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Communication

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

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ABSTRACT

Copper-promoted azide-alkyne cycloadditions were attempted to synthesize a series of variedly functionalized 1*H*-1,2,3-triazole-linked isatin-ferrocene, ferrocenylmethoxy-isatin and isatin-ferrocenylchalcone 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.

1. Introduction

Trichomoniasis is a sexually transmitted disease caused by the protozoan parasite *T. vaginalis* and responsible for 156 million cases world-wide 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 α-glucosidase has been reported by Wang et al. with the most potent compound showed $IC_{50} = 3.8\ \mu M$ [10]. Isatin-Moxifloxacin hybrids were evaluated against MTB $H_{37}Rv$ and MDR-TB strains of *M. tuberculosis* with the most potent compound demonstrating $IC_{50} = 0.05\ \mu M$, 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 ferrocenyl-

Abbreviations: SAR, Structure Activity Relationship.

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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].

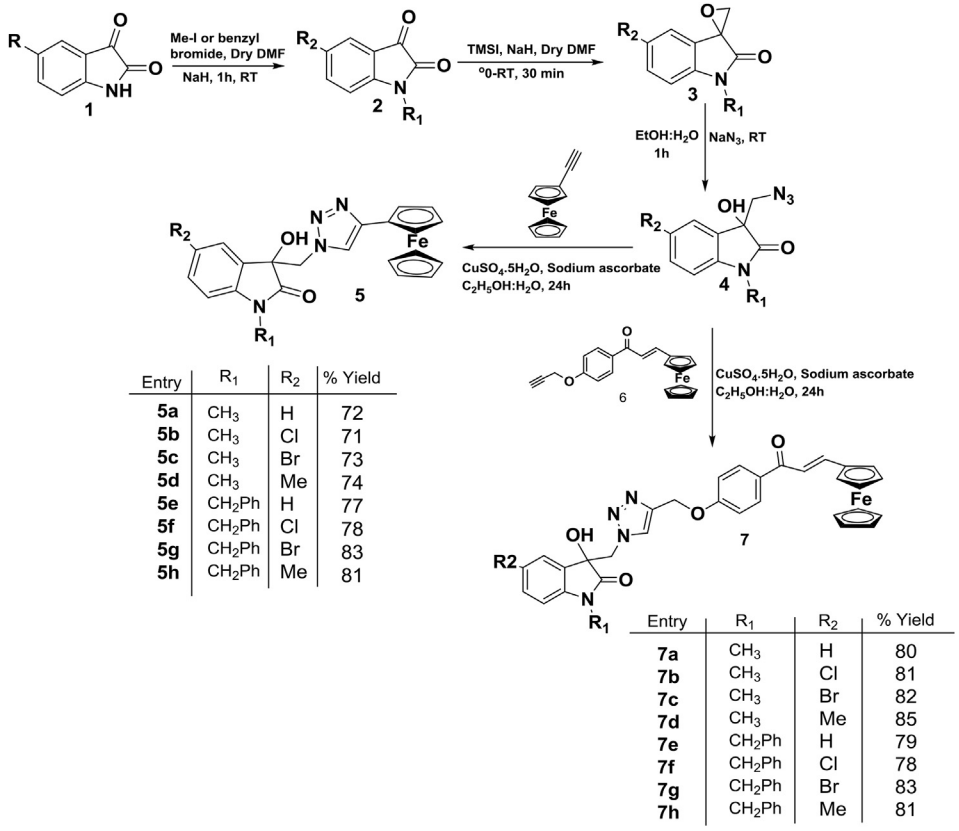
Recent published studies from our laboratories describe the synthesis of isatin-based scaffold *viz*. mono and bis-isatin, isatin-ferrocene, isatin ferrocenyl chalcones, and isatin metronidazole conjugates along with their preliminary *in vitro* evaluation against *T. vaginalis*. [17] With an aim of 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 1*H*-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.[18c] 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 ^{1}H 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 $CHCl_3$: methanol (95:5) mixture as eluent, to yield the desired conjugates **11** in good yields.

