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Synthesis and antiplasmodial activity of new 4-aryl-2-trichloromethylquinazolines

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Abstract—A series of original 4-aryl-substituted 2-trichloromethylquinazoline derivatives was synthesized using a microwave-assisted Suzuki-Miyaura cross-coupling approach. Antiplasmodial activity was evaluated on both chloroquino-resistant and -sensitive *Plasmodium falciparum* strains, and the selectivity indexes for THP1 and HepG2 human cells were also calculated, revealing their antiplasmodial potential.

Malaria remains one of the most lethal diseases, killing more than 1 million people each year according to the WHO. The therapeutic management of this infectious disease has been considerably hindered because of the emergence of chloroquino-resistant strains of *Plasmodium falciparum*. Moreover, malaria mainly affects developing countries in which treatment cost is a major constraint on medical support. In such a context, it appears essential to identify new molecules with antiplasmodial potential, with a view to developing new pharmacological agents which could contribute to solving this public health challenge.

In continuation of previous studies[1–4] resulting from our research program directed toward the preparation of new azaheterocyclic compounds and their anti-infectious evaluation, we synthesized a series of new tricyclic quinazoline derivatives bearing a trichloromethyl group in position 2, via a microwave-assisted Suzuki-Miyaura cross-coupling reaction.

Certain molecules including a 2-trichloromethyl- substituted quinazoline ring present pharmacological properties valuable for oncology[5,6] and infectiology.[7] Furthermore, other quinazoline-derived molecules appear to be antimalarial agents.[8] In parallel, trichloromethylated structures such as triazine derivatives show the same anti-infectious activity.[9]

We recently developed a microwave-assisted chlorination reaction protocol suitable for α-methylazaheterocycles, and one of the molecules prepared during this work was 4-chloro-2-trichloromethyl-quinazoline **1** (Fig. 1).[10] This molecule, obtained in good yields with

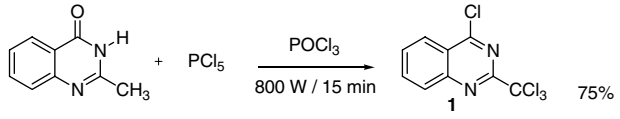

Figure 1. Synthesis of 4-chloro-2-trichloromethylquinazoline **1**.

Keywords: Malaria; Quinazoline; Microwave-assisted chemistry; Suzuki-Miyaura cross-coupling reaction.
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a short reaction time, also presented the advantage of being functionalized in position 4. It was initially evaluated for its antiplasmodial potential, which appeared limited.

We therefore investigated the possibility of using 4-chloro-2-trichloromethylquinazoline **1** as a substrate for a Suzuki-Miyaura cross-coupling reaction, hoping that the substitution of an aryl group for the chlorine atom in position 4 would improve biological activity.

In a recent publication, Connolly and co-workers studied Suzuki-Miyaura cross-coupling reaction from 2-substituted-4-chloroquinazolines, using $Pd(PPh_3)_4$ as a catalyst. This reaction seems to be greatly influenced by the nature of the quinazoline substituent in position 2, varying the reaction yields from 53% to 93%.[11]

A trichloromethyl group was not automatically compatible with such a reaction mainly because of the sensitivity of the trichloromethyl group to alkaline mediums and because of the formation of Csp^3/Csp^3 homocoupling by-products that we observed in nitrotrichloromethylquinoline and trichloromethylbenzoxazole series. These side reactions were also noted in trichloromethylquinoline and trichloromethylbenzene series by Folli and coworkers,[12] in reactions using other metals such as iron.

This cross-coupling reaction was therefore studied with the assistance of a liquid chromatography/mass spectrometry apparatus, affording the possibility of studying the influence of each reaction parameter in an efficient way. All reaction trials were conducted with 4-chloro-2-trichloromethylquinazoline **1** as a substrate and phenylboronic acid as a reagent, in a synthesis microwave oven, inside miniaturized sealed reactors, so as to realize each trial on limited amounts of reagents (Fig. 2).

