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Bistacrine derivatives as new potent antimalarials

Ines Schmidt [a], Gabriele Pradel [b], Ludmilla Sologub [b], Alexandra Golzmann [b], Che J. Ngwa [b], Anna Kucharski [a], Tanja Schirmeister [c], Ulrike Holzgrabe [a,*]

[a] Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
[b] Institute of Molecular Biotechnology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany
[c] Institute of Pharmacy and Biochemistry, University of Mainz, Staudinger Weg 5, 55099 Mainz, Germany

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ABSTRACT

Linking two tacrine molecules results in a tremendous increase of activity against *Plasmodia* in comparison to the monomer. This finding prompted the synthesis of a library of monomeric and dimeric tacrine derivatives in order to derive structure–activity relationships. The most active compounds towards chloroquine sensitive *Plasmodium* strain 3D7 and chloroquine resistant strain Dd2 show IC_{50} values in the nanomolar range of concentration, low cytotoxicity and target the cysteine protease falcipain-2, which is essential for parasite growth.

1. Introduction

In 2013 the World Health Organization estimated 198 million cases of malaria, whereof 584,000 were fatal.[1] The causative pathogens of this disease are four species of the genus *Plasmodium*, i.e., *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, all of them being sporozoic parasites. Malaria—still remaining one of the most deadly diseases—occurs mainly in Africa, where 78% of the deaths apply to children under the age of five.[2] However, the disease also appears in South-East Asia and South America. Nearly 50% of the world population lives in an area of risk. Due to the small number of efficient therapeutics and the increasing rate of resistance the search for new drugs against malaria is urgently needed.

Tacrine **1a** (1,2,3,4-tetrahydroacridine, THA, Fig. 1), which is known as a reversible acetylcholinesterase inhibitor, was in clinical use for the treatment of Alzheimer's disease. It is a common approach to expand the application of drugs that have already been evaluated as drug leads for other diseases.[3–5] Interestingly, in an antiprotozoal screening tacrine was found to exhibit antimalarial activity (IC_{50} = 12.5 μM against chloroquine sensitive strain 3D7). Furthermore, the linking of two tacrine moieties with an alkyl chain of varying length resulted in a tremendous increase of the activity.

These preliminary results prompted the synthesis of a library of monomeric and dimeric tacrine derivatives with different substitution pattern and tether length, and evaluation of the antimalarial activity. Inhibition of β-hematin formation, which is the assumed mechanism of action of chloroquine, was tested because of the similarity of the aminoquinoline moiety of chloroquine and tacrine.[6] Additionally, the inhibition of the cysteine protease falcipain-2 (FP2), which is essential for parasite survival by playing a key role in host hemoglobin degradation, was investigated.

2. Results and discussion

2.1. Chemistry

Monomeric tacrine derivatives were prepared as reported for the unsubstituted compound by means of condensation and subsequent cyclization of 2-aminobenzonitriles and cyclohexanones in toluene with catalytic amounts of *p*-toluene sulfonic acid (Scheme 1).[7] All compounds **1a**–**1f** were obtained in moderate yields after recrystallisation from acetone.

The synthesis of dimers using tacrine derivatives and 1,ω-dibromoalkanes as alkylating reagents failed due to side reactions in addition to low reproducibility. Thus, the dimerisation reaction started off from 9-chloro-1,2,3,4-tetrahydroacridine derivatives, which were obtained by condensation of equimolar amounts of the appropriate 2-aminobenzoic acids with cyclohexanones using an excess of $POCl_3$ (Scheme 2).[8] The excess of $POCl_3$ and high temperatures are necessary to prevent the formation of 4-quinolinones.

The achieved 9-chloro-intermediates (**2a**–**2j**) were heated under reflux with 0.5 equiv of 1,ω-diaminoalkanes for several

* Corresponding author. Tel.: +49 (0) 931/31 85461; fax: +49 (0) 931/31 85494.
E-mail address: ulrike.holzgrabe@uni-wuerzburg.de (U. Holzgrabe).

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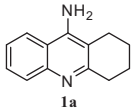


Figure 1. Tacrine (1,2,3,4-tetrahydroacridine, THA).

hours in phenol under an argon atmosphere. Dimeric tacrine compounds **3a**–**3i**, **4a**–**4g**, **5a** and **5b** were obtained in mostly good yields.

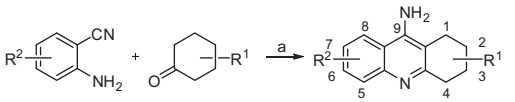
2.2. Biology

2.2.1. In vitro activity against *Plasmodium falciparum*

The antimalarial activity and cytotoxicity of monomeric compounds **1a**–**1f**, unsubstituted dimeric compounds **3a**–**3i** and substituted dimeric compounds **4a**–**4g**, **5a** and **5b** were determined in vitro against *Plasmodium falciparum* chloroquine sensitive strain 3D7, and against J774.1 macrophages by means of the Malstat[9] and the AlamarBlue® assay,[10] respectively. Additionally, the unsubstituted dimeric compounds **3a**–**3i** as well as **5a** and **b** were tested against the chloroquine resistant strain Dd2.

For the monomeric compounds **1a**–**1f**, the following structure–activity relationships could be derived: Introduction of a weak electron-donating propyl group in position 2 leads to an increase of activity to a low, single-digit micromolar IC_{50} value (Table 1).

Besides, exchanging the aromatic proton in position 7 by a halogen substituent as chlorine results in a further significant increase of activity. Of note, electron-withdrawing substituents such as a nitro group as well as electron-donating groups, e.g., methyl groups, in these positions show similar effects. Therefore, a single substitution in position 7 increases activity independently of the electronical structure of the introduced substituent, whereas the combination of chlorine in position 7 and propyl in position 2 is superior. Thus, 7-chloro-2-propyl-1,2,3,4-tetrahydroacridine **1d** exhibited the highest antimalarial activity (IC_{50} = 0.21 μM) (Table 1).



1a: R^1 = H, R^2 = H
1b: R^1 = 2-propyl, R^2 = H
1c: R^1 = H, R^2 = 7-Cl
1d: R^1 = 2-propyl, R^2 = 7-Cl
1e: R^1 = H, R^2 = 7-NO_2
1f: R^1 = H, R^2 = 7-Me

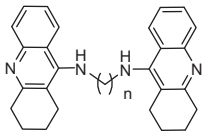
Scheme 1. Reagents and conditions: (a) *p*-TsOH, toluene, 30–192 h, reflux. The description of positions for R^1 and R^2 is outlined.

Table 1
Increasing activities and cytotoxicities in relation to substitution

Compound	R^1	R^2	Yield [%]	IC_{50} (3D7) [μM]	J774.1 [μM]
1a	H	H	68	12.50 ± 4.65	>100
1b	2-Propyl	H	40	1.52 ± 0.28	43.0
1c	H	7-Cl	50	4.02 ± 1.87	43.2
1d	2-Propyl	7-Cl	24	0.21 ± 0.06	8.40
1e	H	7-NO_2	10	3.23 ± 2.73	44.6
1f	H	7-Me	31	2.04 ± 0.03	44.3

Chloroquine: IC_{50} (3D7) = 20 nM, IC_{50} (J774.1) = 18.4 μM.

