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Original article

Synthetic indole and melatonin derivatives exhibit antimalarial activity on the cell cycle of the human malaria parasite *Plasmodium falciparum*

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ABSTRACT

Discovering the mechanisms by which cell signaling controls the cell cycle of the human malaria parasite *Plasmodium falciparum* is fundamental to designing more effective antimalarials. To better understand the impacts of melatonin structure and function on the cell cycle of *P. falciparum*, we have synthesized two families of structurally-related melatonin compounds (**7**–**11** and **12**–**16**). All synthesized melatonin analogs were assayed in *P. falciparum* culture and their antimalarial activities were measured by flow cytometry. We have found that the chemical modification of the carboxamide group attached at C-3 position of the indole ring of melatonin (**6**) was crucial for the action of the indole-related compounds on the *P. falciparum* cell cycle. Among the melatonin derivatives, only the compounds **12, 13** and **14** were capable of inhibiting the *P. falciparum* growth in low micromolar IC_{50}. These results open good perspectives for the development of new drugs with novel mechanisms of action.

1. Introduction

Annually, more than 300 million people are infected by the *Plasmodium* protozoan, the etiological agent of malaria and approximately one million people are expected to die each year according to the World Health Organization (WHO). Whereas chemotherapy has previously been quite successful in the treatment of malaria, the *Plasmodium* parasite currently exhibits an increased resistance to classical antimalarials, hastening the search for new compounds [1].

Examples of drugs used clinically in the treatment of malaria include doxycycline (**1**), a synthetically derived tetracycline antibiotics group, and quinone-related compounds (**2**–**5**) (Fig. 1). Specific treatment options depend on the species of malaria parasite causing the infection, the part of the world in which the infection was acquired, pregnancy status, and the severity of infection.

Our knowledge of the basic biology that underlies the *Plasmodium* development through the stages ring, trophozoite and schizont within the red blood cells (RBCs) is still limited. Therefore, it is important to identify compounds that modulate the RBC stages of the malaria life cycle. Melatonin (*N*-acetyl-5-methoxytryptamine, **6**) is a tryptophan-derived hormone that participates in several physiological activities that are influenced by the light/dark circadian cycle [2]. It is secreted by the pineal gland of all mammals and is also present in plants [2–8]. The effects on *Plasmodium* cell cycle were firstly described by Hotta and collaborators in a work that demonstrated that the synchronization of parasite development is

Abbreviations: FCM, flow cytometry; RBC, Red blood cell.

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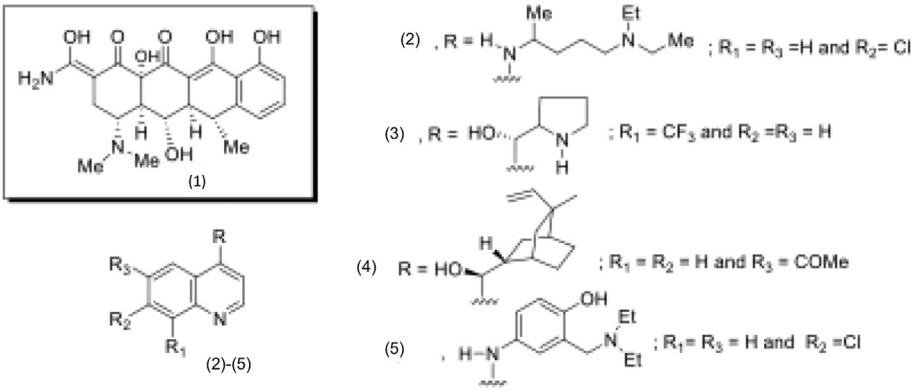

Fig. 1. Some examples of drug used clinically in the treatment of malaria.

lost in pinealectomized mice, but restored when melatonin were administrated [5]. The effects of melatonin in *Plasmodium falciparum* were extensively evaluated in several works [9–11] and include a complex signaling pathway. The molecular mechanism for melatonin action in *P. falciparum* and *Plasmodium chabaudi* is quite complex and includes an increase in cytosolic calcium and cAMP and activation of proteases and PKA [6,8,12,13]. Second messengers play a fundamental role in different biological systems that activate a myriad of cellular components including protozoan [14–19]. Several laboratories have investigated the role of calcium and cAMP in malaria parasite cycle as well as potential molecular targets including kinases and proteases [12,20–24]. For *P. falciparum* melatonin triggers IP_3 generation [25], protease activation [12] and activate a subset of genes for the ubiquitin proteasome-system (UPS) [26]. Recently, we found that the *P. falciparum* transcription factor, Pf NF-YB is modulated by melatonin [27].

