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Article

# *In vitro* and *ex vivo* antiplasmodial activity of 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one) against circulating strains of *Plasmodium* spp. in the state of Rondônia, Brazil

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Malaria is a disease caused by *Plasmodium* spp. protozoa. The ability of *Plasmodium* to develop resistance to current antimalarial drugs makes the study of chemotherapeutic alternatives extremely important. This study aimed to evaluate the antimalarial activity of compound 3286938 (1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one), which presents in its structure a 3,4,5-trimethoxyphenyl group, *in vitro*, using the W2 strain of *P. falciparum* and against circulating strains of *P. vivax* and *P. falciparum* from the state of Rondônia. The compound 3286938 obtained an $IC_{50}$ of 24.4 µM against the W2 strain of *P. falciparum,* and against the circulating strains, it presented a median (MD)=38.7 µM for *P. vivax* and MD=6.7 µM for *P. falciparum*. As for toxicity, 3286938 showed $CC_{50}$ > 500 µM for VERO and HepG2 strains with a selectivity index greater than 12.9, a ratio calculated for *P. falciparum* and *P. vivax* regarding Vero and HepG2 cells. The compound was not considered hemolytic in *in vitro* assays, thus indicating the specificity of its antiplasmodial action. Based on the results presented, and considering the unprecedented character of the compound, it can be concluded that 3286938 was shown to be promising for complementary *in vitro* and *in vivo* studies aiming to produce effective antiplasmodial action.

**Keywords:** Malaria. *P. falciparum*. *P. vivax*. Trimethoxy-phenyl.

## INTRODUCTION

Malaria is considered a serious disease by the World Health Organization (WHO) and it continues to have a major impact on public health, especially in tropical countries. According to WHO, it is estimated that more than one billion cases of malaria occurred worldwide between 2010 and 2018 (WHO, 2020). In Brazil, more than 99% of cases are concentrated in the Legal Amazon Region, due to its tropical climate and socioeconomic conditions which favor the proliferation, growth and survival of the anopheline vector (Lapouble, Santelli, Muniz-Junqueira, 2015; SINAN, 2019). Three species are associated with human infection in the country: *Plasmodium falciparum*, *P. vivax* and *P.*

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*malariae*. *P. vivax* is most predominant, followed by the species *P. falciparum* (Brazil, 2015).

Malaria caused by *P. falciparum* is considered the most serious form of the disease, leading to high mortality rates, with the potential to manifest neurological disorders, respiratory problems and may predispose women to the threat of miscarriage (Mackintosh, Beeson, Marsh, 2004). However, in recent years, *P. vivax* has been identified as the main cause of morbidity and deaths in babies and children in Southeast Asia (Kaushik, Gomber, Dewan, 2012; Singh *et al*., 2013). In addition, the clinical presentation of malaria caused by these two species can be aggravated due to the existence of isolates with remarkable genetic variation that are resistant to the antimalarials currently used, such as chloroquine (CQ) for *P. vivax* and artemisinin for *P. falciparum*; this becomes a threat to the control and elimination of the disease (Dondorp *et al*., 2009; Miotto *et al*., 2013).

The difficulty of controlling the disease and the emergence of strains resistant to current antimalarials makes the search for new drugs important to complement or replace the drugs currently used to treat malaria (Phyo *et al*., 2012; Ashley *et al*., 2014; Imwong *et al*., 2017).

In this context, we can highlight plants of the genus *Piper* L., Piperaceae which have components such as: amides, alkaloids, derivatives of benzoic acid, lignans, flavonoids and cinnamic acid (Facundo *et al*., 2008; Regasini *et al*., 2009). There are reports in the literature of trypanocidal, leishmanicidal and anti-inflammatory bioactivity for this genus (Calderon *et al*., 2009; Santos *et al*., 2018; Oliveira *et al*., 2018). In addition, there are other studies and reviews published in the literature demonstrating specific action of molecules derived from cinnamic acid (Guzman, 2014). These actions include antimalarial (Kanaani, Ginsburg, 1992), antifungal (Tawata *et al.,* 1996), antioxidant, antimicrobial (Sova, 2012) and anticancer activities (Pontiki *et al.,* 2014).