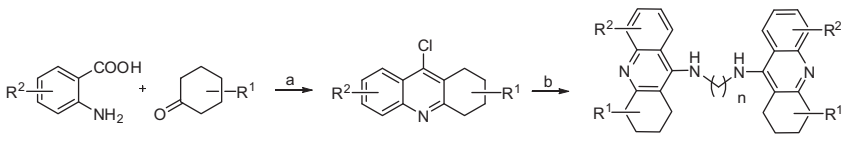
Table 2
In vitro activities against *Plasmodium falciparum* strains 3D7 and Dd2



Compound	*n*	IC_{50} (3D7) [μM]	IC_{50} (Dd2) [μM]	J774.1 [μM]	SI[a/b]
3a	2	0.40 ± 0.07	6.11 ± 2.90	45.2	113 (7.40)
3b	3	0.49 ± 0.16	3.99 ± 3.30	42.6	86.9 (10.7)
3c	4	0.17 ± 0.02	1.39 ± 0.38	8.30	48.8 (5.97)
3d	5	0.63 ± 0.18	0.55 ± 0.09	1.80	2.86 (3.27)
3e	6	0.10 ± 0.03	n.d.	2.97	29.7
3f	7	0.07 ± 0.01	0.22 ± 0.16	1.90	27.1 (8.64)
3g	8	0.11 ± 0.01	0.30 ± 0.02	1.90	17.3 (6.33)
3h	9	0.05 ± 0.02	0.15 ± 0.03	1.80	36.0 (12.0)
3i	10	0.11 ± 0.01	0.14 ± 0.2	1.80	16.4 (12.9)

n.d.: not determined.
[a] IC_{50} (J774.1)/IC_{50}(3D7).
[b] IC_{50} (J774.1)/IC_{50}(Dd2).

The dimerization leads to a substantial increase in activity, depending on the length of the linker (compounds **3a**–**3i**) (Table 2). Interestingly, even a short linker of two methylene units displays a 30-fold enhancement of activity in comparison to THA, which could be increased up to IC_{50} = 50 nM for a chain length of six to nine methylene groups.

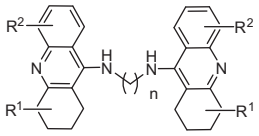


2a: R^1 = H, R^2 = H
2b: R^1 = 3,3-dimethyl, R^2 = H
2c: R^1 = 3-methyl, R^2 = 6-Cl
2d: R^1 = 3,3-dimethyl, R^2 = 6-Cl
2e: R^1 = H, R^2 = 6-Cl
2f: R^1 = 2-methyl, R^2 = 6-Cl
2g: R^1 = 3-methyl, R^2 = 7-NO_2
2h: R^1 = 3,3-dimethyl, R^2 = 7-NO_2
2i: R^1 = H, R^2 = 7-OMe
2j: R^1 = 2-OEt, R^2 = 6-Cl

3a-i: R^1 = H, R^2 = H, n = 2 - 10
4a: R^1 = 3,3-dimethyl, R^2 = H, n = 6
4b: R^1 = 3-methyl, R^2 = 6-Cl, n = 6
4c: R^1 = 3,3-dimethyl, R^2 = 6-Cl, n = 6
4d: R^1 = H, R^2 = 6-Cl, n = 6
4e: R^1 = 2-methyl, R^2 = 6-Cl, n = 6
4f: R^1 = 3-methyl, R^2 = 7-NO_2, n = 6
4g: R^1 = 3,3-dimethyl, R^2 = 7-NO_2, n = 6
5a: R^1 = H, R^2 = 7-OMe, n = 9
5b: R^1 = 2-OEt, R^2 = 6-Cl, n = 9

Scheme 2. Reagents and conditions: (a) excess $POCl_3$, 20 min–4 h, reflux; (b) 1,ω-diaminoalkane, NaI, phenol, 2–4 h, reflux.

Table 3
In vitro activities against *Plasmodium falciparum* strain 3D7 of dimeric, substituted compounds



Compound	*n*	R^1	R^2	IC_{50} (3D7) [μM] (IC_{50} (Dd2) [μM])	J774.1 [μM]	SI
4a	6	3,3-Dimethyl	H	0.03 ± 0.002	1.80	60
4b	6	3-Methyl	6-Cl	0.05 ± 0.003	1.50	30
4c	6	3,3-Dimethyl	6-Cl	0.03 ± 0.007	1.50	50
4d	6	H	6-Cl	0.02 ± 0.007	1.80	90
4e	6	2-Methyl	6-Cl	0.03 ± 0.007	7.80	260
4f	6	3-Methyl	7-NO_2	0.02 ± 0.014	25	1250
4g	6	3,3-Dimethyl	7-NO_2	0.04 ± 0.014	7.90	198
5a	9	H	7-Methoxy	0.11 ± 0.004 (0.16 ± 0.035)*	1.70	15
5b	9	2-Ethoxy	6-Cl	0.20 ± 0.047 (0.16 ± 0.035)*	1.74	9
Chloroquine		—	—	0.02 ± 0.005	18.4	920

* Chloroquine resistant strain Dd2.

A 'the longer the linker, the better the activity'-correlation was found in the chloroquine resistant strain Dd2, too. Of note, whereas unsubstituted dimers with short linkers are less active against chloroquine resistant strain (factor 10), the dimers with longer chains (>7 carbons) exhibit the same activity towards both chloroquine sensitive and resistant strains. This is confirmed by substituted compounds **5a** and **5b** showing almost the same IC_{50} values in both strains (Table 3). This hints to a mode of action which might be different from chloroquine. The cytotoxicity (J774.1) is affected in the same manner. Nevertheless, the most active compound **3h** was found to have an acceptable selectivity index of 36 in case of 3D7 strain and 12 in Dd2.

In order to study the influence of the substituents of the tacrine moiety on the antimalarial activity of the dimers, compounds having a linker of six and nine methylene units were synthesized. These linker lengths showed the highest activities of unsubstituted dimeric tacrine derivatives.

Surprisingly the substituted dimers with a six-membered linker showed a significantly higher antiplasmodial activity in the two-digit nanomolar range of concentration in comparison to compounds **5a** and **5b** with a nine-membered linker, but the tacrine substituents did not influence the activity (Table 3). However, the dimers **4e**, **4f** and **4g** are less toxic resulting in a selectivity index of 1250 for **4f**, which is sufficient for therapeutic use. The cytotoxicity of the other compounds might be due to very high lipophilicity ($clogP \sim 10$), which also has negative impact on oral bioavailability and druggability.

2.2.2. Inhibition of β-hematin formation

Hence inhibiting the formation of β-hematin (hemozoin) is the assumed mode of action for chloroquine[11] and the tacrine skeleton resembles the quinoline moiety of chloroquine, the inhibition of β-hematin formation was investigated.

In the intraerythrocytic stage of plasmodial infection more than 80% of the infected host red blood cells hemoglobin is degraded in the digestive vacuole of the parasites.[12] The resulting oligopeptides and amino acids are transferred back into the cytoplasm, whereas the non-proteinaceous component heme (ferroprotoporphyrin IX) stays in the digestive vacuole. This is toxic for the parasites because huge amounts of reactive oxygen species are being generated. Therefore heme is detoxificated by conversion into nontoxic, insoluble hemozoin (malaria pigment). Chugh et al.[13] assume that chloroquine is able to form a complex with heme and thus hinders the detoxification of heme. Ncokazi and Egan[14] developed a simple colorimetric high-throughput screening assay, which is based on the formation of a low spin complex of pyridine with heme, but not with hemozoin. The absorbance of this complex obeys Lambert–Beer's law, making it useful for quantitating the actual heme concentration in heme/hemozoin mixtures. This tool allows for investigating whether compounds inhibit the formation of β-hematin.

