Antiplasmodial activities of dyes against Plasmodium falciparum asexual and sexual stages: Contrasted uptakes of triarylmethanes Brilliant green, Green S (E142), and Patent Blue V (E131) by erythrocytes

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Antiplasmodial activities of dyes against *Plasmodium falciparum* asexual and sexual stages: Contrasted uptakes of triarylmethanes Brilliant green, Green S (E142), and Patent Blue V (E131) by erythrocytes

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ABSTRACT

The search for safe antimalarial compounds acting against asexual symptom-responsible stages and sexual transmission-responsible forms of *Plasmodium* species is one of the major challenges in malaria elimination programs. So far, among current drugs approved for human use, only primaquine has transmission-blocking activity. The discovery of small molecules targeting different *Plasmodium falciparum* life stages remains a priority in antimalarial drug research. In this context, several independent studies have recently reported antiplasmodial and transmission-blocking activities of commonly used stains, dyes and fluorescent probes against *P. falciparum* including chloroquine-resistant isolates. Herein we have studied the antimalarial activities of dyes with different scaffold and we report that the triarylmethane dye (TRAM) Brilliant green inhibits the growth of asexual stages ($IC_{50} \leq 2$ μM) and has exflagellation-blocking activity ($IC_{50} \leq 800$ nM) against *P. falciparum* reference strains (3D7, 7G8) and chloroquine-resistant clinical isolate (Q206). In a second step we have investigated the antiplasmodial activities of two polysulfonated triarylmethane food dyes. Green S (E142) is weakly active against *P. falciparum* asexual stage ($IC_{50} \simeq 17$ μM) whereas Patent Blue V (E131) is inactive in both antimalarial assays. By applying liquid chromatography techniques for the culture supernatant analysis after cell washings and lysis, we report the detection of Brilliant green in erythrocytes, the selective uptake of Green S (E142) by infected erythrocytes, whereas Patent Blue V (E131) could not be detected within non-infected and 3D7-infected erythrocytes. Overall, our results suggest that two polysulfonated food dyes might display different affinity with transporters or channels on infected RBC membrane.

1. Introduction

The development of new antimalarial drugs targeting both asexual symptoms-responsible stages and sexual transmission-responsible forms of *Plasmodium falciparum* is highly encouraged by the World Health Organization (WHO) and health agencies (Burrows et al., 2013; Delves, 2012). Currently primaquine is the only marketed drug specifically used to prevent malaria transmission from infected humans to mosquitoes (White, 2013). The WHO recommends since 2012 the addition of a single low-dose of primaquine (0.25 mg base/kg) to artemisinin-based combination

Abbreviations: ATP, adenoside triphosphate; DAD, diode array detector; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; HPLC, high-performance liquid chromatography; IC, inhibitory concentration; LD, lethal dose; NMR, nuclear magnetic resonance; ND, not determined; RBC, red blood cells; SD, standard deviation; TRAM, triarylmethane; WHO, World Health Organization.

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therapy (ACTs) in uncomplicated infection (WHO, 2015; Dicko et al., 2016). In contrast to the discovery of new antimalarial compounds targeting asexual blood stages, only few transmission-blocking agents have been identified (Penna-Coutinho et al., 2016) so far, mostly by screening current antimalarial molecules (Delves et al., 2012; Sinden et al., 2012). Synthetic thiazine dye methylene blue is one of them (Guttmann and Ehrlich, 1891; Adjalley et al., 2011). Despite not being prescribed as antimalarial since the 1950s, methylene blue is remarkably safe for humans and at the same time highly active against many *Plasmodium* species including drug-resistant parasites (Schirmer et al., 2003). Noteworthy methylene blue has strong transmission-blocking activity (Coulibaly et al., 2009). Its mode of action remains unclear however disulfide reductase interactions and effect on heme detoxification have been pointed out (Buchholz et al., 2008).

