Bioorganic & Medicinal Chemistry 18 (2010) 2998–3003

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

The bisnaphthalimides as new active lead compounds against *Plasmodium falciparum*

Maximilian Tischer [a], Ludmilla Sologub [b], Gabriele Pradel [b,*], Ulrike Holzgrabe [a,*]

[a] Institute of Pharmacy and Food Chemistry, University of Wuerzburg, Am Hubland, 97074 Wuerzburg, Germany
[b] Research Center for Infectious Diseases, University of Wuerzburg, Joseph-Schneider-Str. 2, Building D15, 97080 Wuerzburg, Germany

ARTICLE INFO

Article history:
Received 14 January 2010
Revised 22 March 2010
Accepted 25 March 2010
Available online 29 March 2010

Keywords:
Malaria
Plasmodium falciparum
Bisquaternary bisnaphthalimides

ABSTRACT

The bisquaternary bisnaphthalimides are a versatile class of compounds being active against the malaria parasite *Plasmodium falciparum* in the lower nanomolar range of concentration combined with no cytotoxicity. The series of compounds is designed as choline analogues and interfering agents of the phosphatidylcholine biosynthesis. The qualitative analysis of the structure–activity relationships (SAR) revealed the importance of a long methylene middle chain of at least 8 methylene groups between the two bisquaternary naphthalimides or a monoquaternary naphthalimide consisting of a long alkyl chain attached to the positively charged nitrogen atom. Since the SARs are different from reported biscationic antimalarial drugs the mode of action remains to be elucidated.

1. Introduction

Malaria is still the most prevalent tropical disease worldwide with its main impact in sub-Saharan Africa. In 2008, 109 countries were endemic for malaria, 45 of them within Africa. Malaria causes an estimated 250 million new infections and almost one million casualties each year, with 85% of them being children under the age of five,[1] Malaria incidence has been recently reduced in some parts of Africa due to anti-mosquito measures and new artemisinin-containing treatments. However, malaria treatment is highly vulnerable to the spread of malaria strain being resistant to the well-established antimalarial drugs, such as chloroquine or mefloquine.[2] The drastic spread of HIV furthermore augments susceptibility for severe malaria infection in HIV patients, with malaria affecting HIV infections by increasing viral load, while HIV increases malaria-related fever and adversely affects malaria during pregnancy.[3]

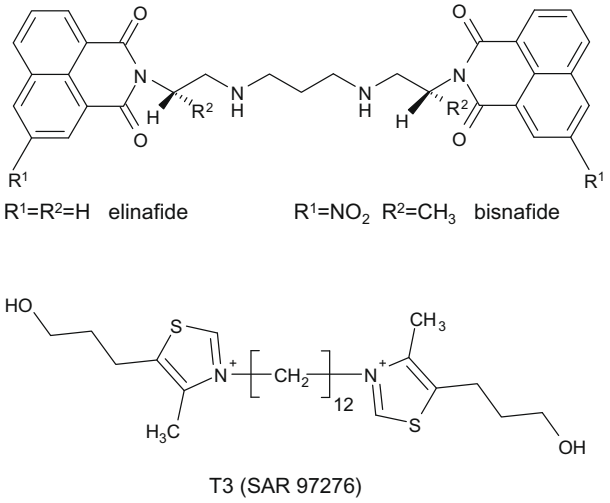
These dramatic conditions emphasize the need for new antiplasmodial compounds. The identification of novel drug targets might lead to the generation of new types of antimalarial compounds not allowing cross-resistance with currently available antimalarials. For example, the phospholipid metabolism of the infected erythrocyte seems to be an promising pharmacological target for a new chemotherapy approach. It is highly specific for a parasite-infected erythrocyte and very important for membrane biosynthesis and parasite growth.[4] The major phospholipid produced in infected erythrocytes is phosphatidylcholine, representing up to 50% of the biosynthesis capacity. It is synthesized by the parasitic enzyme machinery starting from polar groups, especially from choline, which is derived from the host cytoplasma.[5] Calas et al. showed that choline-like compounds are active against trophozoite blood stages of the parasite,[4] probably by interfering with the choline carrier, which is the rate-limiting step in phosphatidylcholine biosynthesis.[6] The bivalent bisthiazolium compound T3 (see Scheme 1) and related compounds,[7–9] being designed as choline analogues, are active in the nanomolar concentration range and were recently found to target the synthesis of the phosphatidylcholine by means of a proteomic analysis.[10] The *Plasmodium falciparum* choline kinase (*Pf*CK) and ethanolamine kinase (PfEK) are inhibited by T3 in the micromolar concentration range.[11] Thus, T3 is currently in preclinical trials.

