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Short communication

Synthesis and antimalarial activity of semicarbazone and thiosemicarbazone derivatives

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Abstract

Seventeen semicarbazone and thiosemicarbazone derivatives were prepared and tested in vitro against a chloroquine resistant strain of *Plasmodium falciparum* (W2) to evaluate their antiplasmodial potential. Three thiosemicarbazones were found to be active against the parasite and non-toxic to human peripheral blood mononuclear cells (PBMC). Among these, compound **5b** presented the lowest IC_{50} value against *P. falciparum* (7.2 μM) and was the least toxic in the PBMC proliferation assay ($IC_{50} = 73.5$ μM). It was selected for in vivo tests on mice infected with *Plasmodium berghei* (strain NK-65). The thiosemicarbazone **5b** was able to reduce the parasitaemia by 61% at 20 mg/kg on day 7 after infection without any sign of toxicity to the animals. In comparison, the standard drug chloroquine at 15 mg/kg showed a reduction around 95%. These in vitro and in vivo results make **5b** an interesting lead for further development.

Keywords: Semicarbazone; Thiosemicarbazone; Antiplasmodial; PBMC

1. Introduction

Malaria is endemic in over 100 countries, affecting especially tropical areas of Africa, Asia and Latin America. It is responsible for high mortality and morbidity in these regions, being especially lethal to children and pregnant women. Additionally, malaria is a risk for travelers and immigrants, with imported cases increasing in non-endemic areas. According to World Health Organization up to one million people die of malaria worldwide, every year [1].

Human malaria is caused by four different species of *Plasmodium*: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. The most severe form is caused by *P. falciparum*, which leads the death of about 1% of infected patients. The mortality among non-immune untreated travelers is significantly higher [1]. Treatment and control have become more difficult due to lack of vaccines, spread of drug-resistant parasites and insecticide-resistant mosquitoes [1]. In order to control this disease new antimalarial drugs have to be developed.

Semicarbazones and thiosemicarbazones are of considerable pharmacological interest since a number of derivatives have shown a broad spectrum of chemotherapeutic properties

Abbreviations: PBMC, peripheral blood mononuclear cells; PHA, phytohaemaglutinin.

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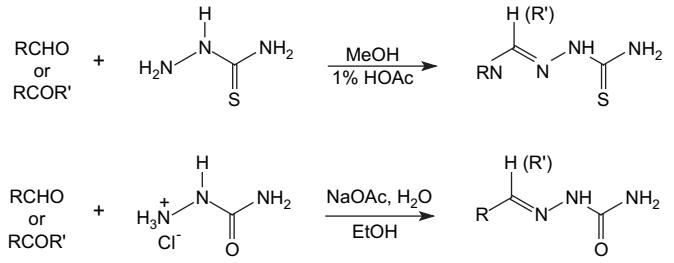
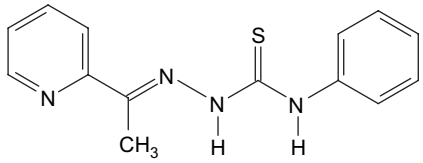


Scheme 1. Synthetic routes for thiosemicarbazones and semicarbazones.

[2]. Antimalarial activity of thiosemicarbazone **1** has been previously reported [3]. Indeed, many thiosemicarbazones exhibit promising anti-protozoan activity through the inhibition of cysteine proteases and other targets [4–7].



1

In this study we reported the synthesis of a series of semicarbazone and thiosemicarbazone derivatives. They were evaluated for their antiplasmodial activity and also for toxicity to human peripheral blood mononuclear cells (PBMC). The most promising compound was tested in mice infected with *Plasmodium berghei*.

2. Chemistry

The semicarbazones and thiosemicarbazones were synthesized in one step by the reaction of corresponding aldehyde or ketone with semi- or thiosemicarbazide [4,8] (Scheme 1).

The compounds prepared and their respective yields are presented in Table 1.

