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Design, synthesis, and biological activities of conformationally restricted analogs of primaquine with a 1,10-phenanthroline framework

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ABSTRACT

A series of primaquine analogs was prepared, according to a conformationally restricted conformation of primaquine. In vitro antiplasmodial activities were evaluated and showed that all compounds were active on different strains of *Plasmodium falciparum*. In particular compounds **5** and **15** possessing a methoxy group were more active than was primaquine. Furthermore, analog **5** displayed good in vitro gametocytocidal activity. In addition selectivity indexes were calculated in respect with cytotoxic activities on Vero cell lines.

Malaria is one of the major life-threatening parasitic disease in tropical and subtropical regions. Worldwide, there are at least 300 million acute cases of malaria and more than 1–2 million deaths each year, mostly among young children infected with *Plasmodium falciparum*.[1] In addition, malaria problem is exacerbated with the spread of drug resistant *P. falciparum* strains.[2,3] In this context, the development of safe and effective antimalarial drugs that prevent transmission, in addition to curing patients, becomes an important strategy toward achieving an effective control of malaria. Currently, primaquine **1** (Fig. 1), an antimalarial drug containing an 8-aminoquinoline skeleton is one of the available transmission-blocking drugs. It displays a marked activity against gametocytes from all species of *Plasmodium* that affects humans, including chloroquine-resistant *P. falciparum*.[4] However, the use of primaquine is limited by its extensive conversion to its inactive metabolite carboxyprimaquine **2**, and by its toxic effects, among them hemolytic anemia, particularly in patients who are deficient in glucose-6-phosphate dehydrogenase.[5–7] The pharmacological profile of this drug should be improved by structural arrangement of the aminoalkyl chain, which could reduce the metabolic inactivation and allow the use of such derivatives in treatment and prevention of malaria. As examples of such improvements, several peptide and amino acid derivatives of primaquine have been synthesized in order to reduce the metabolic oxidative deamination pathway, as well as to reduce toxicity of the parent drug.[8–11] Such derivatives display improved activity/toxicity ratios when compared to primaquine. However, it has been shown that amino acid and peptide derivatives of primaquine are rapidly hydrolyzed to primaquine by aminopeptidases and endopeptidases,[12–14] suggesting that they might undergo extensive hydrolysis to the parent drug in the GI tract when given orally.

Our purpose in these studies concerns the design and synthesis of an original class of primaquine analogs based on a conformational approach. To our knowledge, no studies concerning such approaches have been reported yet. This design was based on our earlier studies exploring the chemistry of azaphenanthridines, where these compounds were screened for antimalarial activity in

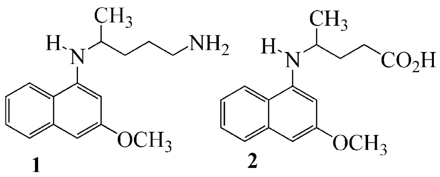

Figure 1. Structures of primaquine **1** and carboxyprimaquine **2**.

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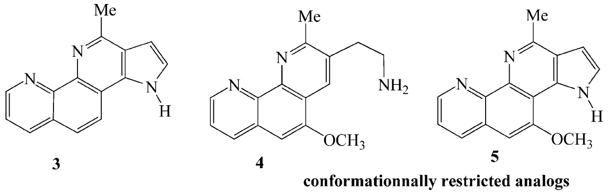


Figure 2. Structures of potential analogs primaquine.

vitro both on a Nigerian chloroquine-sensitive strain and on the chloroquine-resistant FcB1-Columbia and FcM29-Cameroon strains. From these studies, a 1,10-phenanthroline skeleton was shown to be the most effective,[15,16] and among these, compound **3** exhibited the highest biological activities[17,18] (Fig. 2). Simple 2D-juxtaposition of this structure with primaquine showed that compound **3** could be an interesting starting material for elaboration of restricted conformational analogs of primaquine containing a 1,10-phenanthroline skeleton (compound **4**). Furthermore, in order to reduce the metabolic oxidative deamination pathway, cyclization into a pyrolidinic ring was considered (compound **5**).

