Communication

Click-chemistry approach to synthesis of functionalized isatin-ferrocenes and their biological evaluation against the human pathogen *Trichomonas vaginalis*

Amandeep Singh a, David Zhang b, Christina C. Tam c, Luisa W. Cheng c, Kirkwood M. Land b,**, Vipan Kumar a, *

a Department of Chemistry, Guru Nanak Dev University, Amritsar, 143005, Punjab, India
b Department of Biological Sciences, University of the Pacific, Stockton, CA, 95211, USA
c Agricultural Research Service, United States Department of Agriculture, Albany, CA, 94710, USA

**Article Info**

Article history:
Received 19 April 2019
Received in revised form 24 May 2019
Accepted 25 May 2019
Available online 30 May 2019

Keywords:
Isatin-ferrocene conjugates
Ferrocene methoxy-isatin
Isatin-ferrocenylchalcones
Click chemistry
Anti-trichomonad evaluation

**Abstract**

Copper-promoted azide-alkyne cycloadditions were attempted to synthesize a series of variedly functionalized 1H-1,2,3-triazole-linked isatin-ferrocene, ferrocenylmethoxy-isatin and isatin-ferrocenyl-chalcone conjugates. The synthesized scaffolds were assayed for their inhibitory activity against *T. vaginalis* as well as several common normal human flora bacteria. The observed inhibitory activities against *T. vaginalis* and undetectable inhibition of microflora bacteria suggest that these compounds may be specific against trichomonad protozoa and could serve as a new scaffold for synthesis of novel compounds against this important human pathogen.

© 2019 Elsevier B.V. All rights reserved.

**1. Introduction**

Trichomoniasis is a sexually transmitted disease caused by the protozoan parasite *T. vaginalis* and responsible for 156 million cases worldwide in 2017 [1]. The standard treatment for trichomoniasis involves 5-nitro-imidazole based drugs, including the most common metronidazole (MTZ). There has been an increased incidence of clinical cases of trichomoniasis resistant to metronidazole [2]. These recalcitrant cases are often treated with even higher dosages of the MTZ, which in turn may lead to higher levels of resistance and an increase in patient side effects [3]. Undoubtedly, new and alternative therapies are needed for trichomoniasis.

Isatin is an indole chemical moiety containing both keto and lactam groups that has been used extensively in the pharmacological and medicinal chemistry fields [4,5]. Isatin derivatives are known to possess anti-inflammatory, anti-fungal, anti-tubercular, anti-cancer, anti-HIV, anti-viral, and anti-bacterial properties [6]. These biological properties validated isatin as a starting point for the design and preparation biological relevant chemical libraries [7,8]. SU11248 (Sutent), a 5-fluoro-3-substituted isatin derivative, was approved by the FDA in 2006 for the treatment of advanced renal carcinoma and gastrointestinal stromal tumors [9]. Inhibitory activity of isatin-chromone against alpha-glucosidase has been reported by Wang et al. with the most potent compound showed IC50 = 3.8 μM [10]. Isatin-Moxifloxacin hybrids were evaluated against MTB H37Rv and MDR-TB strains of *M. tuberculosis* with the most potent compound demonstrating IC50 = 0.05 μM, two to seven times more active than standard drugs moxifloxacin and rifampicin [11].

In the past decade, the application of organometallic chemistry in the field of biology and medicine has led to a new area called bioorganometallic chemistry [12]. Ferrocene is the most attractive among metallocenes because of its stability, biological activity, and non-toxicity [13]. Ferrocene is an ideal platform for the synthesis of new biologically active compounds. Replacement of purely organic component with ferrocene has shown to improve the biological potency of the target compounds, as demonstrated by ferroceny-
conjugates of commercial anti-estrogen tamoxifen and anti-androgen nilutamide [14]. Ferroquine (FQ) is the most exemplary contribution of ferrocene to the improvement of the anti-plasmodial potential of chloroquine and is under clinical trials [15]. In addition, the high lipophilicity of ferrocene may allow interaction with or permeation of biological membranes, which is essential for achieving their desired biological activities [16].

Recent published studies from our laboratories describe the synthesis of isatin-based scaffold viz. mono and bis-isatin, isatin-ferrocene, isatin ferrocenyl chalcones, and isatin metronidazole conjugates along with their preliminary in vitro evaluation against T. vaginalis [17]. With an aim of discovering varied biologically relevant scaffolds, and extending from previous studies, the present manuscript describes the synthesis of a series of functionalized isatin-ferrocenes. These molecules were then evaluated for their growth inhibition activities against T. vaginalis. The 1H-1,2,3-triazole core has drawn considerable attention in the field of medicinal chemistry mainly due to its capability of forming hydrogen bonds that eventually improves its solubility and interaction with biomolecular targets [18].