Within the frame of our broad screening program bisquaternary naphthalimides were identified to be active against *P. falciparum* strain 3D7 in the low micromolar and nanomolar range of concentration. The bistertiary analogues of this class of compounds are known to have high antitumoral activity against both murine and human tumor cells.[12,13] The bissecondary compounds elinafide and bisnafide, consisting of a basic linker with a C_2–N_{sec}–$C_{2,3}$–N_{sec}–C_2 motive (see Scheme 1), exhibited a high in vitro antitumoral activity intercalating twice with the DNA. Elinafide was transferred to clinical trials against solid tumors but did not succeed.[12]

The bisquaternary naphthalimide derivatives, originally developed as allosteric modulators of muscarinic receptors,[14,15] share some of the aforementioned features. An initial cytotoxicity screening of a series of compounds with a C_3–N_{quart}–C_{3-12}–N_{quart}–C_3 middle chain revealed no cytotoxicity against different cell lines.[16]

* Corresponding authors. Tel.: +49 931 3182174 (G.P.), +49 931 3185461 (U.H.).
E-mail addresses: gabriele.pradel@uni-wuerzburg.de (G. Pradel), u.holzgrabe@pharmazie.uni-wuerzburg.de (U. Holzgrabe).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.03.067



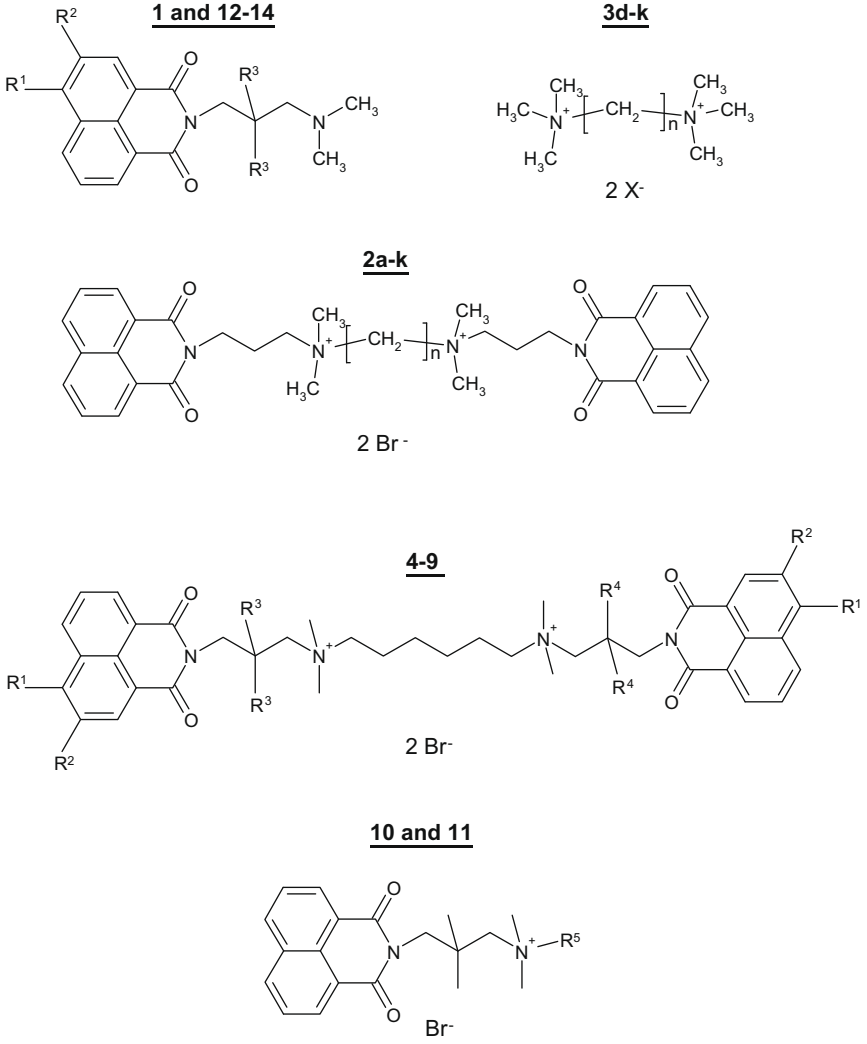
Scheme 1. Antitumoral naphthalimide compounds characterized by an amino group and linker, respectively, and T3, an antimalarial drug in preclinical trials.

