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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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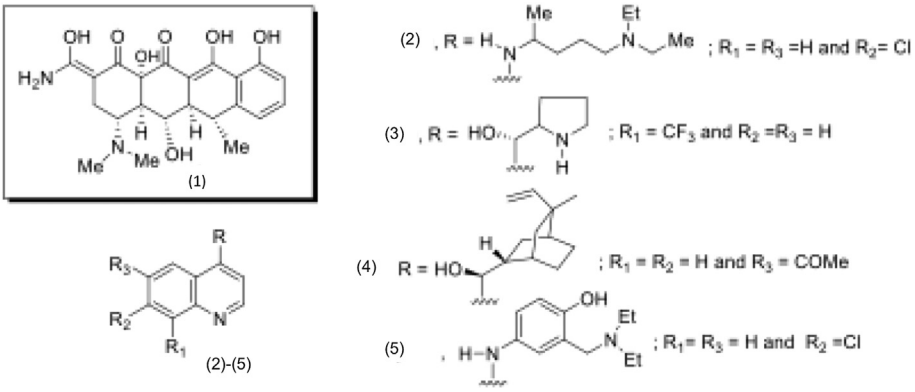


Fig. 1. Some examples of drug used clinically in the treatment of malaria.

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**.

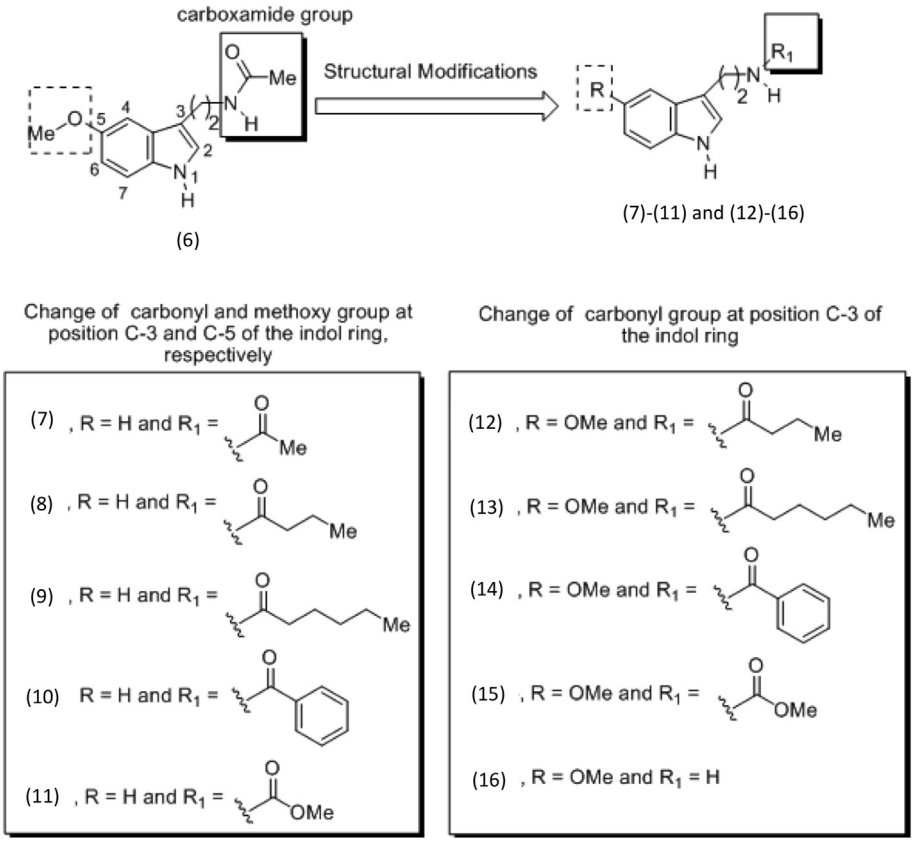
The two series of indole derivatives **8**–**10** and **12**–**14** were synthesized by *N*-acylation reaction of tryptamine (**17**) or 5-methoxytryptamine (**16**) with the appropriate acyl chloride. Finally, the compounds **17** and **16** were easily converted into the corresponding carbamate derivatives **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