For computational analysis, structure of primaquine **1** and compound **5** was built in HyperChem Release 7.5 pro for Windows (Hypercube Inc. Gainesville, Florida).[19] A particular interest arose by considering the open conformation of primaquine **1a** which consisted in the proximity of the oxygen of the methoxy group (potential H acceptor) with the nitrogen of the dihydropyrrolic cycle (H donor), allowing a possible existence of a closed conformer of primaquine stabilized by an intramolecular H-bond. In this context, a closed conformation of primaquine **1b** with a length constraint between the above nitrogen and oxygen was built facilitating this intramolecular H-bonding. The resulting optimized structure was then solvated and this system was optimized. A molecular dynamics program was then run, and finally the geometries were optimized using the semi-empirical PM3 programme. The resulting conformations of primaquines **1a** and **1b** are shown in Figure 3.

Compound **5** was then built and its geometry was optimized following the same methodology. The overlaid three-dimensional structure of the closed conformation of primaquine **1b** and compound **5** is represented in Figure 4. The molecules possess a planar moiety defined by the quinolinic nucleus, the 8-amino group and the 6-methoxy group of the parent primaquine model. The stereoplots also reveal a good overlapping of the methyl groups. From these calculations, some inter-atomic distances quantifying the proximity of the nitrogen atom of the side chain to the quinoline skeleton have been taken as reference. The values reported in Table 1 showed that the distances are similar confirming conformational similarities between the two compounds.

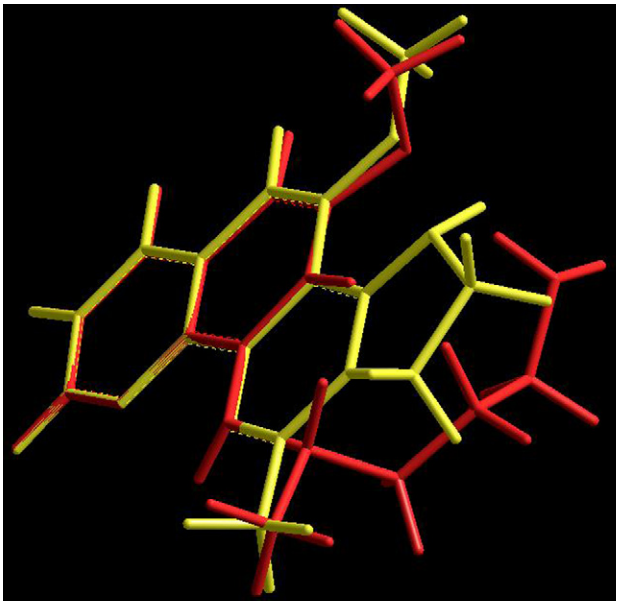


Figure 4. Conformation overlay of primaquine (**1a**, red) and compound (**5**, yellow).

Table 1
Calculated inter-atomic distances (Å) taken for reference of the compact conformer of primaquine and the suggested analog **5**

	Prim	5
NH_2(ter)–NH	5.13	4.18
NH_2(ter)–O(OCH_3)	2.82	2.87
NH_2(ter)–N(quin)	6.65	6.22

Considering the above studies, the principle of creating an additional dihydropyrrolic cycle fused to the initial 1,10-phenanthroline ring was admitted for the synthesis of the new structural primaquine analog **5**.

Compound **3** was prepared according to our previous work.[18] Using the same methodology, compound **10** was prepared in two steps by condensing 8-amino-6-methoxyquinoline **7** with acetylbutyrolactone to give the enaminolactone **9** in 84% yield, which was further treated in refluxing $POCl_3$ leading to compound **10** in

