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3-aryl-indolinones derivatives as antiplasmodial agents: synthesis, biological activity and computational analysis

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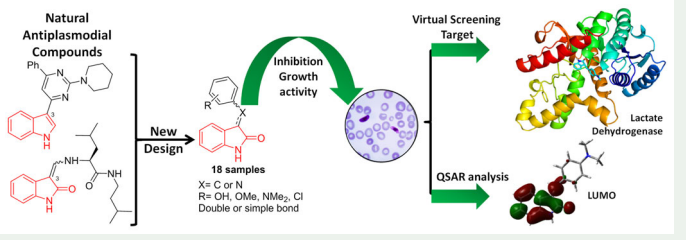
ABSTRACT

Malaria is an infectious illness, affecting vulnerable populations in Third World countries. Inspired by natural products, indole alkaloids have been used as a nucleus to design new antimalarial drugs. So, eighteen oxindole derivatives, *aza* analogues were obtained with moderate to excellent yields. Also, the saturated derivatives of oxindole and *aza* derivatives *via* H_2/Pd/C reduction were obtained in good yields, leading to racemic mixtures of each compound. Next, the inhibitory activity against *P. falciparum* of 18 compounds were tested, founding six compounds with $IC_{50} < 20\,\mu M$. The most active of these compounds was **8c**; however, their unsaturated derivative **7c** was inactive. Then, a structure-activity relationship analysis was done, founding that focused LUMO lobe on the specific molecular zone is related to inhibitory activity against *P. falciparum*. Finally, we found a potential inhibition of lactate dehydrogenase by oxindole derivatives, using molecular docking virtual screening.



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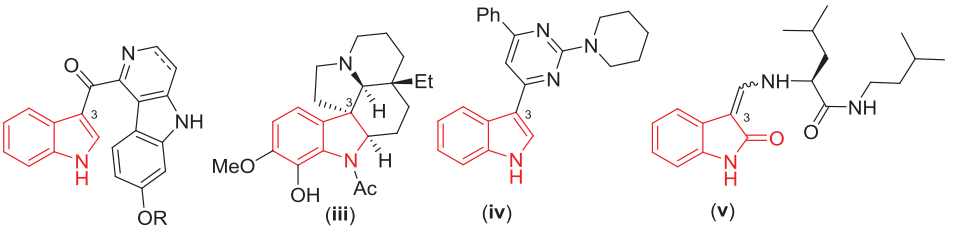


(**i**) R= H, single bond
(**ii**) R= OGlc, double bond

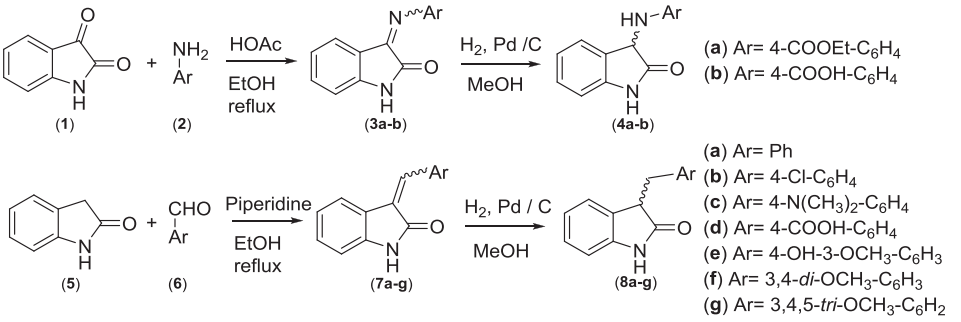
Figure 1. Natural and synthetic indole alkaloids with antiplasmodial activity.

1. Introduction

Malaria is caused by *Plasmodium* parasites which are transmitted to people through the bites of infected female *Anopheles* mosquitoes, and the children are the group most affected by this disease. World Health Organization highlights the critical importance of keeping efforts to prevent, detect, and treat malaria (WHO 2020), owing to this infectious disease affects vulnerable populations in the Third World countries.

In the Third World countries, nature has been used to treat different diseases, for example, *Cryptolepis sanguinolenta*, a medicinal plant frequently used in Central and West Africa, this plant has indole alkaloids with antiplasmodial activities (Forkuo et al. 2017). In this respect, from *Aristolochia cordigera* were isolated indole alkaloids such as 3,4-dihydro-hyrtiosulawesine (i) and 6-*O*-(β-glucopyranosyl)hyrtiosulawesine (ii) (Figure 1), which exhibited antiplasmodial activity in the low micromolar range against *P. falciparum* strain (3D7 strain), and low cytotoxicity on hepatic cells (Pereira et al. 2017). Moreover, in South America *Aspidosperma olivaceum* is used to treat fever and malaria in some regions of Brazil. From *A. olivaceum* has been isolated aspidoscarpine (iii) a promising compound against *P. falciparum* and low cytotoxicity in hepatic cells (Chierrito et al. 2014). Inspired by the antimalarial alkaloids, have been synthesized several indole alkaloids with *in vitro* activity against *P. falciparum*, among them an indole-pyrimidine hybrid (iv) (Agarwal et al. 2005), and 3-methylene-indolinone (v) (Kumar et al. 2011). Then, indole alkaloids represent an interesting class of natural products with antimalarial activity and high similarity to our quinoline derivatives (Muscia, Bollini, Bruno, et al. 2006; Muscia, Bollini, Carnevale, et al. 2006).

