Accepted Manuscript

Synthesis of isatin based N^1-alkylated 3-β-*C*-glycoconjugated-oxopropylidene oxindoles as potent antiplasmodial agents

Ravi Kumar Thakur, Prince Joshi, Kapil Upadhyaya, Kartikey Singh, Gaurav Sharma, Sanjeev K. Shukla, Renu Tripathi, Rama Pati Tripathi

PII: S0223-5234(18)30962-0

DOI: https://doi.org/10.1016/j.ejmech.2018.11.008

Reference: EJMECH 10865

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 23 August 2018

Revised Date: 3 November 2018

Accepted Date: 5 November 2018

Please cite this article as: R.K. Thakur, P. Joshi, K. Upadhyaya, K. Singh, G. Sharma, S.K. Shukla, R. Tripathi, R.P. Tripathi, Synthesis of isatin based N^1-alkylated 3-β-*C*-glycoconjugated-oxopropylidene oxindoles as potent antiplasmodial agents, *European Journal of Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.ejmech.2018.11.008.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Synthesis of isatin based N^1-alkylated 3-β-*C*-glycoconjugated-oxopropylidene oxindoles as potent antiplasmodial agents

Ravi Kumar Thakur,[a,1,3] Prince Joshi,[b,1] Kapil Upadhyaya,[a] Kartikey Singh,[a] Gaurav Sharma,[c,d] Sanjeev K. Shukla,[c,d] Renu Tripathi, [b,c,*] and Rama Pati Tripathi [a,c, 2,*]

[a]*Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, Lucknow-226031, India.*
[b]*Parasitology Division, CSIR-Central Drug Research Institute, Lucknow-226031, India.*
[c]*Academy of Scientific and Innovative Research (AcSIR), New Delhi-110001, India.*
[d]*Sophisticated Analytical Instrument Facility, CSIR-Central Drug Research Institute, Lucknow 226031, India.*
[1]*These authors contributed equally to this work*

Current Addresses:
[2]*Dean, National Institute of Pharmaceutical Education and Research Raebarely, New Transit Campus, Bijnor Road, Sarojani Nagar Near CRPF Base Camp, Lucknow 226002, U.P., India*
[3]*Chandigarh University, NH-95 Chandigarh-Ludhiana Highway, Mohali, Punjab 140413, India.*

*****Corresponding Authors
E-mail addresses: rpt.cdri@gmail.com, (Rama Pati Tripathi), Mobile- 09415004443.
E-mail addresses: renu1113@rediffmail.com, renu_tripathi@cdri.res.in (Renu Tripathi), Tel: +91-(522)-2772450. Fax: +91-(522)-2771941. Ext. 2453, Mobile- 09415349883.

Abstract

In an attempt to develop new antimalarial drugs, we have synthesized a new class of *N*-alkylated 3-glycoconjugated-oxopropylidene oxindoles starting from substituted isatins and glucopyranosyl propanone via a well-known cross-aldol reaction followed by dehydration. The newly synthesised compounds were screened for their *in vitro* antiplasmodial activity, and among all the compounds **9g**, **9f**, **9b**, **8d**, **9d**, **9c**, and **9e** displayed potent activity with the IC_{50} values in the range of 0.1-0.3 µM against Chloroquine (CQ) sensitive *Pf*3D7 strain, while compounds **9d**, **9b**, **9e**, **8c**, **8f**, **9c**, and **9a** have shown promising activity having IC_{50} values in 0.1-0.4 µM range against CQ resistant *Pf*K1 strain, which is even better than the standard drug chloroquine with IC_{50} value of 0.5 µM.

Keywords: Isatin**,** Glucopyranosyl propanone, Cross aldol reaction, Antiplasmodial activity.

Introduction

Malaria remains one of the most prevalent and deadliest parasitic diseases with persistent to the global health problem. Among the five species of *Plasmodium* viz. *Plasmodium ovale, Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax* and, *Plasmodium knowlesi*, the most virulent malarial parasite is *Plasmodium falciparum*, which is responsible for millions of deaths per year[1]. Natural products such as quinine and artemisinin have contributed immensely in the treatment of malaria and proved to be the lead compounds for the development of antimalarials[2]. Despite the availability of various antimalarial drugs like quinine, artemisinin, artemether, chloroquine, chlorproguanil, mefloquine, primaquine, sulfadoxine, amodiaquine, and many others, the widespread emergence of multi-drug resistant strains of *Plasmodium falciparum* to clinically approved drugs has realized the urgent need of highly effective, safe, novel and fast-acting antimalarial agent[3].

Isatin (*1H*-Indole-2,3-dione) is a versatile heterocyclic scaffold endowed with various biological activities and good tolerance in humans [4]. Isatin is present as a main component in many alkaloids[5], drugs[6] as well as dyes[7], pesticides and analytical reagents. Besides, isatins, as well as *N*-alkylated isatins have been used as synthetic building block for the preparation of various important naturally occurring heterocyclic compounds[8], having significant biological activities[9]. The C-3 carbonyl group of isatins is highly reactive and the nucleophilic attack at this position leads to the formation of 2-oxindole derivatives. Oxindoles, basically isatin derivatives are endowed with fascinating pharmacological[10] as well as biological activities such as antimicrobial[11], antitumor[12], antitubercular[13], antimalarial[14], anti-HIV[15] and antibacterial activities[16]. Some of them have been reported to exhibit significant antimalarial activities[17] as shown below (Figure 1). It is known that glucose delivery is crucial for the survival of the malarial parasites and in this context various glycoconjugates[18] and glucosides[19] with potent antimalarial activities have also been reported (Figure 2). These compounds inhibit the *Plasmodium falciparum* Hexose Transporter (*Pf*HT) which plays an important role in glucose uptake and survival of the malarial parasites. Therefore, glucose consumption could be an excellent target for the development of novel antimalarial agents since its elevated presence of glucose uptake and metabolism in infected erythrocytes in all stages of the parasitic life cycle have been reported [19].