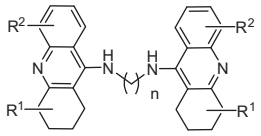
In the experiments chloroquine was used as a positive and DMSO as a negative control. We were able to obtain an IC_{50} value for chloroquine of 3.01 equiv with respect to the inserted heme. This is comparable to the values given in the literature (1.91 equiv).[14] Tacrine derivatives did not inhibit hemozoin formation, so we assert that it is definitely not addressed by the compounds reported herein (data not shown). This is supported by the fact that the activities of these compounds are similar in both chloroquine sensitive and resistant strains.

2.2.3. Inhibition of falcipain-2

Falcipain-2 (FP2) is localized in the acidic food vacuole and is mainly responsible for the host hemoglobin degradation.[15] Within this pathway, the parasites obtain amino acids for their own protein synthesis and thus for their survival.[16] Many chemotypes such as peptides, e.g., peptidyl aziridines,[17] vinyl sulfones,[18] peptidomimetics[19] or nonpeptidic compounds such as chalcones,[20] isoquinolines,[21] and thiosemicarbazones[22] act as FP-2-inhibitors and validate this target for antimalarials.

To examine the inhibitory effect of the compounds against recombinant falcipain-2 Cbz-Phe-Arg-AMC was used as a substrate. Falcipain-2 cleaves the fluorescent aminomethyl-coumarin (AMC) from the substrate. This enables monitoring the enzyme activity by measuring the fluorescence intensity which is directly proportional to the concentration of AMC. The first step was an initial screening using a 20 μM concentration of test compounds, whereas pure DMSO was used as negative control. Only six compounds with good activity in vitro passed this screening with an inhibition >35% and were chosen for further kinetic experiments in order to determine the IC_{50} values (Table 4).[23] Therefore, the substrate was added to a buffer solution containing enzyme and different inhibitor concentrations (compounds **3g**, **5b**: 0–100 μM; **3h**, **4d**: 0–50 μM, **4f**, **5a**: 0–25 μM). Fluorescence was

Table 4
Inhibition of falcipain-2



Compound	R^1	R^2	*n*	% Inhibition [I] = 20 μM	IC_{50} (FP-2) [μM]	IC_{50} (3D7) [μM]
3g	H	H	8	34.4	17.1	0.11
3h	H	H	9	45.9	11.5	0.05
4d	H	6-Cl	6	36.9	17.3	0.02
4f	3-Methyl	7-NO_2	6	56.7	5.2	0.02
5a	H	7-Methoxy	9	62.2	14.9	0.11
5b	2-Ethoxy	6-Cl	9	25	n.d.	0.20

n.d.: not determined.

observed over a period of 10 min. The assays were performed in duplicate. The straight lines of derived progress curves of all tested compounds indicate a non time-dependent, reversible mode of inhibition. IC_{50} values were determined by plotting the inhibitor concentration against ΔF/min and subsequent nonlinear regression analysis.

The derived low micromolar IC_{50} values indicate a good inhibition of the compounds to the enzyme. The IC_{50} values for the falcipain-2-inhibition are substantially higher than those determined for *Plasmodia*, but they are in the similar range of concentration. This might indicate that falcipain-2 represents at least one target of the compounds studied.

3. Conclusion

Mono- and dimeric tacrine derivatives with different substitution patterns of the tacrine moiety and linker lengths were synthesized and tested for their antimalarial activity. Especially dimers were found to have an antiplasmodial activity in the nanomolar concentration range, but this was often associated with cytotoxicity. Nevertheless one compound (**4f**) could be identified which exhibited a very high selectivity index (>1000) and thus can be regarded as a new lead compound. Since the cytotoxicity of the other compounds might be due to their high lipophilicity, the new lead compound will be optimized with regard to an even higher antiplasmodial activity along with a decreased lipophilicity. In addition, the inhibitory activity towards falcipain-2 has to be considered.

4. Experimental

4.1. Chemistry

4.1.1. Instruments and analyses

Melting points were determined with a Sanyo Gallenkamp melting point apparatus MPD350 BM 3.5 and are reported uncorrected. Reactions were monitored by thin-layer chromatography (TLC) using coated silica gel plates (Merck, Darmstadt, Germany), detection under ultraviolet light (λ = 254 nm). Column chromatography was performed on Merck Silica Gel 60 (70–230 mesh; Merck, Darmstadt). IR-Spectra were recorded on a Jasco FT-IR-6100 spectrometer (Jasco, Groß-Umstadt, Germany) using an ATR unit. ^{1}H (400.132 MHz) and ^{13}C (100.613 MHz) NMR spectra were acquired on a Bruker AV 400 instrument (Bruker Biospin, Ettlingen, Germany). Chemical shifts are expressed in δ (ppm) relative to TMS as internal standard. The purities of the synthesized compounds were determined via HPLC (Knauer Eurospher C18-column, 150 × 4.6 mm, 5 μm), mobile phase: 25 mM phosphate buffer, pH 7.4: MeOH 3:7, flow: 1.5 mL/min. LC/MS was conducted on an Agilent 1100 analytical HPLC with DAD detection and an Agilent LC/MSD Trap (Agilent Technologies, Böblingen, Germany). ESI-MS data was conducted in positive mode. For LC/MS, following conditions were used: Agilent Zorbax SB-CN (50 × 4.6 mm, 3.5 μm), (A) CH_3CN + 0.1% acetic acid, (B) H_2O + 0.1% acetic acid, gradient: 95% A (0–5 min), 95% → 10% A (5–10 min), 10% A (10–15 min), flow: 0.4 mL/min. MS-detection: ESI, nebulizer pressure: 40 psi, drying gas: 9 l/min, drying gas temperature: 350 °C. All reagents and solvents were obtained from commercial suppliers and were used without further purification.

Tacrine[7] (**1a**) and compounds **1c**,[24] **1e**,[25] **1f**,[24] **2a**,[8] **2e**,[8] **2f**,[26] **2i**,[27] and **3a**–**i**[28,29] were synthesized as reported.

4.1.2. 2-Propyl-1,2,3,4-tetrahydroacridine-9-amine (1b)

2-Aminobenzonitrile (236 mg, 2.00 mmol) and 4-propyl-cyclohexanone (337 mg, 2.40 mmol) were condensed with *p*-toluene-sulfonic acid (69 mg, 0.34 mmol) in toluene (10 mL) for 96 h under reflux. Isolation was performed as described for the compound **1a**.[7] Yield: 40% (193 mg) yellowish powder. Mp: 186–187 °C. R_f = 0.44 ($CHCl_3$/MeOH, 6:1). Purity: 99 %. IR $\tilde{\nu}$ [cm^{-1}]: 3493, 3301, 3050, 1636, 1424, 1011, 753. ^{1}H NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 7.89 (1H, d, ^3J = 8.4, C^5-*H*), 7.69 (1H, dd, ^3J = 8.3, ^4J = 1.0, C^8-*H*), 7.56 (1H, ddd, ^3J = 8.4, 6.8, ^4J = 1.0, C^6-*H*), 7.36 (1H, dd, ^3J = 8.3, 6.8, ^4J = 1.1, C^7-*H*), 4.69 (2H, br s, NH_2), 3.11 (1H, ddd, ^2J = 17.4, ^3J = 5.0, 3.4, C^4-H_2), 3.00 (1H, ddd, ^2J = 17.4, ^3J = 11.8, 5.5, C^4-H_2), 2.73 (1H, ddd, ^2J = 15.8, ^3J = 5.5, ^4J = 1.3, C^1-H_2), 2.17 (1H, dd, ^2J = 15.8, ^3J = 10.2, C^1-H_2), 2.03–2.10 (1H, m, C^3-H_2), 1.82–1.91 (1H, m, C^2-*H*), 1.41–1.55 (5H, m, C^3-H_2, C^2-CH_2-CH_2-CH_3), 0.97 (3H, t, ^3J = 6.8 Hz, C^2-CH_2-CH_2-CH_3). ^{13}C NMR ($CDCl_3$, δ [ppm]): 158.7 (C^{4a}), 146.6 (C^{10a}), 146.5 (C^9), 128.9 (C^5), 128.6 (C^6), 124.0 (C^7), 119.7 (C^8), 117.2 (C^{8a}), 110.3 (C^{9a}), 39.1 (C^2-CH_2-CH_2-CH_3), 33.9 (C^4), 31.0 (C^2), 30.8 (C^1), 29.1 (C^3), 20.3 (C^2-CH_2-CH_2-CH_3), 14.5 (C^2-CH_2-CH_2-CH_3). ESI-MS: *m/z* 241.2 $[M+H]^+$.