3. Pharmacology

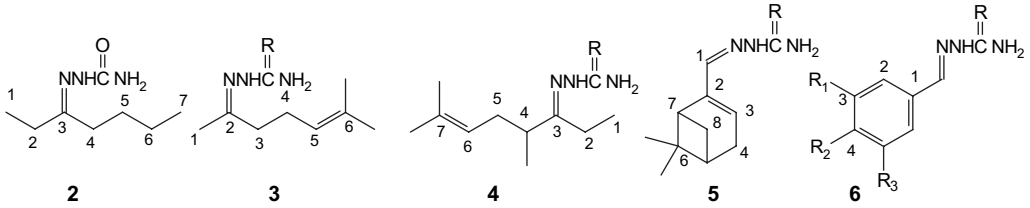
Compounds **2**–**6j** were assayed in vitro at 20 μg/mL against *P. falciparum* chloroquine resistant parasites (W2 strain) [9,10]. The inhibition of parasite growth was evaluated by comparison of [^{3}H]-hypoxanthine incorporation levels between treated infected blood and untreated controls. The results are expressed as percentage of inhibition of parasite growth after treatment with each compound.

All compounds were also tested in vitro with human PBMC stimulated with phytohemaglutinin (PHA) as previously described [11] and proliferation evaluated by MTT method. Dexamethasone was used as a positive control.

The IC_{50} values, concentrations that cause 50% inhibition of *P. falciparum* growth or 50% inhibition of PBMC proliferation, were evaluated for those compounds showing antiplasmodial activity above 50% at 20 μM.

The thiosemicarbazone **5b** was the most effective derivative in the in vitro assays and was thus selected for tests in mice infected with the NK-65 strain of *P. berghei* at doses of 5, 10 and 20 mg/kg, administered orally by gavage. The

Table 1
Chemical structures and reaction yields of synthetic semicarbazones and thiosemicarbazones



2 **3** **4** **5** **6**

Compound	R	R_1	R_2	R_3	Yield (%)
2	–	–	–	–	54
3a	O	–	–	–	81
3b	S	–	–	–	68
4a	O	–	–	–	64
4b	S	–	–	–	82
5a	O	–	–	–	86
5b	S	–	–	–	53
6a	O	H	$(CH_3)_2N$	H	70
6b	S	H	$(CH_3)_2N$	H	54
6c	O	H	OMe	H	48
6d	S	H	OMe	H	86
6e	O	OMe	OH	H	65
6f	S	OMe	OH	H	70
6g	O	OMe	OMe	H	50
6h	S	OMe	OMe	H	84
6i	O	OMe	OMe	OMe	74
6j	S	OMe	OMe	OMe	86

Table 2
In vitro effect of semicarbazone and thiosemicarbazone derivatives on the growth of *Plasmodium falciparum* (W2 strain) and on the proliferation of human lymphocytes stimulated with PHA

Compound[a]	% Inhibition of parasite growth[b]	% Proliferation of PBMC vs control[c]
2	Inactive	89 ± 28*
3a	Inactive	107 ± 18
3b	*100 ± 0.05*	*98 ± 12*
4a	Inactive	0 ± 11
4b	*99 ± 0.64*	60 ± 9*
5a	Inactive	110 ± 14
5b	*95 ± 1*	76 ± 5*
6a	Inactive	84 ± 23*
6b	45 ± 6	98 ± 4
6c	38 ± 14	141 ± 61
6d	Inactive	90 ± 19
6e	Inactive	116 ± 32
6f	23 ± 12	95 ± 4
6g	32 ± 6	80 ± 18
6h	Inactive	84 ± 12
6i	Inactive	107 ± 29
6j	Inactive	78 ± 5*

*Statistically different from the control ($p < 0.005$).
[a] All compounds were tested at 20 μg/mL.
[b] Mean values of two independent experiments ± standard deviation.
[c] Mean values of four independent experiments ± standard deviation.

antimalarial activity was evaluated by counting the infected red blood cells in blood smears taken at days 5 and 7 after parasite inoculation. Results were expressed as the percentage of parasitaemia reduction in comparison with the non-treated group.

4. Results and discussion

The results of the bioassays are summarized in Table 2.