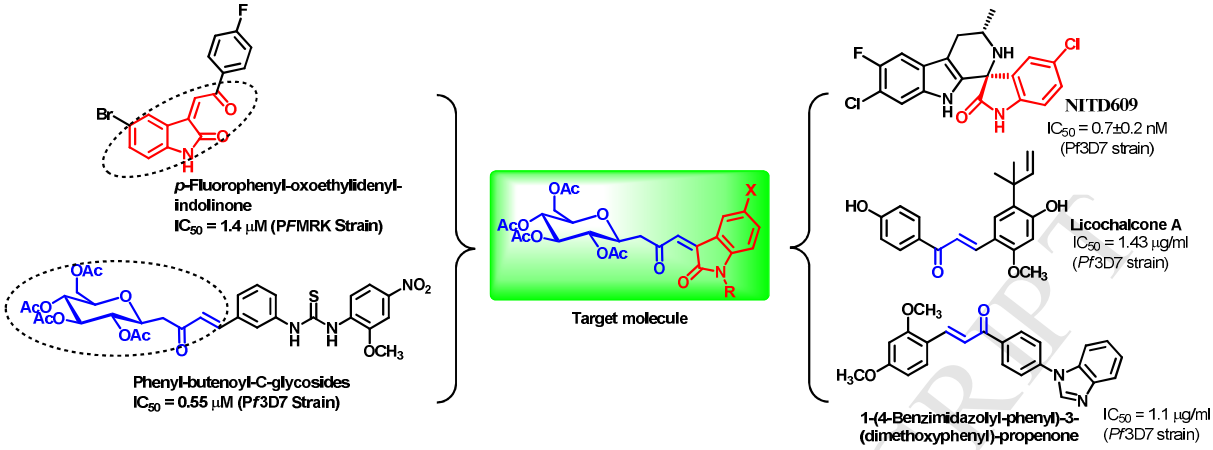


Figure 1: Representative oxindole and chalcone based antimalarial agents and our proposed molecule.

In this context, development of glucose conjugated isatin derivatives as antimalarial agents could be an effective strategy.

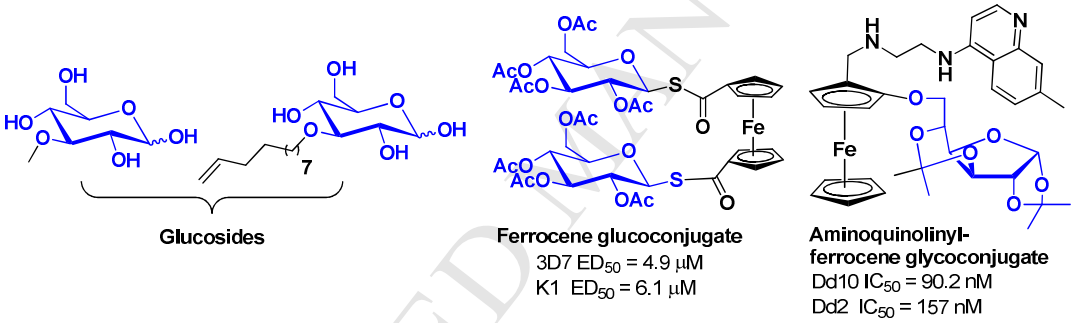


Figure 2: The structures of some carbohydrate-based antimalarials.

We report herein the synthesis of glycohybridised oxopropylidene oxindoles by utilizing the most versatile cross-aldol reaction between substituted isatin and glucosyl propanone. Unlike various reactions between an aldehyde and a ketone, the ketone–ketone cross-aldol reactions are rare [20]. The enone functionality in target compounds have resemblance with chalcones, which are considered as an important motif in medicinal chemistry, endowed with various biological activities such as antimicrobial, anticancer, anti-inflammatory, antibacterial, and antiproliferative[21]. A few of the chalcone derivatives including one of our sugar derived chalcone, have demonstrated potent antimalarial activities [22], (Figure 1). Our recent work showed that glycohybridised isatin hydrazones are potent antiplasmodial agents [23]. Thus, keeping all these facts in mind and in continuation of our previous efforts in the development of

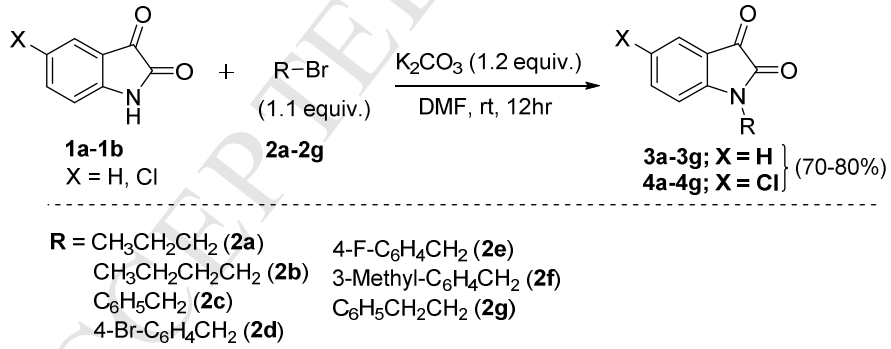
novel antimalarials[22d, 24], we embarked upon a new series of isatin glycoconjugates and evaluated them for their antiplasmodial activity.

Result and discussion

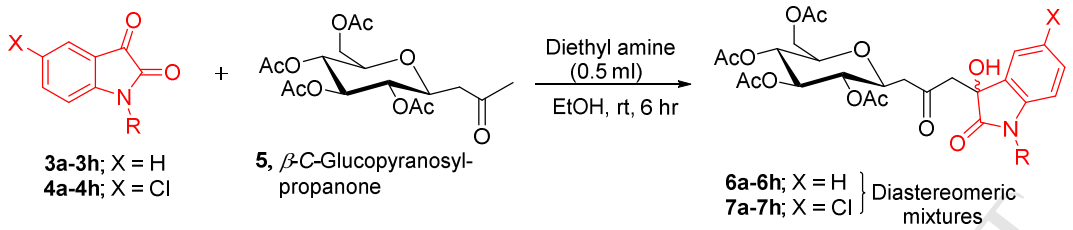
Chemistry

Synthesis of N^1-alkylated-3-(3′-*β*-*C*-glucoconjugated-2′-oxo-propyl)-3-hydroxy-oxindoles:

Our synthetic strategy starts with the alkylation[25] of the commercially available isatin/5-chloroisatin (**1**) with different alkyl bromides (**2**), in the presence of base K_2CO_3 and DMF as the solvent to give the respective N^1-alkylated isatin/5-chloroisatin derivatives (**3** and **4**) in 70-80% yields (Scheme 1). The resulting N^1-alkylated derivatives **3** or **4** on reaction with 1 equiv. of acetylated *β*-*C*-glucopyranosyl-propanone (**5**) in the presence of Hunig's base diethylamine (0.5 ml) in ethanol at room temperature, gave the aldol addition products N^1-alkylated-3-(3′-*β*-*C*-glucoconjugated-2′-oxo-propyl)-3-hydroxy-oxindoles (**6** or **7**) as diastereomeric mixtures[26]. The resulting diastereomeric mixtures thus formed were isolated in 70-85% yields in 53:47 to 63:37 ratios. (Scheme 2, Table 1) Ratios were determined by integration of the ^{1}H NMR spectra of the mixture of compounds. The structure elucidation of the diastereomeric mixtures was carried out based on their spectroscopic data ^{1}H, ^{13}C, and mass.