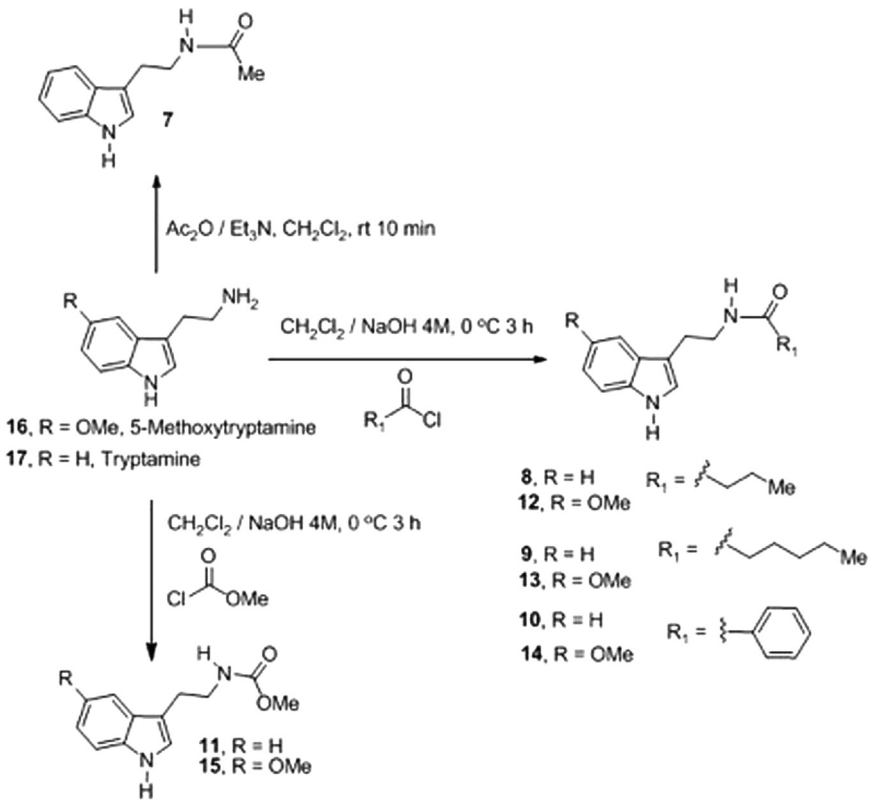
Melatonin and its derivatives control *P. falciparum* and *P. chabaudi* cell cycle [2,4,7]. Given that the potential role of melatonin derivatives and despite the chemical compounds here tested were already knew, we followed their ability to block the human malaria parasite *P. falciparum* cell cycle. 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



Scheme 1. Structural analogy between melatonin and indole derivatives **7**–**11** and **12**–**16**.



Scheme 2. Synthesis of two families of indole derivatives **7**–**11** and **12**–**16**.

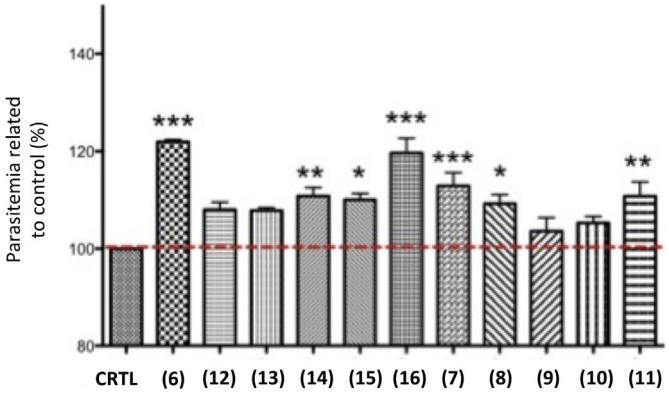


Fig. 2. Effect of indole derivatives **7**–**11** and **12**–**16** (concentration 500 nM) on cell cycle of *P. falciparum*. After incubation for 48 h the parasitemia was measured by flow cytometry. The data represent the percentage of parasitemia compared to control (solvent treatment). (*) One-way ANOVA (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$).