The first promising screening results on bisnaphthalimides exhibiting high antimalarial activity prompted the synthesis and biological characterization of a larger series of bisquaternary bisnaphthalimides of a varying length of the middle chain and varying substitution in addition to bisquaternary compounds missing the naphthalimide moiety and monoquaternary compounds equipped with one lateral naphthalimide.

2. Results and discussion

The synthesis for the bisquaternary naphthalimides started off with the conversion of commercially available correspondingly substituted 1,8-naphthalene dicarboxylic acid anhydrides with equimolar amounts of *N*,*N*-dimethyl-propane-1,3-diamine in presence of a catalytic amount of *p*-toluenesulfonic acid into the *N*-(*N*′,*N*′-dimethylaminopropyl)-naphthalimide **1** and likewise **12–14**. The symmetrical bisquaternary compounds **2a–k**, **4**, **5**, **8** and **9** (see Scheme 2) were synthesized by refluxing **1** and **12–14**, respectively, and a half equivalent of an α,ω-dibromoalkane in acetonitrile either classically by refluxing for hours to days or in the microwave, which speeds up the reaction substantially.[14,17] Compounds **8** and **9** having methyl groups in the lateral propylene chains were synthesized according to Ref. 14. Monoquaternary compounds **10** and **11** were obtained by alkylation of **1** with an excess of the corresponding alkylating agent.

The compounds **3d** and **3g–k** (see Scheme 2) were synthesized by alkylation of an excess of trimethylamine with α,ω-dibromoal-



Scheme 2. Compounds studied here; the substitution patterns are given in Tables 1 and 2

kane in methanol by stirring at room temperature for two days (analogue to Ref. 18). Compounds **3e** and **3f** were synthesized by complete methylation of the α,ω-diaminoalkane with an excess iodomethane in presence of Hünig's base in chloroform. The structure of all compounds was confirmed by means of NMR and IR spectroscopy and found to be in accordance with data reported for related compounds.[14,15]

The antimalarial activity was determined for the human malaria pathogen *P. falciparum* in vitro[19] by means of the Malstat assay, using chloroquine-sensitive strain 3D7 and in selected cases chloroquine-resistant strain Dd2, at concentrations between 10 pM and 100 μM. The Malstat assay measures the activity of the *Plasmodium*-specific enzyme lactate dehydrogenase as described in Refs. 20,21. The antimalarial activity of all compounds is depicted in Tables 1 and 2. The cytotoxicities of all compounds were measured in the macrophage cell line J774.1 using the AlamarBlue-based cytotoxicity test.[22] Each compound was tested in duplicate. None of the compounds showed any cytotoxic activity (IC_{50} value higher than 100 μM after 48 h).

Since the antimalarial activity of, for example, bisthiazolium compounds,[7] bispyridinium[23–25] and bisquaternary ammonium salts[4,18] were found to depend heavily on the distance between the two positive charges, compounds **2** were synthesized with varying length of middle chain from 3 to 12 methylene groups. Comparison of these compounds revealed the antimalarial activity ranging from inactive to (IC_{50} >100 μM) to active in the nanomolar range of concentration (IC_{50} = 60 nM). The antimalarial activity of compounds reaches a maximum at a chain length of 8–9 methylene groups. Whereas compounds with 3–5 methylene groups apparently did not exhibit antimalarial activity, the activity levels off at about 0.2 μM at chain length of 10 methylene groups and longer. This finding is in contrast to the optimal middle chain length reported for the aforementioned bisthiazolium compounds,[7] bispyridinium[24,25,18] and bisquaternary ammonium salts[4,18] which are mostly found to be equivalent to 12 methylene groups, indicating a different binding mode to the target structure or even addressing a different target. Interestingly, the methyl groups in the lateral propylene chains in compounds **8** and **9** exhibit a 10 times higher activity than the parent compound **2d**.

In the case of compounds **2** with a middle chain longer than $n = 9$ (**2h–k**), it might be speculated that only one naphthalimide moiety of the very long molecule may produce the antimalarial activity. This hypothesis is supported by the activity of compound **11**, which consists a sole naphthalimide moiety and one quaternary nitrogen in addition to a six-membered alkyl chain. However, the activity of **11** is some 10 times less than the activity of the corresponding compounds **2f** and **2g**, but similar to **8** and **9** (cf. Tables 1 and 2).