Based on all the active compounds **i-v**, the common shared structural feature is the indole moiety linked to an aromatic ring or an aliphatic group by a single or double bond in position 3 of indole or oxindole nucleus (Figure 1). So, according to this information, we report the synthesis and antiplasmodial activity of thirty-one oxindole (indolin-2-one) derivatives and *aza* analogues. Furthermore, we conducted computational studies, based on a Quantitative Structure-Activity Relationship (QSAR) and docking methods, to investigate both the structure-activity relationships and the identification of the potential molecular target underlying this series of *P. falciparum* inhibitors.



Scheme 1. Synthesis route to obtain of oxindole derivatives

2. Results and discussion

2.1. Chemistry

The condensation reactions of isatin (**1**) with arylamines (**2**) yielded 3-arylimino-indolinones (**3a-b**) under acid catalysis in moderate yields (30–63%), while the oxindole (**5**) with arylaldehydes (**6**) yielded 3-arylidene-indolinones (**7a-g**), through Knoevenagel synthesis using an alkaline catalyst with yields between 45–92% (see Scheme 1).

The ^{1}H-NMR spectra of the compounds **3** and **7** exhibit the typical signals for aromatic hydrogens and the NH signal of the oxindole core (between $\delta = 9.45$–11.03 and $\delta = 8.68$–10.66 ppm, respectively, details in Supplementary Material). In addition, products **7** show the CH signal shift which varies depending on the nature of the aryl moiety next to it. The *E* and *Z* isomer ratio was studied by the chemical shifts of the aromatic '*ortho*' protons on the benzylidene ring. Particularly, the *Z* isomer showed these hydrogens to downfield according to a previous report (Zhang and Go 2009).

Moreover, 3-oxindole saturated analogues **4** and **8** were synthesized by palladium-catalyzed hydrogenation from compounds **3** and **7**, respectively (see Scheme 1). The reduction of the exocyclic double bond afforded the desired products **4** and **8** in racemic forms, with moderate to excellent yields. The ^{1}H-NMR spectra of the compounds **4a-b** showed two signals at $\delta \sim 5.97$ and 4.00 ppm of the NH and CH fragments. While the compounds **8a-g** were confirmed by signals to high-field $\delta \sim 3.50$ and 3.00 ppm corresponding to the CH_2 group bonded to the oxindole nucleus, as well as the two signals $\delta \sim 47.0$ and $\delta \sim 35.0$ ppm in the ^{13}C-NMR spectra.

2.2. Antiplasmodial activity

The inhibitory activity of the eighteen oxindole derivatives was tested against the *P. falciparum* (3D7 strain) using the SYBR Green assay. Several oxindole derivatives exhibited poor inhibitory activity between 10–40 μM (see Table S1), thus the IC_{50} values greater than 20 μM were considered inactive. Interestingly, only six compounds (**3a**, **7b**, **7e**, **7f**, **7g**, and **8c**) showed inhibitory activity between $IC_{50} = 19$ to 5.8 μM (see Table 1).

The compound **3a** (4-CO_2Et) showed decreased inhibitory activity amongst the active compounds. Three 3-aryliden-indolin-2-ones derivatives possess one or more methoxy groups (**7e-g**), and compound **7b** (4-Cl) has an electron-attracting group, and they showed modest antiplasmodial activity ($IC_{50} = 13$–18 μM). Interestingly, the

Table 1. Half inhibitory concentration (IC_{50}) against *P. falciparum* (3D7 strain).

Comp.	IC_{50}^{3D7} (μM) (Avg ± SD)	IC_{50}^{HepG2} (μM) (Avg ± SD)	Comp.	IC_{50}^{3D7} (μM) (Avg ± SD)	IC_{50}^{HepG2} (μM) (Avg ± SD)
3a	19 ± 1	ND	**4a**	>20	ND
3b	>20	ND	**4b**	>20	ND
7a	>20	ND	**8a**	>20	ND
7b	13 ± 2	ND	**8b**	>20	ND
7c	>20	ND	**8c**	5.8 ± 0.3	>100
7d	>20	ND	**8d**	>20	ND
7e	18 ± 1	ND	**8e**	>20	ND
7f	15 ± 2	ND	**8f**	>20	ND
7g	14 ± 1	ND	**8g**	>20	ND
C+	0.006 ± 0.002	305 ± 24			

ND = not determinate, C+ = Artesunate

compound **8c** was the most potent among the tested compounds ($IC_{50} = 5.8\,\mu M$), however, its unsaturated analogue **7c** (4-NMe_2) was inactive ($IC_{50} > 20\,\mu M$). This finding suggests that the bond between the oxindole core and the aromatic substituent is important to the antiplasmodial activity. Additionally, the cytotoxicity of compound **8c** was determined against Human Hepatoma cells (HepG2), because this cell line acts as a surrogate for effects of toxicity on human liver (Hughes et al. 2011). The inhibitor exhibited a very low cytotoxic effect on the cells ($IC_{50} > 100\,\mu M$) and a reasonable selectivity index (SI > 18).