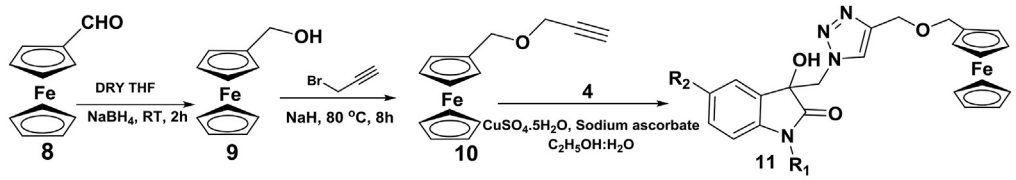
The synthesized conjugates *viz.* **5a-h**, **7a-h**, **11a-h** were evaluated for their growth inhibitory activity against *T. vaginalis* as previously described [17a]. As evident from Table 1, the activity of the synthesized was found to be depend upon the nature of the substituent at C-5 and *N*-1 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



Entry	R_1	R_2	% Yield
5a	CH_3	H	72
5b	CH_3	Cl	71
5c	CH_3	Br	73
5d	CH_3	Me	74
5e	CH_2Ph	H	77
5f	CH_2Ph	Cl	78
5g	CH_2Ph	Br	83
5h	CH_2Ph	Me	81

Entry	R_1	R_2	% Yield
7a	CH_3	H	80
7b	CH_3	Cl	81
7c	CH_3	Br	82
7d	CH_3	Me	85
7e	CH_2Ph	H	79
7f	CH_2Ph	Cl	78
7g	CH_2Ph	Br	83
7h	CH_2Ph	Me	81

Scheme 1. Synthesis of 1*H*-1,2,3-triazole-linked Isatin-ferrocene conjugates **5a-h** and **7a-h**.



Entry	R_1	R_2	% Yield
11a	CH_3	H	64
11b	CH_3	Cl	62
11c	CH_3	Br	65
11d	CH_3	Me	68
11e	CH_2Ph	H	61
11f	CH_2Ph	Cl	72
11g	CH_2Ph	Br	70
11h	CH_2Ph	Me	71

Scheme 2. Synthesis of 1*H*-1,2,3-triazole-linked ferrocene methoxy-isatin conjugates **11a-h**.

Table 1
Preliminary screening of isatin conjugates **5a-h**, **7a-h,** and **11a-h** for growth inhibition of the human pathogen *T. vaginalis*.

Entry	% Inhibition (+/-SE)	Entry	% Inhibition (+/− SE)	Entry	% Inhibition (+/-SE)
5a	39.7 (1.4)	**7a**	82.9 (2.1)	**11a**	19.8 (1.1)
5b	84.8 (1.5)	**7b**	74.7 (2.2)	**11b**	24.6 (2.1)
5c	84.3 (2.2)	**7c**	75.6 (1.1)	**11c**	46.9 (0.8)
5d	49.8 (1.2)	**7d**	73.9 (1.8)	**11d**	49.3 (0.4)
5e	85.7 (1.6)	**7e**	60.4 (2.1)	**11e**	N.D
5f	89.0 (1.2)	**7f**	57.4 (2.2)	**11f**	84.4 (1.3)
5g	93.6 (2.2)	**7g**	50.0 (1.8)	**11g**	87.6 (2.3)
5h	86.7 (2.1)	**7h**	39.3 (1.2)	**11h**	85.1 (1.1)

replacement of *N*–CH_3 with *N*-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*-benzyl substituted scaffolds. The *N*-methylated counterparts exhibited good inhibition with compound **7a** (R = H, N–CH_3) proving 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*–CH_3 proved to be the ineffective in inhibiting the growth. The replacement of *N*–CH_3 to *N*-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

Table 2
IC_{50} values of the most potent conjugates.

Compounds	IC_{50} (μM)
5b	52.5
5c	51.6
5e	50.4
5f	28.9
5g	30.1
5h	28.3
7a	55.2
11f	27.0
11g	32.5
11h	59.1
MTZ	0.7

28.9, 30.1, 28.3, 27.0, and 32.5 μM that were higher than the standard FDA-approved drug metronidazole.

In order to determine if the observed activities are specific to the parasite *T. 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 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. 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 μM. This new scaffold can serve as a platform for the synthesis of new lead compounds against this human pathogen.

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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.

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