of water (20 mL) and subsequently extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine solution, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Purification of the reaction mixture *via* column chromatography using hexane: ethyl acetate (4:1) mixture furnished the desired *N*-propargylated isatin derivatives in good yields.

3.2. Procedure for the synthesis of 7-chloro-4-piperazin-1-yl-quinoline **6**

To a well stirred solution of 4,7-dichloroquinoline (1 mmol) and triethylamine (1.2 mmol), piperazine (1 mmol) was added and the reaction mixture was allowed to stir at 110 °C for 8 h. The progress of the reaction mixture was monitored with TLC and on completion; it was washed with 2 N NaOH solution with subsequent extraction with ethy lacetate (3 \times 30 mL). The combined organic layers were washed with brine solution, dried over anhydrous Na₂SO₄ and concentrated under vacuum resulting in the desired product.

3.3. Typical procedure for the synthesis of N-propargylated-isatin-Mannich adducts **3a–3p**

To a well stirred solution of *N*-propargylated isatin (1 mmol) in dry dioxane, catalytic amount of copper chloride and *p*-formaldehyde was added in succession. The reaction mixture was stirred for 10 min with subsequent addition of secondary amine (2 mmol) and refluxed at 120 °C for 8 h. After the completion of reaction as evident from TLC, the reaction mixture was filtered and subsequently extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to result in the desired Mannich adducts in good yields.

3.3.1. 1-(4-Piperadin-1-yl-but-2-ynyl)-1H-indole-2,3-dione (3a)

Orange solid, yield 87%, m.p. 149 °C: ¹H NMR (300 MHz, CDCl₃): δ 1.14–1.74 (m, 6H, –CH₂–CH₂–CH₂–); 2.40 (s, 4H, –CH₂–N–CH₂–); 3.21 (s, 2H, –CH₂); 4.54 (s, 2H, –CH₂); 7.10–7.17 (m, 2H Ar–H); 7.58–7.63 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 23.6, 25.6, 29.6, 47.5, 53.2, 80.7, 111.2, 117.4, 123.9, 125.1, 138.1, 149.7, 157.0, 182.6, HRMS calculated for C₁₇H₁₈N₂O₂ 282.1368 [M⁺] found 282.1375; Analysis calculated for C₁₇H₁₈N₂O₂ C, 72.32; H, 6.43; N, 9.92; found C, 72.39; H, 6.37; N, 9.97.

3.3.2. 1-(4-Pyrrolidin-1-yl-but-2-ynyl)-1H-indole-2,3-dione (3b)

Orange solid, yield 86%, m.p. 136 °C: ¹H NMR (300 MHz, CDCl₃): δ 1.79–1.83 (m, 4H, –CH₂–CH₂); 2.56–2.64 (m, 4H, –CH₂–N–CH–); 3.42 (s, 2H, –CH₂); 4.59 (s, 2H, –CH₂); 7.15–7.24 (m, 2H, Ar–H); 7.62–7.69 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz) 23.6, 29.8, 43.1, 52.7, 81.6, 111.2, 117.4, 123.8, 125.1, 138.1, 149.6, 149.8, 151.6, 182.6, HRMS calculated for C₁₆H₁₆N₂O₂ 268.1212 [M⁺] found 268.1219; Analysis calculated for C₁₆H₁₆N₂O₂: C, 71.62; H, 6.01; N, 10.44; found C, 71.58; H, 6.08; N, 10.39.

3.3.3. 1-(4-Morpholin-4-yl-but-2-ynyl)-1H-indole-2,3-dione (3c)

Orange solid, yield 88%, m.p. 158 °C: ¹H NMR (300 MHz, CDCl₃): δ 2.49 (t, J = 4.4 Hz, 4H, $-CH_2-N-CH_2-$); 3.25 (s, 2H, -CH); 3.69 (t, J = 4.4 Hz, 4H, $-CH_2-O-CH_2-$); 4.55 (s, 2H, -CH); 7.07–7.18 (m, 2H, Ar–H); 7.60–7.65 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 29.4, 47.4, 52.6, 66.5, 79.7, 110.9, 117.4, 124.0, 125.2, 138.1, 149.6, 157.0, 182.5, HRMS calculated for C₁₆H₁₆N₂O₃ 284.1161 [M⁺] found 284.1169; Analysis calculated for C₁₆H₁₆N₂O₃: C, 67.59; H, 5.67; N, 9.85; found C, 67.65; H, 5.72, N, 9.78.

3.3.4. 1-(4-Diethylamino-but-2-ynyl)-1H-indole-2,3-dione (3d)

Viscous liquid, yield 76%, ¹H NMR (300 MHz, CDCl₃): δ 1.05 (t, J = 7.1 Hz, 6H, CH₃); 2.52–2.60 (m, 4H, –CH₂–N–CH₂–); 3.25 (s, 2H, –CH₂); 4.54 (s, 2H, –CH₂); 7.07–7.16 (m, 2H, Ar–H); 7.56–7.65 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 14.8, 29.5, 46.7, 47.4, 80.1, 110.9, 117.7, 124.1, 125.3, 138.2, 149.7, 157.1, 182.5, HRMS calculated for C₁₆H₁₆N₂O₂ 270.1368 [M⁺] found 270.1359; Analysis calculated for C₁₆H₁₆N₂O₂: C, 71.09; H, 6.71; N, 10.36 found C, 71.01; H, 6.83; N, 10.47.