Comparison of the activities of pairs of compounds **2** and the corresponding molecules **3** reveals a constantly lower activity of the naphthalimide-free derivatives indicating the importance of the lateral, flat, aromatic area. However, the longer the middle chain the higher and closer the activities are in both series, which thwart the aforementioned hypothesis of the importance of the naphthalimides.

In order to find out whether the lateral naphthalimide moieties are essential for a high antimalarial activity of our series of compounds, the naphthalimides with a six-membered middle chain were substituted with nitro and amino groups, which had previously increased the antitumoral activity of the corresponding tertiary compounds synthesized by Brana and Ramos.[12] Whereas the nitro and amino substitution in *para*-position to the carbonyl group (cf. **2d** with **4** and **6**, respectively) does not have a significant influence on the activity, the nitro group in *meta*-position enhances the activity by a factor of five and the amino substituent in this position decreases the activity by a factor of 3 (cf. **2d** with **5** and **7**, respectively). These results, which do not fit with the SAR of antitumoral activity (being higher for both nitro and amino substituted tertiary naphthalimides), clearly indicate that, firstly, the mode of action is different from the antitumoral action and, secondly, the naphthalimide seems to play a pivotal role for a high antimalarial activity.

The question remained whether two naphthalimides are necessary for a high antimalarial activity. Therefore, the tertiary synthesis precursor **1** and additionally the monoquaternary compounds **10** and **11** were tested. Compound **1** does not have any antimalarial activity (but shows cytotoxicity) whereas a corresponding bistertiary compound with a six-membered middle chain was active in the submicromolar concentration range (~0.6 μM). In contrast, the monoquaternary compounds **10** and **11** are active, and a long alkyl chain seems to augment the activity to the level of compounds **2h** to **2k**. These results emphasize that at least one naphthalimide is necessary for a high antimalarial activity.

Notably, the antiplasmodial activity of selected compounds, which was tested in the chloroquine-resistant strain Dd2, was comparable with the antiplasmodial activity against the chloroquine-sensitive strain 3D7 (cf. both activities of compounds **6**, **10** and **11**, Table 2) indicating that the mode of action is different from the chloroquine mechanism of action. This is in line with the previous report on bisquaternary compounds by Calas et al.[4]

For two of the compounds, **5** and **8**, we aimed to obtain first indications on the possible mode of action by investigating ultrastructural changes of compound-treated blood stage parasites by means of electron microscopy. Interestingly, a number of compound-treated parasites revealed intracellular membrane whirls, which are indicative of a dysfunctional membrane biosynthesis. No such whirls were observed in untreated or DMSO-treated parasites (see Fig. 1).

Table 1
Compound class **2** and **3** and their antimalarial activity obtained from the Malstat assay, performed in triplicate

Compounds[a]	*n*	IC_{50} (μM) 3D7 of **2**	IC_{50} (μM) 3D7 of **3**[a]
2a	3	>100	
2b	4	>100	
2c	5	>100	
2d/**3d**	6	7.99 ± 4.55	>100 (700)
2e/**3e**	7	0.51 ± 032	>100
2f/**3f**	8	0.064 ± 0.007	>100 (12)
2g/**3g**	9	0.06 ± 0.009	0.892
2h/**3h**	10	0.145 ± 0.058	0.911 (1.7)
2i/**3i**	11	0.17 ± 0.0917	0.755 ± 0.318
2k/**3k**	12	0.219 ± 0.19	0.395 ± 0.176 (0.09)

[a] In brackets, the antiplasmodial activities reported by Calas et al.[18] using a semi-automated microdilution assay are indicated.

Table 2
Antimalarial activity of compounds **1** and **4**–**11** obtained from the Malstat assay, performed in triplicate

Compound[a]	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM) 3D7[a]
1	—	—	—	—	—	>100
4	NO_2	H	CH_3	CH_3	—	3.48 ± 0.12
5	H	NO_2	CH_3	CH_3	—	0.66 ± 0.03
6	NH_2	H	CH_3	CH_3	—	6.76 ± 2.39 (7.39 ± 0.04)
7	H	NH_2	CH_3	CH_3	—	22.63 ± 2.80
8	H	H	CH_3	H	—	0.40 ± 0.18
9	H	H	CH_3	CH_3	—	0.80 ± 0.38
10	—	—	—	—	C_3H_7	9.81 ± 7.83 (8.11 ± 0.08)
11	—	—	—	—	$(CH_2)_6Br$	0.43 ± 0.25 (0.89 ± 0.54)
12	H	H	CH_3	—	—	
13	H	NO_2	CH_3	—	—	
14	NO_2	H	CH_3	—	—	

[a] In brackets the IC_{50} values for the chloroquine-resistant strain Dd2 are indicated.