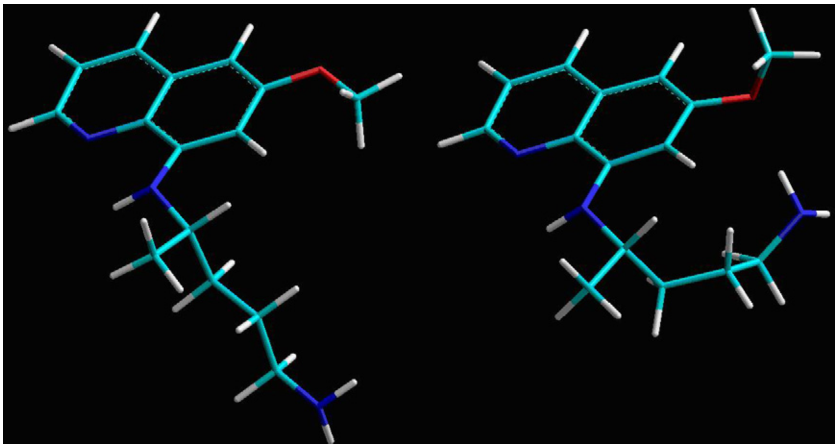


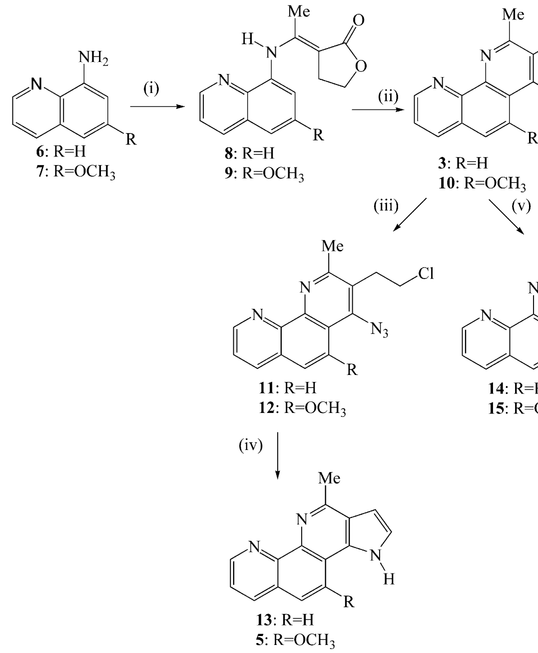
Figure 3. "Open conformation" of primaquine (**1a**, left) and "closed conformation" of primaquine (**1b**, right).

62% yield. Treatment of **3** and **10** by sodium azide gave the corresponding azido compounds **11 and 12** in good yield (90% and 91%).[20,21] The final compounds 2,3-dihydro-4-methyl-1*H*-pyrrolo[3,2-*c*]-1,10-phenanthroline **13** and 2,3-dihydro-11-methoxy-4-methyl-1*H*-pyrrolo[3,2-*c*]-1,10-phenanthroline **5** were then obtained in 69% and 72% yield, respectively, by refluxing this synthetic intermediate in acetone with a 1.5 equiv of triphenylphosphine[22] (Scheme 1).

In addition to assess the impact of the heteroatoms on the biological activities, the furano derivatives 2,3-dihydro-14-methylfuro[3,2-*c*]-1,10-phenanthroline **14** and its 11-methoxy derivative **15** were synthesized. To that end, the dichloro derivatives **3 and 10** were treated in refluxing acetic acid to give **14 and 15** in 67% and 89% yield, respectively.[20,23]

All structural determinations were made on the basis of mass spectroscopy ^{1}H NMR and ^{13}C NMR studies.

Antiplasmodial activities:[24] Four strains of *P. falciparum* were used to evaluate the in vitro antiplasmodial activities of compounds **5–14**: The chloroquine-resistant W2, FcB1-Columbia and FcM29-Cameron strains, and the chloroquine-sensitive F32 strain. Parasites were cultured according to the method originally described by Trager and Jensen.[25] For each in vitro test, the parasite culture was incubated with the tested compound with increased dilutions for 48 h. Results are summarized in Table 2.



Scheme 1. Reagents and conditions: (i) 2-acetylbutyrolactone, toluene, reflux (Dean-stark); (ii) POCl$_3$, reflux; (iii) NaN$_3$, acetone, reflux; (iv) P(Ph)$_3$, acetone, reflux; (v) acetic acid, reflux.

These results showed that all tetracyclic compounds exhibit interesting in vitro antiplasmodial activities while the tricyclic derivative **10** is less active. All compounds were equally active on both chloroquine-sensitive and resistant strains. In terms of structure–activity relationships (SAR), the effectivity of the dihydropyrrolo compounds **5** and **13** is markedly influenced by the presence of the methoxy group (compound **5** is about 10-fold more active than **13** on the different strains). In the case of the furan derivatives **14** and **15,** no significant differences were observed. Cytotoxic activities of the restricted conformational primaquine analogs were also evaluated in vitro against the Vero cell line, and the selectivity indexes (cytotoxic/antiplasmodial ratio (CAR)) have been calculated. The results indicated that the antiplasmodial activities of the pyrrolo derivatives **5 and 13** are closely related to their cytotoxic effect (the more active the compounds are, the higher the cytotoxicity is and the lower the selectivity indexes are), except for compound **5** on the FcB1 strain which showed a selectivity index of 132. Interestingly, in the furano derivatives, **14** (containing a methoxy group) is twofold more cytotoxic than the compound **15**. Finally, from these experiments, compound **15** appears to be the most potent and selective antiplasmodial compound.