Drawing on the 19th century results, several dyes are endowed with scaffolds originating from azine, benzoquinoline, oxazine, phenazine, thiazine, triarylmethane, xanthene chemical families and they have been revisited as potential antimalarial drugs. Rhodamine B and Eosin B dyes (xanthenes) were found to be highly active against asexual forms of chloroquine-resistant *P. falciparum* ($IC_{50} \leq 10$ nM) (Vennerstrom et al., 1995; Massimine et al., 2006). More recently Acridine orange (benzoquinoline), MitoRed and Rhodamine B (xanthenes) have been identified as gametocytocidal compounds ($100 \leq IC_{50} \leq 800$ nM) on stage V gametocytes using an ATP-based viability bioassay (Gebru et al., 2014). It is also worth noting that the triarylmethane clotrimazol (antifungal) inhibited the growth of several sensitive and resistant *P. falciparum* ($0.2 \leq IC_{50} \leq 1.1$ μM) (Saliba and Kirk, 1998; Tiffert et al., 2000). In the context of antimalarial discovery, the screening of structurally different dyes remains therefore relevant. Herein we investigate the antimalarial activities of several dyes against asexual and sexual forms of two *P. falciparum* reference strains and one culture-adapted clinical isolate.

2. Materials and methods

2.1. Chemicals, dyes and stock solutions

Analytical grade acetonitrile (MeCN) was obtained from Carlo Erba. Patent blue V (HPLC purity ≥ 97%, CAS N°: 3536-49-0), Green S (HPLC purity ≥ 98%, CAS N°: 3087-16-9), Basic blue 3 (dye content 25%, CAS N°: 33203-82-6), methylene blue (HPLC purity ≥ 82%, CAS N°: 7220-79-3), and Acridine orange (dye content 75%, CAS N°: 494-38-2) were all purchased from Sigma-Aldrich. Brilliant green (^{1}H NMR purity > 98%, CAS N°: 633-03-4) and Neutral red (^{1}H NMR purity ≥ 92%, CAS N°: 553-24-2) were purified by crystallization from laboratory source. Azocarmine G (^{1}H NMR purity ≥ 90%, CAS N°: 25641-18-3), Thymol (^{1}H NMR purity ≥ 87%, CAS N°: 76-61-9) and Auramine O (^{1}H NMR purity ≥ 80%, CAS N°: 2465-27-2) were used without purification. Proton Nuclear Magnetic Resonance analysis (^{1}H NMR) was performed on a Varian 400 MHz spectrometer. Stock solutions (10 mM) were prepared by dissolving dyes into sterile water.

2.2. Reference strains and clinical isolate

The *P. falciparum* South American chloroquine-resistant reference strain 7G8 (MRA-926) and the African chloroquine-sensitive strain 3D7 (MRA-102) were obtained from the MR4 (www.beiresources.org/MR4Home.aspx). The *P. falciparum* isolate Q206 was collected from a mono-infected patient seeking treatment in 2013 in French Guiana. The adaptation to *in vitro* multiplication of Q206 isolate was performed using standard protocols (Trager and Jensen, 1976). Parasitemia was checked daily and kept below 2% by adding fresh red blood cells (RBC). The isolate was considered culture-adapted after 3 weeks of uninterrupted *in vitro* cultivation with growing parasitemia.