Only the thiosemicarbazones **3b**, **4b** and **5b** were active against *P. falciparum*. The lack of activity of the corresponding semicarbazones (**3a**, **4a** and **5a**) as well as all aromatic derivatives (**6a**–**j**) was taken as an evidence that the combination of both the thiocarbonyl functionality and the aliphatic moiety are critical for the observed antiplasmodial activity. When tested at 20 μg/mL in the PBMC assay, compounds **3b**, **4b**, and **5b** showed only weak inhibitory effect on the PHA-stimulated blastogenesis. A negative result in this assay can be interpreted as an indication of lack of cytotoxic effects to normal human cells in a high replication rate [11]. In order to better compare the antiplasmodial activity and the toxicity to PBMC of these compounds, their IC_{50} values were determined (Table 3).

Table 3
IC_{50} values of selected compounds in the assays with *Plasmodium falciparum* (W2 strain) and human PBMC stimulated with PHA

Compound	Antiplasmodial activity IC_{50} (μM)[a]	PBMC proliferation IC_{50} (μM)[b]
3b	12.6 ± 0.3	35.2 ± 2.1[c]
4b	9.2 ± 0.3	31.3 ± 1.3
5b	7.2 ± 0.09	73.5 ± 4.3
Chloroquine	0.20 ± 0.01	5.1 ± 1.2

[a] IC_{50} represents the concentration of drug able to inhibit by 50% the in vitro the parasitaemia.
[b] IC_{50} represents the concentration of drug able to inhibit by 50% the lymphocyte proliferation in PBMC stimulated with phytohemaglutinin A.
[c] The results are expressed as the mean ± standard error of three independent experiments.

Table 4
Antimalarial activity of thiosemicarbazone **5b** on BALB/c mice infected with *Plasmodium berghei* (NK-65 strain)

Compound	Dose (mg/kg)	Parasitaemia reduction (%)[a]	
		Day 5 after parasite inoculation	Day 7 after parasite inoculation
5b	5	0[b]	8[b]
	10	36.0 ± 5.7[c]	32.0 ± 13.0[c]
	20	77.5 ± 17.7[c]	61.0 ± 4.2[c]
Chloroquine	15	93.0 ± 2.8[c]	94.5 ± 4.7[c]

[a] Calculated as percentage in relation to the parasitaemia in the non-treated control group ($N = 5$).
[b] Value from one experiment.
[c] Mean values of two independent experiments ± standard error.

Compound **5b** exhibited the lowest IC_{50} value against *P. falciparum* (7.2 μM) and the highest IC_{50} value in the PBMC proliferation assay (73.5 μM) (Table 3) and was then selected for tests in mice infected with the NK-65 strain of *P. berghei*. Then, **5b** was tested at doses of 5, 10 and 20 mg/kg, administered orally. Results are summarized in Table 4.

Compound **5b** reduced the parasitaemia by 61% at 20 mg/kg on day 7 after infection, without any signal of toxicity to the mice. Under the same conditions, mice treated with chloroquine at 15 mg/kg had their parasitaemia reduced by 95%.

5. Conclusion

In conclusion, thiosemicarbazones **3b**, **4b** and **5b** displayed significant inhibition of *P. falciparum* in vitro. Compound **5b** showed considerable activity in mice infected with *P. berghei* while causing no significant toxic effects to human PBMC or to the treated mice. These results make compound **5b** an interesting lead for drug development and encourage further investigation on thiosemicarbazone derivatives of naturally occurring aldehydes and ketones aiming at the discovery of more potent antimalarial compounds.

6. Experimental

6.1. Chemistry

All melting points were determined on Electrothermal IA9000 series digital melting point apparatus and are uncorrected. NMR spectra were measured in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard with a Bruker Avance 200 instruments. Chemical shifts are given in δ scale and J values are given in hertz. All starting materials were commercially available research grade chemicals and used without further purification. The semicarbazone and thiosemicarbazone derivatives were prepared essentially as reported [4,8].

6.1.1. Data for heptan-3-one semicarbazone ***2*** *[12]*

M.p.: 105–105.6 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.08–9.05 (m, 1H, –N*H*–), 6.17 (s, 2H, –NH_2), 2.28–2.11 (m, 4H, 2 × H-2 and 2 × H-4), 1.53–1.18 (m, 4H, 2 × H-5 and 2 × H-6), 1.04–0.83 (m, 6H, 2 × CH_3).