Activity against gametocytes:[29] As primaquine displays a marked activity against gametocytes, we investigated the in vitro gametocytocidal activity of compounds **5–15**. The gametocytes used for these studies were obtained from the chloroquine-resistant W2 strain. After 13 days of maturation, the gametocytes were exposed for 48 h to the tested compounds and gametocytaemia was evaluated by microscopy. The results showed that all compounds exhibited marked gametocytocidal activities against *P. falciparum*. Compound **5**, the direct analog of primaquine, showed an activity similar to primaquine, and **14, 15** showed higher activities than primaquine. The furan derivative **15** was the most potent antiplasmodial agent in both sexual and asexual stages.

In conclusion, new tetracyclic 1,10-phenanthrolines related to primaquine have been designed and synthesized. All compounds showed marked antiplasmodial activities in vitro on both sensitive and chloroquine-resistant strains of *P. falciparum*. More interestingly, one derivative (compound **15**) exhibited high selectivity indexes and the highest antigametocytocidal activity. It can be concluded that 2,3-dihydrofuro[3,2-*c*]-1,10-phenanthrolines provide interesting lead in our search for new antiplasmodial agents and should be potentially useful in malaria chemotherapy.

Table 2

IC$_{50}$ of compounds **5–15** (μM) on four *P. falciparum* Strains tested and cytotoxicity on Vero cell line

Compound	F32	W2	FcM$_{29}$	FcB$_1$	Vero	Gametocytes (W2 strain)
10	7.71 ± 3.15	6.56 ± 1.78	5.0 ± 0.81	4.84 ± 0.65	NT	NT
5	0.41 ± 0.2 (72)	1.28 ± 0.15 (23)	1.28 ± 0.03 (23)	0.22 ± 0.07 (132)	29.8 ± 6.3	9.8
13	1.31 ± 0.08 (47)	4.17 ± 1.36 (15)	9.36 ± 1.48 (7)	1.14 ± 0.17 (54)	61.6 ± 3.4	10.6
14	1.18 ± 0.5 (32)	1.10 ± 0.04 (34)	0.38 ± 0.16 (99)	0.97 ± 0.04 (39)	37.6 ± 4.2	5.5
15	0.86 ± 0.5 (72)	0.93 ± 0.07 (66)	0.48 ± 0.15 (129)	0.93 ± 0.26 (39)	62.2 ± 6.6	3.75
PQ	6.83 ± 1.66 (49)	7.70 ± 1.46 (44)	7.25 ± 0.84 (47)	6.87 ± 1.69 (49)	339.7 ± 35.1	9.65

PQ, primaquine; NT, not tested. Numbers in brackets: selectivity index.