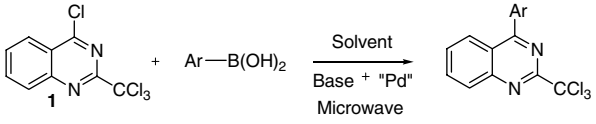


Figure 2. General Suzuki-Miyaura cross-coupling reaction for the preparation of 4-aryl-2-trichloromethylquinazolines from **1**.

Eleven reaction parameters were tested through 50 trials and the best conditions were finally defined (Table 1), permitting the synthesis of 4-phenyl-2-trichloromethylquinazoline **2**[13] with 65% yield in only 2 h, using the simple $Pd(OAc)_2$ catalyst.

Using microwave-assisted general operating procedure, we prepared a series of 4-aryl-2-trichloromethylquinazolines[14] by varying the boronic acid reagents (Table 2). The reaction yields obtained were little influenced by the nature of the boronic acid (from 50% to 65%).

In order to demonstrate that the trichloromethyl group was mainly responsible for the biological activity of our molecules, we evaluated a dehalogenated analog, 2-methyl-4-phenylquinazoline **13**,[13] and we also included 4-phenyl-2-trichloromethylquinazoline **2** in a nucleophilic substitution with methylamine, leading to the amidine *N*,*N*′-dimethyl-4-phenylquinazoline-2-carboximidamide hydrochloride **14**[15] (Fig. 3).

All molecules were assessed for their in vitro antiplasmodial activity against two different strains of *P. falciparum*: W2 chloroquino-resistant and 3D7 chloroquino-sensitive. In order to identify our molecules' potential, a drug compound of reference, chloroquine, was tested in the same conditions. The IC_{50} values were then calculated (Table 2).[2,16,17]

Usually, *gem*-trichloromethylated compounds exert an anti-infectious activity which is directly linked to their toxicity. To synthesize molecules of therapeutic interest, it was necessary for us to determine whether our molecules were presenting a toxic profile on human cells, or if they could offer in vitro selective antiparasitic properties. Thus, the IC_{50} were determined for two different human cell lines (THP1 and HepG2), using doxorubicin as reference of cellular toxicity.[17–19]

Then the selectivity indexes of all compounds and reference drug compounds were calculated (Table 2).[20] Some of the tested molecules emerge as interesting antiplasmodial agents because of their safe and active profile.

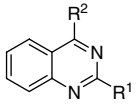
Apart from molecule **12**, these quinazoline derivatives do not display any in vitro toxic properties neither

Table 1. Parameters studied for the Suzuki-Miyaura reaction optimization

Type of parameter studied	Trials	Best reaction conditions
Solvent	DME, dioxane, DMF	DMF
Base	CsF, K_3PO_4, Na_2CO_3, $NaHCO_3$, Cs_2CO_3	Cs_2CO_3
Catalyst	$PdP(Ph_3)_4$, $Pd(OAc)_2$	$Pd(OAc)_2$
Co-catalyst	None or CuI	None
Nb equiv boronic acid (equiv)	1, 1.3, 1.5	1.3
Nb equiv base (equiv)	2, 2.5, 3	2.5
Nb equiv catalyst (%)	2.5, 5	2.5
Temperature (°C)	100, 150, 175	150
M.W. power (W)	100, 150, 200, 250	150
Pressure (bar)	+0, 0.5, 1, 4	+0.5
Time (h)	0.5, 1, 1.5, 2, 2.5, 3	2

Reactions were performed in a Biotage Initiator® monomode microwave reactor.
The analytical LC/MS system used was a Waters Alliance® 2695.