3.3.5. 5-Chloro-1-(4-piperidin-1-yl-but-2-ynyl)-1H-indole-2,3dione (**3e**)

Orange solid, yield 83%, m.p. 147 °C: ¹H NMR (300 MHz, CDCl₃): δ 1.17–1.56 (m, 6H, $-CH_2-CH_2-CH_2-$); 2.41 (s, 4H, $-CH_2-N-CH-$); 3.23 (s, 2H, $-CH_2$); 4.54 (s, 2H, $-CH_2$); 7.08–7.11 (m, 1H, Ar–H); 7.57–7.60 (m, 2H, Ar–H): ¹³C NMR (CDCl₃, 75 MHz): 23.6, 25.6, 29.6, 47.5, 53.2, 80.1, 112.4, 118.4, 125.1, 129.8, 137.6, 148.0, 151.4, 181.6, HRMS calculated for C₁₇H₁₇ClN₂O₂ 316.0979 [M⁺] found 316.0987; Analysis calculated for C₁₇H₁₇ClN₂O₂: C, 64.46; H, 5.41; N, 9.84 found C, 64.51; H, 5.49; N, 9.78.

3.3.6. 5-Chloro-1-(4-pyrrolidin-1-yl-but-2-ynyl)-1H-indole-2,3dione (**3f**)

Orange solid, yield 87%, m.p. 139 °C: ¹H NMR (300 MHz, CDCl₃): δ 1.75–1.77 (m, 4H, $-CH_2-CH_2-$); 2.51–2.55 (m, 4H, $-CH_2-N-CH-$); 3.36 (s, 2H, $-CH_2$); 4.54 (s, 2H, $-CH_2$); 7.07–7.10 (m, 1H, Ar–H); 7.56–7.59 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 23.6, 29.9, 43.2, 52.8, 81.6, 112.48, 118.5, 125.2, 129.9, 137.6, 148.1, 156.5, 181.8, HRMS calculated for C₁₆H₁₅ClN₂O₂ 302.0822 [M⁺] found 302.0831; Analysis calculated for C₁₆H₁₅ClN₂O₂: C, 63.47; H, 4.99; N, 9.25 found C, 63.52; H, 4.91; N, 9.31.

3.3.7. 5-Chloro-1-(4-morpholin-4-yl-but-2-ynyl)-1H-indole-2,3dione (**3g**)

Orange solid, yield 85%, m.p. 160 °C: ¹H NMR (300 MHz, CDCl₃): δ 2.50 (t, *J* = 4.5 Hz, 4H, $-CH_2-N-CH_2-$); 3.26 (t, *J* = 2.0 Hz, 2H, -CH); 3.71 (t, *J* = 4.5 Hz, 4H, $-CH_2-O-CH_2-$); 4.56 (t, *J* = 2.0 Hz, 2H, $-CH_2$); 7.07–7.10 (m, 1H, Ar–H); 7.58–7.61 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz) 29.8, 47.2, 52.3, 66.7, 80.3, 112.3, 118.4, 125.3, 130.0, 137.6, 147.9, 156.5, 181.6, HRMS calculated for C₁₆H₁₅ClN₂O₃; C, 60.29; H, 4.74; N, 8.79 found C, 60.35; H, 4.79; N 8.65.

3.3.8. 5-Chloro-1-(4-diethylamino-but-2-ynyl)-1H-indole-2,3dione (**3h**)

Viscous liquid, yield 78%, ¹H NMR (300 MHz, CDCl₃): δ 1.07 (t, J = 7.1 Hz, 6H, CH₃); 2.51–2.59 (m, 4H, –CH₂–N–CH₂–); 3.6 (s, 2H, –CH₂); 4.54 (s, 2H, –CH₂); 7.07–7.10 (m, 1H, Ar–H); 7.56–7.63 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 14.8, 29.5, 46.7, 47.4, 81.6, 110.9, 117.7, 124.1, 125.3, 138.2, 149.7, 157.1, 181.7, HRMS calculated for C₁₆H₁₇ClN₂O₂ 304.0979 [M⁺] found 304.0986; Analysis calculated for C₁₆H₁₇ClN₂O₂: C, 63.05; H, 5.62; N, 9.19; found C, 63.13; H, 5.75; N, 9.07.