2.3. Quantitative structure-activity relationship (QSAR)

The multilinear equations between the activity and various descriptors were developed according to our previous reports (Mellado et al. 2018, 2020). The QSAR model showed a correlation of the LUMO and ω descriptors show with the biological activity ($r^2 = 0.96$, see Tables S2 and S3).

$$pIC_{50} = 5.22(0.07) + 0.41(0.12)\ \mathbf{LUMO} + 0.11(0.07)\boldsymbol{\omega} \quad (1)$$

The descriptor LUMO (Lowest Unoccupied Molecular Orbital) is the equivalent to Lewis acid (Lopez et al. 2013). This descriptor has been used to explain the inhibitory activity of several compounds against *P. falciparum*. For instance, the antiplasmodial activity of tryptanthrin derivatives was related to the focused LUMO lobe of the molecule (Olson et al. 2018). On the other hand, the electrophilic global index (ω), is a concept of measure for energy stabilization, such as when systems receive additional electronic charges from underground (Pearson 1993). However, there is no information about ω related to the inhibitory activity of molecules against *P. falciparum*.

The Equation (1) showed the LUMO descriptor weight is ~3.7-fold greater than ω descriptor. Thus, the modification of LUMO descriptor is gravitating for modulating the inhibitory activity of oxindole derivatives against *P. falciparum*. For example, the LUMO plot of compounds **7c** ($IC_{50} > 20\,\mu M$) and **8c** ($IC_{50} = 5.8\,\mu M$) shows a LUMO concentrated on the oxindole nucleus in the compound **8c** (Figure S1B); however, the LUMO in the compound **7c** is expanded on the entire structure (Figure S1A). These results are in the way of the focused LUMO on the specific molecular zone (Olson et al. 2018).

2.4. Molecular docking study

Since the molecular mechanism of antiplasmodial action of oxindole derivatives is still unknown, the molecular docking technique was used to predict their possible target (Łączkowski et al. 2017). Then, six enzymes crucial for the parasite development were selected: falcipain-2, falcipain-3, dihydroorotate dehydrogenase, lactate dehydrogenase, macrophage migration inhibitory factor, and SUB1 protease (Njogu et al. 2016).

The calculated affinity energies of all active compounds are summarized in Table S4. The results on the SUB1 Protease showed a positive value of docking score, thus this molecular target was ruled out. The results on falcipain-2 showed a narrow energy affinity between selected compounds and native ligand ($\Delta E = 0.5$ kcal/mol), thus the displacement of native ligand by active compounds is unlikely. In the case of the proteins dihydroorotate dehydrogenase, falcipain-3, and lactate dehydrogenase, the native ligand has decreased affinity energy than all compounds. Finally, based on the linear relationship between the affinity energy and the experimental IC_{50} values, lactate dehydrogenase stood out as a promising molecular target of oxindole derivatives ($r = 0.777$), while the dihydroorotate dehydrogenase ($r = 0.158$) and falcipain-3 ($r = 0.100$) were ruled out due to low significance.

The analysis of the modelled binding mode of **3a**, **7b**, **7e**, **7f**, **7g**, and **8c** to the lactate dehydrogenase indicated that the oxindole nucleus of all compounds binds in the same site of the pyruvate (native ligand). The oxindole core is bound between the ASN-126 and ALA-79 residues at the catalytic binding site (see Figure S2). In this binding mode, the oxindole ring of compound **8c** forms a hydrogen-bond with ALA-79 backbone (blue dashes, Figure S3), while the electron-rich 4-NMe_2-Ph substituent undergoes π-stacking with the amide side-chain of ASN-126 (blue dashes, Figure S3). These findings are in agreement with the results obtained from the QSAR study, which indicated that the increment of the LUMO electronic density on the oxygen atom of the oxindole nucleus is favourable for the inhibitory activity of this series, due to the hydrogen bonding acceptor role. In addition, a 100 ns molecular dynamic simulation was carried out, finding stable hydrogen bond interactions during the simulation time, between the compound **8c** and the residues ALA-79 and ASN-126.

3. Experimental

Experimental details relating to this paper are in the supplementary material.

4. Conclusion

Some oxindole derivatives synthesized showed antimalarial activity. The best inhibitor, **8c**, showed antiplasmodial activity in the low micromolar range ($IC_{50} = 5.8\ \mu M$) and low cytotoxic effect on HepG2 cells ($IC_{50} > 100\ \mu M$). The computational studies suggested that the focused LUMO properties of the oxindole ring are important for antiparasitic activity and the *P. falciparum* lactate dehydrogenase might be a molecular target underlying oxindole derivatives inhibitory activity. Our findings suggest that **8c** is an attractive candidate for hit-to-lead development.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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