3. Conclusion

Taken together, bisquaternary bisnaphthalimides are promising candidates for the development of new active compounds against *P. falciparum*, the causative agent of malaria tropica. Since the exact mode of action is not yet elucidated, a detailed target analysis has to be performed and is currently in progress. This is the first time that the bivalent ligands are composed of two positively charged nitrogens in a distance of 8–9 methylene groups and that flat, aromatic moieties are attached to the nitrogen instead of long alkyl chains reported so far. Since the substitution of the naphthalimides seems to be critical for a high

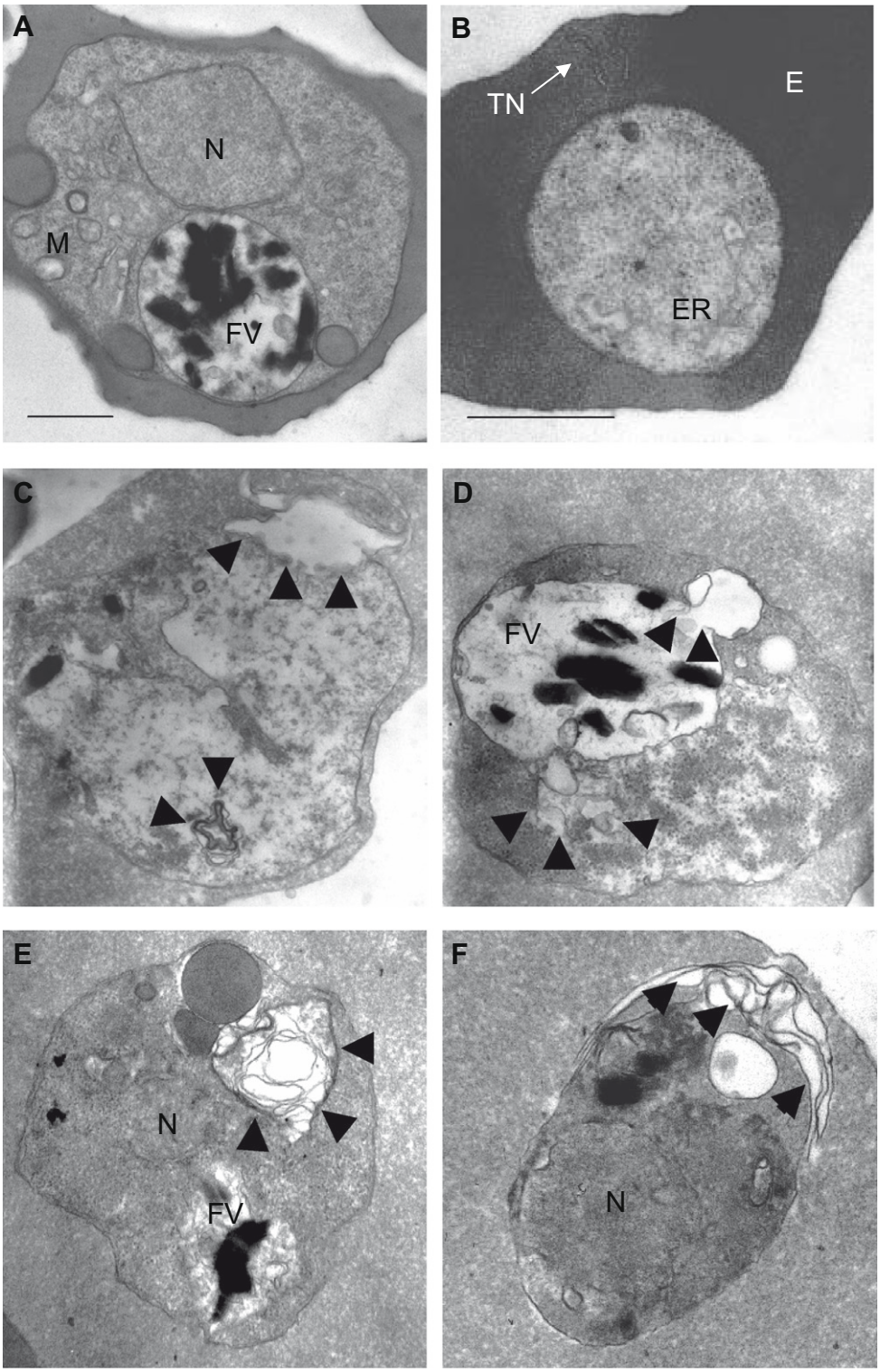


Figure 1. Electron micrographs depicting blood stage parasites, which are either untreated (A), treated with 0.5 vol %. DMSO (B), with compound **8** (C, D) or with compound **5** (E, F). Compound-treated blood stages exhibit intracellular membrane whirls (arrowheads), which are not present in the control parasites. E, erythrocyte; ER, endoplasmic reticulum; FV, food vacuole; M, mitochondrion; N, nucleus; TN, tubular network. Bar, 0.5 μm.

antimalarial activity, further variation of this moiety is in progress.