In addition to melatonin, its precursor *N*-acetylserotonin, serotonin, tryptamine, also affect parasite cell cycle [2]. In other way, another indole compound, IAA (indole 3-acetic acid) that plays an important role in physiological process of plants were unable to modulate the cell cycle of *P. falciparum* or regulate the UPS genes as observed for melatonin, indicating some level of specificity of tryptophan compounds on *Plasmodium* cell cycle control [28].

Melatonin (**6**) has a central role in the control of parasite replication and establishment of parasitemia, so targeting and blocking this hormone pathway can contribute to the discovery of new antimalarial drugs. Bagnaresi and collaborators have shown that when parasite's synchronicity is disrupted by the addiction of the melatonin receptor blocker Luzindole, the antimalarial activity of chloroquine is enhanced at suboptimal doses [29]. Increased resistance to classical antimalarials urges the discovery of new compounds that can be used in the clinical arsenal against malaria. Our interest in the development of new melatonin antagonists prompted us to synthesize and test the ability of new melatonin-related compounds **7**–**11** and **12**–**16** to modulate the human malaria parasite cell cycle and block parasite's development acting as antimalarials. These *N*-heterocyclic derivatives were designed by molecular modifications in the structure of the lead compound melatonin, as shown in Scheme 1. The hydrogen substitution on the methoxy group and the introduction of the different substituents attached to the carboxamide at the C-3 position of the indole ring of melatonin resulted in family **7**–**11**. In the second family, we have investigated the substitution pattern of the amide function (for compounds **12**–**15**) and presence of a primary amine group (for compound **16**) attachment to the indole system of melatonin on the cell cycle of *P. falciparum* (Scheme 1).

2. Results and discussion

2.1. Chemistry

The compounds **7**–**11** and **12**–**16** were prepared according to the synthetic pathways described in Scheme 2. Tryptamine (**17**) was reacted with acetic anhydride to give the *N*-acetyl compound **7**.

The two series of indole derivatives **8**–**10** and **12**–**14** were synthesized by *N*-acylation reaction of tryptamine (**17**) or 5-methoxytryptamine (**16**) with the appropriate acyl chloride. Finally, the compounds **17** and **16** were easily converted into the corresponding carbamate derivatives **11** and **15**, on treatment with methyl chloroformate an aqueous solution of NaOH at 0 °C [30–33].

2.2. Effect of two families of indole derivatives 7–11 and 12–16 on cell cycle of P. falciparum

Melatonin and its derivatives control *P. falciparum* and *P. chabaudi* cell cycle [2,4,7]. Given that the potential role of melatonin derivatives and despite the chemical compounds here tested were already knew, we followed their ability to block the human malaria parasite *P. falciparum* cell cycle. To interfere with signaling pathways and parasite replication inside RBCs we next investigated the synthetic melatonin derivatives and search for their potential pharmacological activity. The two series of synthetic indole compounds **7**–**11** and **12**–**16** were incubated with *P. falciparum*-infected using different combination as well as alone cell for their *in vitro* antimalarial activity.

After compounds incubation in the culture, we assess their action by following parasitemia using flow cytometry and the DNA-binding fluorescent dye YOYO-1 [34]. When compared to the control (solvent treated), the following compounds significantly increased parasitemia: **(6)** 21.9 ± 0.8% ($p < 0.001$), **(7)** 12.9 ± 4.6 ($p < 0.001$); **(8)** 9.2 ± 3.1 ($p < 0.05$) and **(11)** 11.7 ± 5 ($p < 0.01$), **(14)** 10.8 ± 3% ($P < 0.01$), **(15)** 10 ± 2% ($p < 0.05$) and **(16)** 19.7 ± 5.2 ($p < 0.001$). The other compounds showed no significant increase in parasitemia compared to control: **(9)** 6 ± 4.8, **(10)** 5.3 ± 2.2%, **(12)** 8 ± 2.6%, **(13)** 7.8 ± 1%, as shown in Fig. 2. Among the compounds tested, only **16** showed an increase of parasitemia similar to melatonin.

The change of a methoxy substituent attachment to the indole ring of melatonin (*e.g.* **7**–**11**) caused a decrease in the modulation of cell cycle of *P. falciparum*, showing an important function of this radical in the indole cellular responses. In the same way, the chain elongation of the amino functional group (**8**–**10**) decreases the reactivity of this compound in the cell cycle of *P. falciparum*. The presence of a carbamate moiety (**11**) at the C-3 position of the