Table 2. Antiplasmodial activity and human cell toxicity of quinazoline derivatives



Drugs	Quinazoline substituents		Antiplasmodial activity[a] (μM)		Human cell toxicity[a] (μM)			W2 strain selectivity index toward HepG2[b]	W2 strain selectivity index toward THP1[b]
	R^1	R^2	W2 IC_{50}	3D7 IC_{50}	MTT (HEP G2) IC_{50}	THP1 IC_{50}	THP1 LC_{50}		
1	CCl_3	Cl	54.5	73.2	>125[e]	>500[e]	>500[e]	>2.3	>9.2
2	CCl_3	Ph	8.0	20.4	>125[e]	125	>500[e]	>15.6	15.6
3	CCl_3	2-CH_3-Ph	Insufficient solubility[f]						
4	CCl_3	4-F-Ph	2.5	3.0	>125[e]	125	500	>50.0	50.0
5	CCl_3	3,4,5-tri-OCH_3-Ph	28.9	23.3	>125[e]	>250[e]	>250[e]	>4.3	>8.7
6	CCl_3	4-Cl-Ph	4.7	2.2	>125[e]	73.2	>500[e]	>26.6	15.6
7	CCl_3	3-CF_3-Ph	40.4	36.6	>125[e]	78.6	>500[e]	>3.1	1.9
8	CCl_3	4-OCH_3-Ph	10.9	25.0	125	250	>250[e]	11.5	22.9
9	CCl_3	2-Naphthyl	8.1	23.3	>125[e]	>500[e]	>500[e]	>15.4	>61.7
10	CCl_3	1-Naphthyl	Insufficient solubility[f]						
11	CCl_3	2-Furyl	20.2	30.0	>125[e]	61.6	>500[e]	>6.2	3.1
12	CCl_3	5-CH_3-2-thienyl	8.7	24.0	25	50	125	2.9	5.7
13	CH_3	Ph	62.4	>125	>125[e]	125	>500[e]	>2	2.0
14	*N*,*N*′-Dimethyl-amidinyl	Ph	>100	>100	>125[e]	>500[e]	>500[e]	~1.25	~5
Ref.	Chloroquine[c]		0.7	0.08	30	40	250	42.9	57.1
Ref.	Doxorubicin[d]				0.2	0.05	2.5		

[a] Mean of three independent experiments.

[b] Selectivity index was calculated according to the following formula: $SI_{W2 \textit{Plasmodium}}$ = human cell IC_{50}/W2 *Plasmodium* IC_{50}.

[c] Chloroquine was used as the antiplasmodial drug compound of reference.

[d] Doxorubicin was used as the drug compound of reference for human cell toxicity.

[e] No toxicity at the highest tested concentration.

[f] Products **3** and **10** could not be evaluated because of their lack of solubility in the culture medium.

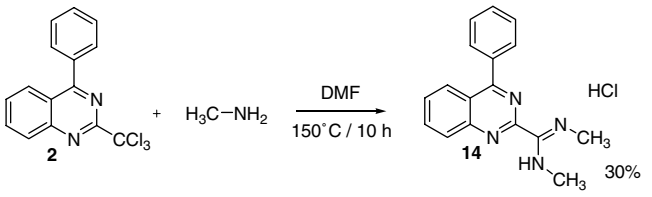


Figure 3. Preparation of amidine hydrochloride **14**.

toward THP1 cell line nor toward HepG2. HepG2 is a commonly used human-derived hepatoma cell line that has shown characteristics similar to those of primary hepatocytes. The goal of cytotoxic assay using HepG2, in addition to classic THP1 cells, was to evaluate the impact of metabolic activation of the tested compounds on the viability of a human hepatic cell line.[21,22]

For most of these molecules, the comparison of the two IC_{50} values calculated for each *Plasmodium* strain seems to indicate that their mode of action is not affected by the resistance to chloroquine, as both chloroquino-resistant and -sensitive strains require roughly the same IC_{50}.

Moreover, an antileshmanial evaluation was performed on *Leishmania donovani* promastigotes and gave IC_{50} values >100 μM for all molecules (reference drug compound being amphotericin B with a $IC_{50} = 0.2$ μM), indicating that these structures do not display any antileishmanial activity.[23–25] This difference observed between antiplasmodial and antileishmanial activities suggests that these molecules may exert specific antiparasitic effects on coccidies, with no effect on kinetoplastids. We are planning to check this hypothesis by testing the same molecules on other plasmatic coccidies such as *Toxoplasma*.