6.1.2. Data for 6-methylhept-5-en-2-one semicarbazone ***3a*** *[13]*

M.p.: 129.9–131.4 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.90 (s, 1H, –N*H*–), 6.17 (s, 2H, –NH_2), 5.08 (s, 1H, H-5), 2.16 (m, 4H, 2 × H-3 and 2 × H-4), 1.76 (s, 3H, –CH_3), 1.64 (s, 3H, –CH_3), 1.57 (s, 3H, –CH_3).

6.1.3. Data for 6-methylhept-5-en-2-one thiosemicarbazone ***3b***

M.p.: 87.7–88.7 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.91 (s, 1H, –N*H*–), 8.03 (s, 1H, –N*H*–), 7.44 (s, 1H, –N*H*–), 5.07 (br s, 1H, H-5), 2.25–2.13 (m, 4H, 2 × H-3 and 2 × H-4), 1.88 (s, 3H, –CH_3), 1.63 (s, 3H, –CH_3), 1.57 (s, 3H, –CH_3); HR-ESI-MS $[M+H]^+$ 200.1612 ($C_9H_{17}N_3S$ requires 200.1221).

6.1.4. Data for 4,7-dimethyloct-6-en-3-one semicarbazone ***4a*** *[14]*

M.p.: 57.7–59.1 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.02 (s, 1H, –N*H*–), 6.18 (s, 2H, –NH_2), 5.09–5.02 (m, 1H, H-6), 2.36–1.94 (m, 5H, 2 × H-2, H-4 and 2 × H-5), 1.64 (s, 3H, –CH_3), 1.56 (s, 3H, –CH_3), 1.99 (d, 3H, $J_{CH_3,H4}$ = 6.6 Hz, –CH_3), 0.93 (t, 3H, $J_{CH_3,H2}$ = 7.6 Hz, –CH_3).

6.1.5. Data for 4,7-dimethyloct-6-en-3-one thiosemicarbazone ***4b***

M.p.: 153.2–153.8 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.79 (s, 1H, –N*H*–), 7.23 (s, 1H, –N*H*–), 6.69 (s, 1H, –N*H*–), 5.01 (t, 1H, $J_{H6,H5}$ = 6.4 Hz, H-6), 2.58–1.99 (m, 5H, 2 × H-2, H-4 and 2 × H-5), 1.66 (s, 3H, –CH_3), 1.57 (s, 3H, –CH_3), 1.12–0.97 (m, 6H, 2 × –CH_3); ESI-MS $[M+H]^+$ 228.3.

6.1.6. Data for 6,6-dimethylnorpin-2-ene-2-carboxaldehyde semicarbazone ***5a*** *[15]*

M.p.: 192.9–197.3 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.93 (s, 1H, –C*H*=N–), 7.46 (s, 1H, –N*H*–), 6.21 (s, 2H, –NH_2), 5.81 (s, 1H, H-3), 2.99 (m, 1H, H-7), 2.45–2.35 (m, 3H, 2 × H-4 and H-8), 2.10 (br s, 1H, H-5), 1.30 (s, 3H, –CH_3), 1.03 (d, 1H, $J_{H7',H7}$ = 8.7 Hz, H-8′), 0.75 (s, 3H, –CH_3).

6.1.7. Data for 6,6-dimethylnorpin-2-ene-2-carboxaldehyde thiosemicarbazone ***5b***

M.p.: 156.1–157.4 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.12 (s, 1H, –C*H*=N–), 8.02 (s, 1H, –N*H*–), 7.68 (s, 1H, –N*H*), 7.55 (s, 1H, –N*H*), 5.97 (br s, 1H, H-3), 3.07 (m, 1H, H-7); 2.45–2.36 (m, 3H, 2 × H-4 and H-8), 2.11 (br s, 1H, H-5), 1.31 (s, 3H, –CH_3), 1.03 (d, 1H, $J_{H7',H7}$ = 8.8 Hz, H-8′), 0.74 (s, 3H, –CH_3); HR-ESI-MS $[M+H]^+$ 224.1525 ($C_{11}H_{17}N_3S$ requires 224.1221).

6.1.8. Data for 4-(dimethylamino)benzaldehyde semicarbazone ***6a*** *[16]*

M.p.: 233.9–236.6 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.97 (s, 1H, –C*H*=N–), 7.72 (s, 1H, –N*H*–), 7.50 (d, 2H, $J_{H2,H3}$ = 8.6 Hz, 2 × H-2), 6.68 (d, 2H, $J_{H3,H2}$ = 8.6 Hz, 2 × H-3), 6.35 (s, 2H, –NH_2), 2.93 (s, 6H, 2 × –CH_3).