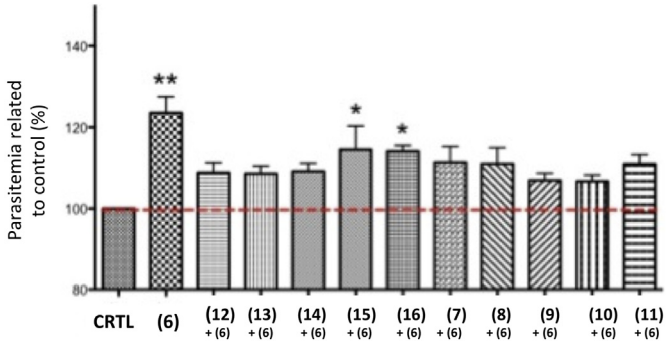


Fig. 3. Effect of indole derivatives on melatonin action in the *P. falciparum* cell cycle. Compounds **7**–**11** and **12**–**16** (concentration 500 nM) were incubated along with **6** (100 nM) for 48 h. The parasitemia was measured by flow cytometry. The data represent the percentage of parasitemia compared to control (solvent). (*) One-way ANOVA (**$p < 0.001$, *$p < 0.05$).

indole ring resulted lower effect on parasite cycle when compared to **7** and melatonin (**6**).

The compounds structurally related to melatonin **12**–**14** presented a significantly lower effect on parasite cycle when compared to melatonin, suggesting a decrease in potency of these ligands due to bulky side chains at the C-3 position of the indole ring.

Among structurally related compounds studied, **16** induced an increase in parasitemia similar to the melatonin effect on *P. falciparum* cycle (19.7 ± 5.2%). This compound differs from melatonin structure by absence of the acetyl group at the C-3 position of the indole system. The active biological profile of this compound is probably due to number of hydrogen acceptors of the amine group.

These results are consistent with those of Sugden et al. (1997), which evaluated the affinity of different compounds with a structure based on melatonin with modifications at the radical's methoxy and N-acyl in *Xenopus leavis* melanophores, Cos-7 and NIH 3T3. The removal of the group 5-methoxy or *N*-acyl led to a reduced in the receptor binding affinity in all compounds. The addition of up to two carbons in the N-acyl side chain retained the binding affinity of the compounds to receptors MT1/MT2/MT3, but longer chains with 4–5 carbons significantly reduced the binding affinity. Such modifications performed by Sugden et al. (1997) are similar to those modifications made at **9** and **13** which similarly exhibited a reduced response to *P. falciparum* compared to the effect of melatonin on parasite cycle (Fig. 2).

We next have incubated the compounds **7**–**11** and **12**–**16** along with the hormone melatonin **(6)** in an asynchronous culture of *P. falciparum* 3D7 to investigate their potential ability to interfere with melatonin effect on *P. falciparum* cell cycle acting as an inhibitor of the hormone effect. After 48 h of incubation the parasitemia was checked by flow cytometry. Compared with the control (solvent) the following compounds significantly increased the parasitemia: **(6)** 23.5 ± 6.8% ($p < 0.0001$), **(15)** 14.5 ± 5.8% ($p < 0$, 05) and **(16)** 14.1 ± 2.4% ($p < 0.05$). The other compounds showed no significant increase in parasitemia compared to control: **(12)** 8.8 ± 4.2%, **(13)** 8.6 ± 3.2%, **(14)** 9.1 ± 3.4%, **(7)** 11.3 ± 6, 8%, **(8)** 11 ± 6.9%, **(9)** 6.9 ± 3%, **(10)** 6.7 ± 2.6% and **(11)** 10.9 ± 4.1% as shown in Fig. 3. These compounds **(7–11)** and **(12–14)** were able to inhibit the effect of melatonin **(6)** in the parasite by acting as blockers of the modulation induced by hormone.

From the analysis of the structures we can conclude that an increase in the number of carbon atoms in structures of **9, 10, 12** and **13** has a deleterious effect on cell cycle modulation, even keeping the methoxy group at position 5′. Furthermore, as also verified by other works [35–40], we note that the methoxy group at position 5′ plays an important role for the activity of melatonin on its receptor, since the compounds that do not present this group (**7**–**11**) generally presents a minor effect on parasitemia (Fig. 2). Although the importance of the methoxy group 5′ on the receptor activity, this radical is not essential for the compound binding, as compounds lacking the methoxy 5′group were capable of inhibit the melatonin effect on the cell cycle progression of parasite (Fig. 3).