Scheme 1: Synthesis of N^1-alkylated-isatin/N^1-alkylated-5-chloroisatin derivatives.



Scheme 2: Synthesis of N^1-alkylated-3-(3′-*β*-*C*-glucoconjugated-2′-oxo-propyl)-3-hydroxy-oxindoles.

Table 1: Synthesis of N^1-alkylated-3-(3′-*β*-*C*-glucoconjugated-2′-oxo-propyl)-3-hydroxy-oxindoles (**6** and **7**) by the reaction of different N^1-alkylated-isatin/N^1-alkylated-5-chloroisatin derivatives (**3** or **4**) and acetylated *β*-*C*-glucopyranosyl-propanone (**5**).

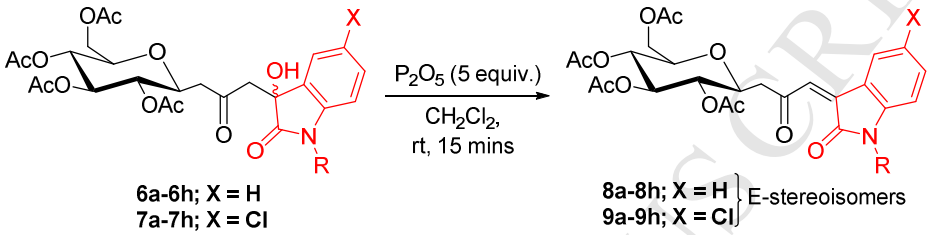
S.No.	Compound No.	R	X	Yield (%)	Ratio[a]
1.	6a	Propyl-	H	78	60:40
2.	6b	Butyl-	H	83	63:37
3.	6c	$PhCH_2$-	H	82	61:39
4.	6d	*p*-Br-$C_6H_4CH_2$-	H	80	59:41
5.	6e	*p*-F-$C_6H_4CH_2$-	H	79	62:38
6.	6f	*m*-Me-$C_6H_4CH_2$-	H	78	61:39
7.	6g	$PhCH_2CH_2$-	H	75	61:39
8.	6h	H-	H	82	61:39
9.	7a	Propyl-	Cl	80	53:47
10.	7b	Butyl-	Cl	82	56:44
11.	7c	$PhCH_2$-	Cl	74	61:39
12.	7d	*p*-Br-$C_6H_4CH_2$-	Cl	81	63:37
13.	7e	*p*-F-$C_6H_4CH_2$-	Cl	71	59:41
14.	7f	*m*-Me-$C_6H_4CH_2$-	Cl	80	60:40
15.	7g	$PhCH_2CH_2$-	Cl	76	61:39
16.	7h	H-	Cl	79	60:40

[a]The diastereomeric ratios were determined from ^{1}H NMR.

Synthesis of N^1-alkylated-3-*β*-*C*-glucoconjugated-oxopropylidene oxindoles:

The target compounds N^1-alkylated-3-*β*-*C*-glucoconjugated-oxopropylidene oxindoles (**8** & **9**) were accessed by dehydration of synthesized aldol addition products N^1-alkylated-3-(3′-*β*-*C*-glucoconjugated-2′-oxo-propyl)-3-hydroxy-oxindoles (**6** & **7**) with an appropriate dehydrating agent. The screening of different acidic dehydrating agents such as 25% dil. HCl, acetic acid, and *p*-TSA under various reaction conditions resulted into a complex mixture of compounds which are difficult to isolate. Our base mediated dehydration methodology[27] resulted into a mixture

of compounds albeit in poor yield of desired dehydrated product. Interestingly, the addition of a well-known dehydrating agent P_2O_5 (5 equiv.)[28] to the stirring solution of N^1-alkylated-3-(3′-*β*-*C*-glucoconjugated-2′-oxo-propyl)-3-hydroxy-oxindoles (**6** or **7**,1 equiv.) in dichloromethane at 0°C afforded a single stereoisomer of N^1-alkylated-3-*β*-*C*-glucoconjugated-oxopropylidene oxindoles **8** and **9** in 52-72% yields (Scheme 3, Table 2). The non-nucleophilic nature of the P_2O_5 makes it an efficient dehydrating agent for the desired transformation.



Scheme 3: Synthesis of N^1-alkylated-3-*β*-*C*-glucoconjugated-oxopropylidene oxindoles.

Table 2: Synthesis of N^1-alkylated-3-*β*-*C*-glucoconjugated-oxopropylidene oxindoles (**8** & **9**) by treating the diastereomeric mixtures N^1-alkylated-3-(3′-*β*-*C*-glucoconjugated-2′-oxo-propyl)-3-hydroxy-oxindoles (**6** & **7**) with dehydrating agent P_2O_5.

S.No.	Compound No.	R	X	Yield (%)
1.	8a	Propyl-	H	69
2.	8b	Butyl-	H	65
3.	8c	$PhCH_2$-	H	55
4.	8d	p-Br-$C_6H_4CH_2$-	H	88
5.	8e	p-F-$C_6H_4CH_2$-	H	52
6.	8f	m-Me-$C_6H_4CH_2$-	H	60
7.	8g	$PhCH_2CH_2$-	H	58
8.	8h	H-	H	64
9.	9a	Propyl-	Cl	72
10.	9b	Butyl-	Cl	70
11.	9c	$PhCH_2$-	Cl	60
12.	9d	p-Br-$C_6H_4CH_2$-	Cl	62
13.	9e	p-F-$C_6H_4CH_2$-	Cl	57
14.	9f	m-Me-$C_6H_4CH_2$-	Cl	64
15.	9g	$PhCH_2CH_2$-	Cl	63
16.	9h	H-	Cl	68

The structure elucidation of the target compounds (**9** & **10**) was done on the basis of their spectroscopic data ^1H, ^{13}C, and HRMS. To confirm the configuration of the isolated single isomer, detailed chemical shift assignment of ^1H and ^{13}C NMR signals was performed by using

various 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, TOCSY, NOESY, HSQC, and HMBC) NMR experiments for one of the above prototypes (**9a**). HMBC correlation of H-1' & H-4 with C-3 and H-1' & H-8 with C-2 and H-1' & H-3' with C-2' showed their connectivity (Figure 3). It has been reported in the literature that 3-substituted indolin-2-ones may exist as either the *Z* or *E* isomer depending on the characteristics of the substituents at the C-3 position of the 3-substituted indolin-2-one[29]. In the ^{1}H, ^{1}H - NOESY spectra, the missing correlation between H-1′ and H-4 strongly supported the formation of *E* configuration of the double bond between C-3 and C-1′ in **9a**. Here, we observed only the nOe correlation between H-1′ and H-3′. Further, the characteristic peaks (H-1′ and C-1′) in all the final compounds for ^{1}H and ^{13}C NMR spectra appeared at around δ7.10-7.20 ppm and δ133 ppm, respectively.