4.1.3. 7-Chloro-2-propyl-1,2,3,4-tetrahydroacridine-9-amine (1d)

2-Amino-5-chlorobenzonitrile (305 mg, 2.00 mmol) and 4-propyl-cyclohexanone (337 mg, 2.40 mmol) were condensed with *p*-toluene-sulfonic acid (69 mg, 0.34 mmol) in toluene (10 mL) for 72 h under reflux. Isolation was performed as described for compound **1a**. Yield: 24 % (132 mg) yellow solid. Mp: 208209 °C. R_f = 0.53 ($CHCl_3$/MeOH, 6:1). Purity: >99%. IR $\tilde{\nu}$ [cm^{-1}]: 3493, 3299, 3126, 1639, 1445, 1128, 678. ^{1}H NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 8.27 (1H, d, ^4J = 2.3, C^8-*H*), 7.62 (1H, d, ^3J = 9.0, C^5-*H*), 7.46 (1H, dd, ^3J = 9.0, ^4J = 2.3, C^6-*H*), 6.44 (2H, br s, NH_2), 2.85 (2H, m, C^4-H_2), 2.75 (1H, dd, ^2J = 17.0, ^3J = 5.0, C^1-H_2), 2.06 (1H, dd, ^2J = 17.0, ^3J = 10.5, C^1-H_2), 1.94–1.90 (1H, m, C^3-H_2), 1.79–1.68

(1H, m, C^2-*H*), 1.54–1.44 (2H, m, C^2-CH_2-*CH*$_2$-CH_3), 1.43–1.34 (3H, m, C^3-*H*$_2$, C^2-*CH*$_2$-CH_2-CH_3), 0.93 (3H, t, ^3J = 7.1, C^2-CH_2-CH_2-*CH*$_3$). ^{13}C NMR (DMSO-d_6, δ [ppm]): 158.1 (C^{4a}), 147.5 (C^{10a}), 144.9 (C^9), 130.1 (C^5), 128.3 (C^6), 127.0 (C^7), 121.1 (C^8), 117.7 (C^{8a}), 109.5 (C^{9a}), 38.6 (C^2-*CH*$_2$-CH_2-CH_3), 33.3 (C^2), 33.2 (C^4), 30.4 (C^1), 28.8 (C^3), 19.5 (C^2-CH_2-*CH*$_2$-CH_3), 14.2 (C^2-CH_2-CH_2-*CH*$_3$). ESI-MS: *m*/*z* 275.2 $[M+H]^+$.

4.1.4. 9-Chloro-3,3-dimethyl-1,2,3,4-tetrahydroacridine (2b)

Anthranilic acid (1.00 g, 7.29 mmol) and 3,3-dimethylcyclohexanone (1.01 g, 8.02 mmol) were refluxed in 8 mL $POCl_3$ for 6 h. The reaction was cooled to room temperature and kept in an ice bath. The solution was carefully neutralized with saturated $NaHCO_3$-solution. The mixture was then extracted three times with 40 mL chloroform and the combined organic phases were dried over $MgSO_4$ and concentrated in vacuo. The crude product was recrystallized from acetone to afford the pure compound **2b**.[8] Yield: 50 % (896 mg) yellow solid. Mp: 63–64 °C. R_f = 0.76 (EtOAc/PE, 1:2). IR $\tilde{\nu}$ [cm^{-1}]: 3059, 1633, 1583, 1482, 1136, 1315, 762. ^{1}H NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 8.18 (1H, dd, ^3J = 8.3, ^4J = 1.2, C^8-*H*), 8.00 (1H, d, ^3J = 8.4, C^5-*H*), 7.67 (1H, ddd, ^3J = 8.4, 7.0, ^4J = 1.2, C^6-*H*), 7.55 (1H, ddd, ^3J = 8.3, 7.0, ^4J = 1.1, C^7-*H*), 3.05 (2H, t, ^3J = 6.9, C^1-*H*$_2$), 2.92 (2H, br s, C^4-*H*$_2$), 1.75 (2H, t, ^3J = 6.9, C^2-*H*$_2$), 1.07 (6H, br s, C^3-(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 159.3 (C^{4a}), 147.5 (C^{10a}), 141.9 (C^9), 129.4 (C^{8a}), 128.8 (C^5), 127.8 (C^6), 126.7 (C^7), 125.6 (C^{9a}), 123.8 (C^8), 48.0 (C^4), 35.3 (C^3), 30.2 (C^2), 28.0 (C^3-(CH_3)$_2$), 25.0 (C^1). ESI-MS: *m*/*z* 246.1 $[M+H]^+$.

4.1.5. 6,9-Dichloro-3-methyl-1,2,3,4-tetrahydroacridine (2c)

2-Amino-4-chlorobenzoic acid (686 mg, 4.00 mmol) and 3-methylcyclohexanone (494 mg, 4.40 mmol) were refluxed in 10 mL $POCl_3$ for 2 h. Isolation was performed as described for compound **2b**. Yield: 54% (574 mg) yellowish solid. Mp: 92–93 °C. R_f = 0.89 (EtOAc/PE, 1:2). IR $\tilde{\nu}$ [cm^{-1}]: 2929, 1653, 1542, 1474, 1315, 875, 812. ^{1}H NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 8.10 (1H, d, ^3J = 9.0, C^8-*H*), 8.00 (1H, s, C^5-*H*), 7.48 (1H, dd, ^3J = 9.0, ^4J = 2.0, C^7-*H*), 3.24–3.16 (2H, m, C^1-*H*$_2$, C^4-*H*$_2$), 2.88 (1H, ddd, ^2J = 17.7, ^3J = 11.1, 6.1, C^1-*H*$_2$), 2.71 (1H, dd, ^2J = 17.6, ^3J = 10.8, C^4-*H*$_2$), 2.10–1.96 (2H, m, C^2-*H*$_2$, C^3-*H*), 1.51 (1H, dtd, ^2J = 13.7, ^3J = 11.1, 6.3, C^2-*H*$_2$), 1.15 (3H, d, ^3J = 6.5 Hz, C^3-*CH*$_3$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 160.8 (C^{4a}), 146.7 (C^{10a}), 141.7 (C^9), 135.2 (C^6), 128.9 (C^{9a}), 127.7 (C^7), 127.6 (C^5), 125.3 (C^8), 124.1 (C^{8a}), 42.5 (C^4), 30.7 (C^2), 28.9 (C^3), 27.2 (C^1), 21.6 (C^3-*CH*$_3$). ESI-MS: *m*/*z* 266.1 $[M+H]^+$.