3.3.9. 5-Fluoro-1-(4-piperidin-1-yl-but-2-ynyl)-1H-indole-2,3dione (**3i**)

Orange solid, yield 85%, m.p. 149 °C: ¹H NMR (300 MHz, CDCl₃): δ 1.41–1.72 (m, 6H, $-CH_2-CH_2-CH_2-$); 2.42 (s, 4H, $-CH_2-N-CH-$); 3.22 (t, *J* = 2.0 Hz, 2H, $-CH_2$); 4.55 (t, *J* = 2.0 Hz, 2H, $-CH_2$); 7.09– 7.14 (m, 1H, Ar–H); 7.31–7.38 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz) 23.7, 25.7, 29.9, 47.7, 53.4, 81.2, 112.2, 118.4, 124.4, 124.7, 138.3, 149.6, 156.6, 181.6, HRMS calculated for C₁₇H₁₇FN₂O₂ 300.1274 [M⁺] found 300.1268; Analysis calculated for C₁₇H₁₇FN₂O₂: C, 67.99; H, 5.71; N, 9.33 found C, 67.92; H, 5.79; N, 9.41.

3.3.10. 5-Fluoro-1-(4-pyrrolidin-1-yl-but-2-ynyl)-1H-indole-2,3dione (**3***j*)

Orange solid, yield 81%, m.p. 141 °C: ¹H NMR (300 MHz, CDCl₃): δ 1.74–1.77 (m, 4H, –CH₂–CH₂–); 2.52–2.55 (m, 4H, –CH₂–N– CH–); 3.36 (s, 2H, –CH₂); 4.53 (s, 2H, –CH₂); 7.07–7.10 (m, 1H, Ar– H); 7.57–7.59 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 23.6, 29.9, 43.2, 52.8, 81.6, 112.5, 118.5, 125.2, 129.9, 137.6, 148.1, 156.5, 181.8, HRMS calculated for C₁₆H₁₅ClN₂O₂ 302.0822 [M⁺] found 302.0815; Analysis calculated for C₁₆H₁₅ClN₂O₂: C, 63.47; H, 4.99; N, 9.25 found C, 63.53; H, 4.85; N, 9.33.

3.3.11. 5-Fluoro-1-(4-morpholin-4-yl-but-2-ynyl)-1H-indole-2,3dione (**3k**)

Orange solid, yield 85%, m.p. 157 °C ¹H NMR (300 MHz, CDCl₃): δ 2.50 (t, J = 4.5 Hz, 4H, -CH₂-N-CH₂-); 3.27 (t, J = 2.0 Hz, 2H, -CH₂); 3.71 (t, J = 4.5 Hz, 4H, -CH₂-O-CH₂-); 4.56 (t, J = 2.0 Hz, 2H, -CH₂); 7.07-7.10 (m, 1H, Ar-H); 7.34-7.39 (m, 2H, Ar-H): ¹³C NMR (CDCl₃, 75 MHz): 29.8, 47.3, 52.3, 66.7, 80.2, 112.3, 118.5, 125.3, 130.0, 137.6, 148.0, 156.5, 181.7, HRMS calculated for C₁₆H₁₅FN₂O₃ 302.1067 [M⁺] found 302.1074; Analysis calculated for C₁₆H₁₅ClN₂O₃: C, 63.57; H, 5.00; N, 9.27 found C, 63.66; H, 5.09; N, 9.36.

3.3.12. 1-(4-Diethylamino-but-2-ynyl)-5-fluoro-1H-indole-2,3dione (**3l**)

Viscous liquid, yield 78%, ¹H NMR (300 MHz, CDCl₃): δ 1.05 (t, J = 7.1 Hz, 6H, CH₃); 2.53–2.62 (m, 4H, –CH₂–N–CH₂); 3.62 (s, 2H, –CH₂); 4.55 (s, 2H, –CH₂); 7.07–7.11 (m, 1H, Ar–H); 7.56–7.65 (m, 2H, Ar–H):¹³C NMR (CDCl₃, 75 MHz): 14.8, 29.4, 46.7, 47.3, 80.3, 112.5, 118.7, 125.3, 130.3, 138.2, 148.2, 157.1, 181.7, HRMS calculated for C₁₆H₁₇FN₂O₂ 288.3168 [M⁺] found 288.3157; Analysis calculated for C₁₆H₁₇ClN₂O₂: C, 66.65; H, 5.94; N, 9.72; found C, 66.73; H, 5.85, N, 9.79.

3.3.13. 5-Methyl-1-(4-pyrrolidin-1-yl-but-2-ynyl)-1H-indole-2,3dione (**3m**)

Orange solid, yield 82%, m.p. 148 °C: ¹H NMR (300 MHz, CDCl₃): δ 1.17–1.57 (m, 6H, –CH₂–CH₂–CH₂–); 2.29 (s, 3H, CH₃); 2.45 (s, 4H, –CH₂–N–CH–); 3.25 (s, 2H, –CH₂); 4.54 (s, 2H, –CH₂); 7.05–7.10 (m, 1H, Ar–H); 7.55–7.60 (m, 2H, Ar–H): ¹³C NMR (CDCl₃, 75 MHz): 20.9, 23.6, 25.7, 29.6, 47.7, 53.2, 81.0, 112.4, 118.4, 125.1, 129.8, 137.6, 148.0, 149.7, 157.2, 182.6, HRMS calculated for C₁₈H₂₀N₂O₂ 296.1525 [M⁺] found 296.1517; Analysis calculated for C₁₈H₂₀N₂O₂: C, 72.95; H, 6.80; N, 9.45 found C, 72.82; H, 6.89; N, 9.37.