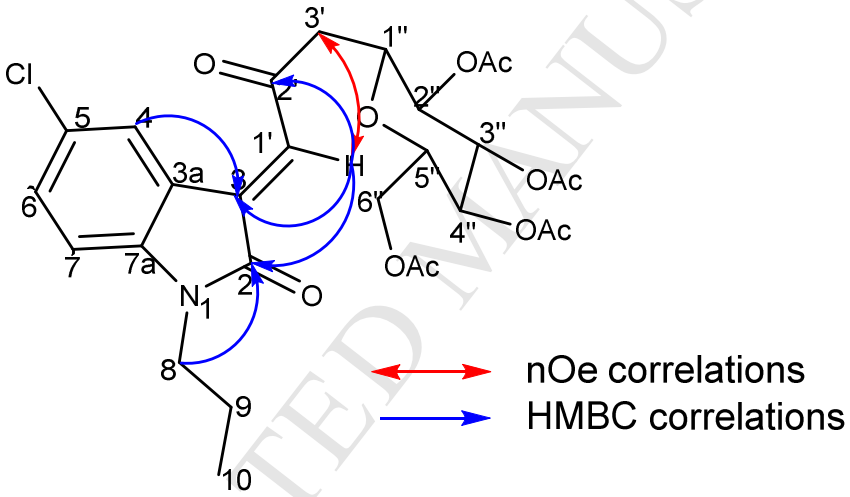


Figure 3: Relevant nOe and HMBC correlations of **9a**.

Use of Zemplén deacetylation reaction condition was found to be unsuitable for the deprotection of the compounds **8** & **9**[30] as well as methanolic-HCl proved to be inefficient. This may probably be due to the retro-aldol reaction taking place leading to the formation of the corresponding N^{1}-alkylated isatin derivative and *β-C*-glucopyranosyl-propanone. Earlier, we have reported acetyl protected phenyl butenonyl-*C*-glycosides[23d] as potent antimalarial agents than their deacetylated counterpart. Apart from this, the literature survey revealed that acetyl-protected glycosylated isoindigo, indirubin derivatives, and other glycoconjugates have shown better antiproliferative activity than their deacetylated counterparts[31]. Screening of these

acetylated analogues **8** & **9** has shown excellent *in vitro* antiplasmodial activity against *P. falciparum*.

Biology

The synthesized N^1-alkylated-3-*β*-*C*-glucoconjugated-oxopropylidene oxindoles (**8** & **9**) were evaluated for their *in vitro* antiplasmodial activity against Chloroquine-sensitive (3D7) and Chloroquine-resistant (K1) strains of *P. falciparum* as compared to the reference drug chloroquine and the respective values for inhibitory concentration 50% (IC_{50} in µM), cytotoxic concentrations 50% (CC_{50} in µM) and selectivity indices (SI) against both *Pf* strains are enlisted in Table 3.

Table 3: *In vitro* antiplasmodial activity and cytotoxicity of targeted compounds (**8** & **9**) and diastereomeric mixtures (**6** & **7**).

Entry	IC_{50} (µM)[a,b]		CC_{50} (µM)[b,d]	SI(µM)[c]		Entry	IC_{50} (µM)[a,b]		CC_{50} (µM)[b,d]
	***Pf*3D7**	***Pf*K1**		***Pf*3D7**	***Pf*K1**		***Pf*3D7**	***Pf*K1**	
8a	3.59	>5	124.74	34.74	24.94	**6a**	>5	>5	ND
8b	2.61	1.84	125.56	48.10	68.23	**6b**	>5	>5	ND
8c	**0.77**	**0.29**	71.18	92.44	245.44	**6c**	>5	>5	ND
8d	**0.23**	**0.80**	76.54	332.78	95.67	**6d**	>5	>5	ND
8e	**0.47**	1.19	119.07	253.34	100.05	**6e**	>5	>5	ND
8f	**0.53**	**0.30**	72.46	136.71	241.53	**6f**	>5	>5	ND
8g	**0.95**	**0.75**	64.69	68.09	86.25	**6g**	>5	>5	ND
8h	4.27	>5	127.20	29.78	25.44	**6h**	>5	>5	ND
9a	1.18	**0.40**	95.55	80.97	238.87	**7a**	>5	>5	ND
9b	**0.18**	**0.22**	88.90	493.88	404.09	**7b**	>5	>5	ND
9c	**0.26**	**0.31**	91.21	350.80	294.22	**7c**	>5	>5	ND
9d	**0.24**	**0.15**	93.33	388.87	622.20	**7d**	>5	4.81	ND
9e	**0.32**	**0.25**	107.26	335.18	429.04	**7e**	3.54	2.26	ND
9f	**0.17**	1.04	90.96	535.05	87.46	**7f**	>5	>5	ND
9g	**0.11**	**0.94**	92.97	845.18	98.90	**7g**	>5	>5	ND
9h	2.68	2.52	121.94	45.50	48.38	**7h**	>5	>5	ND
CQ	**0.007**	**0.5**	**>500**	**>71428**	**>1000**				

ACCEPTED MANUSCRIPT

[a]50% Inhibitory concentration against chloroquine sensitive (3D7) and resistant (K1) strains of *P. falciparum*. [b]values are represented as the average of at least duplicate determinations. [c]Selectivity index (SI) = CC_{50}/IC_{50}. [d]50% Cytotoxic concentrations. ND = Not done.