2.3. Antimalarial activity of compounds *12–14*

Among the indole analogues of two series **7**–**11** and **12**–**16**, compounds **12, 13** and **14** showed potential antimalarial activity. We performed a dose–response curve to obtain the IC_{50} value of these compounds using ring-stage synchronized parasites. The compounds showed the following IC_{50}: **(12)** (IC_{50} = 19.17 ± 0.08 μM), **(13)** (IC_{50} = 19.10 ± 0.09 μM) and **(14)** (IC_{50} = 2.93 ± 0.064 μM) (Fig. 4).

There are currently no available drugs of the class of indole derivatives for the treatment of malaria. NITD609 is proposed as a new antimalarial compounds belonging to the spiroindolone class and showed IC_{50} = 10 nM in the *P. falciparum* cycle and was able to interfere with the transmission of the parasite to the vector *Anopheles* [41]. This compound is currently in phase I of clinical trials as an antimalarial. Besides this compound, other indolic compounds presented antimalarial activity [42,43], at low micromolar concentration, ranging between 39 μM and 0.65 μM, similar results obtained in this work, IC_{50} range of 19 to 2.9 μM. These result shown the importance of indole nucleus for the development of a new class of antimalarial.

The development of a new drugs class that act through different mechanisms of action it is of great importance to circumvent the increasing resistance of *P. falciparum* to existing antimalarials. Accordingly, the description of three novel indole with very significant antimalarial activity opening good perspectives for development of new drugs with novel mechanisms of action.

3. Experimental protocols

The chemical reagents and all solvents used in this study were purchased from Merck AG (Darmstadt, Germany) and VETEC LTDA (Rio de Janeiro, Brazil). Melatonin was purchased from Sigma–Aldrich Brazil LTDA (São Paulo, Brazil).

Melting points were determined with a Fisher–Johns instrument and are uncorrected. Infrared (IR) spectra were recorded on an ABB FTLA2000-100 spectrophotometer in KBr pellets (Quebec,

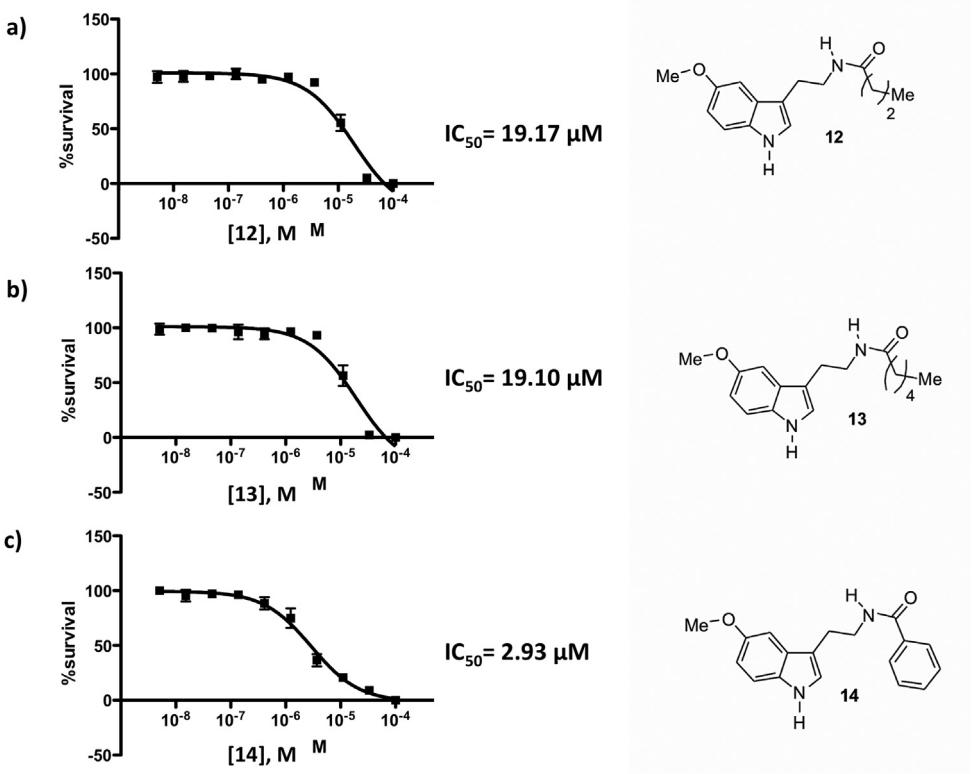


Fig. 4. Dose–response curve with different concentrations used to calculate the IC_{50} of the compounds **12** (a), **13** (b) and **14** (c) after 48 h incubation with *P. falciparum*.