6.1.9. Data for 4-(dimethylamino)benzaldehyde thiosemicarbazone ***6b*** *[16]*

M.p.: 199–201.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.17 (s, 1H, –C*H*=N–), 7.99 (br s, 1H, –N*H*–), 7.93 (s, 1H, –N*H*–), 7.75 (br s, 1H, –N*H*–), 7.57 (d, 2H, $J_{H2,H3}$ = 8.6 Hz, 2 × H-2), 6.68 (d, 2H, $J_{H3,H2}$ = 8.6 Hz, 2 × H-3), 2.95 (s, 6H, 2 × –CH_3).

6.1.10. Data 4-methoxybenzaldehyde semicarbazone ***6c*** *[17]*

M.p.: 195–197 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.07 (s, 1H, –C*H*=N–), 7.80 (s, 1H, –N*H*–), 7.61 (d, 2H, $J_{H2,H3}$ = 8.8 Hz, 2 × H-2), 6.92 (d, 2H, $J_{H3,H2}$ = 8.8 Hz, 2 × H-3), 6.42 (br s, 2H, –NH_2), 3.74 (s, 3H, –OCH_3).

6.1.11. Data for 4-methoxybenzaldehyde thiosemicarbazone ***6d*** *[18]*

M.p.: 166.1–167.1 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.35 (s, 1H, –C*H*=N–), 8.14 (br s, 1H, –N*H*–), 8.03 (s, 1H, –N*H*), 7.94 (br s, 1H, –N*H*), 7.76 (d, 2H, $J_{H2,H3}$ = 8.7 Hz, 2 × H-2), 6.98 (d, 2H, $J_{H3,H2}$ = 8.7 Hz, 2 × H-3), 3.81 (s, 3H, –OCH_3).

6.1.12. Data for 4-hydroxy-3-methoxybenzaldehyde semicarbazone ***6e*** *[17]*

M.p.: 218.3–219.4 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.08 (s, 1H, –C*H*=N–), 9.33 (s, 1H, –N*H*–), 7.73 (s, 1H, –O*H*), 7.38 (d, 1H, $J_{H2,H6}$ = 1.7 Hz, H-2), 6.98 (dd, 1H, $J_{H6,H2}$ = 1.7 Hz and $J_{H6,H5}$ = 8.1 Hz, H-6), 6.75 (d, 1H, $J_{H5,H6}$ = 8.1 Hz, H-5), 6.50 (br s, 2H, –NH_2), 3.81 (s, 3H, –OCH_3).

6.1.13. Data for 4-hydroxy-3-methoxybenzaldehyde thiosemicarbazone ***6f*** *[19]*

M.p.: 187.3–188.8 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.26 (s, 1H, –C*H*=N–), 9.46 (s, 1H, –N*H*–), 8.11 (s, 1H, –O*H*), 7.93 (br s, 2H, –NH_2), 7.47 (s, 1H, H-2), 7.03 (d, 1H, $J_{H6,H5}$ = 8.0 Hz, H-6), 6.77 (d, 1H, $J_{H5,H6}$ = 8.0 Hz, H-5), 3.82 (s, 3H, –OCH_3).

6.1.14. Data for 3,4-dimethoxybenzaldehyde semicarbazone ***6g*** *[20]*

M.p.: 179.7–180.5 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.12 (s, 1H, –C*H*=N–), 7.76 (s, 1H, –N*H*–), 7.43 (d, 1H, $J_{H2,H6}$ = 1.6 Hz, H-2), 7.07 (dd, 1H, $J_{H6,H2}$ = 1.6 Hz, $J_{H6,H5}$ = 8.3 Hz, H-6), 6.92 (d, 1H, $J_{H5,H6}$ = 8.3 Hz, H-5), 6.52 (br s, 2H, –NH_2), 3.81 (s, 3H, –OCH_3), 3.77 (s, 3H, –OCH_3).