3.3.14. 5-Methyl-1-(4-pyrrolidin-1-yl-but-2-ynyl)-1H-indole-2,3dione (**3n**)

Orange solid, yield 84%, m.p. 139 °C: ¹H NMR (300 MHz, CDCl₃): δ 1.73–1.77 (m, 4H, –CH₂–CH₂–); 2.29 (s, 3H, CH₃); 2.52–2.57 (m, 4H, –CH₂–N–CH–); 3.36 (s, 2H, –CH₂); 4.55 (s, 2H, –CH₂); 7.07–7.12 (m, 1H, Ar–H); 7.57–7.59 (m, 2H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 20.9, 23.6, 29.8, 43.2, 52.8, 81.6, 112.4, 118.5, 125.2, 129.9, 137.5, 148.0, 149.9, 156.56, 181.7, HRMS calculated for $C_{17}H_{18}N_2O_2$ 282.1368 [M⁺] found 282.1374; Analysis calculated for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92 found C, 72.39; H, 6.37; N, 9.86.

3.3.15. 5-Methyl-1-(4-morpholin-4yl-but-2-ynyl)-1H-indole-2,3dione (**30**)

Orange solid, yield 87%, m.p. 159 °C ¹H NMR (300 MHz, CDCl₃): δ 2.29 (s, 3H, CH₃); 2.51 (t, *J* = 4.5 Hz, 4H, -CH₂-N-CH₂-); 3.32 (s, 2H, -CH); 3.75 (t, *J* = 4.5 Hz, 4H, -CH₂-O-CH₂-); 4.53 (s, 2H, -CH₂); 7.07-7.10 (m, 1H, Ar-H); 7.32-7.39 (m, 2H, Ar-H): ¹³C NMR (CDCl₃, 75 MHz): 20.9, 29.8, 47.2, 52.3, 66.7, 81.6, 112.3, 118.4, 125.3, 129.9, 137.6, 147.9, 149.7, 156.5, 181.6, HRMS calculated for C₁₇H₁₈N₂O₃ 298.1317 [M⁺] found 298.1328; Analysis calculated for C₁₇H₁₈N₂O₃: C, 68.44; H, 6.08; N, 9.39 found C, 68.53; H, 6.14, N, 9.45.

3.3.16. 1-(4-Diethylamino-but-2-ynyl)-5-methyl-1H-indole-2,3dione (**3p**)

Viscous liquid, yield 78%, ¹H NMR (300 MHz, CDCl₃): δ 1.07 (t, J = 7.1 Hz, 6H, CH₃); 2.27 (s, 3H, CH₃); 2.52–2.60 (m, 4H, -CH₂-N–CH₂); 3.62 (s, 2H, -CH₂); 4.55 (s, 2H, -CH₂); 7.06–7.10 (m, 1H, Ar–H); 7.56–7.65 (m, 2H, Ar–H): ¹³C NMR (CDCl₃, 75 MHz): 14.8, 20.9, 29.4, 46.7, 47.3, 80.3, 112.5, 118.7, 125.3, 130.3, 138.2, 148.2, 157.1, 181.7, HRMS calculated for C₁₇H₂₀N₂O₂ 288.3168 [M⁺] found 288.3159; Analysis calculated for C₁₇H₂₀N₂O₂: C, 71.81; H, 7.09; N, 9.85; found C, 71.95; H, 7.17, N, 9.77.

3.4. Typical procedure for the synthesis of N-propargylated-isatin-Mannich-bis-adducts **5a–5d**

To a well stirred solution of *N*-propargylated isatin (1 mmol) in dry dioxane, catalytic amount of copper chloride and *p*-formaldehyde was added in succession. The reaction mixture was stirred for 10 min with subsequent addition of piperazine (0.5 mmol) and refluxed at 120 °C for 8 h. After the completion of reaction as evident from TLC, the reaction mixture was filtered and subsequently extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to result in the desired *bis*-Mannich adducts in good yields.

3.4.1. Bis-{1-(yl-but-2-ynyl)-1H-indole-2,3-dione} piperazine (5a)

Orange solid, yield 67%, m.p. >240 °C: ¹H NMR (300 MHz, CDCl₃): δ 2.55 (s, 8H, piperazine); 3.26 (s, 4H, –CH₂); 4.54 (s, 4H, CH₂); 7.11–7.20 (m, 4H, Ar–H); 7.60–7.64 (m, 4H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 29.7, 46.8, 51.8, 80.0, 111.0, 117.6, 124.1, 125.4, 138.3, 149.7, 157.1, 182.7, HRMS calculated for C₂₈H₂₄N₄O₄ 480.1798 [M⁺] found 480.1805; Analysis calculated for C₂₈H₂₄N₄O₄ C, 69.99; H, 5.03; N, 11.66; found C,69.82; H, 5.09; N, 11.73.