Canada). NMR spectra, unless otherwise stated, were obtained in deuterated Me_2SO-d_6 using a Varian Unity Plus 300 MHz spectrometer. Chemical shifts (δ) are expressed in ppm and the coupling constant (J) in Hertz. Reactions were routinely monitored by thin layer chromatography (TLC) on silica gel pre-coated F_{254} Merck plates. Column chromatography was performed on silica gel flash from Across. The developed chromatograms were viewed under ultraviolet light at 254 nm.

The compound **16** has been synthesized using Venkatachalam and co-workers protocol [31].

3.1. Chemistry

3.1.1. General procedure for the preparation of the melatonin derivatives *8*–*10* and *12*–*14*

An aqueous solution of 4 M sodium hydroxide (1.2 mmol) was added slowly to a stirred solution of tryptamine (**17**) (1.2 mmol, for compounds **8**–**10**) or 5-methoxytryptamine (**16**) (1.2 mmol, for compounds **12**–**14**) in dichloromethane (3 mL) at 0 °C. After 5 min of stirring at 0 °C, the appropriate chloride derivative (1.2 mmol) was added dropwise. The mixture was stirred for 5 min at 0 °C and then for 3 h at room temperature. H_2O (20 mL) was added. The two layers were separated, and the aqueous phase was extracted with dichloromethane (3 × 20 mL). The organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel. Elution was made successively with ethyl acetate/hexane (1:1) to give the desired compound.

*3.1.1.1. N-(2-(5-methoxy-1H-indol-3-yl)ethyl)butanamide (**12**).* Obtained in 17% yield as a pale yellow solid; m.p. 104–105 °C; IR (KBr) ν_{max} (cm^{-1}) 3382 (N–H); 1677 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3H, CH_3, J = 4.5 Hz); 1.63 (q, 2H, CH_2, J = 4.5 Hz); 2.09 (t, 2H, CH_2, J = 4.5 Hz); 2.93 (t, 2H, CH_2, J = 4.5 Hz); 3.59 (t, 2H, CH_2, J = 4.5 Hz); 3.85 (s, 3H, OCH_3); 6.85–6.87 (m, 1H, H-3′); 6.97–6.98 (m, 1H, H-5′); 7.03–7.04 (m, 1H, H-2′); 7.24–7.28 (m, 1H, H-2); 8.56 (bs, 1H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.6 (CH_3); 19.1 (CH_2); 25.3 (CH_2); 38.7 (CH_2); 39.6 (CH_2); 55.9 (CH_3); 100.4 (CH); 112.0 (CH); 112.3 (C); 122.9 (CH); 127.6 (C); 131.6 (C); 153.8 (C); 173.1 (C=O) ppm.

*3.1.1.2. N-[2-(5-methoxy-1H-indol-3-yl)ethyl]hexanamide (**13**).* Obtained in 92% yield as a pale yellow solid; m.p. 71–72 °C; IR (KBr) ν_{max} (cm^{-1}) 3319 (N–H); 1679 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 3H, CH_3, J = 4.2 Hz); 1.22–1.29 (m, 4H); 1.58–1.61 (m, 2H, CH_2); 2.58–2.61 (m, 2H, CH_2); 2.97–3.00 (m, 2H, CH_2); 3.86 (s, 3H, OCH_3); 3.93 (t, 2H, CH_2, J = 4.2 Hz); 6.86–6.88 (m, 1H, H-3′); 6.97–6.98 (m, 1H, H-5′); 7.10–7.11 (m, 1H, H-2); 7.24–7.28 (m, 1H, H-2′); 7.90 (bs, 1H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9 (CH_3); 22.4 (CH_2); 24.6 (CH_2); 25.1 (CH_2); 31.3 (CH_2) 37.9 (CH_2); 44.9 (CH_2); 55.9 (CH_3); 100.4 (CH); 111.9 (CH); 112.1 (C); 112.4 (CH); 123.1 (CH); 127.6 (C); 131.4 (C); 154.2 (C); 176.5 (C=O) ppm.