2.3. Isotopic incorporation assay

Antimalarial activities were assessed by calculating the concentration of drugs inhibiting 50% of growth (IC50), compared to the drug-free control. IC_{50} of dyes were measured on the reference strains (3D7, 7G8) and the clinical isolate Q206 with a modified growth inhibition assay (Desjardins et al., 1979; Le Bras and Deloron, 1983). Briefly parasites are cultivated using asexual culture medium (RPMI 1640 medium with 25 mM HEPES, 25 mM sodium bicarbonate, gentamicin 20 mM, glucose 11 mM and L-glutamine 2 mM) supplemented with 10% decomplemented human serum, at a 5% hematocrit and incubated at 10% O_2, 5% CO_2 and 85% N_2 gas. Once a parasitemia of 3–4% is reached with more than 80% of rings, a 96 well plate (0.75% parasitemia, 2% hematocrit) was plotted with ^{3}H-hypoxanthine (0.8 mCi) and various dye concentrations. After culturing for 42 h at 37 °C under the same atmosphere conditions, parasite growth was determined based on the amount of [^{3}H]-hypoxanthine incorporated using a multidetector scintillation counter (PerkinElmer 1450 Microbeta). Six concentrations (100 μM–1 nM) were tested to calculate IC_{50} of active compounds using the ICEstimator 1.2 online software (www.antimalarial-icestimator.net) (Le Nagard et al., 2010; Kaddouri et al., 2006). Each concentration was tested in triplicate for each compound. We define the biological activities as follows: very good activity, $IC_{50} < 0.2$ μM; good activity, $0.2 < IC_{50} < 1$ μM; moderate activity, $1 < IC_{50} < 10$ μM; weak activity, $10 < IC_{50} < 20$ μM; inactive, $IC_{50} > 20$ μM.

2.4. In vitro gametocyte production and maturation

Gametocyte cultures were performed following published protocols (Delves et al., 2013). Briefly, a culture of asexual parasites at approximately 5% parasitemia was used to seed gametocyte production at 0.5–1% parasitemia and 4% hematocrit under 10% O_2, 5% CO_2 and 85% N_2 atmosphere. Culture medium (RPMI 1640 medium with 25 mM HEPES, 50 mg/L hypoxanthine, 2 g/L sodium bicarbonate, 10% human serum) was replaced daily but without any further addition of red blood cells. Maturation of gametocytes was evaluated morphologically using Giemsa-stained blood films. Stage V male gametocyte functional maturity was controlled by observing exflagellation under bright-field microscope. Briefly, a 30 μL aliquot of gametocyte culture was centrifuged and the cell pellet was resuspended in 15 μL of ookinete medium (RPMI medium with 25 mM HEPES, 50 mg/L hypoxanthine, 2 g/L sodium bicarbonate, 100 μM xanthurenic acid, 10% human serum) and finally introduced into a chamber of a FastRead disposable hemocytometer slide (Immune Systems). Exflagellation centres were observed at 10× or 20× magnification.

2.5. Exflagellation inhibition assay

Exflagellation inhibition assays were performed using published protocols (Leba et al., 2015; Bhattacharyya and Kumar, 2001). Briefly, 1.5 mL tube containing 170 μL of culture medium (RPMI 1640 medium with 25 mM HEPES, 50 mg/L hypoxanthine, 2 g/L sodium bicarbonate, 10% human serum) was preheated at 37 °C with the dye pre-dissolved in water for a final concentration of 1 μM. 30 μL of 7G8 or Q206 stage V mature gametocytes (12–15 days of culture) were then added and placed into a 37 °C incubator. After 24 h, cells were centrifuged (200 g), the supernatants were removed and cell pellets were suspended in ookinete medium

(15 μL) with a drop in temperature from 37 °C to 25 °C to induce exflagellation. The exflagellation centres were recorded within 40 min in 10 microscopic fields using chambers of a FastRead disposable hemocytometer slide (Immune Systems) at a magnification of 10×. For meaningful measures, exflagellation measurements were performed on at least 100 exflagellation centres in the drug-free controls and all the measurements were performed in triplicate. The percentage of exflagellation inhibition was determined by dividing drug assay exflagellation values of the drug-treated by the drug-free controls. For asexual growth inhibition, IC_{50} were determined as described earlier. Biological activities were defined as detailed in Section 2.3.