3.4.2. Bis-{5-chloro-1-(yl-but-2-ynyl)-1H-indole-2,3-dione} piperazine (**5b**)

Orange solid, yield 70%, m.p. >240 °C: ¹H NMR (300 MHz, CDCl₃): δ 2.56 (s, 8H, piperazine); 3.30 (s, 4H, –CH₂); 4.55 (s, 4H, –CH₂); 7.07–7.11 (m, 2H, Ar–H); 7.59–7.61 (m, 4H, Ar–H): ¹³C NMR (CDCl₃, 75 MHz): 29.7, 46.8, 51.7, 80.2, 112.5, 118.6, 125.1, 126.4, 138.3, 149.7, 157.2, 182.7, HRMS calculated for C₂₈H₂₂Cl₂N₄O₄ 548.1018 [M⁺] found 548.1025; Analysis calculated for C₂₈H₂₂Cl₂N₄O₄: C, 61.21; H, 4.04; N, 10.20; found C,61.30; H, 4.13; N, 10.15.

3.4.3. Bis-{5-fluoro-1-(yl-but-2-ynyl)-1H-indole-2,3-dione} piperazine (**5c**)

Orange solid, yield 65%, m.p. >240 °C: ¹H NMR (300 MHz, CDCl₃): δ 2.55 (s, 8H, piperazine); 3.35 (s, 4H, –CH₂); 4.54 (s, 4H, –

CH₂); 7.06–7.11 (m, 2H, Ar–H); 7.58–7.62 (m, 4H, Ar–H) 13 C NMR (CDCl₃, 75 MHz): 29.7, 46.8, 51.8, 80.2, 111.0, 117.6, 124.1, 125.4, 138.3, 149.7, 157.1, 181.6, HRMS calculated for C₂₈H₂₂F₂N₄O₄ 516.1609 [M⁺] found 516.1615; Analysis calculated for C₂₈H₂₂F₂N₄O₄: C, 65.11; H, 4.29; N, 10.85; found C, 65.23; H, 4.35; N, 10.77.

3.4.4. Bis-{5-methyl-1-(yl-but-2-ynyl)-1H-indole-2,3-dione} piperazine (5d)

Orange solid, yield 60%, m.p. >240 °C: ¹H NMR (300 MHz, CDCl₃): δ 2.56 (s, 8H, piperazine); 2.29 (s, 6H, CH₃); 3.31 (s, 4H, – CH₂); 4.55 (s, 4H, –CH₂); 7.07–7.11 (m, 2H, Ar–H); 7.59–7.61 (m, 4H, Ar–H) ¹³C NMR (CDCl₃, 75 MHz): 20.9, 29.5, 46.8, 51.8, 80.2, 111.0, 117.6, 124.1, 125.5, 138.3, 149.7, 157.1, 182.7, HRMS calculated for C₃₀H₂₈N₄O₄ 508.2111 [M⁺] found 508.2122; Analysis calculated for C₃₀H₂₈N₄O₄ C, 70.85; H, 5.55; N, 11.02; found C, 70.79; H, 5.62; N, 11.14.

3.5. Typical procedure for the synthesis of Mannich adducts 7a-7e

A mixture of *N*-propargylated isatin **2** (1 mmol), paraformaldehyde (2.5 mmol), 7-chloro-4-piperazin-1-yl-quinoline **7** (1 mmol) and anhydrous cupric chloride (0.5 mmol) in dry 1,4-dioxane was heated at 100 °C for 3 h. After completion of reaction as evidenced by TLC, the reaction mixture was filtered and the filtrate was treated with brine solution and extracted with ethyl acetate (3 × 60 mL). Combined organic layers were dried over Na₂SO₄ and the solvent was evaporated under reduced pressure resulting in a crude product which was purified by column chromatography using a chloroform:methanol (95:5) mixture.

3.5.1. 1-{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-but-2ynyl}-1H-indole-2,3-dione (**7a**)

Red solid; yield 88%; m.p. 115–116 °C: ¹H NMR (CDCl₃, 300 MHz): δ 2.79 (s, 4H, 2x-CH₂–); 3.24 (s, 4H, 2x-CH₂–); 3.42 (s, 2H, –N–CH₂–); 4.60 (s, 2H, –N–CH₂–); 6.82 (d, *J* = 5.1 Hz, 1H, H²); 7.15 (t, *J* = 7.8 Hz, 1H, ArH); 7.41 (dd, *J* = 2.1, 9.0 Hz, 1H, H⁴); 7.59–7.65 (m, 3H, ArH); 7.89 (d, *J* = 8.7 Hz, 1H, H³); 8.03 (d, *J* = 1.8 Hz, 1H, H⁵); 8.70 (d, *J* = 5.1 Hz, 1H, H¹); ¹³C NMR (DMSO-*d*₆, 100 MHz): δ ppm = 29.3, 45.9, 50.9, 51.4, 78.3, 79.2, 109.3, 111.6, 112.6, 121.2, 123.8, 124.0, 125.7, 125.9, 127.8, 133.5, 145.7, 149.4, 151.9, 156.1, 157.3, 159.8, 182.0, HRMS Calculated for C₂₅H₂₁ClN₄O₂ [M]⁺444.1353 found 444.1345; Analysis calculated for C₂₅H₂₁ClN₄O₂: C, 67.49; H, 4.76; N, 12.59, found: C, 67.57; H, 4.66; N, 12.52.