4.1.6. 6,9-Dichloro-3,3-dimethyl-1,2,3,4-tetrahydroacridine (2d)

2-Amino-4-chlorobenzoic acid (1.03 g, 6.00 mmol) and 3,3-dimethylcyclohexanone (833 mg, 6.60 mmol) were refluxed in 10 mL $POCl_3$ for 2 h. Isolation was performed as described for compound **2b**. Yield: 63% (1.06 g) yellowish solid. Mp: 102–103 °C. R_f = 0.92 (EtOAc/PE, 1:1). IR $\tilde{\nu}$ [cm^{-1}]: 3064, 1634, 1544, 1473, 1362, 902, 812. ^{1}H NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 8.10 (1H, d, ^3J = 9.0, C^8-*H*), 7.99 (1H, s, C^5-*H*), 7.49 (1H, dd, ^3J = 9.0, ^4J = 2.0, C^7-*H*), 3.02 (2H, t, ^3J = 6.9, C^1-*H*$_2$), 2.89 (2H, br s, C^4-*H*$_2$), 1.74 (2H, t, ^3J = 6.9, C^2-*H*$_2$), 1.06 (6H, br s, C^3-(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 160.6 (C^{4a}), 147.3 (C^{10a}), 141.5 (C^9), 135.4 (C^6), 128.1 (C^{9a}), 127.7 (C^7), 127.7 (C^5), 125.3 (C^8), 124.1 (C^{8a}), 47.9 (C^4), 35.2 (C^2), 30.1 (C^3), 27.9 (C^3-(CH_3)$_2$), 24.95 (C^1). ESI-MS: *m*/*z* 280.1 $[M+H]^+$.

4.1.7. 9-Chloro-3-methyl-7-nitro-1,2,3,4-tetrahydroacridine (2g)

2-Amino-5-nitrobenzoic acid (911 mg, 5.00 mmol) and 3-methylcyclohexanone (561 mg, 5.00 mmol) were refluxed in 10 mL $POCl_3$ for 1 h. Isolation was performed as described for compound **2b**. Yield: 53% (735 mg) beige solid. Mp: 122–123 °C. R_f = 0.63 (EtOAc/PE, 1:1). IR $\tilde{\nu}$ [cm^{-1}]: 3031, 1617, 1517, 1476, 1301, 848, 741. ^{1}H NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 9.13 (1H, d, ^4J = 2.5, C^8-*H*), 8.42 (1H, dd, ^3J = 9.2, ^4J = 2.5, C^6-*H*), 8.08 (1H, d, ^3J = 9.2, C^5-*H*), 3.30–3.21 (2H, m, C^1-*H*$_2$, C^4-*H*$_2$), 2.93 (1H, ddd, ^2J = 17.7, ^3J = 11.1, 6.1, C^1-*H*$_2$), 2.75 (1H, dd, ^2J = 17.9, ^3J = 10.8, C^4-*H*$_2$), 2.15–1.98 (2H, m, C^2-*H*$_2$, C^3-*H*), 1.55 (1H, dtd, ^2J = 13.8, ^3J = 11.1, 6.3, C^2-$_2$), 1.17 (3H, d, ^3J = 6.5, C^3-*CH*$_3$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 164.0 (C^{4a}), 148.8 (C^{10a}), 145.8 (C^7), 143.2 (C^9), 131.9 (C^{9a}), 130.7 (C^5), 124.8 (C^{8a}), 122.9 (C^6), 121.2 (C^8), 42.9 (C^4), 30.4 (C^2), 28.8 (C^3), 27.3 (C^1), 21.5 (C^3-*CH*$_3$).

ESI-MS: *m*/*z* 277.1 $[M+H]^+$.

4.1.8. 9-Chloro-3,3-dimethyl-7-nitro-1,2,3,4-tetrahydroacridine (2h)

2-Amino-5-nitrobenzoic acid (728 mg, 4.00 mmol) and 3,3-dimethylcyclohexanone (505 mg, 4.00 mmol) were refluxed in 10 mL $POCl_3$ for 1 h. Isolation was performed as described for compound **2b**. Yield: 52% (607 mg) beige solid. Mp: 162–163 °C. R_f = 0.83 (EtOAc/PE, 1:1). IR $\tilde{\nu}$ [cm^{-1}]: 3083, 1617, 1519, 1473, 1311, 899, 738. ^{1}H NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 9.12 (1H, d, ^4J = 2.5, C^8-*H*), 8.42 (1H, dd, ^3J = 9.2, ^4J = 2.5, C^6-*H*), 8.09 (1H, d, ^4J = 9.2, C^5-*H*), 3.08 (2H, t, ^3J = 6.9, C^1-*H*$_2$), 2.95 (2H, s, C^4-*H*$_2$), 1.78 (2H, t, ^3J = 6.9, C^2-*H*$_2$), 1.08 (6H, s, C^3-(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 163.8 (C^{4a}), 149.0 (C^{10a}), 145.8 (C^7), 143.1 (C^9), 130.7 (C^5), 130.2 (C^{9a}), 124.8 (C^{8a}), 122.9 (C^6), 121.2 (C^8), 47.2 (C^4), 35.0 (C^2), 30.2 (C^3), 27.9 (C^3-(CH_3)$_2$), 25.1 (C^1). ESI-MS: *m*/*z* 291.1 $[M+H]^+$.

4.1.9. 6,9-Dichloro-2-ethoxy-1,2,3,4-tetrahydroacridine (2j)

2-Amino-4-chlorobenzoic acid (1.20 g, 7.00 mmol) and *p*-ethoxycyclohexanone[30] (1.00 g, 7.93 mmol) were refluxed in 20 mL $POCl_3$ for 2 h. Isolation was performed as described for compound **2b**. Yield: 80% (1.66 g) greyish solid. Mp: 59–60 °C. R_f = 0.34 (EtOAc/PE, 1:5). IR $\tilde{\nu}$ [cm^{-1}]: 3057, 1603, 1545, 1474, 1095, 936, 735. ^{1}H NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 8.05 (1H, d, ^3J = 9.0, C^8-*H*), 7.96 (1H, d, ^4J = 2.1, C^5-*H*), 7.45 (1H, dd, ^3J = 9.0, ^4J = 2.1, C^7-*H*), 3.95–3.90 (1H, m, C^2-*H*), 3.69–3.55 (2H, m, C^2-O-*CH*$_2$-CH_3), 3.32–3.19 (2H, m, C^1-*H*$_2$, C^4-*H*$_2$), 3.10–3.02 (2H, m, C^1-*H*$_2$, C^4-*H*$_2$), 2.13–2.08 (2H, m, C^3-*H*$_2$), 1.23 (3H, t, ^3J = 7.0, C^2-O-CH_2-*CH*$_3$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 160.1 (C^{4a}), 147.1 (C^{10a}), 142.1 (C^9), 135.6 (C^6), 127.7 (C^7), 127.6 (C^5), 127.1 (C^{9a}), 125.3 (C^8), 123.9 (C^{8a}), 72.4 (C^2), 63.8 (O-*CH*$_2$-CH_3), 33.8 (C^4), 30.5 (C^1), 27.1 (C^3), 15.7 (O-CH_2-*CH*$_3$). ESI-MS: *m*/*z* 296.1 $[M+H]^+$.