References

1. Hoffman, L. S.; Subramanian, M. G.; Collins, F. H.; Craig, V. *Nature* **2002**, *415*, 702.
2. Greenwood, B.; Mutabingwa, T. *Nature* **2002**, *415*, 670.
3. Beales, P. F. In *Malaria Principles and Practice of Malariology*; Wernsdofer, W. H., McGregor, I., Eds.; Churchill Livingstone: New York, 1988. pp 1263–1285.
4. Rieckmann, K. H.; McNamara, J. V.; Frischer, H.; Stockert, T. A.; Carson, P. E.; Powel, R. D. *Bull. W.H.O.* **1968**, *38*, 625–632.
5. Brueckner, R. P.; Ohrt, C.; Baird, J. K.; Milhous, W. K. In *Antimalarial Chemotherapy*; Rosenthal, P. J., Ed.; Humana Press: Totowa, 2001; pp 123–151.
6. Mihaly, G. W.; Ward, S. A.; Edwards, G.; Orme, M. L'E.; Breckenridge, A. M. *Br. J. Clin. Pharmacol.* **1984**, *17*, 441.
7. Constantino, L.; Paixao, P.; Moreira, R.; Portela, M. J.; Rosario, V. E.; Iley, J. *Exp. Toxicol. Pathol.* **1999**, *51*, 299.
8. Trouet, A.; Pirson, P.; Steiger, R.; Masquelier, M.; Baurain, R.; Gillet, J. *Bull. W.H.O.* **1981**, *59*, 449.
9. Philip, A.; Kepler, J. A.; Johnson, B. H.; Carroll, F. Y. *J. Med. Chem.* **1988**, *31*, 870.
10. Portela, M. J.; Moreira, R.; Valente, E.; Constantino, L.; Iley, J.; Pinto, J.; Rosa, R.; Cravo, P.; do Rosario, V. E. *Pharm. Res.* **1999**, *16*, 949.
11. Vangapandu, S.; Sachdeva, S.; Jain, R.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. *Bioorg. Med. Chem.* **2004**, *12*, 239.
12. Portela, M. J.; Moreira, R.; Valente, E.; Constantino, L.; Iley, J.; Pinto, J.; Rosa, R.; Cravo, P.; do Rosario, V. E. *Pharm. Res.* **1999**, *16*, 949.
13. Vangapandu, S.; Sachdeva, S.; Jain, R.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. *Bioorg. Med. Chem.* **2004**, *12*, 239.
14. Borissova, R.; Stjarnkvist, P.; Karlsson, M. O.; Sjoholm, I. *J. Pharm. Sci.* **1995**, *84*, 256.
15. Kitjaroentham, A.; Suthiphongchai, T.; P.Wilairat *Acta Trop.* **2006**, *97*, 5.
16. Wijayanti, M. A.; Sholikhah, E. N.; Tahir, I.; Hadanu, R.; Jumina, S. ; Mustofa, M. *J. Health Sci.* **2006**, *52*, 794.
17. Yapi, A. D.; Mustofa, M.; Valentin, A.; Chavignon, O.; Teulade, J. C.; Mallié, M.; Chapat, J. P.; Blache, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1886.
18. Yapi, A. D.; Valentin, A.; Chezal, J. M.; Chavignon, O.; Chaillot, B.; Gerhardt, R.; Teulade, J. C.; Blache, Y. *Arch. Pharm.* **2006**, *339*, 201.
19. Molecular modeling: The geometry was optimized to an rms (root mean square) gradient of 0.001 in vacuo (Polak–Ribière method). A periodic box, 15 × 15 × 15 Å around the drug was then set up, containing 112 water molecules. The system was optimized in MM+ using switched cut-offs (outer 7.5 and inner 3.5 Å) to an rms gradient of 0.5. Then a molecular dynamics program was run for 1 ps, with 0.001 ps steps, relaxation time 0.1 ps, to a simulation temperature of 300 K. This was followed by MM+ geometry optimization to an rms gradient of 0.2. The molecular dynamics run was repeated and a further MM+ protocol was carried out to a gradient of rms 0.004 on the selected drug. Finally, the geometries were optimized using the semi-empirical PM3 programme in singly excited configuration interaction. (RHF [Restricted Hartree–Fock], charge 0, spin multiplicity 1, lowest state, orbital criterion, five occupied and five unoccupied orbitals). Compact conformation of primaquine model was built with a length constraint between the nitrogen and oxygen atom of 1.5 Å.
20. Badawey, E. S.; Kappe, T. *Eur. J. Med. Chem.* **1997**, *32*, 815.
21. 4-Azido-3-(2-chloroethyl)-2-methyl-1,10-phenanthroline **11** and 4-azido-3-(2-chloroethyl)-5-methoxy-2-methyl-1,10-phenanthroline **12** were used without purification. Yields are the crude yields after precipitation in ether and filtration. Analytical samples were obtained by chromatography and structures were determined by NMR and IR spectroscopy.