Having a look at results observed with test compounds **13** and **14** (without a CCl_3 group), it appears that the molecular skeleton responsible for the antiplasmodial activity is the 2-trichloromethylquinazoline. However, this skeleton, chlorinated in position 4 (test compound **1**), does not present satisfying IC_{50} values on *Plasmodium* strains.

From the results obtained, it appears that antiplasmodial activity can be significantly improved by adding an aryl group in position 4 of the quinazoline ring.

Among 4-aryl-2-trichloromethylquinazolines, the best activity values are noted with substituted phenyl groups. Note that the nature and the position of substituents on the phenyl group play an important role in antiplasmodial activity. Thus, favorable influence is noted with halogen atoms in the *para* position, especially with fluorine (test compound **4**).

In comparison with the commercial drug compound of reference, chloroquine, the best IC_{50} values obtained on the chloroquino-resistant W2 strain with 4-aryl-2-trichloromethylquinazolines are about 3–6 times higher. However, if we consider the 9 μM W2 IC_{50} value of doxycycline, another common commercial antimalarial agent,[26] we note that molecules **2**, **4**, **6**, **8**, **9**, and **12** work in the same concentration range.

Finally, molecules **2**, **4**, **6**, **8**, and **9** show a favorable antiplasmodial activity profile toward THP1 and HepG2 human cell lines. Molecule **4**, hit compound in this series (W2 $IC_{50} = 2.5$ μM), reaches quite good selectivity indexes (50), similar to those of chloroquine, and so, despite superior IC_{50} values because of its safer profile toward human cells.

The next step of our research program will consist in studying the mechanism by which these molecules produce their antiplasmodial effect.

Acknowledgments

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14. *Compound* **3**: Yield 60%; white powder, mp 142 °C; ^1H NMR (200 MHz; $CDCl_3$) δ 2.25 (3H, s), 7.37–7.47 (4H, m), 7.66–7.71 (1H, m), 7.80–7.84 (1H, m), 7.96–8.03 (1H,

m), 8.24–8.28 (1H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 20.0 (CH_3), 97.4 (C), 122.9 (C), 125.6 (CH), 127.2 (CH), 129.2 (CH), 129.6 (CH), 129.8 (CH), 129.9 (CH), 131.1 (CH), 134.7 (CH), 135.5 (C), 136.9 (C), 150.2 (C), 160.5 (C), 171.4 (C). Anal. Calcd for $C_{16}H_{11}Cl_3N_2$ (337.63) C, 56.92; H, 3.28; N, 8.30; found C, 56.85; H, 3.36; N, 8.76.

Compound **4**: Yield 60%; white powder, mp 130 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.25–7.34 (2H, m), 7.70–7.78 (1H, m), 7.87–7.94 (2H, m), 7.97–8.05 (1H, m), 8.18–8.27 (2H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 97.3 (C), 115.9 (2CH, d, J = 21.9 Hz), 121.7 (C), 126.7 (CH), 129.4 (CH), 129.8 (CH), 132.50 (2CH, d, J = 8.8 Hz), 132.54 (C, d, J = 3.6 Hz), 134.58 (CH), 150.75 (C), 160.7 (C), 164.3 (C, d, J = 251.7 Hz), 168.6 (C). Anal. Calcd for $C_{15}H_8Cl_3FN_2$ (341.59) C, 52.74; H, 2.36; N, 8.20; found C, 52.33; H, 2.38; N, 8.63.

Compound **5**: Yield 55%; yellow crystals, mp 151 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 3.94 (6H, s), 3.96 (3H, s), 7.08 (2H, s), 7.70–7.78 (1H, m), 7.97–8.05 (1H, m), 8.23–8.31 (2H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 56.4 ($2CH_3$), 60.97 (CH_3), 97.3 (C), 108.0 (2CH), 121.9 (C), 127.1 (CH), 129.2 (CH), 129.8 (CH), 131.7 (C), 134.5 (CH), 140.4 (C), 150.8 (C), 153.5 (2C), 160.7 (C), 169.6 (C). Anal. Calcd for $C_{18}H_{15}Cl_3N_2O_3$ (413.68) C, 52.26; H, 3.65; N, 6.77; found C, 52.40; H, 3.66; N, 6.86.