4. Experimental

^{1}H and ^{13}C NMR spectra were recorded on an *Avance 400* nuclear magnetic resonance spectrometer, Bruker Biospin GmbH, Rheinstetten, Germany (^{1}H 400.132 MHz, ^{13}C 100.613 MHz). As internal standard the signals of the deuterated solvents were used (DMSO-d_6: ^{1}H 2.5 ppm, ^{13}C 39.52 ppm, methanol-d_4: ^{1}H 3.31 ppm, ^{13}C 49.0 ppm). The following abbreviations describing the multiplicity are used: (s) singlet, (d) doublet, (t) triplet, (dd) doublet of doublets, (br) broad signal, (m) multiplet. Coupling constants are given in hertz. IR-spectra were obtained with a Biorad PharmalyzIR FT-IR spectrometer (Biorad, Cambridge, MA, USA). Melting points were measured using an apparature Sanyo Gallenkamp (Sanyo Gallenkamp, Loughborough, UK), and are not corrected. TLC was carried out on TLC aluminum sheets, silica gel F_{254} (Merck KGaA, Darmstadt, Germany). All chemicals were purchased from Sigma–Aldrich Chemicals (Deisenhofen, Germany), Acros Organics (Geel, Belgium) and VWR International (Darmstadt, Germany) and were used without further purification.

The syntheses of compounds **1** and **12**, and **2d**, **8** and **9** were performed according to Refs. 16,14, respectively, of compounds **3d**, **3f**, **3h** and **3k** according to Ref. 18 and of compound **3e** according to Ref. 26.

4.1. General synthesis procedure for compounds 1 and 12–14

One equivalent of a substituted 1,8-naphthalenedicarboxylic acid anhydride was suspended in 100 mL of toluene abs. One equiv of the correspondingly substituted *N*,*N*-dimethylpropane-1,3-diamine and a catalytic amount of *p*-toluenesulfonic acid was added and the mixture was refluxed for 6 h. The reaction mixture was allowed to cool to room temperature and extracted with 300 mL (6×50 mL) of aqueous sodium hydrogencarbonate solution pH 9. Precipitated *p*-toluenesulfonic acid was filtered off, the solution dried over anhydrous Na_2SO_4 and the solvent removed in vacuo to give compounds **1** and **12–14**. Recrystallization from ethanol may be applied. Spectroscopic and analytical data see Supplementary data.

4.2. General synthesis procedure for compounds 2a–k, 4, 5, 8, and 9

One equivalent of **1, 12, 13**, and **14**, respectively, 0.5 equiv of the corresponding α,ω-dibromoalkane and a catalytic amount of KI/K_2CO_3 were dissolved in 20–40 mL of acetonitrile. The reaction mixture was refluxed for several days or in an overpressure tube in a synthetic microwave oven (30 °C/min, 500 W, 90 °C) and than cooled to 4 °C for 2 h. The precipitate was filtered off and washed with acetonitrile and pentane, and dried over P_4O_{10}. For spectroscopic and analytical data see Supplementary data.

4.3. General synthesis procedure for compounds 3d and 3g–k

One equivalent of the corresponding α,ω-dibromoalkane was suspended in 50 mL of methanol. A fivefold excess of 2 M solution of triethylamine in methanol was added and the mixture stirred at room temperature for two days. The volume was reduced in vacuo and the obtained oil cooled to 0 °C. The precipitation was initiated by adding some drops of acetone. The precipitate was filtered and washed with acetone and pentane, and dried over P_4O_{10}. For spectroscopic and analytical data see Supplementary data.

4.4. General synthesis procedure for compounds 3e and 3f

One equivalent of the corresponding α,ω-diaminoalkane, 6.5 equiv of methyliodide and 4 equiv of *N*-ethyldiisopropylamine were dissolved in 30 mL of chloroform and stirred at room temperature for two days. The precipitate was filtered off and extracted with acetone in a Soxleth extractor for 10 h to give **3e** and **3f**. For spectroscopic and analytical data see Supplementary data.