The activity result shows that among all the synthesized targeted compounds **9g**, **9f**, **9b**, **8d**, **9d**, **9c**, and **9e** have exhibited potent antiplasmodial activity having IC_{50} values in the range of 0.1-0.3 µM as 0.11, 0.17, 0.18, 0.23, 0.24, 0.26 and 0.32 µM against chloroquine-sensitive *Pf*3D7 strain, respectively. Apart from this, compounds **9d**, **9b**, **9e**, **8c**, **8f**, **9c**, and **9a** have shown excellent promising activity having IC_{50} values in the range of 0.1-0.4 µM as 0.15, 0.22, 0.25, 0.29, 0.30, 0.31 and 0.40 µM, respectively against chloroquine-resistant *Pf*K1 strain, which are even better than the standard drug chloroquine showing IC_{50} value 0.5 µM. Moreover, compounds **8e**, **8f**, **8c** and **8g** have also shown significant activity against *Pf*3D7 strain with IC_{50} values 0.47, 0.53, 0.77 and 0.95 µM, respectively while compounds **8g**, **8d**, and **9g** displayed very good activity against *Pf*K1 strain with IC_{50} values 0.75, 0.80 and 0.94 µM, respectively. As shown in Table 3 most of the compounds are active having IC_{50} values falling in the range of 0-1 µM, against both the strains. The most active series of compounds against both the strains is **9a-h** which is 5-chloroisatin derivative, while in the case of isatin derivatives **8a-h** the antiplasmodial activity comparatively reduced against both the strains. The latter indicates that the *in vitro* antiplasmodial activity is highly enhanced by 5-chloro substitution at isatin core. The effect of substitution at the N-1 position of isatin can also be studied in displaying the antiplasmodial activity. The N-1 unsubstituted compounds such as **8h** were found inactive at higher concentration, while **9h** being a 5-chloroisatin derivative showed moderate activity with IC_{50} values 2.68 µM against *Pf*3D7 strain and 2.52 µM against the *Pf*K1 strain. These compounds showed the comparatively poor result as compared to other compounds with substitution at N-1. In addition to this, the pure diastereomeric mixtures (**6** & **7**) have also been evaluated for *in vitro* antiplasmodial activity against both *Pf*3D7 and *Pf*K1 strains (Table 3). It was found that almost all of these diastereomeric mixtures were inactive at a higher concentration with IC_{50} >5 µM against any of the strains, except compound **7e** which showed moderate activity against *Pf*K1 strain with IC_{50} value 2.26 µM. This clearly signifies the importance of exocyclic double bond at the C-3 position of the isatin ring, for the antimalarial activity. All the compounds having IC_{50} <5 µM against either of the *Pf* strains were evaluated for their cytotoxicity against monkey kidney cell line (VERO) and these were found to be non-cytotoxic with very good SI values. The most active compounds with IC_{50} values in 0.1-0.3 µM

range against *Pf*3D7 strain have very good CC_{50} and SI values in 76.54-107.26 µM and 332.78-845.18 µM range, respectively. Similarly, the compounds with IC_{50} values in 0.1-0.4 µM range against *Pf*K1 strain also have very good SI values in 238.87-622.20 µM. Therefore, these compounds hold the potential to be developed as novel antimalarial drug candidates.

Conclusion

In conclusion, the present study describes the synthesis of a novel series of N^1-alkylated-3-*β*-*C*-glucoconjugated-oxopropylidene oxindoles **8** and **9** utilizing cross-aldol chemistry followed by dehydration along with their antiplasmodial activity against *3D7* and *K1* strains of *P. falciparum*. Among all the synthesized targeted glucoconjugates, ten compounds have shown significantly potent results with IC_{50} in the range of 0.11-0.77 µM against the chloroquine-sensitive *Pf*3D7 strain. Further, these compounds have also exhibited excellent promising results against chloroquine-resistant *Pf*K1 strain having IC_{50} values in 0.15-0.94 µM range. These compounds were found to be more selective for plasmodial over mammalian cells. Our study reveals that the exocyclic double bond at the C-3 position of isatin has played a crucial role in displaying the antiplasmodial activity, as none of the diastereomeric mixtures (**6** or **7**) were active against *Plasmodium* parasites. The preference for chloro substituent at C-5 position (**9a-h**) to exhibit antiplasmodial activity is also evident.

Materials & Methods

In Vitro Cultivation of *P. falciparum*

The Chloroquine (CQ) sensitive (3D7) and resistant (K1) strains of *P. falciparum* were cultured *in vitro* as per Trager and Jensen (1976) method with some modifications[32]. Cultures were maintained in fresh human erythrocytes at 5% hematocrit in complete medium. RPMI-1640 (HEPES modified) medium (Sigma) supplemented with 0.5% AlbuMaxII, 0.2% glucose, sodium bicarbonate and 15 µM hypoxanthine and incubated at 37 °C in a CO_2 incubator. Parasite growth rate and the stage were determined by the test of Giemsa's stained thin smears of the RBCs. All the above mentioned final compounds were tested over a concentration range of 150 nM to 10 µM. *P. falciparum* drug susceptibility test was carried out by determining fifty percent inhibitory concentration (IC_{50}) according to the method of Johnson *et al.* (2007) with some modifications[33]. Briefly, two-fold serial dilutions of compounds and chloroquine were

prepared in 96 well plates and then 50 µL asynchronous cultures of infected erythrocytes with 0.8-1% parasitaemia and 1% haematocrit was added to each well (100 µL-final volume). Eight wells were treated as a positive control (without drug) and 4 wells as a negative control (without parasite and drug). These plates were incubated in a CO_2 incubator maintained at 37 °C for 72 h. Then 100 µL lytic buffer containing SYBR Green 1x final concentration was added to each well and incubated for 2 h at room temperature in dark place. Plates were read under fluorescence reader at Ex. 485 nm, Em. 535 nm. Fifty percent inhibitory concentration (IC_{50}) was determined by quantifying DNA in treated and control cultures of parasites in human erythrocytes according to the SYBR Green I (Sigma Aldrich) fluorescence-based method.

Cytotoxicity Assay

Cytotoxic level of compounds was determined according to O'Brien *et. al*. (2000) method with few modifications[35]. The monkey kidney cell line (VERO) was maintained *in vitro* in MEM medium (Sigma) supplied with 15% fetal bovine serum (FBS) and 5% CO_2 at 37 °C. An appropriate serial drug dilution was prepared in culture plates and the cells were exposed to the concentrations of particular compounds for three days, 10% resazurin, a cell viability marker, was added and read under fluorescent reader at excitation of 530 ± 25 nm and emission of 590 ± 25 nm wavelength for calculation of the median cytotoxic concentration (CC_{50}).

Author contributions

The manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript. R.K.T. and P.J. contributed equally to this work.

Conflicts Of Interest

The authors declare that they have no conflicts of interest with the contents of this article.