4.1.10. N^1,N^6-Bis(3,3-dimethyl-1,2,3,4-tetrahydroacridine-9-yl)-hexane-1,6-diamine (4a)

Compound **2b** (300 mg, 1.22 mmol) was heated with hexane-1,6-diamine (71 mg, 0.61 mmol), NaI (10 mg) and phenol (500 mg) at 180 °C for 2 h under argon atmosphere. After cooling to room temperature, 20 mL of a 3 N KOH solution were added and the crude product was extracted three times with 30 mL EtOAc. The organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography to afford pure compound **4a**.[28] Yield: 59% (194 mg) yellowish solid. Mp: 170–172 °C. R_f = 0.42 ($CHCl_3$/MeOH, 10:2). Purity: >99%. IR $\tilde{\nu}$ [cm^{-1}]: 3057, 1603, 1545, 1474, 1095, 936, 735. ^{1}H NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 7.95 (2H, d, ^3J = 8.3, ^4J = 1.1, C^8-*H*), 7.90 (2H, dd, ^3J = 8.4, ^4J = 1.0, C^5-*H*), 7.54 (2H, ddd, ^3J = 8.4, 6.8, ^4J = 1.1, C^6-*H*), 7.33 (2H, ddd, ^3J = 8.3, 6.8, ^4J = 1.0, C^7-*H*), 3.89 (2H, br s, N*H*), 3.50 (4H, t, ^3J = 7.1, C^a-*H*$_2$), 2.84 (4H, s, C^4-*H*$_2$), 2.71 (4H, t, ^3J = 6.8, C^1-*H*$_2$), 1.71 (4H, t, ^3J = 6.8, C^2-*H*$_2$), 1.68–1.62 (4H, m, C^b-*H*$_2$), 1.44–1.41 (4H, m, C^c-*H*$_2$), 1.04 (12H, s, C^3-(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 158.3 (C^{4a}), 150.6 (C^9), 148.1 (C^{10a}), 129.1 (C^5), 128.3 (C^6), 123.7 (C^7), 123.1 (C^8), 120.4 (C^{8a}), 114.6 (C^{9a}), 49.5 (C^a), 48.0 (C^4), 35.6 (C^2), 31.9 (C^b), 30.0 (C^3), 28.0 (C^3-(CH_3)$_2$), 26.9 (C^c), 22.2 (C^1). ESI-MS: *m*/*z* 268.2 $[M+2H]^{2+}$.

4.1.11. N^1,N^6-Bis(6-chloro-3-methyl-1,2,3,4-tetrahydro-acridine-9-yl)hexane-1,6-diamine (4b)

Compound **2c** (200 mg, 0.76 mmol) was heated with hexane-1,6-diamine (44 mg, 0.38 mmol), NaI (10 mg) and phenol (800 mg) under reflux for 3 h. Isolation was performed as described for compound **4a**. Yield: 63% (136 mg) brownish solid. Mp: 156–157 °C. R_f = 0.49 ($CHCl_3$/MeOH, 10:2). Purity: 92%. IR $\tilde{\nu}$ [cm^{-1}]: 3052, 1603, 1552, 1489, 1154, 895, 734. ^{1}H NMR ($CDCl_3$, δ [ppm], J [Hz]): 7.89 (2H, d, ^4J = 1.8, C^5-*H*), 7.80 (2H, d, ^3J = 9.0, C^8-*H*), 7.19 (2H, dd, ^3J = 9.0, 1.8, C^7-*H*), 3.84 (2H, br s, N*H*), 3.40 (4H, t, ^3J = 6.6, C^a-H_2), 3.04 (2H, ddd, ^2J = 17.0, ^3J = 4.2, ^4J = 1.4, C^4-H_2), 2.69 (2H, ddd, ^2J = 15.9, ^3J = 5.7, 3.2, C^1-H_2), 2.59–2.51 (4H, m, C^1-H_2, C^4-H_2), 1.99–1.86 (4H, m, C^2-H_2, C^3-*H*), 1.60–1.55 (4H, C^b-H_2), 1.39 (2H, dtd, ^2J = 12.9, ^3J = 10.9, 5.7, C^2-H_2), 1.37–1.33 (4H, C^c-H_2), 1.12 (6H, d, ^3J = 6.5, C^3-CH_3). ^{13}C NMR ($CDCl_3$, δ [ppm]): 159.6 (C^{4a}), 150.7 (C^9), 148.0 (C^{10a}), 134.2 (C^6), 127.9 (C^5), 124.6 (C^8), 124.5 (C^7), 118.6 (C^{8a}), 115.5 (C^{9a}), 49.6 (C^a), 42.2 (C^4), 31.8 (C^b), 31.0 (C^2), 28.9 (C^3), 26.8 (C^c), 24.3 (C^1), 21.6 (C^3-CH_3). ESI-MS: m/z 288.2 $[M+2H]^{2+}$.

4.1.12. N^1,N^6-Bis(6-chloro-3,3-dimethyl-1,2,3,4-tetrahydro-acridine-9-yl)hexane-1,6-diamine (4c)

Compound **2d** (280 mg, 1.00 mmol) was heated with hexane-1,6-diamine (58 mg, 0.50 mmol), NaI (10 mg) and phenol (500 mg) under reflux for 2 h. Isolation was performed as described for compound **4a**. Yield: 38% (117 mg) yellowish solid. Mp: 102–103 °C. R_f = 0.53 ($CHCl_3$/MeOH, 10:2). Purity: 98%. IR $\tilde{\nu}$ [cm^{-1}]: 3052, 1605, 1555, 1488, 1076, 876, 737. ^{1}H NMR ($CDCl_3$, δ [ppm], J [Hz]): 7.92 (2H, br s, C^5-*H*), 7.90 (2H, d, ^3J = 9.1, C^8-*H*), 7.27 (2H, ddd, ^3J = 9.1, ^4J = 2.2, C^7-*H*), 3.96 (2H, br s, N*H*), 3.54–3.49 (4H, m, C^a-H_2), 2.82 (4H, s, C^4-H_2), 2.66 (4H, t, ^3J = 6.8, C^1-H_2), 1.71 (4H, t, ^3J = 6.8, C^2-H_2), 1.69–1.63 (4H, m, C^b-H_2), 1.44–1.41 (4H, m, C^c-H_2), 1.04 (12H, s, C^3-(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 159.2 (C^{4a}), 150.5 (C^9), 149.2 (C^{10a}), 134.7 (C^6), 127.5 (C^5), 124.8 (C^8), 124.5 (C^7), 116.3 (C^{8a}), 114.3 (C^{9a}), 49.6 (C^a), 47.8 (C^4), 35.4 (C^2), 31.9 (C^b), 29.9 (C^3), 27.9 (C^3-(CH_3)$_2$), 26.8 (C^c), 22.0 (C^1). ESI-MS: m/z 302.2 $[M+2H]^{2+}$.

4.1.13. N^1,N^6-Bis(6-chloro-1,2,3,4-tetrahydroacridine-9-yl)-hexan-1,6-diamine (4d)

Compound **2e** (300 mg, 1.19 mmol) was heated with hexane-1,6-diamine (70 mg, 0.60 mmol), NaI (10 mg) and phenol (500 mg) under reflux for 2 h. Isolation was performed as described for compound **4a**. Yield: 35% (116 mg) yellow solid. Mp: 125–126 °C. R_f = 0.31 ($CHCl_3$/MeOH, 10:2). Purity: 98 %. IR $\tilde{\nu}$ [cm^{-1}]: 3122, 1604, 1556, 1505, 1077, 803, 672. ^{1}H NMR ($CDCl_3$, δ [ppm], J [Hz]): 7.89 (2H, d, ^4J = 2.2, C^5-*H*), 7.86 (2H, d, ^3J = 9.0, C^8-*H*), 7.26 (2H, dd, ^3J = 9.0, ^4J = 2.2, C^7-*H*), 3.48–3.44 (4H, m, C^a-H_2), 3.03–3.01 (4H, m, C^4-H_2), 2.66–2.64 (4H, m, C^1-H_2), 1.92–1.89 (8H, m, C^2-H_2, C^3-H_2), 1.67–1.62 (4H, m, C^b-H_2), 1.43–1.40 (4H, m, C^c-H_2). ^{13}C NMR ($CDCl_3$, δ [ppm]): 159. 8 (C^{4a}), 150.8 (C^9), 148.3 (C^{10a}), 134.2 (C^6), 127.8 (C^5), 124.6 (C^8), 124.5 (C^7), 118.6 (C^{8a}), 116.1 (C^{9a}), 49.6 (C^a), 34.2 (C^4), 31.8 (C^b), 26.9 (C^c), 24.8 (C^1), 23.1, 22.8 (C^2, C^3). ESI-MS: m/z 274.2 $[M+2H]^{2+}$.