*3.1.1.3. N-[2-(5-methoxy-1H-indol-3-yl)ethyl]benzamide (**14**).* Obtained in 28% yield as a pale yellow solid; m.p. 107–109 °C; IR (KBr) ν_{max} (cm^{-1}) 3299, 3059 (N–H); 1635 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.98 (t, 3H, CH_2, J = 4.2 Hz); 3.69 (s, 3H, OCH_3); 3.71–3.72 (m, 2H, CH_2); 6.64–6,65 (m, 1H, H-3′); 6.78–6.80 (m, 1H, H-5′); 6.88–6.89 (m, 1H, H-2′); 7.00–7.01 (m, 1H, NH); 7.15–7.16 (m, 1H, H-2); 7.26–7.29 (m, 2H, H-3″ and H-5″); 7.36–7.39 (m, 1H, H-4″); 7.64–7.65 (m, 2H, H-2″ and H-6″); 8.75 (bs, 1H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6 (CH_2); 41.2 (CH_2); 55.7 (CH_3); 100.3 (CH); 111.9 (CH); 112.0 (CH); 122.2 (C); 122.9 (CH); 127.5 (C); 131.5 (C); 153.7 (C); 157.1 (C=O) ppm.

*3.1.1.4. N-[2-(1H-indol-3-yl)ethyl]butanamide (**8**).* Obtained in 34% yield as a pale yellow solid; m.p. 84–86 °C; IR (KBr) ν_{max} (cm^{-1}) 3406, 3300 (N–H); 1645 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3H, CH_3, J = 7.3 Hz); 1.62 (q, 2H, CH_2, J = 7.3 Hz); 2.09 (t, 2H, CH_2, J = 7.3 Hz); 2.98 (t, 2H, CH_2, J = 6.3 Hz); 3.61 (t, 2H, CH_2, J = 6.3 Hz); 5.59 (bs, 1H, N–H); 7.03–7.04 (m, 1H, H-3′); 7.11–7.14 (m, 1H, H-4′); 7.19–7.22 (m, 1H, H-2′); 7.36–7.38 (m, 1H, H-2), 7.59–7.61 (m, 1H, H-5′); 8.15 (bs, 1H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.6 (CH_3); 19.1 (CH_2); 25.3 (CH_2); 38.7 (CH_2); 39.6 (CH_2); 111.3 (CH); 112.6 (C); 118.5 (CH); 119.2 (CH); 122.1 (CH); 127.2 (C); 136.4 (C); 173.1 (C=O) ppm.

*3.1.1.5. N-[2-(1H-indol-3-yl)ethyl]hexanamide (**9**).* Obtained in 34% yield as a pale yellow solid; m.p. 90–92 °C; IR (KBr) ν_{max} (cm^{-1}) 3397, 3256 (N–H); 1631 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, CH_3, J = 7.3 Hz); 1.23–1.30 (m, 4H); 1.55–1.61 (m, 2H, CH_2); 2.08–2.11 (m, 2H, CH_2); 2.96–2.98 (m, 2H, CH_2); 3.61 (t, 2H, CH_2, J = 6.3 Hz); 5.59 (bs, 1H, N–H); 7.03–7.04 (m, 1H, H-3′); 7.11–7.14 (m, 1H, H-4′); 7.19–7.22 (m, 1H, H-2′); 7.37–7.38 (m, 1H, H-2), 7.59–7.61 (m, 1H, H-5′); 8.17 (bs, 1H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8 (CH_3); 22.3 (CH_2); 25.2 (CH_2); 31.3 (CH_2) 36.3 (CH_2); 39.6 (CH_2); 111.2 (CH); 112.7 (C); 118.5 (CH); 119.3 (CH); 122.0 (CH); 127.2 (C); 136.4 (C); 173.3 (C=O) ppm.