2.6. Permeability assay

Dyes ability to permeate into RBC was assessed by HPLC analysis. Briefly, 1.5 mL tubes containing 100 μL of non-infected RBC or 3D7-infected RBC at 15–20% of parasitemia with 50% of rings were incubated at 37 °C for 2 h with 200 μL of compound diluted in medium at 100 μM. Each tube was then centrifuged (400 g, 5 min) before removing the supernatant and washing the pellet 3 times with PBS and the full removal of the dye is checking by controlling absence of dyes in supernatants by HPLC analysis. To release dyes from RBC, 500 μL of double-distilled water was added followed by a freeze-thaw step. Hemoglobin and others protein were precipitated for 24 h by adding 1.5 mL of acetonitrile. Finally, the supernatant was filtered through a 0.22 μm membrane before injection on a Varian 920-LC system (C18 Discovery column Sigma, 5 μm, 15 cm × 4.6 mm) equipped with DAD detector (UV–Visible absorbance detector). All samples were analyzed using a linear gradient (flow rate 1 mL/min) of water/acetonitrile/formic acid (95:5:0.1 to 5: 95:0.1).

3. Results and discussion

3.1. Antiplasmodial screening of dyes

The chemical diversity of dyes is clustered in a few chemical families including azine, azo, benzoquinoline, oxazine, phenazine, thiazine, bi and triarylmethane, xanthene. Given this chemical diversity, we decided in an initial screening to choose randomly different scaffolds within different chemical families to test their activities against *P. falciparum* asexual stages, and for a subset of compounds, against sexual stages (inhibition of gametes' exflagellation). Table 1 presents the results obtained using chloroquine-sensitive *P. falciparum* strain 3D7, chloroquine-resistant strain 7G8 and clinical isolate Q206. Methylene blue is used as a positive control of inhibition (Table 1, entry 1). First the lipophilic phenazine Neutral red is weakly active in asexual stage bioassay (3D7, 7G8, Q206; 13.4 μM ≤ IC_{50} ≤ 18.6 μM) and does not induce any gametocytes' exflagellation inhibition at 1 μM (Table 1, entry 2). In comparison, the phenazine dye Azocarmine G is inactive against asexual forms of *P. falciparum,* possibly because of the presence of hydrophilic sulfonate groups that significantly limits its entry through the RBC membrane therefore preventing access to potential intracellular targets (Table 1, entry 3). In the case of oxazine Basic blue 3, IC_{50} values were measured at 0.7 μM, 1.3 μM and 1.1 μM using 3D7, 7G8 and Q206 respectively (Table 1, entry 4). However, a major issue concerns its purity since like other dyes this compound is not commercially available at suitable purity for biological assays. It should be reminded that previous studies do not mention dye purity (Vennerstrom et al., 1995; Massimine et al., 2006) making difficult the comparaison between results. Benzoquinoline Acridine orange has been identified recently as a potent transmission blocking compounds acting against mature gametocytes in an ATP-based bioluminescence bioassay (Gebru et al., 2014) and we report herein nanomolar activity against asexual forms (270 nM ≤ IC_{50} ≤ 780 nM) (Table 1, entry 5). However, Acridine orange has mutagenic effect on bacteria and mammal cells (Ferguson and Denny, 1991), thus preventing further development in medicinal chemistry. Moderate activities were found for diarylmethane Auramine O with IC_{50} values ranging from 6.3 to 10.9 μM in asexual stage bioassays whereas triarylmethane Thymol blue was found to be inactive (IC_{50} > 20 μM) (Table 1, entries 6, 7). Finally, the triarylmethane dye Brilliant green shows good to moderate antimalarial activity (0.8 μM ≤ IC_{50} ≤ 1.5 μM) in asexual bioassays and inhibits 90.5% of gametocytes exflagellation at 1 μM using chloroquine-resistant clinical isolate Q206 (Table 1, entries 8). Based on the later result we conducted further investigations using triarylmethane dyes.