Many phenylpropanoids are derived from cinnamic acid, through reduction, hydroxylation and methylation reactions (Dewick, 2009). Studies and reviews published in the literature have demonstrated specific action of molecules derived from cinnamic acid (Guzman, 2014). The compounds that make up this family of metabolites were extracted, modified and synthesized for future studies. Santos *et al*. (2018) demonstrated that synthetic compounds derived from trimethoxyphenylpropanoic acid showed antiprotozoal action against *Leishmania* spp. and *T. cruzi*; study conducted by Ferreira *et al*. (2010) also reinforced the 3,4,5-trimethoxyphenyl group's activity.

Therefore, due to the biological characteristics described above, this group can be an alternative in the search for new synthetics that can be used in the treatment of malaria. The compound 3286938 (Figure 1) presents in its structure the 3,4,5-trimethoxyphenyl group. Therefore, it was chosen for study due to its structural similarities with phenylpropanoid derivatives.

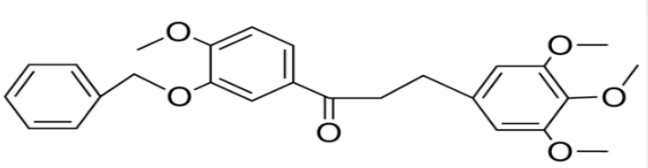

**FIGURE 1 -** 11-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one (CID: 3286938).

In this article, we report the activity of the synthetic compound 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one), a derivative of 3,4,5-trimethoxyphenyl propanoic acid, against *P. falciparum* strains *in vitro* and against *P. vivax* and *P. falciparum* strains circulating in the state of Rondônia.

## MATERIAL AND METHODS

### Ethical approval

This study was approved by the Research Ethics Committee of the Oswaldo Cruz Foundation - Rondônia, according to protocol numbers CAAE: 44899715.2.0000.0011 and CAAE 58738416.1.0000.0011.

### Obtaining the compound

3286938 was obtained commercially by the company Sigma-Aldrich; its solubilization was carried out in dimethylsulfoxide (DMSO-Sigma-Aldrich) at a final concentration of 0.5%. Solubilization of the compound occurred on the day of the respective experiments.

### *In vitro* assay against W2 *P. falciparum*

W2 *P. falciparum* parasites were grown in human erythrocytes according to criteria adapted from Trager, Jensen (1976). With the predominance of young trophozoites, the culture was synchronized using 5% D-sorbitol (Sigma-Aldrich) and the hematocrit was adjusted to 5%, according to conditions described by Lambros, Vanderberg (1979). The compound was tested in serial concentrations of 1000-1.5 µM. As a control of parasite death, artemisinin was used in serial concentrations of 0.17-0.001 µM, dihydroartemisinin (0.5-0.0002 µM). Infected red blood cells were used as a positive control and uninfected red blood cells as a negative control. The plates were maintained in an incubator at 37 °C for 48 hours (in a standard gas mixture consisting of 5% $O_2$, 5% $CO_2$ and 90% $N_2$) and stained using Sybr Green I (Invitrogen), which is a DNA intercalator. It is known that erythrocytes do not have nucleic acids, while parasites of *Plasmodium* spp. contain both DNA and RNA, so when parasites grow, there is also an increase in DNA capable of intercalating with Sybr Green I, resulting in increased fluorescence as described by Smilkstein *et al.* (2004).

### Cytotoxicity Assay

The cytotoxic effect of the compound was evaluated against adherent HepG2 cells (derived from a human hepatoma) and VERO cells (kidney cells of the African monkey), using an MTT (3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide - Sigma-Aldrich) colorimetric assay, as described by Mosmann (1983). Cultivation took place in RPMI-1640 medium (Gibco) supplemented with 10% Bovine Fetal Serum (Gibco), followed by incubation at 37 °C with 5% $CO_2$, at 95% humidity (Calvo-Calle *et al.,* 1994). For the toxicity tests, the cell suspension was adjusted to a proportion of $1x10^4$ cells/well in microplates and incubated again for 12 hours in order to achieve cell adhesion to the wells. After this incubation time, the cells were treated for 72 hours in serial concentrations from 500-3.9 µM for 3286938, artemisinin and dihydroartemisinin. Absorbance readings were obtained using a spectrophotometer (Biochrom Asys, Expert Plus, Holliston, USA) at 570 nm wavelengths. Untreated cells were used as the positive control and cells treated with 0.05% saponin (Sigma-Aldrich) were used as the negative control. The concentration cytotoxic to 50% of viable cells ($CC_{50}$) was calculated compared to cells with no treatment.