4.1.14. N^1,N^6-Bis(6-chloro-2-methyl-1,2,3,4-tetrahydro-acridine-9-yl)hexane-1,6-diamine (4e)

Compound **2f** (266 mg, 1.00 mmol) was heated with hexane-1,6-diamine (58 mg, 0.50 mmol), NaI (15 mg) and phenol (1.00 g) under reflux for 3 h. Isolation was performed as described for compound **4a**. Yield: 60% (175 mg) yellowish solid. Mp: 188–189 °C. R_f = 0.46 ($CHCl_3$/MeOH, 10:2). Purity: >99%. IR $\tilde{\nu}$ [cm^{-1}]: 3063, 1605, 1555, 1502, 1137, 923, 871. ^{1}H NMR ($CDCl_3$, δ [ppm], J [Hz]): 7.85 (2H, d, ^3J = 9.0, C^8-*H*), 7.84 (2H, d, ^4J = 2.2, C^5-*H*), 7.24 (2H, dd, ^3J = 9.0, ^4J = 2.2, C^7-*H*), 3.98 (2H, br s, N*H*), 3.50–3.39 (4H, m, C^a-H_2), 3.07 (2H, ddd, ^2J = 17.6, ^3J = 5.5, 3.2, C^4-H_2), 2.98 (2H, ddd, ^2J = 17.6, ^3J = 11.5, 5.7, C^4-H_2), 2.73 (2H, ddd, ^2J = 15.6, ^3J = 5.1, ^4J = 1.3, C^1-H_2), 2.16 (2H, dd, ^2J = 15.6, ^3J = 10.4, C^1-H_2), 2.00–1.86 (2H, m, C^2-*H*, C^3-H_2), 1.66–1.63 (4H, m, C^b-H_2), 1.48 (2H, dtd, ^2J = 12.7, ^3J = 11.5, 5.5, C^3-H_2), 1.42–1.38 (4H, m, C^c-H_2), 1.13 (6H, d, ^3J = 6.5, C^2-CH_3). ^{13}C NMR ($CDCl_3$, δ [ppm]): 159.3 (C^{4a}), 151.0 (C^9), 148.0 (C^{10a}), 134.4 (C^6), 127.2 (C^5), 124.6 (C^8), 124.5 (C^7), 118.3 (C^{8a}), 115.6 (C^{9a}), 49.4 (C^a), 33.4 (C^4), 33.3 (C^1), 31.7 (C^b), 30.7 (C^3), 29.3 (C^2), 26.8 (C^c), 22.1 (C^2-CH_3). ESI-MS: m/z 289.1 $[M+2H]^{2+}$.

4.1.15. N^1,N^6-Bis(3-methyl-7-nitro-1,2,3,4-tetrahydro-acridine-9-yl)hexane-1,6-diamine (4f)

Compound **2g** (300 mg, 1.08 mmol) was heated with hexane-1,6-diamine (63 mg, 0.54 mmol), NaI (10 mg) and phenol (500 mg) under reflux for 2 h. Isolation was performed as described for compound **4a**. Yield: 35% (116 mg) brownish solid. Mp: 195–196 °C. R_f = 0.65 ($CHCl_3$/MeOH, 10:2). Purity: >99 %. IR $\tilde{\nu}$ [cm^{-1}]: 3101, 1609, 1558, 1490, 1254, 1142, 834. ^{1}H NMR ($CDCl_3$, δ [ppm], J [Hz]): 9.03 (2H, d, ^4J = 2.3, C^8-*H*), 8.26 (2H, dd, ^3J = 9.3, ^4J = 2.3, C^6-*H*), 7.91 (2H, d, ^3J = 2.3, C^5-*H*), 4.30 (2H, br s, N*H*), 3.70–3.65 (4H, m, C^a-H_2), 3.14 (2H, dd, ^2J = 17.5, ^3J = 2.9, C^4-H_2), 2.75 (2H, ddd, ^2J = 15.9, ^3J = 5.8, 3.2, C^1-H_2), 2.69–2.58 (4H, m, C^1-H_2, C^4-H_2), 2.11–2.06 (2H, m, C^2-H_2), 2.03–1.92 (2H, m, C^3-*H*), 1.86–1.76 (4H, m, C^b-H_2), 1.57 - 1.45 (6H, m, C^2-H_2, C^c-H_2), 1.13 (6H, d, ^3J = 6.5, C^3-CH_3). ^{13}C NMR ($CDCl_3$, δ [ppm]): 178.8 (C^{4a}), 152.1 (C^9), 150.5 (C^{10a}), 142.8 (C^7), 130.3 (C^5), 121.9 (C^6), 121.6 (C^8), 117.7 (C^{8a}), 115.4 (C^9), 49.5 (C^a), 42.7 (C^4), 31.7 (C^b), 30.7 (C^2), 28.7 (C^3), 26.7 (C^c), 24.1 (C^1), 21.5 (C^3-CH_3). ESI-MS: m/z 299.2 $[M+2H]^{2+}$.

4.1.16. N^1,N^6-Bis(3,3-dimethyl-7-nitro-1,2,3,4-tetrahydro-acridine-9-yl)hexane-1,6-diamine (4g)

Compound **2h** (300 mg, 1.03 mmol) was heated with hexane-1,6-diamine (60 mg, 0.52 mmol), NaI (50 mg) and phenol (1.00 g) under reflux for 2 h. Isolation was performed as described for compound **4a**. Yield: 52% (169 mg) orange solid. Mp: 165–166 °C. R_f = 0.76 ($CHCl_3$/MeOH, 10:2). Purity: 92%. IR $\tilde{\nu}$ [cm^{-1}]: 3041, 1613, 1583, 1493, 1260, 1151, 832. ^{1}H NMR ($CDCl_3$, δ [ppm], J [Hz]): 9.06 (2H, d, ^4J = 2.3, C^8-*H*), 8.28 (2H, dd, ^3J = 9.3, ^4J = 2.3, C^7-*H*), 7.92 (2H, d, ^3J = 9.3, C^5-*H*), 4.31 (2H, br s, N*H*), 3.74–3.68 (4H, m, C^a-H_2), 2.84 (4H, s, C^4-H_2), 2.67 (4H, t, ^3J = 6.7, C^2-H_2), 1.83 (4H, m, C^b-H_2), 1.75 (4H, t, ^3J = 6.7, C^1-H_2), 1.56 (4H, m, C^c-H_2), 1.05 (6H, s, C^3-(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 161.8 (C^{4a}), 152.0 (C^9), 150.9 (C^{10a}), 142.8 (C^7), 130.4 (C^5), 121.9 (C^6), 121.8 (C^8), 117.8 (C^{8a}), 114.4 (C^{9a}), 49.5 (C^a), 47.7 (C^4), 35.2 (C^1), 31.8 (C^b), 29.9 (C^3), 27.9 (C^3-(CH_3)$_2$), 26.7 (C^c), 21.9 (C^2). ESI-MS: m/z 313.3 $[M+2H]^{2+}$.