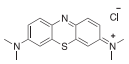
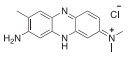
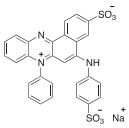
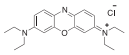
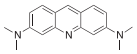
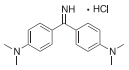
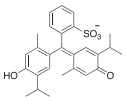
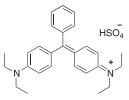
3.2. Triarylmethane dyes (TRAM)

It should be underlined that dyes' toxicity is potentially a major issue since most of these compounds have not been fully tested in toxicological studies. Brilliant green has been used on humans as a topical antiseptic (Reynolds and Prasad, 1992; Bakker et al., 1992) and against intestinal anthelmintic (Gosselin et al., 1984) in the 80's. However, side effects such as diarrhea and abdominal pain have quickly limited its use in medicine. Brilliant green has a lowest lethal dose (LD_{Lo}) value of 75 mg/kg on rabbit (Sax, 1984), which prevents its development in drug discovery. On the other hand several TRAM are currently used for the treatment of constipation (sodium picosulfate), against fungal infection (clotrimazole) and in food industry (Patent blue V, Green S). Table 2 presents a second series of results including Brilliant green and two other TRAM dyes, Patent blue V (E131) and Green S (E142), which are currently used as food dyes and approved by the European Food Safety Authority (EFSA). Only the chloroquine-resistant 7G8 strain and the chloroquine-resistant clinical isolate Q206 were used for sexual bioassays in this series as 3D7 showed significant phenotypic variability in its capacity for successful exflagellation. Methylene blue is used as drug control (Table 2, entry 1). Results confirm the moderate activity of Brilliant green against asexual forms of *P. falciparum* 7G8 strain and Q206 isolate (1.4 μM ≤ IC_{50} ≤ 1.5 μM) and noteworthy we report for the first time its inhibiting effect on gametocytes' exflagellation (472 nM ≤ IC_{50} ≤ 778 nM) (Table 2, entry 2). Patent blue V is inactive in both bioassays (Table 2, entry 3), whereas Green S is weakly active (16.5 μM ≤ IC_{50} ≤ 17.2 μM) only in the growth inhibition using 7G8 strain and Q206 clinical isolate (Table 2, entry 4). Green S has an intriguing antimalarial activity considering that food dyes are safe chemicals by ingestion unable to permeate cell membranes (TPSA > 140 $Å^2$).

3.3. Uptake of TRAM dyes by erythrocytes

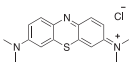
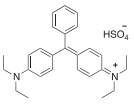
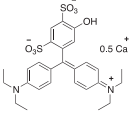
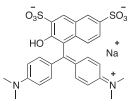
To provide additional information related to TRAM uptake by red blood cells (RBC), further experiments were conducted. We first checked successfully that high concentration of TRAM dyes in the medium (100 μM) does not induce the lysis of erythrocytes via a membrane disruption mechanism. To evaluate dye ability to permeate erythrocyte membrane or to stick to their cell surface, we attempted to detect by HPLC-DAD analysis dyes in the supernatant after cell lysis. Using uninfected erythrocytes, Brilliant green and methylene blue are detected whereas Green S and Patent Blue V could not be observed (Fig. 1). We next assessed dyes uptake in infected RBC. As expected Brilliant green and methylene blue are detected in supernatants (results not shown), Patent Blue V failed once more to detection however Green S (E142) is surprisingly detected (Table 2). This result suggests that Green S (E142), a

Table 1
Dyes antiplasmodial activity against *Plasmodium falciparum* asexual and sexual stages.