3.5.2. 1-{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-but-2ynyl}-5-fluoro-1H-indole-2,3-dione (**7b**)

Red Solid; Yield: 90%; m.p. 135–136 °C: ¹H NMR (CDCl₃, 300 MHz): δ 2.78 (s, 4H, 2x-CH₂–); 3.20 (s, 4H, 2x-CH₂–); 3.42 (s, 2H, –N–CH₂–); 4.57 (s, 2H, –N–CH₂–); 6.73 (d, *J* = 5.1 Hz, 1H, H²); 6.86–6.89 (m, 1H, ArH); 7.16–7.23 (m, 2H, ArH); 7.39 (d, *J* = 9.0 Hz, 1H, H⁴); 7.88 (d, *J* = 9.0 Hz, 1H, H³); 7.99 (s, 1H, H⁵); 8.66 (d, *J* = 5.1 Hz, 1H, H¹); ¹³C NMR (CDCl₃, 75 MHz): δ ppm = 29.9, 45.6, 51.2, 52.5, 78.4, 79.3, 108.9, 111.3, 112.4, 118.1, 121.7, 124.2, 124.9, 125.2, 126.6, 128.7, 134.8, 146.7, 150.0, 151.6, 156.4, 157.9, 181.9, HRMS Calculated for C₂₅H₂₀CIFN₄O₂ [M]⁺462.1259 found 462.1252; Analysis calculated for C₂₅H₂₀CIFN₄O₂: C, 64.87; H, 4.35; N, 12.10, found: C, 64.80; H, 4.41; N, 12.01.

3.5.3. 5-Chloro-1-{4-[4-(7-chloro-quinolin-4-yl)-piperazin-1-yl]but-2-ynyl}-1H-indole-2,3-dione (**7c**)

Red solid; yield: 92%; m.p. 124–125 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.80 (s, 4H, 2x-CH₂–); 3.21 (s, 4H, 2x-CH₂–); 3.40 (s, 2H, -N-CH₂–); 4.58 (s, 2H, -N-CH₂–); 6.74 (d, *J* = 5.1 Hz, 1H, H²); 6.85–6.88 (m, 1H, ArH); 7.18–7.25 (m, 2H, ArH); 7.39 (d, *J* = 9.0 Hz,

1H, H⁴); 7.81 (d, J = 9.0 Hz, 1H, H³); 8.00 (s, 1H, H⁵); 8.67 (d, J = 5.1 Hz, 1H, H¹); ¹³C NMR (CDCl₃, 75 MHz): δ ppm = 29.5, 45.4, 51.0, 52.2, 78.3, 79.4, 108.8, 111.2, 112.6, 118.2, 121.6, 124.3, 124.8, 125.1, 126.7, 128.5, 134.6, 146.4, 150.1, 151.9, 156.2, 157.7, 182.1, HRMS Calculated for C₂₅H₂₀Cl₂N₄O₂ [M]⁺478.0963 found 478.0971; Analysis calculated for Anal. C₂₅H₂₀Cl₂N₄O₂: C, 62.64; H, 4.21; N, 11.69, found: C, 62.54; H, 4.31; N, 11.76.

3.5.4. 1-{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-but-2ynyl}-5-methyl-1H-indole-2,3-dione (7d)

Red solid; yield: 82%; m.p. 114-115 °C: ¹H NMR (CDCl₃, 300 MHz): δ 2.23 (s, 3H, -CH₃); 2.77 (s, 4H, 2x-CH₂-); 3.18 (s, 4H, 2x-CH₂-); 3.38 (s, 2H, -N-CH₂-); 4.55 (s, 2H, -N-CH₂-); 6.69 (d, J = 5.1 Hz, 1H, H²); 6.82–6.85 (m, 1H, ArH); 7.17–7.24 (m, 2H, ArH); 7.31 (d, J = 9.0 Hz, 1H, H⁴); 7.81 (d, J = 9.0 Hz, 1H, H³); 8.01 (s, 1H, H^{5}); 8.63 (d, J = 5.1 Hz, 1H, H^{1}); ¹³C NMR (CDCl₃, 75 MHz): δ ppm = 21.1, 29.4, 45.7, 51.2, 52.0, 78.2, 79.6, 108.7, 111.2, 113.5, 118.0, 121.7, 124.2, 124.6, 125.8, 126.5, 128.9, 134.9, 136.3, 147.1, 149.6, 151.0, 157.7, 181.9, HRMS Calculated for C₂₆H₂₃ClN₄O₂ [M]⁺458.1510found 458.1518; Analysis calculated for C₂₆H₂₃ClN₄O₂: C, 68.04; H, 5.05; N, 12.10, found: C, 68.14; H, 5.11; N, 12.15.