### Hemolytic Assay

The hemolytic assay was performed with a suspension of 180 µL of human erythrocytes (1%) distributed in a 96-well microplate with a "U" bottom and subjected to treatment with the compound at serial concentrations (1000-1.5 µM). As a positive control of hemolysis, 0.05% saponin was used, while 1% red blood cells in incomplete RPMI-1640 medium were used as the negative control and RPMI-1640 alone was used as background. The plates were incubated in an incubator at 37°C for 30 minutes with shaking every 5 minutes (Wang *et al.,* 2010). Then, the absorbance was read on a spectrophotometer at 540 nm wavelengths.

### *Ex vivo* testing

Blood samples from patients infected with *P. falciparum* and *P. vivax,* with parasitemia between 11-100 parasites per 100 oil-immersion thick film fields, were collected between June 2017 and October 2018 at the Tropical Medicine Research Center (CEPEM) in the city of Porto Velho, state of Rondônia. Underage patients, indigenous people, pregnant women, people with mental disabilities and comorbidities, and patients who had used any antimalarial drug in the previous month or presented severe malaria were excluded from the study.

For *ex vivo* chemosensitivity assays, plates were prepared with the drug dihydroartemisinin tested at serial dilutions of 1-0.00024 µM and 3286938 at concentrations of 1000-1.5 µM; for both tests, the dilutions were done at proportions of 1:4. Initial parasitemia was verified using thick smear slides (stained using Giemsa). The white blood cells were removed by centrifugation, and the red cells were filtered through a CF11 cellulose column (Moody, 2002). The parasite culture was supplemented with McCoy medium for *P. vivax* and RPMI-1640 for *P. falciparum* (Marfurt *et al.,* 2011). Parasite maturation was monitored after intervals of 24 and 48 hours of

incubation, using thick blood films, from the untreated control. Incubation with antimalarials and compound 3286938 was stopped when at least 40% of schizonts were reached in 200 forms of parasites; the slide count was performed using optical microscopy.

### Statistical analysis

The inhibitory concentration for 50% of parasites ($IC_{50}$) and the cytotoxicity concentration for 50% of cells ($CC_{50}$) were determined using dose-response curves based on non-linear regression; the curves were plotted using the program OriginPro. The selectivity index (SI) was obtained by calculating the ratio between $CC_{50}/IC_{50}$, where a value >10 is considered selective, and a value <10 is considered non-selective to parasites (Nava-Zuazo *et al.,* 2010). Hemolytic activity was analyzed in GraphPad Prism and validated by analysis of variance (ANOVA), followed by Tukey's post-test with a significance of $p < 0.05$. In the descriptive analysis of the circulating strains, the measure of central tendency MD = median was used, being determined from the N sample of patients and calculating the interquartile interval and then plotted on GraphPad Prism.

## RESULTS

Analogue 3286938 was initially tested *in vitro* against the W2 strain of *P. falciparum*. The $IC_{50}$ value obtained for this test was 24.4 µM (±1.4). The $IC_{50}$ value for the reference drugs (Figure 1 and Table I) used as a control showed results of 0.00026 µM (± 0.0) for artemisinin (ART) and 0.00065 µM (± 0.0) for dihydroartemisinin (DHART). Against circulating strains, the median $IC_{50}$ for compound 3286938 was 38.7 µM (MD 59.4) for *P. vivax* and 6.7 µM (MD 4) for *P. falciparum*. The median $IC_{50}$ for DHART, the reference drug used in the *ex vivo* assay, was 0.0011 µM (MD 0.001) against *P. falciparum* and 0.0051 µM (MD 0.003) against *P. vivax* (Figure 2 and Table I). The reference drug, artemisinin, was not tested against circulating strains.