Entry	Chemical family	Dye (purity %)	Chemical structure	Asexual growth inhibition IC_{50} (μM) ± SD[a]			Gametocyte exflagellation inhibition (% ± SD)[b]
				3D7	7G8	Q206	Q206
1	Thiazine	Methylene blue (82%)		1×10^{-3}	1×10^{-3}	1×10^{-3}	100
2	Phenazine	Neutral red (92%)		13.4 ± 0.45	15.9 ± 0.66	18.6 ± 0.9	−9.4 ± 19.7
3	Phenazine	Azocarmine G (90%)		>100	>100	>100	8.9 ± 3.0
4	Oxazine	Basic blue 3 (25%)		0.7 ± 0.04	1.3 ± 0.11	1.1 ± 0.09	ND
5	Benzoquinoline	Acridine orange (75%)		0.3 ± 0.01	0.8 ± 0.02	0.7 ± 0.02	ND
6	Diarylmethane	Auramine O (80%)		10.9 ± 0.54	6.3 ± 0.27	7.0 ± 0.36	ND
7	Triarylmethane	Thymol blue (87%)		68.2 ± 5.84	20.3 ± 2.1	33.9 ± 3.70	ND
8	Triarylmethane	Brilliant green (95%)		0.8 ± 0.06	1.4 ± 0.10	1.2 ± 0.09	90.5 ± 4.4

[a] Hypoxanthin incorporation assay (mean ± SD, tested in triplicate).
[b] Exflagellation inhibition assay at 1 μM expressed as a percentage of inhibition compared to the drug-free control (mean ± SD, tested in triplicate).

Table 2
Comparison of antiplasmodial activity of triarylmethanes with Methylene Blue against *Plasmodium falciparum* asexual and sexual stages.

Entry	Dye (purity %)	Chemical structure	TPSA[c]	WLOGP[c]	Asexual/IC_{50} (μM) ± SD[a]		Sexual/IC_{50} (μM) ± SD[b]	
					7G8	Q206	7G8	Q206
1	Methylene blue (82%)		47.38	2.5	1×10^{-3}	5×10^{-3}	ND	23×10^{-3}
2	Brilliant green (95%)		6.25	5.95	1.4 ± 0.10	1.4 ± 0.20	0.8 ± 0.02	0.5 ± 0.04
3	Patent blue V E131 (98%)		157.64	5.63	>100	>100	>100	>100
4	Green S E142 (97%)		157.64	5.22	16.5 ± 1.53	17.2 ± 2.09	>100	>100

[a] Hypoxanthin incorporation assay (mean ± SD, tested in triplicate).
[b] Exflagellation inhibition assay (mean ± SD, tested in triplicate).
[c] Calculated using SwissADME (Daina et al., 2017).

hydrophilic water-soluble food dye permeates selectively membrane of infected erythrocytes and has a weak activity against *P. falciparum*. Alternatively, Green S can also stick to the cell surface instead of permeating the cell membrane but our data cannot discriminate between these two mechanisms. It is known that *P. falciparum* has the capacity to alter the membrane properties by increasing its permeability to numerous solutes including nutrients required for intracellular parasite growth. Sugars, amino acids, purines, vitamins are needed for *in vitro* propagation and have increased permeability after infection (Ginsburg, 1994; Saliba and