3.5.5. 1-{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-but-2ynyl}-5-nitro-1H-indole-2,3-dione (**7e**)

Red solid; yield: 82%; m.p. 116–118 °C. ¹H NMR (CDCl₃, 300 MHz): $\delta 2.75$ (s, 4H, 2x-CH₂–); 3.18 (s, 4H, 2x-CH₂–); 3.36 (s, 2H, $-N-CH_2-$); 4.55 (s, 2H, $-N-CH_2-$); 6.67 (d, J = 5.1 Hz, 1H, H²); 6.82–6.85 (m, 1H, ArH); 7.16–7.25 (m, 2H, ArH); 7.31 (d, J = 9.0 Hz, 1H, H⁴); 7.81 (d, J = 9.0 Hz, 1H, H³); 8.01 (s, 1H, H⁵); 8.63 (d, J = 5.1 Hz, 1H, H¹); ¹³C NMR (CDCl₃, 75 MHz): δ 29.4, 45.7, 51.2, 52.2, 78.2, 79.6, 108.7, 111.2, 113.5, 118.0, 121.7, 124.3, 124.6, 125.9, 126.5, 128.9, 134.9, 136.3, 147.1, 149.6, 151.1, 157.7, 181.9, HRMS Calculated for C₂₅H₂₀ClN₅O₄ [M]⁺489.9104 found 489.9117; Analysis calculated for C₂₅H₂₀ClN₅O₄: C, 61.29; H, 4.11; N, 14.30, found: C, 61.38; H, 4.03; N, 14.42.

3.6. In vitro protozoal parasite susceptibility assay

To perform the initial susceptibility screens on *T. foetus* strain D1, compounds were dissolved in DMSO to obtain concentrations of 100 μ M; 5 μ L aliquots of these suspensions were diluted in 5 mL of TYM Diamond's media to obtain a final concentration of 100 μ M. After 24 h, cells were counted using a hemacytometer. The IC₅₀ value for compounds **7a**, **7c** and **7d** was determined by running assays of increasing drug concentrations, 5 μ M–40 μ M, and performing a regression analysis using Prism software, from GraphPad.

3.7. In vitro cytotoxic studies

PC-3 cells were cultured in DMEM supplemented with 10% FBS. Cells were plated in 96 well tissue culture plates and treated with compounds in the concentration range 20 μ M -0.001μ M for 72 h 10 μ l MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) 5 mg/ml in PBS was added to wells. Plates were incubated for 4 h. Plates were centrifuged at 1000 rpm for 5 min. Supernatant was removed; 100 μ l DMSO was added and mixed on shaker for 30 min. Absorbance was measured at 550 nm in Envision Multilabel Plate Reader (Perkin Elmer). Percentage inhibition was calculated and dose response curve was plotted using Graph pad Prism.

Acknowledgements

Financial assistance from University Grants Commission (UGC) under Rajiv Gandhi fellowship for SC/ST students (Nisha) is gratefully acknowledged. KL would like to acknowledge 2012 Pacific Fund Summer Undergraduate Research Fellowship at the University of Pacific. We would also like to thank Dr. Lydia K. Fox, Director of Undergraduate Research for her support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.015.

References

- [1] S.Z. Skirrow, R.H. BonDurant, Vet. Bull. 58 (1988) 591-603.
- [2] A. Yule, S.Z. Skirrow, R.H. BonDurant, Parasitol. Today 5 (1989) 373–377.
- [3] B.L. Clark, Aust. Vet. J. 47 (1971) 103–107.
- [4] R.J. Stewart, J.R. Campbell, E.D. Janzen, J. McKinnon, Can. Vet. J. 39 (1998) 638– 641.
- [5] S. Parker, J. Campbell, C. Ribble, A. Gajadhar, J. Am. Vet. Med. Assoc. 215 (1999) 231–235.
- [6] E.R. Cobo, C. Morsella, D. Cano, A. Cipolla, C.M. Campero, Theriogenology 62 (2004) 1367–1382.
- [7] D.O. Rae, J.E. Crews, Vet. Clin. North Am. Food Anim. Pract. 22 (2006) 595-611.
- [8] V. Midlej, R. Vilela, A.B. Dias, M. Benchimol, Vet. Parasitol. 165 (2009) 216–230.
 [9] C.M. Ribeiro, M.B. Falleiros, S.D. Bicudo, J.P. Junior, M.A. Golim, F.C. Filho,
- Theriogenology 73 (2010) 64–70. [10] B.T. Ratan, B. Anand, P. Yogeeswari, D. Sriram, Bioorg. Med. Chem. Lett. 15
- [10] B.1. Ratan, B. Anand, P. Yogeeswari, D. Shiram, Bioorg. Med. Chem. Lett. 15 (2005) 4451–4455.
- [11] T. Jiang, K.L. Kuhen, K. Wolff, H. Yin, K. Bieza, J. Caldwell, B. Bursulaya, T. Tuntland, K. Zhang, D. Karanewsky, Y. He, Bioorg. Med. Chem. Lett. 16 (2006) 2109–2112.
- [12] R. Tripathy, A. Reiboldt, P.A. Messina, M. Iqbal, J. Singh, E.R. Bacon, Th. S. Angeles, Sh. X. Yang, M.S. Albom, C. Robinson, H. Chang, B.A. Ruggeri, J.P. Mallamo, Bioorg. Med. Chem. Lett. 16 (2006) 2158–2162.
- [13] A. Cane, M.C. Tournaire, D. Barritault, M. Crumeyrolle-Arias, Biochem. Biophys. Res. Commun. 276 (2000) 379–384.
- [14] V. Ch. Silveira, J.S. Luz, C.C. Oliveira, I. Graziani, M.R. Ciriolo, A.M. Costa-Ferreira, J. Inorg. Biochem 102 (2008) 1090–1103.