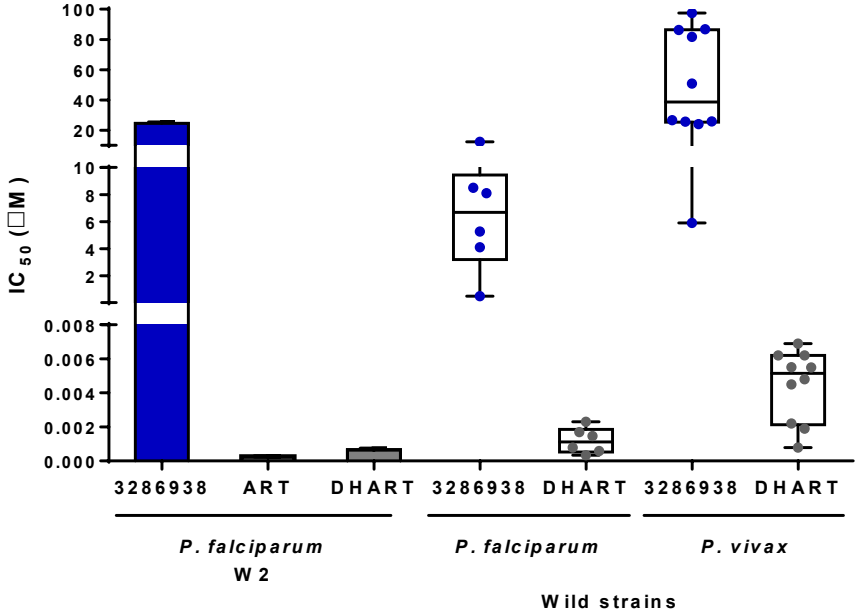


**FIGURE 2 -** Evaluation of *in vitro* and *ex vivo* activity of 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one) against *Plasmodium falciparum* (W2) and *Plasmodium vivax*. Note: 3286938= 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one); ART= artemisinin, reference drug used in the treatment of malaria; DHART= dihydroartemisinin, reference drug used in the treatment of malaria; *P. falciparum* (W2)= adapted laboratory strain; *P. vivax*= *P. vivax* strains isolated from patients; *P. falciparum*= *P. falciparum* strains isolated from patients. The $IC_{50}$ values for *in vitro* assays were obtained through the means and standard deviations of three independent experiments. The $IC_{50}$ values for the *ex vivo* tests with 3286938 and DHART were obtained using the median with a sample number of 10 patients for *P. vivax* and 6 patients for *P. falciparum*.

**TABLE I -** *In vitro* antimalarial activity

| Compound | W2 Strains | Wild Strains | | Toxicity | | Selectivity index | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (µM) (±) | $IC_{50}$ (µM) (MD quartile) | | $CC_{50}$ (µM) (±) | | HepG2 | | | VERO | | |
| | *P. falciparum* | *P. falciparum* | *P. vivax* | HepG2 | VERO | $SI^a$ | $SI^b$ | $SI^c$ | $SI^a$ | $SI^b$ | $SI^c$ |
| 3286938 | 24.4 ± 1.4 | 6.7 MD 4 | 38.7 MD 59.4 | > 500 | > 500 | > 20.5 | > 74.6 | > 12.9 | > 20.5 | > 74.6 | > 12.9 |
| ART | 0.00026 ± 0.0 | NT | NT | > 500 | > 500 | >1.9 | NC | NC | >1.923 | NC | NC |
| DHART | 0.00065 ± 0.0 | 0.0011 MD 0.001 | 0.0051 MD 0.003 | 32.05 ± 5 | 170.35 ± 1.34 | 49.3 | 29.1 | 6.284 | 26.2 | 154.9 | 33.4 |

$IC_{50}$, $CC_{50}$ and SI values for the compound 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one) and reference drugs against *Plasmodium falciparum* (W2) and circulating strains of *Plasmodium vivax* and *Plasmodium falciparum*.