*3.1.1.6. N-[2-(1H-indol-3-yl)ethyl]benzamide (**10**).* Obtained in 52% yield as a pale yellow solid; m.p. 130–132 °C; IR (KBr) ν_{max} (cm^{-1}) 3406, 3300 (N–H); 1645 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.11 (t, 3H, CH_2, J = 6.3 Hz); 3.81 (q, 2H, CH_2, J = 6.3 Hz); 6.33 (bs, 1H, N–H); 7.04–7.07 (m, 1H, H-3′); 7.11–7.16 (m, 1H, H-4′); 7.19–7.24 (m, 1H, H-2′); 7.35–7.40 (m, 3H, H-2, H-5′ and H-3″); 7.44–7.49 (m, 1H, H-5″); 7.64–7.68 (m, 3H, H-2″, H-4″ and H-6″); 8.31 (bs, 1H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.2 (CH_2); 40.2 (CH_2); 111.3 (CH); 112.9 (C); 118.7 (CH); 119.5 (CH); 122.1 (CH); 122.2 (CH); 126.8 (CH); 127.3 (C); 128.5 (CH); 131.3 (CH); 134.6 (C); 136.4(C) ppm.

*3.1.1.7. N-(2-(1H-indol-3-yl)ethyl)acetamide (**7**).* A solution of acetic anhydride (0.3 mL, 0.318 mmol) and triethylamine (0.52 mL, 3.74 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a stirred suspension of tryptamine (**17**) (0.3 g, 1.87 mmol) in dry CH_2Cl_2 (4.5 mL). After 10 min of stirring at room temperature, the reaction mixture was treated with a saturated aqueous $NaHCO_3$ solution, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The organic phase was washed with brine, dried with $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography using $CHCl_3$:CH_3OH (95:5) afforded compound **7** (370 mg, 98%) as a pale yellow solid: 78–79 °C; IR (KBr) ν_{max} (cm^{-1}) 3397, 3257 (N–H); 1631 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.92 (s, 3H, CH_3); 2.97 (t, 2H, CH_2, J = 7.2 Hz); 3.60 (t, 2H, CH_2, J = 7.2 Hz); 5.73 (bs, 1H, N–H); 7.03–7.04 (m, 1H, H-3′); 7.10–7.15 (m, 1H, H-4′); 7.18–7.23 (m, 1H, H-2′); 7.36–7.39 (m, 1H, H-2), 7.58–7.61 (m, 1H, H-5′); 8.22 (bs, 1H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.1 (CH_3); 25.1 (CH_2); 39.9 (CH_2); 111.3 (CH); 112.7 (C); 118.5 (CH); 119.3 (CH); 122.1 (CH); 127.3 (C); 136.4 (C); 170.3 (C=O) ppm.

*3.1.2. General procedure for the preparation of the melatonin derivatives **11** and **15***

Methyl chloroformate (0.0597 mL, 0.624 mmol) and an aqueous solution of NaOH (0.156 mL, 4 M, 0.624 mmol) were added to a solution of tryptamine (**17**) (0.100 g, 0.624 mmol) or 5-methoxytryptamine (**16**) (0.118 g, 0.624 mmol) in $CHCl_3$ (1.56 mL) at 0 °C. The mixture was then stirred for 3 h at room temperature and washed with water (1.5 mL). The aqueous phase was extracted with dichloromethane (2 × 1.5 mL), and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give orange oil. The crude product was purified by flash column chromatography on silica gel. Elution was made successively with ethyl acetate/hexane (1:1) to give the desired compound.

*3.1.2.1. Methyl [2-(5-methoxy-1H-indol-3-yl)ethyl]carbamate (**15**).* Obtained in 57% yield as a pale yellow solid; m.p. 76–77 °C; IR (KBr) ν_{max} (cm^{-1}) 3322 (N–H); 1670 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.81 (t, 2H, CH_2, J = 6.6 Hz); 3.38–3.39 (m, 2H, CH_2); 3.55 (s, 3H, CH_3); 3.74 (s, 3H, OCH_3); 6.73–6.77 (m, 1H, H-3′); 6.82–6.83 (m, 1H, H-5′); 6.92–6.93 (m, 1H, H-2′); 7.10–7.12 (m, 1H, H-2); 8.29 (bs, 1H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6 (CH_2); 41.2 (CH_2); 51.9 (CH_3); 55.8 (CH_3); 100.3 (CH); 111.9 (CH); 112.0 (CH); 112.2 (C); 122.9 (CH); 127.5 (C); 131.5 (C); 153.7 (C); 157.1 (C=O) ppm.