Acknowledgments

RKT and PJ are thankful to UGC, New Delhi and DBT for providing fellowships. We thank SAIF-CDRI for the analytical facilities and Mrs. Shashi Rastogi for technical assistance.

References

[1] (a) R.G. Ridley, Medical need, scientific opportunity and the drive for antimalarial drugs, Nature, 415 (2002) 686-693; (b) N. Kumar, R. Singh, D.S. Rawat, Tetraoxanes: synthetic and medicinal chemistry perspective, Med Res Rev, 32 (2012) 581-610.

[2] (a) M.H. Gelb, Drug discovery for malaria: a very challenging and timely endeavor, Curr Opin Chem Biol, 11 (2007) 440-445; (b) J. Wiesner, R. Ortmann, H. Jomaa, M. Schlitzer, New antimalarial drugs, Angew Chem Internat Ed Eng, 42 (2003) 5274-5293; (c) G.A. Biagini, P.M. O'Neill, P.G. Bray, S.A. Ward, Current drug development portfolio for antimalarial therapies, Curr Opin Pharmacol, 5 (2005) 473-478; (d) R. Gaur, M.P. Darokar, P.V. Ajayakumar, R.S. Shukla, R.S. Bhakuni, In vitro antimalarial studies of novel artemisinin biotransformed products and its derivatives, Phytochemistry, 107 (2014) 135-140; (e) C.A. Morris, S. Duparc, I. Borghini-Fuhrer, D. Jung, C.S. Shin, L. Fleckenstein, Review of the clinical pharmacokinetics of artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration, Malaria J, 10 (2011) 263

[3] (a) P.F. Salas, C. Herrmann, C. Orvig, Metalloantimalarials, Chem Rev, 113 (2013) 3450-3492; (b) D.A. Fidock, P.J. Rosenthal, S.L. Croft, R. Brun, S. Nwaka, Antimalarial drug discovery: efficacy models for compound screening, Nat Rev Drug Discov, 3 (2004) 509-520. .

[4] (a) J.F.M.d. Silva, S.J. Garden, A.C. Pinto, The chemistry of isatins: a review from 1975 to 1999, J Braz Chem Soc, 12 (2001) 273-324; (b) S.N. Pandeya, S. Smitha, M. Jyoti, S.K. Sridhar, Biological activities of isatin and its derivatives, Acta Pharm. 55 (2005) 27-46; (b) K.L. Vine, L. Matesic, J.M. Locke, M. Ranson, D. Skropeta, AntiCancer Agents Med. Chem. 9 (2009) 397-414; (c) P. Davidovich, V. Aksenova, V. Petrova, D. Tentler, D. Orlova, S. Smirnov, V. Gurzhiy, A.L. Okorokov, A. Garabadzhiu, G. Melino, N. Barlev, V. Tribulovich, Discovery of Novel Isatin-Based p53 Inducers, ACS Med Chem Lett, 6 (2015) 856-860

[5] (a) B. Batanero, F. Barba, Electrosynthesis of tryptanthrin, Tet Lett, 47 (2006) 8201-8203; (b) H. Deng, J.P. Konopelski, Aryllead(IV) reagents in synthesis: formation of the C11 quaternary center of N-methylwelwitindolinone C isothiocyanate, Org Lett, 3 (2001) 3001-3004.

[6] T. Aboul-Fadl, F.A. Bin-Jubair, O. Aboul-Wafa, Schiff bases of indoline-2,3-dione (isatin) derivatives and nalidixic acid carbohydrazide, synthesis, antitubercular activity and pharmacophoric model building, Eur J Med Chem, 45 (2010) 4578-4586.

[7] E.S. Ferreira, A.N. Hulme, H. McNab, A. Quye, The natural constituents of historical textile dyes, Chem Soc Rev, 33 (2004) 329-336.

[8] (a) R. Dalpozzo, G. Bartoli, G. Bencivenni, Recent advances in organocatalytic methods for the synthesis of disubstituted 2- and 3-indolinones, Chem Soc Rev, 41 (2012) 7247-7290; (b) H. Liang, W. Rui, Recent Advances in Asymmetric Organocatalytic Construction of 3,3′-Spirocyclic Oxindoles, Adv Syn Catal, 355 (2013) 1023-1052; (c) M.S. Shmidt, A.M. Reverdito, L. Kremenchuzky, I.A. Perillo, M.M. Blanco, Simple and efficient microwave assisted N-alkylation of isatin, Molecules, (Basel, Switzerland), 13 (2008) 831-840.

[9] (a) W.M. J., I. Steen, v.M.J. H., H. Henk, Total Synthesis of the Spirocyclic Oxindole Alkaloids Corynoxine, Corynoxine B, Corynoxeine, and Rhynchophylline, Eur J Org Chem, 2013 (2013) 1100-1106; (b) M. Amat, C. Ramos, M. Perez, E. Molins, P. Florindo, M.M. Santos, J. Bosch, Enantioselective formal synthesis of ent-

rhynchophylline and ent-isorhynchophylline, Chem Comm (Cambridge, England), 49 (2013) 1954-1956.

[10] (a) R. Fortt, G. Smith, R.O. Awais, S.K. Luthra, E.O. Aboagye, Automated GMP synthesis of [(18)F]ICMT-11 for in vivo imaging of caspase-3 activity, Nucl Med Biol, 39 (2012) 1000-1005; (b) N.K. Görkem Ermut, İdil Çetin, Mehmet Topçul, Seher Birteksöz, Synthesis and chemotherapeutic activities of 5-chloro-1H-indole-2,3-dione 3-thiosemicarbazones, Marmara Pharm J, 17 (2013) 147-154.

[11] M. Kumar, K. Ramasamy, V. Mani, R.K. Mishra, A.B.A. Majeed, E.D. Clercq, B. Narasimhan, Synthesis, antimicrobial, anticancer, antiviral evaluation and QSAR studies of 4-(1-aryl-2-oxo-1,2-dihydro-indol-3-ylideneamino)-N-substituted benzene sulfonamides, Arab J Chem, 7 (2014) 396-408.

[12] (a) D. Havrylyuk, B. Zimenkovsky, O. Vasylenko, A. Gzella, R. Lesyk, Synthesis of new 4-thiazolidinone-, pyrazoline-, and isatin-based conjugates with promising antitumor activity, J Med Chem, 55 (2012) 8630-8641; (b) S.B. Kumar, M. Ravinder, G. Kishore, V. Jayathirtha Rao, P. Yogeeswari, D. Sriram, Synthesis, antitubercular and anticancer activity of new Baylis–Hillman adduct-derived N-cinnamyl-substituted isatin derivatives, Med Chem Res, 23 (2014) 1934-1940.