2. Result and discussion

The synthetic methodology for the synthesis of precursor C-5 substituted azido-hydroxy isatin involved the initial base-promoted N-alkylation with methyl iodide or benzyl bromide to yield 2. The treatment of 2 with trimethyl silyl iodide in dry DMF afforded spiro-isatin 3. Epoxide ring opening of 3 with sodium azide led to the formation of 3-(azidomethyl)-3-hydroxy-indolin-2-one 4. Cu-promoted click chemistry of 4 with ethynyl ferrocene with subsequent purification via column chromatography using (5:95) methanol: chloroform mixture as eluent gave the desired isatin-ferrocene conjugate 5 in good yields (Scheme 1). Synthesis of isatin-ferrocenyl chalcones conjugates 7 involved the click reaction between 3-(azidomethyl)-3-hydroxy-indolin-2-one 4 and ferrocenyl chalcone 6, synthesized via reported protocols. The structure of conjugate 7 was characterized using spectral data and analytical evidences. For example, 7d exhibited a molecular ion peak at [M]+ 602.1662. Its 1H NMR showed singlet at δ 3.10 of three protons corresponding to N-methyl substituent. The presence of sharp singlet at δ 4.15 of 5H corresponds to the cyclopentadienyl ring of ferrocene along with two singlets at δ 4.50 and at δ 4.61. The presence of absorption signals at 175.0 and 188.1 ppm corresponding to the carbonyl carbon of isatin and chalcone respectively, further confirmed the assigned structure.

The sequence of synthetic steps leading to ferrocene methoxy-isatin conjugates 11 is depicted in Scheme 2. Ferrocene carboxaldehyde 8 on reaction with sodium borohydride yielded ferrocene methanol 9 that was further treated with propargyl bromide in the presence of sodium hydride as a base to result in O-propargylated ferrocene methanol 10. Cu-promoted azide-alkyne cycloaddition reaction of 4 with 10 led to the isolation of crude solid, which was purified via column chromatography using CHCl3: methanol (95:5) mixture as eluent, to yield the desired conjugates 11 in good yields. The synthesized conjugates viz. 5a-h, 7a-h, 11a-h were evaluated for their growth inhibitory activity against T. vaginalis as previously described [17a]. As evident from Table 1, the activity of the synthesized was found to be depend upon the nature of the substituent at C-5 and N-1 position of the isatin ring as well as nature of linker used. A careful analysis of observed inhibition data among isatin-ferrocene conjugates 5a-h revealed the presence of halogen substituent (Cl, Br) for good activities as evident by 5b and 5c. The

![Scheme 1. Synthesis of 1H-1,2,3-triazole-linked Isatin-ferrocene conjugates 5a-h and 7a-h.](image)
replacement of \(N\text{-CH}_3\) with \(N\text{-benzyl}\) improved the activities, in general, with conjugate 5g exhibiting approximately 94% growth inhibition.

Induction of ferrocenyl-chalcone moiety among the synthesized hybrids (7a-h) resulted in the loss of activity especially in case of \(N\text{-benzyl}\) substituents. The \(N\text{-methylated}\) counterparts exhibited good inhibition with compound 7a (R = H, \(N\text{-CH}_3\)) proved to be the most potent in the series with growth inhibition of approximately 83%. Comparing the observed activity profiles with ferrocene-methoxy isatin conjugates (11a-h) revealed an interesting structure activity relationship with dependence on the substituent at N-1 of isatin ring. As evident, the compounds (11a-d) with \(N\text{-CH}_3\) proved to be ineffective in inhibiting the growth. The replacement of \(N\text{-CH}_3\) to \(N\text{-benzyl}\) resulted in improvement in activities with percentage inhibition in the range of 84.43–87.63 irrespective of the nature of substituent at C-5 position of isatin ring.

The most potent conjugates, identified from the percentage inhibition studies were evaluated for their IC\(_{50}\) values (concentration that inhibited 50% parasite growth) and the results are enlisted in Table 2. Conjugates 5f, 5g, 5h, 11f, and 11g showed IC\(_{50}\) values of 28.9, 30.1, 28.3, 27.0, and 32.5 \(\mu\)M that were higher than the standard FDA-approved drug metronidazole.

In order to determine if the observed activities are specific to the parasite \(T.\text{vaginalis}\) or non-specific because of their cytotoxicity, it was considered worthwhile to evaluate the effect of these compounds on normal human flora since they occupy same niche as this protozoal pathogen. Using the classic disc diffusion assay, we tested these compounds against a panel of normal human flora consisting of the non-pathogenic strains, \(Escherichia\text{ coli K12 MG1655, Lactobacillus acidophilus, Lactobacillus rhamnosus LGG, and Lactobacillus reuteri.}\) No cytotoxic effects from any of these synthesized compounds were observed for any of the normal flora.

### 3. Conclusion
A series of isatin-ferrocene conjugates linked with different functionalities were synthesized and assayed for their inhibitory activities against \(T.\text{vaginalis}\). As evident from the biological data, the synthesized scaffolds are less active than standard drug metronidazole. The most active conjugate of the series 11f with a benzyl substituent at the N-1 position and chloro at C-5 position proved to be most active and non-cytotoxic with an IC\(_{50}\) value of 27 \(\mu\)M. This new scaffold can serve as a platform for the synthesis of new lead compounds against this human pathogen.

### Acknowledgements
VK acknowledges Council of Scientific and Industrial Research (CSIR) New Delhi grant No. 02(0293)/17/EMR-I for providing financial assistance. The Department of Biological Sciences, University of the Pacific, also provided support to KML and DZ for this research. LWC and CCT were also partially funded by the United States Department of Agriculture, Agricultural Research Service, National Program project NP108, CRIS 2030–42000–039–00D.
Appendix A. Supplementary data

Text and structures giving general experimental procedures, characterization data of (5a-h, 7a-h, 11a-h) compounds, and scanned \(^{1}H, ^{13}C\) NMR spectra for the compounds viz. 5f, 7a, 7f, 11a, 11c, 11d.

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jorganchem.2019.05.025.

References