Note: $IC_{50}$= Inhibitory Concentration of 50% of the parasites; $CC_{50}$= Cytotoxic concentration for 50% of the cell population. SI= Selectivity index (ratio between $CC_{50}/IC_{50}$); = average of $IC_{50}$ and $CC_{50}$; NT= not tested; NC= not calculated; quartile= median of $IC_{50}$ of circulating *P. falciparum* and *P. vivax* strains and interquartile range; ± standard deviation of the average of three experiments. 3286938= 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one), ART= artemisinin, DHART= dihydroartemisinin. $SI^a$= Selectivity index calculated for *P. falciparum* (W2). $SI^b$= Selectivity index calculated for circulating strains of *P. falciparum*. $SI^c$= Selectivity index calculated for circulating strains of *P. vivax*.

The compound 3286938 demonstrated an absence of toxicity at concentrations below 500 µM against HepG2 and VERO cell lines; the same result was observed for ART, with 100% cell viability at a concentration of 500 µM. For the reference drug DHART, the $CC_{50}$ value for HepG2 was 32.05 (± 5) and for VERO it was 170.35 (± 1.34) (Table I).

According to Table I, the selectivity index (SI) of the compound 3286938 was greater than 20.5 for both cells against the W2 strain in *in vitro* assays. For circulating strains of *P. falciparum,* the SI value was > 74.6.6 for HepG2 and VERO cells, and against *P. vivax* the SI value was >12.9 for both strains. Artemisinin, used as a control drug, obtained an SI value >1.923 (HepG2, VERO); this drug has not been tested against circulating strains. For the reference drug, DHART, the SI *in vitro* was 49.307 against HepG2 and 26.207 against VERO; for circulating strains of *P. falciparum* the SI obtained was 29.136 (HepG2) and 154.9 (VERO), and for *P. vivax,* SI of 6.284 (HepG2) and 33.401 (VERO) were obtained. Compound 3286938 was not considered hemolytic at a concentration of 1000 µM, the highest concentration used for the hemolysis assay.

The circulating *P. vivax* isolates showed $IC_{50}$ values for compound 3286938 ranging from 5.9 µM to 97.43 µM; the reference drug DHART ranged from 0.00078 to 0.0069 µM and 100% of the patients were male with ages ranging from 21 to 57 years old. Among the *P. falciparum* isolates, there was a smaller number of infected patients, with variation in the $IC_{50}$ values for DHART from 0.00034 µM to 0.0023 µM. For the compound 3286938, $IC_{50}$ varied between 0.49 µM and 12.3 µM. Of six isolated patients, four were male and two were female, with ages ranging from 21 to 57 years old (Table II).

Upon comparison of the incubation time of the isolates from the two species, it is noted that the incubation time for *P. falciparum* was shorter than that of *P. vivax*, due to the fact that *P. falciparum* has an adapted culture that is well described in the literature (Trager, Jensen, 1976), while *P. vivax,* in addition to having few previous studies, is difficult to maintain in a continuous culture both *in vitro* and *ex vivo* (Rangel *et al.,* 2018).

**TABLE II -** Profile of patients infected with *P. falciparum* and *P. vivax* with lower sensitivity of the compound 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one) and Dihydroartemisinin detected by the *ex vivo* assay isolated from patients seen at CEPEM/RO in the state of Rondônia, from June 2017 to October 2018