[13] (a) K. Kumar, S. Carrere-Kremer, L. Kremer, Y. Guerardel, C. Biot, V. Kumar, Azide-alkyne cycloaddition en route towards 1H-1,2,3-triazole-tethered beta-lactam-ferrocene and beta-lactam-ferrocenylchalcone conjugates: synthesis and in vitro anti-tubercular evaluation, Dalton Trans (Cambridge, England : 2003), 42 (2013) 1492-1500; (b) P. Mondal, S. Jana, A. Balaji, R. Ramakrishna, L. Kanthal, Synthesis of Some New Isoxazoline Derivatives of Chalconised Indoline 2-one as a Potential Analgesic, Antibacterial and Anthelmimtic Agents, J Young Pharm, 4 (2012) 38-41.

[14] R. Raj, P. Singh, P. Singh, J. Gut, P.J. Rosenthal, V. Kumar, Azide-alkyne cycloaddition en route to 1H-1,2,3-triazole-tethered 7-chloroquinoline-isatin chimeras: synthesis and antimalarial evaluation, Eur J Med Chem, 62 (2013) 590-596.

[15] B.K. Paul, D. Ray, N. Guchhait, Unraveling the binding interaction and kinetics of a prospective anti-HIV drug with a model transport protein: results and challenges, Phys Chem Chem Phys, 15 (2013) 1275-1287.

[16] G. Kiran, T. Maneshwar, Y. Rajeshwar, M. Sarangapani, Microwave-Assisted Synthesis, Characterization, Antimicrobial and Antioxidant Activity of Some New Isatin Derivatives, J Chem, 2013 (2013) 1-7.

[17] (a) M. Rottmann, C. McNamara, B.K. Yeung, M.C. Lee, B. Zou, B. Russell, P. Seitz, D.M. Plouffe, N.V. Dharia, J. Tan, S.B. Cohen, K.R. Spencer, G.E. Gonzalez-Paez, S.B. Lakshminarayana, A. Goh, R. Suwanarusk, T. Jegla, E.K. Schmitt, H.P. Beck, R. Brun, F. Nosten, L. Renia, V. Dartois, T.H. Keller, D.A. Fidock, E.A. Winzeler, T.T. Diagana, Spiroindolones, a potent compound class for the treatment of malaria, Science, 329 (2010) 1175-1180; (b) C.L. Woodard, Z. Li, A.K. Kathcart, J. Terrell, L. Gerena, M. Lopez-Sanchez, D.E. Kyle, A.K. Bhattacharjee, D.A. Nichols, W. Ellis, S.T. Prigge, J.A. Geyer, N.C. Waters, Oxindole-based compounds are selective inhibitors of Plasmodium falciparum cyclin dependent protein kinases, J Med Chem, 46 (2003) 3877-3882; (c) K.R. Butsi, (2017) 'Synthesis and characterization of spirooxindole derivatives as potential antimalarial agents' (Master of Science dissertation), submitted to the Faculty of Science University of the Witwatersrand Johannesburg, wiredspace.

[18] (a) C.L. Ferreira, C.B. Ewart, C.A. Barta, S. Little, V. Yardley, C. Martins, E. Polishchuk, P.J. Smith, J.R. Moss, M. Merkel, M.J. Adam, C. Orvig, Synthesis, structure, and biological activity of ferrocenyl carbohydrate conjugates, Inorg Chem, 45 (2006) 8414-8422; (b) C. Herrmann, P.F. Salas, B.O. Patrick, C. de Kock, P.J. Smith, M.J. Adam, C. Orvig, 1,2-disubstituted ferrocenyl carbohydrate chloroquine conjugates as potential antimalarial agents, Dalton Trans (Cambridge, England : 2003), 41 (2012) 6431-6442.

[19] T. Joet, U. Eckstein-Ludwig, C. Morin, S. Krishna, Validation of the hexose transporter of Plasmodium falciparum as a novel drug target, Proc Natl Acad Sci U S A, 100 (2003) 7476-7479.

[20] M.A. Kabeshov, O. Kysilka, L. Rulisek, Y.V. Suleimanov, M. Bella, A.V. Malkov, P. Kocovsky, Cross-Aldol Reaction of Isatin with Acetone Catalyzed by Leucinol: A Mechanistic Investigation, Chemistry (Weinheim an der Bergstrasse, Germany), 21 (2015) 12026-12033.

[21] (a) P. Singh, A. Anand, V. Kumar, Recent developments in biological activities of chalcones: a mini review, Eur J Med Chem, 85 (2014) 758-777; (b) Z. Nowakowska, A review of anti-infective and anti-inflammatory chalcones, Eur J Med Chem, 42 (2007) 125-137; (c) L. Ni, C.Q. Meng, J.A. Sikorski, Recent advances in therapeutic chalcones, Exp Opin Ther Pat, 14 (2004) 1669-1691.

[22] (a) M. Chen, T.G. Theander, S.B. Christensen, L. Hviid, L. Zhai, A. Kharazmi, Licochalcone A, a new antimalarial agent, inhibits in vitro growth of the human malaria parasite Plasmodium falciparum and protects mice from P. yoelii infection, Antimicrobl Agents Chemother, 38 (1994) 1470-1475; (b) N. Yadav, S.K. Dixit, A. Bhattacharya, L.C. Mishra, M. Sharma, S.K. Awasthi, V.K. Bhasin, Antimalarial activity of newly synthesized chalcone derivatives in vitro, Chem Biology Drug Des, 80 (2012) 340-347; (c) N. Mishra, P. Arora, B. Kumar, L.C. Mishra, A. Bhattacharya, S.K. Awasthi, V.K. Bhasin, Synthesis of novel substituted 1,3-diaryl propenone derivatives and their antimalarial activity in vitro, Eur J Med Chem, 43 (2008) 1530-1535; (d) K.K. Ramakrishna, S. Gunjan, A.K. Shukla, V.R. Pasam, V.M. Balaramnavar, A. Sharma, S. Jaiswal, J. Lal, R. Tripathi, Anubhooti, R. Ramachandran, R.P. Tripathi, Identification of novel phenyl butenonyl C-glycosides with ureidyl and sulfonamidyl moieties as antimalarial agents, ACS Med Chem Lett, 5 (2014) 878-883.