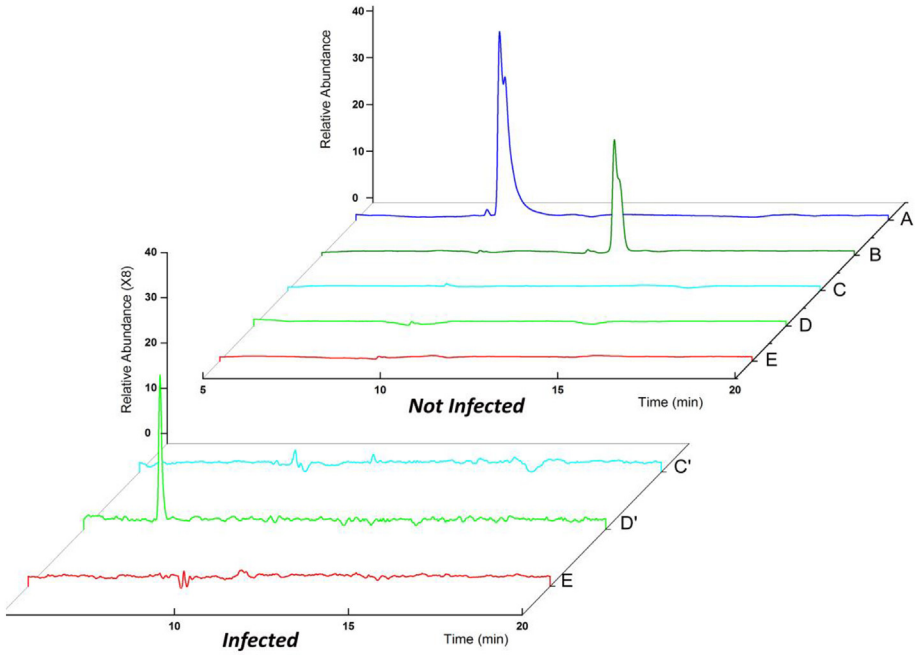


Fig. 1. HPLC chromatograms of RBC cellular content after cell lysis.
A–D: non-infected RBC; C′-D′: 3D7-infected RBC after lysis. A: Methylene Blue; B: Brilliant green; C, C′: Patent Blue V; D, D′: Green S; E: no-dye control detected at λ = 625 nm. Relative abundance of C-E chromatograms were amplified (×8).

Kirk, 1998; Kirk and Lehane, 2014). It is also now well-established that the parasite inserts its own proteins (CLAG3, RhopH2, RhopH3) into the host erythrocyte membrane to form a channel known as the plasmodial surface anion channel (PSAC). (Desai et al., 2000; Nguitragool et al., 2011; Ito et al., 2017; Sherling et al., 2017). This unusual channel mediates uptake of nutrients, toxins and several antimalarial agents (Sharma et al., 2013; Mira-Martínez et al., 2013). In this study we have compared Patent Blue V (E131), a tertiary carbon bonding two *N*,*N*-diethylaniline groups and a 4-hydroxybenzene-1,3-disulfonic acid moiety, and Green S (E142), a tertiary carbon bonding two *N*,*N*-methylaniline groups and a 3-hydroxynaphthalene-2,7-disulfonic acid moiety. These structural features are too closely related to suggest obvious difference in their physicochemical properties that could explain the absorption selectivity between Patent Blue V (E131) or Green S (E142) by the PSAC. Drugs may enter via lipid-diffusion or active transport across the erythrocyte membrane, before crossing the parasitophorous vacuolar membrane and the parasite plasma membrane. Computational approach shows that most existing compounds with antiplasmodial activity have physicochemical properties consistent with lipid-diffusion through the infected erythrocytes membranes with few exceptions for doxycycline, azithromycin, lumefantrine, blasticidin S, leupeptin and fosmidomycin that are predicted to be poorly absorbed but could cross the host erythrocyte membrane through a channel or a transporter (Ghose et al., 1998; Egan et al., 2000; Basore et al., 2015). We have investigated this approach by calculating the partition coefficient (WLOGP) and the topological polar surface area (TPSA) for triarylmethane compounds (Table 2 and Fig. 2). Brilliant green is found inside the good absorption 95% confidence ellipse, whereas both hydrophilic food dyes Green S (E142) and Patent Blue V (E131) are predicted to be poorly absorbed with a high TPSA (≃ 160). Considering the fact that Green S (E142) can be found in infected but not uninfected erythrocytes, these physicochemical predictions suggest an active transport inside the RBC, presumably by the PSAC. However, the contrasted selectivity in RBC absorption for Patent Blue V (E131) and Green S (E142) remains unclear and could reflect a difference between dyes in their affinity with the PSAC.