| Patient ID | Gender/ age | Parasites/ µL | (% of Trophozoites at 0h) | Mature schizonts (%) | Incubation time (h) | $IC_{50}$ µM | |
|---|---|---|---|---|---|---|---|
| | | | | | | DHART | 3286938 |
| ***Plasmodium vivax*** | | | | | | | |
| 170612 (01) | M/35 | 3000 | 94 | 56 | 24 | 0.0055 | 24 |
| 170711 (01) | M/41 | 3000 | 93 | 40 | 24 | 0.00078 | 97.43 |
| 170717 (02) | M/24 | 3000 | 93 | 66 | 24 | 0.00551 | 50.84 |
| 180202 (01) | M/51 | 2958.4 | 98.6 | 78 | 24 | 0.0062 | 86.8 |
| 180221 (01) | M/45 | 3000 | 100 | 40 | 48 | 0.0048 | 86.3 |
| 180312 (01) | M/43 | 2966.7 | 98.9 | 40 | 24 | 0.0019 | 5.9 |
| 180312 (02) | M/21 | 3000 | 100 | 40 | 30 | 0.0022 | 81.8 |
| 180328 (01) | M/37 | 3000 | 100 | 40 | 32 | 0.0062 | 25.7 |
| 180424 (01) | M/36 | 3000 | 100 | 45 | 36 | 0.0069 | 25.8 |
| 181015 (01) | M/57 | 3000 | 100 | 58 | 40 | 0.0045 | 26.61 |
| ***Plasmodium falciparum*** | | | | | | | |
| 170614 (01) | M/30 | 3000 | 100 | 40 | 24 | 0.00078 | 5.27 |
| 170629 (02) | F/57 | 3000 | 100 | 40 | 24 | 0.0023 | 12.3 |
| 180730 (01) | F/36 | 3000 | 100 | 51 | 24 | 0.0017 | 8.1 |
| 180802 (01) | M/19 | 3000 | 100 | 45 | 24 | 0.00034 | 8.5 |
| 180815 (01) | M/51 | 2996.4 | 99.9 | 57 | 24 | 0.00146 | 4.1 |
| 180912 (01) | M/21 | 3000 | 100 | 40 | 27 | 0.00058 | 0.49 |

Note: ID= Code; $IC_{50}$= Inhibitory Concentration of 50% of the parasites; DHART= dihydroartemisinin; 3286938= 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one).

## DISCUSSION

Antimalarial activity of phenylpropene derivatives is poorly described in the literature. This class was studied by Palaniswamy *et al.* (2010); the authors mention that the ethanolic extract of *Trigonella foenum-graecum L.* showed $IC_{50}$ values of 8.75 ± 0.35 µg/mL and 10.25 ± 0.35 µg/mL against chloroquine-sensitive and resistant strains, respectively. According to the authors, after a phytochemical screening, tannin-phenolic compounds were found in the ethanolic extract; these compounds may have contributed to the antiplasmodial activity of *Trigonella foenum-graecum L*. The majority of phenolic compounds, like 3286938, the target of this study, are derived from plants' metabolic pathways of phenylpropanoids.

A complementary study on antimalarial activity developed by Sharma *et al.* (2011), with methoxylated phenylpropenes against Dd2 (CQ-Resistant) *P. falciparum*, describes promising Inhibitory Concentrations for two phenylpropene derivatives with $IC_{50}$ values ranging from 4 to 6.8 µM. The results described by these authors, as well as the results observed by our group, with the derivative 3286938 ($IC_{50}$ 24.4 µM) against the W2 (CQ-Resistant) strain of *P. falciparum* illustrate the importance of this class of molecules in the search for new antimalarial prototypes.

Results of *ex vivo* screening with 3286938 against isolates from the region such as *P. falciparum* ($IC_{50}$ of 6.7 µM) and *P. vivax* ($IC_{50}$ of 38.7 µM) reinforce that this synthetic compound is a candidate for structural analysis seeking to improve and diversify a series of new derivatives.

Regarding studies involving the movement of the methoxy group in research on antimalarial activity, Liu, Wilairat, Go (2001) shows the antiplasmodial activity of the compound 1-(2,3,4-trimethoxyphenil)-3-(3-quinolinyl)-2-propen-1-one, with an $IC_{50}$ value of 2 µM against the K1 Chloroquine-resistant strain of *P. falciparum*. Casertano *et al.* (2020), like the authors mentioned above, worked with the methoxy group, more specifically with trimethoxybenzene, inserting substituents in the benzyl side chain. The results demonstrate $IC_{50}$ values from 5.02 to 0.81 µM and SI ranging from 45 to 11.1 against *P. falciparum* (CQ-Resistant) and HMEC-1 cells (human microvascular endothelial cells). The molecule from the present study, 3286938 1-(3-benzyloxy-4-methoxy-phenyl)-3-(3,4,5-trimethoxy-phenyl)-propan-1-one), which contains trimethoxy benzene as one of its groups, did not present such low $IC_{50}$ values; however, the SI values for HepG2 and VERO cells were higher than 20.5 when antimalarial activity against the W2 strain was considered; an SI value of 74.6 was observed for circulating strains of *P. falciparum* and an SI of 12.9 for circulating strains of *P. vivax*.