6.1.15. Data for 3,4-dimethoxybenzaldehyde thiosemicarbazone ***6h*** *[19]*

M.p.: 193.2–195 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.32 (s, 1H, −C*H*═N−), 8.16 (br s, 1H, −N*H*−), 8.02 (br s, 1H, −N*H*), 7.97 (s, 1H, −N*H*), 7.51 (d, 1H, $J_{H2,H6} = 1.5$ Hz, H-2), 7.13 (dd, 1H, $J_{H6,H2} = 1.5$ Hz, $J_{H6,H5} = 8.3$ Hz, H-6), 6.94 (d, 1H, $J_{H5,H6} = 8.3$ Hz, H-5), 3.81 (s, 3H, −OCH_3), 3.78 (s, 3H, −OCH_3).

6.1.16. Data for 3,4,5-trimethoxybenzaldehyde semicarbazone ***6i*** *[21]*

M.p.: 202.4–203.4 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.23 (s, 1H, −C*H*═N−), 7.77 (s, 1H, −N*H*−), 7.00 (s, 2H, 2 × H-2), 6.58 (br s, 2H, −NH_2), 3.79 (s, 6H, 2 × −OCH_3), 3.65 (s, 3H, −OCH_3).

6.1.17. Data for 3,4,5-trimethoxybenzaldehyde semicarbazone ***6j*** *[22]*

M.p.: 187.9–188.2 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.44 (s, 1H, −C*H*═N−), 8.23 (br s, 1H, −N*H*−), 8.10 (br s, 1H, −N*H*), 7.95 (s, 1H, −N*H*), 7.08 (s, 2H, 2 × H-2), 3.82 (s, 6H, 2 × −OCH_3), 3.67 (s, 3H, −OCH_3).

6.2. In vitro antimalarial assay

The assay was based on the procedures previously described [9,10]. Briefly, trophozoite stages of *P. falciparum* in sorbitol-synchronized culture were grown at 2% parasitaemia and 2.5% haematocrit [9]. The culture was diluted with culture medium (RPMI 1640) without hypoxanthine and incubated with the compounds at 20 μg/mL final concentration for screening and at various concentrations for IC_{50} determination. Controls without drugs and with chloroquine were run in parallel. Inhibition of parasite growth was evaluated by comparison of [^{3}H]-hypoxanthine incorporation levels between treated and untreated experiments [10]. The half-maximal inhibitory concentration (IC_{50}) was estimated by curve fitting using the Microcal software (Origin Software, Inc., Northampton, MA, USA). All assays were performed in triplicate.

6.3. In vivo antimalarial assay

The suppressive test described by Peters [23] and modified by Carvalho et al. [24], was performed in mice infected with *P. berghei*, strain NK-65. Briefly, BALB/c mice were infected via intraperitoneal injection of 10^5 infected red blood cells. Animals were randomly separated into groups of five for each treatment and the drugs were administered daily for 4 consecutive days with compound **5b** at doses of 5, 10 and 20 mg/kg, administered in a 0.2 mL volume per animal, via gavage. Controls included a non-treated group and a group that received the standard antimalarial drug chloroquine (as its diphosphate salt) at 15 mg/kg. Antimalarial activity was evaluated by counting the infected red blood cells in blood smears taken at days 5 and 7 after parasite inoculation. Results were expressed as a percentage of parasitaemia reduction in comparison with the non-treated group. Animal use was approved by the Animal Experimentation Ethics Committee (CEUA-P0094-01 − Fundação Oswaldo Cruz).

6.4. Lymphocyte assays

Assay with peripheral blood mononuclear cells (PBMC) was run as previously, with modifications [11]. Shortly, PBMC of health adults were separated by Ficoll gradient. PBMC (1.5×10^5 cells per well) were cultured in RPMI 1640 medium, supplemented with 5% (v/v) heat-inactivated, pooled AB serum and 2 mM L-glutamine and antibiotic/antimicotic solution containing 1000 U/ml penicillin, 1000 μg/ml streptomycin and 25 μg/ml fungisone in flat bottomed microtiter plates. The cultures were stimulated with 2.5 μg/ml of phytohemaglutinin (PHA) in presence of different compounds at a final concentration of 20 μg/mL and incubated for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2. Proliferation was quantified using MTT. Dexamethasone was used as positive control in this assay. PBMC assay were performed in triplicate.

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