- [15] R.A. Amal, R. Raghunathan, M.R. Sridevikumaria, N. Raman, Bioorg. Med. Chem. 11 (2003) 407–419.
- [16] M.C. Rodriguez-Arguelles, S. Mosquera-Vazaquez, P. Touron-Touceda, J. Sanmartin-Matalobos, A.M. Garcia-Deibe, M. Belicchi-Ferraris, G. Pelosi, C. Pelizzi, F. Zani, J. Inorg. Biochem 101 (2007) 138–147.
- [17] L. Maskell, E. A Blanche, M.A. Colucci, J.L. Whatmore, Ch. J. Moody, Bioorg. Med. Chem. Lett. 17 (2007) 1575–1578.
- [18] M. Verma, P.S. Nath, S.K. Nand, J.P. Stables, Acta Pharm. 54 (2004) 49-56.
- [19] N. Igosheva, C. Lorz, E. O'Conner, V. Glover, H. Mehmet, Neurochem. Int. 47 (2005) 216–224.
- [20] L.R. Chen, Y. Ch. Wang, Y.W. Lin, Sh. Y. Chou, Sh. F. Chen, L.T. Liu, Y.T. Wu, Ch. J. Kuo, T. Sh. Sh. Chen, Sh. H. Juang, Bioorg. Med. Chem. Lett. 15 (2005) 3058–3062.
- [21] J.F.M. Da Silva, S.J. Garden, A.C.J. Pinto, Braz. Chem. Soc. 12 (2001) 273–324 and references therein.
- [22] (a) K.L. Vine, J.M. Locke, M. Ranson, K. Benkendorff, S.G. Pyne, J.B. Bremner, Bioorg. Med. Chem. 15 (2007) 931–938;
 (b) K.L. Vine, J.M. Locke, M. Ranson, S.G. Pyne, J.B. Bremner, J. Med. Chem. 50 (2007) 5109–5117;
 (c) L. Matesic, J.M. Locke, J.B. Bremner, S.G. Pyne, D. Skropeta, M. Ranson, K.L. Vine, Bioorg. Med.Chem. 16 (2008) 3118–3124.
- [23] P. Singh, S. Kaur, V. Kumar, P.M.S. Bedi, M.P. Mahajan, I. Sehar, H.C. Pal, A.K. Saxena, Bioorg. Med. Chem. Lett. 21 (2011) 3017–3020.
- [24] P. Singh, P. Sharma, A. Anand, P.M.S. Bedi, T. Kaur, A.K. Saxena, Eur. J. Med. Chem. 55 (2012) 455–461.
- [25] (a) K. Kumar, S. Carrère-Kremer, L. Kremer, Y. Guérardel, C. Biot, V. Kumar, Organometallics (2013), http://dx.doi.org/10.1021/om301157z;
 (b) K. Kumar, S. Sagar, L. Esau, M. Kaur, V. Kumar, Eur. J. Med. Chem. 58 (2012) 153–159;
 (c) K. Kumar, S.C. Kremer, L. Kremer, Y. Guérardel, C. Biot, V. Kumar, Dalton Trans 42 (2013) 1492–1500;

(d) Nisha, P. Singh, D.T. Hendricks, K. Bisetty, V. Kumar, Synlett 24 (2013) 1865-1869.

- [26] R. Raj, P. Singh, N.T. Haberkern, R.M. Faucher, N. Patel, K.M. Land, V. Kumar, Eur. J. Med. Chem. 63 (2013) 897–906.
- [27] Nisha, V. Mehra, M. Hopper, N. Patel, D. Hall, L.A. Wrischnik, K.M. Land, V. Kumar, Med. Chem. Comm. 4 (2013) 1018-1024.
- [28] R.R. Pandey, A. Srivastava, R. Malasoni, A. Naqvi, A. Jain, J.P. Maikhuri, S. Paliwal, G. Gupta, A.K. Dwivedi, Bioorg. Med. Chem. Lett. 22 (2012) 5735– 5738.