[23] R.K. Thakur, P. Joshi, P. Baranwal, G. Sharma, S.K. Shukla, R. Tripathi, R.P. Tripathi, Synthesis and antiplasmodial activity of glyco-conjugate hybrids of phenylhydrazono-indolinones and glycosylated 1,2,3-triazolyl-methyl-indoline-2,3-diones, Eur J Med Chem, 155 (2018) 764-771.

[24] (a) N. Devender, S. Gunjan, R. Tripathi, R.P. Tripathi, Synthesis and antiplasmodial activity of novel indoleamide derivatives bearing sulfonamide and triazole pharmacophores, Eur J Med Chem, 131 (2017) 171-184; (b) K. Singh, P. Joshi, R. Mahar, P. Baranwal, S.K. Shukla, R. Tripathi, R.P. Tripathi, Synthesis and antiplasmodial activity of purine-based C-nucleoside analogues, Med Chem Comm, 7 (2018) 1232-1238; (c) N. Devender, S. Gunjan, S. Chhabra, K. Singh, V.R. Pasam, S.K. Shukla, A. Sharma, S. Jaiswal, S.K. Singh, Y. Kumar, J. Lal, A.K. Trivedi, R. Tripathi, R.P. Tripathi, Identification of beta-Amino alcohol grafted 1,4,5 trisubstituted 1,2,3-triazoles as potent antimalarial agents, Eur J Med Chem, 109 (2016) 187-198.

[25] T.M. Bridges, J.E. Marlo, C.M. Niswender, C.K. Jones, S.B. Jadhav, P.R. Gentry, H.C. Plumley, C.D. Weaver, P.J. Conn, C.W. Lindsley, Discovery of the first highly M5-preferring muscarinic acetylcholine receptor ligand, an M5 positive allosteric modulator derived from a series of 5-trifluoromethoxy N-benzyl isatins, J Med Chem, 52 (2009) 3445-3448.

[26] D. Kleeblatt, M. Becker, M. Plotz, M. Schonherr, A. Villinger, M. Hein, J. Eberle, M. Kunz, Q. Rahman, P. Langer, Synthesis and bioactivity of N-glycosylated 3-(2-oxo-2-arylethylidene)-indolin-2-ones, RSC Adv, 5 (2015) 20769-20782.

[27] N. Anand, K. Upadhyaya, A. Ajay, R. Mahar, S.K. Shukla, B. Kumar, R.P. Tripathi, A strategy for the synthesis of anthraquinone-based aryl-C-glycosides, J Org Chem, 78 (2013) 4685-4696.

[28] V. Khatri, A. Kumar, B. Singh, S. Malhotra, A.K. Prasad, Synthesis of beta-C-Glycopyranosyl Aldehydes and 2,6-Anhydro-heptitols, J Org Chem, 80 (2015) 11169-11174.

[29] L. Sun, N. Tran, F. Tang, H. App, P. Hirth, G. McMahon, C. Tang, Synthesis and Biological Evaluations of 3-Substituted Indolin-2-ones: A Novel Class of Tyrosine Kinase Inhibitors That Exhibit Selectivity toward Particular Receptor Tyrosine Kinases, J Med Chem, 41 (1998) 2588-2603.

[30] R.K. Thakur, A. Mishra, K.K.G. Ramakrishna, R. Mahar, S.K. Shukla, A.K. Srivastava, R.P. Tripathi, Synthesis of novel pyrimidine nucleoside analogues owning multiple bases/sugars and their glycosidase inhibitory activity, Tetrahedron, 70 (2014) 8462-8473.

[31] (a) L.W.L. Chen, Derivatives of isoindigo, indigo and indirubin and use in treating cancer, WO Patent 03051900, (2003); (b) K. Upadhyaya, Hamidullah, K. Singh, N.. Srivastava, R. Ashraf, A. Sharma, R. Mahar, S.K. Shukla, J. Sarkar, R. Ramachandran, J. Lal, R. Konwar, R. P. Tripathi, Identification of gallic acid-based glycoconjugates as a novel tubulin polymerization inhibitors Org. Biomol. Chem. 14 (2016) 1338-1358.

[32] W. Trager, J.B. Jensen, Human malaria parasites in continuous culture, Science, 193 (1976) 673-675.

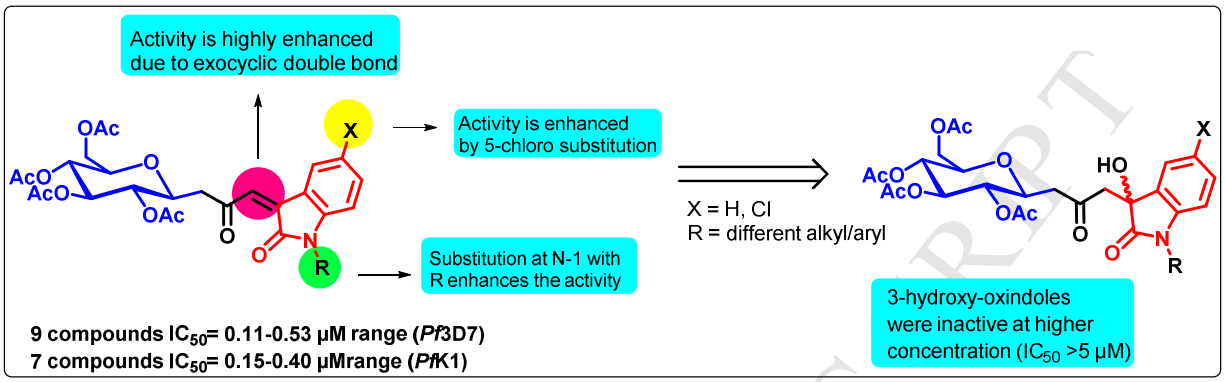
[33] J.D. Johnson, R.A. Dennull, L. Gerena, M. Lopez-Sanchez, N.E. Roncal, N.C. Waters, Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening, Antimicrob Agents Chemother, 51 (2007) 1926-1933.

[34] J. O'Brien, I. Wilson, T. Orton, F. Pognan, Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity, Eur J Biochem, 267 (2000) 5421-5426.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Graphical Abstract



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Research highlights

- A series of glucoconjugated oxindoles were accessed from glucosyl propanone and isatin.
- These compounds were accessed by simple cross-aldol reaction followed by dehydration.
- **10** compounds showed potent activity with IC_{50} in **0.11-0.77** μM against *Pf*3D7 strain.
- **10** compounds showed promising activity with IC_{50} in **0.15-0.94** μM against *Pf*K1 strain.
- Activity is enhanced in compounds having exocyclic double bond and 5-chloro substituent.

ACCEPTED MANUSCRIPT