Compound **6**: Yield 57%; white powder, mp 127 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.54–7.60 (2H, m), 7.69–7.77 (1H, m), 7.80–7.87 (2H, m), 7.96–8.04 (1H, m), 8.15–8.26 (2H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 97.2 (C), 121.6 (C), 126.6 (CH), 129.0 (2CH), 129.5 (CH), 129.8 (CH), 131.7 (2CH), 13.6 (CH), 134.8 (C), 137.1 (C), 150.7 (C), 160.7 (C), 168.5 (C). Anal. Calcd for $C_{15}H_8Cl_4N_2$ (358.05) C, 50.32; H, 2.25; N, 7.82; found C, 50.00; H, 2.18; N, 7.73.

Compound **7**: Yield 50%; white powder, mp 108 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.72–7.82 (2H, m), 7.86–7.90 (1H, m), 8.00–8.16 (4H, m), 8.27–8.31 (1H, m). ^{13}C NMR (50 MHz; $CDCl_3$)δ 97.1 (C), 121.7 (C), 123.8 (C, q, J = 272.6 Hz), 126.4 (CH), 127.1 (CH, q, J = 4 Hz), 127.2 (CH, q, J = 3.5 Hz), 129.4 (CH), 129.8 (CH), 130 (CH), 131.3 (C, q, J = 32.9 Hz), 133.6 (CH), 134.9 (CH), 137.2 (C), 150.8 (C), 160.8 (C), 168.3 (C). Anal. Calcd for $C_{16}H_8Cl_3F_3N_2$ (391.6) C, 49.07; H, 2.06; N, 7.15; found C, 48.91; H, 1.97; N, 7.24.

Compound **8**: Yield 55%; pale yellow powder, mp 138 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 3.83–4.02 (3H, m), 7.09–7.15 (2H, m), 7.66–7.75 (1H, m), 7.87–8.01 (3H, m), 8.20–8.29 (2H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 55.5 (CH_3), 97.5 (C), 114.3 (2CH), 121.8 (C), 127.2 (CH), 128.9 (C), 129.0 (CH), 129.7 (CH), 132.5 (2CH), 134.3 (CH), 150.8 (C), 160.8 (C), 161.9 (C), 169.2 (C). Anal. Calcd for $C_{16}H_{11}Cl_3N_2O$ (353.63) C, 54.34; H, 3.14; N, 7.92; found C, 54.14; H, 3.13; N, 7.87.

Compound **9**: Yield 56%; white powder, mp 95 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.55–7.67 (2H, m), 7.70–7.78 (1H, m), 7.94–8.09 (5H, m), 8.26–8.36 (3H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 97.4 (C), 122.1 (C), 126.9 (CH), 127.1 (CH), 127.2 (CH), 127.7 (CH), 127.8 (CH), 128.6 (CH), 128.9 (CH), 129.3 (CH),129.8 (CH), 130.9 (CH), 132.9 (C), 133.8 (C), 134.2 (C), 134.5 (CH), 150.79 (C), 160.8 (C), 169.9 (C). Anal. Calcd for $C_{19}H_{11}Cl_3N_2$ (373.66) C, 61.07; H, 2.97; N, 7.50; found C, 60.68; H, 2.99; N, 7.29.

Compound **10**: Yield 50%; white powder, mp 209 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.38–7.80 (7H, m), 7.96–8.10 (3H, m), 8.28–8.32 (1H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 97.4 (C), 123.62 (C), 124.9 (CH), 125.4 (CH), 126.4 (CH), 127.0 (CH), 127.4 (CH), 128.5 (CH), 128.6 (CH), 129.3 (CH),129.6 (CH), 130.4 (CH), 131.5 (C), 133.5 (C), 133.8 (C), 134.8 (CH), 150.22 (C), 160.8 (C), 170.7 (C). Anal. Calcd for $C_{19}H_{11}Cl_3N_2$ (373.66) C, 61.07; H, 2.97; N, 7.50; found C, 60.72; H, 3.06; N, 7.57.