4.5. General synthesis procedure for compounds 6 and 7

0.2 Mmol of **4** and **5**, respectively, were dissolved in a mixture of 40 mL ethanol, 20 mL water and 5 mL of acetic acid. To this solution 0.08 g of palladium 10% on activated carbon were added and heated in a synthetic microwave hydrogenation reactor (12.5 bar H_2, 800 W, 30 °C/min, 90 °C) for 1.5 h. The catalyst was filtered and the solvent removed in vacuo. For spectroscopic and analytical data see Supplementary data.

4.6. Malstat assay

The compounds were screened on the human malaria pathogen *P. falciparum* at concentrations between 10 pM and 100 μM. The antimalarial activity was determined for the human malaria pathogen *P. falciparum* in vitro,[19] using chloroquine-sensitive strain 3D7 and in selected cases chloroquine-resistant strain Dd2. Synchronized ring stages of *P. falciparum* strain 3D7 were plated in 96-well-plates at a parasitemia of 1% in the presence of the compounds (dissolved either in H_2O or in dimethyl sulfoxide, DMSO). Incubation of parasites with DMSO alone at a concentration of 0.5% was used for negative control. The parasites were cultivated in triplicates in vitro as described for 72 h. Nineteen parasite viability were screened subsequently using the Malstat assay, which measures the activity of the *Plasmodium*-specific enzyme lactate dehydrogenase as described.[20,21] Each compound was tested two to four times and the IC_{50} inhibitory effect and the standard deviation were calculated.

4.7. Cytotoxicity assay

The cytotoxicities of all compounds were measured in the macrophage cell line J774.1 using the AlamarBlue-based cytotoxicity test.[22] Macrophages were cultured in $NaHCO_3$-buffered RPMI medium containing 10% FCS, 2 mM glutamine, 10 mM Hepes pH 7.2, 100 U/mL penicillin, 50 μg/mL gentamicin, and 50 μM 2-mercaptoethanol without phenol red in the absence or presence of increasing concentrations of the compounds at a cell density of 1×10^5 cells/mL for 24 h at 37 °C, 5% CO_2, and 95% humidity. Following the addition of 10% of AlamarBlue solution, the plates were incubated for 24 and 48 h and the optical densities measured with a Multiscan Ascent enzyme-linked immunosorbent assay (ELISA) reader (Thermo Electron Corporation, Dreieich, Germany) using a test wavelength of 540 nm and a reference wavelength of 630 nm. The final concentration of DMSO in the medium never exceeded 1% (v/v) and had no effect on the proliferation of extracellular or intracellular parasites. Each compound was tested in duplicate. None of the compounds showed any cytotoxic activity (IC_{50} value higher than 100 μM after 48 h).

4.8. Electron microscopy

Blood stages of in vitro cultivated *P. falciparum* parasites were incubated with compounds **5** and **8** at IC_{50} concentrations or with 0.5 vol % DMSO for control for 72 h and fixed in 1% glutaraldehyde and 4% paraformaldehyde in PBS for five days. The samples were post fixed in 1% osmium tetroxide and 1.5% $K_3[Fe(CN)_6]$ in PBS

for 2 h, followed by incubation in 0.5% uranyl acetate for 1 h. Samples were dehydrated in increasing concentrations of ethanol and then incubated for 1 h in propylene oxide, followed by another incubation for 1 h in a 1:1 mixture of propylene oxide and Epon (Sigma–Aldrich). Specimens were subsequently embedded in Epon at 60 °C for 2 d and cut using an RMC MT-7000 ultramicrotome. Post-staining of sections was done with 1% uranyl acetate for 30 min. Photographs were taken with a Zeiss EM10 transmission electron microscope (Carl Zeiss MicroImaging GmbH, Goettingen, Germany) and digital electron micrographs were processed using Adobe Photoshop (Adobe Systems, San Jose, California, USA).

Acknowledgments

We thank Elena Katzowitsch and Tobias Ölschläger (Institute for Molecular Infection Biology of the University of Wuerzburg) for the cytotoxicity screening. This work was funded by the SFB 630 (U.H. and M.T.) and the Emmy-Noether programme (G.P.) of the Deutsche Forschungsgemeinschaft.

Supplementary data

Supplementary data (synthesis information, and analytical and spectroscopic data) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.067.