4.1.17. N^1,N^9-Bis(7-methoxy-1,2,3,4-tetrahydroacridine-9-yl)-nonane-1,9-diamine (5a)

Compound **2i** (1.00 g, 4.04 mmol) was heated with nonane-1,9-diamine (320 mg, 2.02 mmol), NaI (100 mg) and phenol (2.00 g) under reflux for 5 h. Isolation was performed as described for compound **4a**. Yield: 28% (330 mg) brownish foam. Mp: 61–62 °C. R_f = 0.42 ($CHCl_3$/MeOH, 10:2). Purity: 99%. IR $\tilde{\nu}$ [cm^{-1}]: 3002, 1623, 1580, 1500, 1226, 1107, 1031, 828. ^{1}H NMR (DMSO-d_6, δ [ppm], J [Hz]): 7.65 (2H, d, ^3J = 9.1, C^5-*H*), 7.44 (2H, d, ^4J = 2.7, C^8-*H*), 7.23 (2H, dd, ^3J = 9.1, ^4J = 2.7, C^6-*H*), 5.61 (2H, br s, N*H*), 3.85 (6H, s, C^7-O-CH_3), 3.37–3.34 (4H, m, C^a-H_2), 2.88 (4H, t, ^3J = 6.2, C^4-H_2), 2.70 (4H, t, ^3J = 6.0, C^1-H_2), 1.84–1.73 (8H, C^2-H_2, C^3-H_2), 1.54–1.47 (4H, m, C^b-H_2), 1.26–1.20 (4H, m, C^c-H_2), 1.20–1.13 (6H, m, C^d-H_2, C^e-H_2). ^{13}C NMR (DMSO-d_6, δ [ppm]): 155.5 (C^7), 154.7 (C^{4a}), 150.3 (C^9), 141.1 (C^{10a}), 128.5 (C^5), 120.6 (C^{8a}), 120.4 (C^6), 116.1 (C^{9a}), 101.9 (C^8), 55.4 (C^7-O-CH_3), 47.3 (C^a), 32.4 (C^4), 30.5 (C^b), 28.8 (C^d), 28.6 (C^e), 26.2 (C^c), 25.2 (C^1), 22.6, 22.2 (C^2, C^3). ESI-MS: m/z 291.3 $[M+2H]^{2+}$.

4.1.18. N^1,N^9-Bis(6-chloro-2-ethoxy-1,2,3,4-tetrahydro-acridine-9-yl)nonane-1,9-diamine (5b)

Compound **2j** (1.00 g, 3.37 mmol) was heated with nonane-1,9-diamine (268 mg, 1.69 mmol), NaI (100 mg) and phenol (3.00 g) under reflux for 2 h. Isolation was performed as described for compound **4a**. Yield: 53% (603 mg) brownish foam. Mp: 152–153 °C (as dihydrochloride). R_f = 0.45 ($CHCl_3$/MeOH, 10:2). Purity: 97%. IR $\tilde{\nu}$ [cm^{-1}]: 3071, 1606, 1577, 1556, 1345, 1090, 802. ^{1}H NMR ($CDCl_3$, δ [ppm], J [Hz]): 7.89 (4H, m, C^5-*H*, C^8-*H*), 7.27 (2H, dd, ^3J = 9.0, ^4J = 2.1, C^7-*H*), 3.98 (2H, br s, N*H*), 3.87–3.81 (2H, m, C^2-*H*), 3.74–3.67 (2H, m, C^2-O-C*H*$_2$-CH_3), 3.61–3.54 (2H, m, C^2-O-C*H*$_2$-CH_3), 3.50–3.46 (4H, m, C^a-*H*$_2$), 3.20 (2H, dt, ^2J = 17.9, ^3J = 5.5, C^3-*H*$_2$), 3.05–2.96 (4H, m, C^1-*H*$_2$, C^3-*H*$_2$), 2.64 (2H, dd, ^2J = 15.4, ^3J = 7.6, C^1-*H*$_2$), 2.26–2.19 (2H, m, C^4-*H*$_2$), 1.97–1.87 (2H, m, C^4-*H*$_2$), 1.68–1.61 (4H, m, C^b-*H*$_2$), 1.38–1.33 (4H, m, C^c-*H*$_2$), 1.30–1.26 (6H, m, C^d-*H*$_2$, C^e-*H*$_2$), 1.25 (6H, t, ^3J = 7.0, C^2-O-CH_2-C*H*$_3$). ^{13}C NMR ($CDCl_3$, δ [ppm]): 158.4 (C^{4a}), 151.4 (C^9), 148.2 (C^{10a}), 134.4 (C^6), 127.8 (C^5), 124.6 (C^8), 124.5 (C^7), 118.1 (C^{8a}), 113.3 (C^{9a}), 74.0 (C^2), 63.9 (C^2-O-C*H*$_2$-CH_3), 49.7 (C^a), 31.9 (C^b), 31.6 (C^1), 31.4 (C^3), 29.5 (C^e), 29.4 (C^d), 27.6 (C^4), 27.0 (C^c), 15.8 (C^2-O-CH_2-C*H*$_3$). ESI-MS: m/z 339.2 $[M+2H]^{2+}$.

4.2. Biological assays

4.2.1. In vitro activity against *Plasmodium falciparum*

Compound concentrations between 10 nM and 100 μM were used to screen on *Plasmodium falciparum* chloroquine-sensitive strain 3D7 and, in selected cases, on 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 in DMSO). Incubation of parasites with pure DMSO at a concentration of 0.5% was used for negative control. The parasites were cultivated in triplicate for 72 h. Parasite viability was subsequently screened using the Malstat assay, which measures the activity of the *Plasmodium*-specific enzyme lactate dehydrogenase as described.[9] Each compound was tested twice, and the IC_{50} inhibitory activity as well as the standard deviation were calculated.

4.2.2. Falcipain-2 enzyme assay

The assays were performed twice in a Varian Cary Eclipse spectrofluorometer in 96-well plates using a microplate reader (excitation 380 nm, emission 460 nm) and a total volume of 200 μl. Cbz-Phe-Arg-AMC was used as substrate. To 180 μL of sodium acetate buffer (50 mM, pH 5.5, containing 5 mM EDTA and 200 mM NaCl) 5 μL enzyme solution, 10 μL inhibitor stock solution (400 μM in DMSO) and 5 μL substrate stock solution (1 mM) were added and the increase of the fluorescence was observed over 5 min. The enzyme activity was calculated from the slopes of the curves obtained (ΔF vs time). Compounds having an inhibitory effect greater than 35 % at 20 μM were chosen for detailed assays. Therefore 185 μL buffer, 5 μL enzyme solution, 5 μL inhibitor solution and 5 μL substrate solution were mixed and the fluorescence intensity was recorded over 10 min. Inhibitor concentrations were in the range of 0–100 μM in case of compounds **3g** and **5b**, 0–50 μM in case of compounds **3h** und **4d**, and 0–25 μM in case of compounds **4f** and **5a**. IC_{50} values were obtained by nonlinear regression analysis using Origin® Pro 2015.

4.2.3. Cytotoxicity assay

The cytotoxicity of all compounds was determined in the macrophage cell line J774.1 using the AlamarBlue®-based cytotoxicity test according to Ahmed et al.[10]. Macrophages were cultured in RPMI medium containing 10% FCS. The compounds were dissolved in DMSO and serial dilutions ranging from 100 μM to 1.28 nM were prepared. Incubation was done at 37 °C with 5% CO_2 for 24 h. After adding 20 μL AlamarBlue® solution, the plates were incubated for further 24 and 48 h. Optical densities were measured with a Multiscan Ascent enzyme-linked immunosorbent assay (ELISA) reader (Thermo Electron Corporation, Dreieich, Germany) applying a wavelength of λ = 540 nm and a reference wavelength of λ = 630 nm.

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