Compound **11**: Yield 64%; yellow powder, mp 120 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 6.70–7.72 (1H, m), 7.72–7.83 (3H, m), 7.92–8.00 (1H, m), 8.14–8.18 (1H, m), 9.01–9.06 (1H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 97.4 (C), 112.8 (CH), 117.9 (CH), 119.6 (C), 126.9 (CH), 129.4 (CH), 129.7 (CH), 134.4 (CH), 146.7 (CH), 151.6 (C), 153.4 (C), 156.1 (C), 160.8 (C). Anal. Calcd for $C_{13}H_7Cl_3N_2O$ (313.57) C, 49.79; H, 2.25; N, 8.93; found C, 49.75; H, 2.26; N, 8.80.

Compound **12**: Yield 65%; yellow powder, mp 123 °C; ^{1}H NMR (200 MHz; $CDCl_3$) δ 2.62 (3H,s), 6.95 (1H, dd, J = 3.7 Hz, J = 1Hz), 7.71–7.79 (1H, m), 7.83 (1H, d, J = 3.7 Hz), 7.92–8.01 (1H, m), 8.17–8.20 (1H, m), 8.56–8.60 (1H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 15.7 (CH_3), 97.3 (C), 120.5 (C), 126,1 (CH), 127.2 (CH), 129.3 (CH), 129.8 (CH), 132.8 (CH), 134.3 (CH), 138.3 (C), 147.7 (C), 151.2 (C), 160.4 (C), 161.5 (C). Anal. Calcd for $C_{14}H_9Cl_3N_2S$ (343.66) C, 48.93; H, 2.64; N, 8.15; found C, 48.90; H, 2.65; N, 8.04.

15. *Compound* **14**: Yield 30%; yellow powder, mp 149 °C, ^{1}H NMR (200 MHz; $CDCl_3$) δ 3.05 (3H, s), 3.20 (3H, s), 7.54–7.61 (2H, m), 7.62–7.70 (2H, m), 7.78–7.83 (2H, m), 7.92–8.00 (1H, m), 8.14–8.20 (2H, m). ^{13}C NMR (50 MHz; $CDCl_3$) δ 35.0 (CH_3), 38.4 (CH_3), 122.4 (C), 127.2 (CH), 128,4 (CH), 128.6 (2CH), 128.9 (CH), 130.2 (2CH), 130.3 (CH), 134.3 (CH), 136.6 (C), 150.7 (C), 157.9 (C), 167.1 (C), 169.4 (C).
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19. MTT assay was done according to the method of Mosmann with slight modifications: Briefly, 5×10^3 cells in 100 µl of culture medium (RPMI + 10% CO_2) were inoculated into each well of 96-well plates. After 24-h incubation, 100 µl of medium with various product concentrations was added and the plates were incubated for 72 h. At the end of the treatment and incubation, the medium was aspirated from the wells and 10 µl MTT solution (5 mg MTT/mL in PBS) was added to each well with 100 µl of medium without fetal calf serum. Cells were incubated for 2 h at 37 °C in a humidified 6% CO_2 with 95% air atmosphere to allow MTT oxidation by mitochondrial dehydrogenase in the viable cells. After 2 h, the MTT solution was aspirated and DMSO (100 µl) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (300 rpm) for 5 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength. DMSO was used as blank. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration was determined from the dose–response curve.
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25. *Leishmania donovani* promastigotes (strain MHOM/IN/80/DD8) in log-phase were incubated at an average density of 10^5 cells/ml. Various concentrations of active compounds were aseptically dissolved in DMSO (final concentration less than 0.5% v/v) and incorporated in triplicate cell cultures. After a 48-h incubation period at 25 °C, parasite growth and viability were determined by flow cytometry after staining DNA by 5 μl propidium iodide (1 mg/ml, Sigma). Amphotericin B was used as reference drug.
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