4. Conclusion

The quest for identifying new drugs able to act on different parasite's targets is currently a leading strategy in the field of malaria research and requires the implementation of at least two antimalarials bioassays. Combining this strategy with the screening of revisited or newly tested molecules increases the chances to identify therapeutic candidate to treat malaria symptoms or fight against malaria transmission. In this context, using growth and exflagellation inhibition assays, we report the antimalarial activities of the dye Brilliant green on both asexual and sexual stages using strains of *P. falciparum* (3D7, 7G8) and one clinical isolate Q206. Issues related to dyes toxicity are of concern and the screening of food additives previously untested is relevant as it was demonstrated recently in the screening of 2816 compounds (NIH collection) registered or approved for human or animal use (Yuan et al., 2011). In this work, food dye Green S (E142), a TRAM parent of Brilliant green, was found to have weak antiplasmodial activity associated with an unexpected specific interaction with infected RBC membrane. Additionally, the closely related TRAM food dye Patent Blue V is inactive in antimalarial assays and shows no interaction with both non-infected and 3D7-infected RBC membrane despite having similar physicochemical properties. These results point out an intriguing contrasted selectivity in TRAM uptake by infected RBC that could suggest different molecular affinities between TRAM and PSAC. Overall to improve TRAM antiplasmodial activities future directions should rely on the synthesis of analogues with the perspective of evaluating the structure-transport relationship

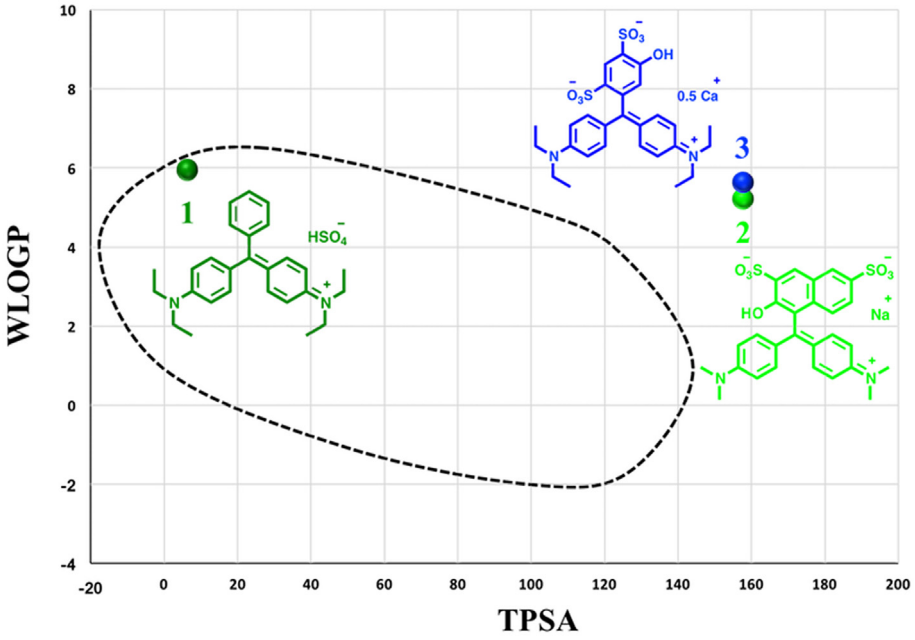


Fig. 2. Plot of TPSA vs. WLOGP for triarylmethane compounds.
TPSA and WLOGP were calculated using SwissADME (Daina et al., 2017) for Brilliant green (1), Green S (2), Patent Blue V (3). Dashed line represents the 95% confidence ellipse for drugs with good absorption as determined by Egan et al. (2000), Daina and Zoete (2016).

in order to design more active and more selective antimalarials.

Competing interests

The authors have declared that they have no competing interests.

Authors' contributions

LJL carried out parasite cultures, experiments and analyzed the data. YE helped with the exflagellation experiments, designed and conducted permeability experiments and analyzed the data. JP contributed to the protocol design as well as SP, EL, and LM who supplied culture-adapted clinical isolates. CD supervised this work, conceived the study and its design with the help of LJL. CD wrote the manuscript and all authors contributed to the manuscript.

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