References and notes

1. WHO Malaria Report **2009**, World Health Organization, Geneve, Switzerland; http://apps.who.int/malaria/wmr2008 (last accessed Nov 18th, 2008).
2. Greenwood, B. M.; Fidock, D. A.; Kyle, D. E.; Kappe, S. H.; Alonso, P. L.; Collins, F. H.; Duffy, P. E. *J. Clin. Invest.* **2008**, *118*, 1266.
3. Greenwood, B. M.; Bojang, K.; Whitty, C. J. M.; Targett, G. A. T. *Malaria, Lancet* **2005**, *365*, 1487.
4. Calas, M.; Ancelin, M. L.; Cordina, G.; Portefaix, P.; Piquet, G.; Vidal-Sailhan, V.; Vial, H. *J. Med. Chem.* **2000**, *43*, 505.
5. Vial, H.; Ancelin, M. L. *Malarial Lipids Subcellu. Biochem.* **1992**, 259.
6. Ancelin, M. L.; Parant, M.; Thuet, M. J.; Philippot, J. R.; Vial, H. *Biochem. J.* **1991**, *273*, 701.
7. Hamzé, A.; Rubi, E.; Arnal, P.; Boisbrun, M.; Carcel, C.; Salom-Roig, X.; Maynadier, M.; Wein, S.; Vial, H.; Calas, M. *J. Med. Chem.* **2005**, *48*, 3639.
8. Degardin, M.; Wein, S.; Durand, T.; Escale, R.; Vial, H.; Vo-Hoang, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5233.
9. Ortial, S.; Denoyelle, S.; Wein, S.; Berger, O.; Durand, T.; Escale, R.; Pellet, A.; Vial, H.; Vo-Hoang, Y. *ChemMedChem* **2010**, *5*, 52.
10. Le Roch, K. G.; Johnson, J. R.; Ahiboh, H.; Chung, D-W. D.; Prudhomme, J.; Plouffe, D.; Henson, K.; Zhou, Y.; Witola, W.; Yates, J. R.; Mamoun, C. B.; Winzeler, E. A.; Vial, H. *BMC Genomics* **2008**, *9*, 513.
11. Alberge, B.; Gannoun-Zaki, L.; Bascunana, C.; Tran Wa Ba, C.; Vial, H.; Cerdan, R. *Biochem. J.* **2010**, *425*, 149.
12. Brana, M. F.; Ramos, A. *Curr. Med. Chem. Anticancer Agents* **2001**, *1*, 237.
13. Sami, S. M.; Dorr, R. T.; Alberts, D. S.; Solyom, A. M.; Remers, W. A. *J. Med. Chem.* **2000**, *43*, 3067.
14. Muth, M.; Bender, W.; Scharfenstein, O.; Holzgrabe, U.; Balatkova, E.; Tränkle, C.; Mohr, K. *J. Med. Chem.* **2003**, *46*, 1031.
15. Bender, W.; Staudt, M.; Tränkle, C.; Mohr, K.; Holzgrabe, U. *Life Sci.* **2000**, *46*, 1031.
16. Muth, M.; Hoerr, V.; Glaser, M.; Ponte-Sucre, A.; Moll, H.; Stich, A.; Holzgrabe, U. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1590.
17. Schmitz, J.; Heller, E.; Holzgrabe, U. *Monatsh. Chem.* **2007**, *138*, 171.
18. Calas, M.; Cordina, G.; Bompart, J.; Bari, M. B.; Jei, T.; Ancelin, M. L.; Vial, H. *J. Med. Chem.* **1997**, *40*, 3557.
19. Ifediba, T.; Vanderberg, J. P. *Nature* **1981**, *294*, 364.
20. Makler, M. T.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 205.
21. Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, L. B.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739.
22. Ahmed, S. A.; Gogal, R. M.; Walsh, J. E. *J. Immunol. Methods* **1994**, *170*, 211.
23. Fuijimoto, K.; Morisaki, D.; Yoshida, M.; Namba, T.; Hye-Sook, K.; Wataya, Y.; Kourai, H.; Kakuta, H.; Sasaki, K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2758.
24. Calas, M.; Ouattara, M.; Piquet, G.; Ziora, Z.; Bordat, Y.; Ancelin, M. L.; Escale, R.; Vial, H. *J. Med. Chem.* **2007**, *50*, 6307.
25. Motoshima, K.; Hiwasa, Y.; Yoshikawa, M.; Fuijimoto, K.; Tai, A.; Kakuta, H.; Sasaki, K. *ChemMedChem* **2007**, *2*, 1527.
26. Burton, A. W. *J. Am. Chem. Soc.* **2007**, *129*, 7627.