Regarding the structure of the molecules evaluated in the study by Yadav *et al.* (2012) and the compound 3286938, one similarity is the presence of a 3,4,5-trimethoxyphenyl group as a radical of the main molecules from the cited studies. Yadav *et al.* (2012) worked with chalcone derivatives, where the methoxy groups were inserted in different positions. Notably, 1-(4-benzimidazol-1-yl-phenyl)-3-(2,4-dimethoxy-phenyl)-propen-1-one presented an $IC_{50}$ value of 1.1 µg/mL against the 3D7 (CQ-sensitive) strain of *P. falciparum*. The authors concluded that the presence of two methoxy groups in positions 2 and 4 was excellent for antimalarial activity; in the sequence they had 3.4 dimethoxy and 2.5 dimethoxy with moderate activity. However, the 3,4,5-trimethoxy segment showed weaker activity, probably because it caused steric impediment in binding to the active site of the cysteine protease enzyme studied. This information indicates the possibility of more precise future investigations regarding structure–activity relationship (SAR) studies on the molecule 3286938, which presents the trimethoxy group in the same position as the study by Yadav *et al.* (2012).

When analyzing the results of the *ex vivo* antiplasmodial assays, it was observed that the circulating strains of *P. falciparum* and *P. vivax* had an $IC_{50}$ within expectations for the region in relation to DHART, with sensitivity for both (Aguiar *et al.,* 2014). However, the $IC_{50}$ of the circulating strain of *P. falciparum* showed 4x less inhibition of parasitic growth than the W2 strain of the same species. It is noted that the W2 strain has been adapted to culture for years (Oduola *et al.,* 1998), which is why these plasmodia naturally survive better in the mimetic conditions of the culture media and supplements, unlike the newly isolated strain. Another factor that influences the discrepancy between the $IC_{50}$

values is the fact that the antiplasmodial assay using the Sybr Green I technique (Smilkstein *et al*., 2004) takes into account parasitic growth as a function of the mass of DNA produced in the schizogony process was performed with W2 which lasted for 48 hours, while the schizont maturation test was performed in 24-27 hours with different parasitemias.

The literature points to the importance of the search for new antimalarial molecules due to the development of resistant strains. In this respect, *P. vivax* stands out for, despite few cases, already showing a loss of sensitivity to antimalarials in Brazil and worldwide (Brega *et al.,* 2005; Suwanarusk *et al.,* 2007). In this study, we observed that despite having a median of 38 μM against *P. vivax*, this was a pioneer study carried out with this class of molecules in the Amazon region as well as in the state of Rondônia. With attention increasing to finding new compounds with antimalarial effects, effective and low-cost synthetic molecules have become the target of antimalarial chemotherapy. The synthesis of derivatives of phenylpropanoids and substances that present the 3,4,5-trimethoxyphenyl group follows this pattern. The promising results obtained in this study demonstrate the potential of these substances in the development of new antimalarials.

Thus, we conclude that the molecule in question, 3286938, is a potential prototype for chemical improvement aiming to further increase the antiplasmodial activity against the two *Plasmodium* species tested and improve the cytotoxicity score and thus succeed in the *in vivo* and mechanism of action tests.

## CONCLUSIONS

The results obtained with compound 3286938 showed antimalarial activity against W2 *P. falciparum in vitro* tests and *ex vivo* against circulating strains of *P. falciparum* and *P. vivax* in Porto Velho, Rondônia. In addition, it is possible to observe this molecule's absence of cytotoxicity against HepG2 and VERO cells, which demonstrates possible specificity of this compound, highlighting it as an interesting prototype for further *in vitro* and *in vivo* investigations against protozoa that cause malaria.

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## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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