22. Typical procedure for preparation of **5**: Compound **12** (4 mmol) was refluxed in phosphorus oxychloride ($POCl_3$, 25 mL) for 4 h. Excess phosphorus oxychloride was removed, water was added, and the mixture was neutralized with a saturated solution of sodium carbonate. After extraction with dichloromethane, the organic layers were washed with brine (50 mL), then dried (Na_2SO_4). Solvent evaporation gave a black oily product which was purified on a neutral alumina and eluted with dichloromethane to afford **5** in 72% yield; mp 210–218 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.66 (s, 3H, CH_3); 3.14 (t, 2H, $J = 9$ Hz, H_3), 3.85 (t, 2H, 9 Hz, H_2), 4.00 (s, 3H, OCH_3), 5.99 (se, 1H, NH), 6.62 (s, 1H, H_{10}), 7.41 (dd, 1H, $J = 4.5$ Hz, $J = 8$ Hz, H_8), 7.95 (d, 1H, $J = 8$ Hz, H_9), 8.91 (d, 1H, $J = 4.5$ Hz, H_7). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 22.8, 27.0, 47.2, 55.6, 97.6, 108.2, 120.2, 122.5, 129.0, 134.3, 143.2, 147.4, 147.6, 154.1, 155.0, 155.2. ν NH = 3490 cm^{-1}.
23. Typical procedure for preparation of **15**: A solution of **10** (0.35 mmol) in acetic acid (3 mL) was refluxed for 6 h. The mixture was then basified with Na_2CO_3 and extracted with dichloromethane. After evaporation of the organic layers, the residue was chromatographed on silica gel (elution; CH_2Cl_2) to give **15** in 89% yield; mp 254–255 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.23 (s, 3H, CH_3); 3.80 (t, 2H, $J = 9$ Hz, H_3); 4.51 (s, 3H, OCH_3), 5.36 (t, 2H, $J = 9$ Hz, H_2), 7.25 (s, 1H, H_{10}), 7.93 (dd, 1H, $J = 4.5$ Hz, $J = 8$ Hz, H_8), 8.48 (d, 1H, $J = 8$ Hz, H_9), 9.43 (d, 1H, $J = 4.5$ Hz, H_7). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 23.4, 27.7, 56.0, 73.2, 99.8, 110.8, 120.7, 123.0, 129.1, 134.5, 142.5, 147.8, 148.2, 153.6, 155.8, 163.5.
24. *Plasmodium* falciparum was cultured according to the method described by Trager and Jensen[25] with modifications.[26] Cultures were synchronized by 5% D-sorbitol lysis (Merck, Darmstadt, Germany). In vitro antimalarial activity was evaluated by $[^3H]$hypoxanthine (ICN, France) incorporation as already.[27] Incubation time between parasite culture and the drugs was 48 h. The cytotoxicity of the drugs was estimated in Vero cell line which was cultured in the same conditions as *P. falciparum*, except for the 5% human serum which was replaced by 5% fetal calf serum (Boehringer). After addition of drugs at various concentrations, cell growth was estimated by $[^3H]$hypoxanthine incorporation after 48 h incubation.[28]
25. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673.
26. Benoit, F.; Valentin, A.; Pélissier, Y.; Marion, C.; Dakuyo, Z.; Mallié, M.; Bastide, J.-M. *Trans. R. Soc. Trop. Med. Hyg.* **1995**, *89*, 217.
27. Valentin, A.; Benoit-Vical, F.; Moulis, C.; Stanislas, E.; Mallié, M.; Fourasté, I.; Bastide, J.-M. *Antimicrob. Agents Chemother.* **1997**, *41*, 2305.
28. Jullian, V.; Bonduelle, C.; Valentin, A.; Acebey, L.; Duigou, A. G.; Prévost, M. F.; Sauvain, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5065.
29. Gametocytogenesis was induced in W2 strain as described by Ifediba[30], with modifications.[31] The parasites were matured for 13 days before testing the compounds at various dilutions. Three days before the test, 50 mM *N*-acetyl-D-glucosamine (Sigma) was added in order to remove asexual stages.[32] After a 48 h incubation, smears were made for every drug concentration and Giemsa stained. Optical determination of gametocytaemia was performed by at least two independent readers who counted at least 10,000 red blood cells. Graphs giving the gametocytaemia reduction versus drug concentration were plotted, and the IC_{50} was graphically determined. Controls were parasites without drugs (0% inhibition).
30. Ifediba, T.; Vanderberg, J. P. *Nature* **1981**, *294*, 364.
31. Benoit-Vical, F.; Lelievre, J.; Berry, A.; Deymier, C.; Dechy-Cabaret, O.; Cazelles, J.; Loup, C.; Robert, A.; Magnaval, J. F.; Meunier, B. *Antimicrob. Agents Chemother.* **2007**, *51*, 1463.
32. Ribaut, C.; Berry, A.; Chevalley, S.; Reybier, K.; Morlais, I.; Parzy, D.; Nepveu, F.; Benoit-Vical, F.; Valentin, A. *Malar. J.* **2008**, *7*, 45.