*3.1.2.2. Methyl [2-(1H-indol-3-yl)ethyl]carbamate (**11**).* Obtained in 68% yield as a pale yellow solid; m.p. 66–67 °C; IR (KBr) ν_{max} (cm^{-1}) 3398, 3283 (N–H); 1688 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.97 (t, 3H, CH_2, J = 6.8 Hz); 3.51–3.52 (m, 2H, CH_2); 3.66 (s, 3H, CH_3); 4.75 (bs, 1H, N–H); 7.03–7.04 (m, 1H, H-3′); 7.11–7.14 (m, 1H, H-4′); 7.19–7.22 (m, 1H, H-2′); 7.36–7.38 (m, 1H, H-2), 7.59–7.61 (m, 1H, H-5′); 8.06 (bs, 1H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.7 (CH_2); 41.2 (CH_2); 51.9 (CH_3); 111.1 (CH); 112.7 (C) 118.6 (CH); 119.3 (CH); 122.0 (CH); 122.0 (CH); 127.2 (C); 136.4 (C); 157.1 (C=O) ppm.

3.2. Biological evaluation

3.2.1. P. falciparum (3D7) in vitro culture

Parasites were cultured according to the method of Trager and Jensen and were synchronized according to the method of Lambros [44,45]. Briefly, parasites were routinely maintained in O^+ human erythrocytes (parasitaemia: 5%, hematocrit: 2%) in RPMI-1640 media supplemented with 0.21% sodium bicarbonate, 50 mg/L hypoxanthine and 10% O^+ human serum in a 92% N_2, 5% CO_2, and 3% O_2 atmosphere.

3.2.2. Flow cytometry analysis

Infected erythrocytes were incubated with 500 nM of the test compounds (**7**–**11** and **12**–**16**) and 100 nM of melatonin **(6)** for 48 h and then fixed in 2% paraformaldehyde in phosphate-buffered saline (PBS) for 24 h; permeabilized and stained with 0.1% Triton X-100 and 5 nM YOYO-1 (Molecular Probes) incubated for 30 min at 37 °C. Parasitemia was determined from dot plots (side scatter versus fluorescence) of 10^5 cells acquired on a FACSCalibur flow cytometer using CELLQUEST software (Becton Dickinson). Initial gating was carried out with unstained, uninfected erythrocytes to account for erythrocyte autofluorescence.

In the experiments of dose–response curves the compounds **12**–**14** were incubated with infected erythrocytes at the ring stage with different concentrations of the compounds: 5 nM, 15 nM, 45 nM, 137 nM, 411 nM, 1.23 μM, 3.70 μM, 11.11 μM, 33.33 μM and 100 μM and incubated for 48 h. The following flow cytometry were performed as described above.

3.2.3. Statistics

Analyses of parasitemia were performed by a one-way analysis of variance test followed by post hoc analysis by the Dunnett's Multiple Comparison Test using GraphPad Prism software. IC_{50} values were produced using sigmoid dose–response curves on GraphPad software. At least three independent experiments were performed for each assay.

4. Conclusions

In summary, two series of 2-(indol-3-yl)ethylamine derivatives **7**–**11** and (2-(5-methoxy-1H-indol-3-yl)ethyl)amine derivatives **12**–**16** have been evaluated for their antimalarial activity against *P. falciparum* (3D7) *in vitro* culture.

We tested the ability of these compounds to modulate the cycle of the parasite, similarly to melatonin **(6)**, as well assessed the ability of these compounds to block the effect of the hormone on cell cycle of *P. falciparum*, acting as inhibitors. Compounds **9** and **10** shown promising results, not being able to modulate cycle of the parasite but being able to block the effect of melatonin in *Plasmodium*.

Compounds **12**–**14** are promising lead structures for the development of new derivatives with antimalarial activity. We are currently working with these compounds to increase their activities, in particular by introducing structural changes at position *N*-1 of the indole ring.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.055.

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