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New derivatives of 7-chloroquinolin-4-amine with antiprotozoal activity

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Abstract Novel ω-aminoacyl and -alkyl derivatives of 7-chloroquinolin-4-amine were prepared and their structures confirmed by NMR spectroscopy. Their antiprotozoal activities were examined in vitro against the sensitive NF54 strain as well as against the multiresistant K_1 strain of *Plasmodium falciparum* and against *Trypanosoma brucei rhodesiense* (STIB 900). The results were compared with the activities of clinically used drugs. Their antitrypanosomal activities were only moderate whereas their antiplasmodial activities looked very promising. Some were equal or slightly more active than chloroquine against the sensitive strain.

However, in comparison to chloroquine, the activity of the new compounds was decreased much less in the resistant strain. Several possessed activity against both strains in low nanomolar concentration.

Keywords Amines; Antiplasmodial activity; Heterocycles; NMR spectroscopy; Nucleophilic substitutions; Quinoline

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Introduction

The tropical infection Malaria and the neglected tropical disease Human African Trypanosomiasis (HAT) represent great health issues in recent years.

Malaria is caused by the bite of a female anopheles mosquito which is infected with the protozoan genus *Plasmodium*. According to the World Health Organization, there were about 214 million cases of malaria and estimated 438 000 deaths in 2015 [1]. Four types of malaria parasites are pathogenic for humans: *Plasmodium falciparum, P. malariae, P. ovale and P. vivax*. The majority of infections are caused by *Plasmodium falciparum*,

the most deadly malaria parasite. The main problem is the major increase of *Plasmodium falciparum* strains which have become resistant to previous generations of medicines. In recent years resistance even to recommended artemisinin-based therapies has become prevalent across an expanding area of Southeast Asia [2]. Therefore, the development of new drugs for the fight against the most deadly drug-resistant strains of *Plasmodium falciparum* is absolutely essential [3].

HAT, also known as sleeping sickness, is transmitted by the bite of tsetse flies that are infected with the protozoan *Trypanosoma brucei*. For the first time in the last 50 years, the number of cases reported has dropped below 10 000 in 2009 after continued control efforts. This decline of number of cases has continued with 6314 new infections reported in 2013. However, the estimated population at different levels of risk is about 70 million people [4]. HAT arises in two forms: Infections with *T. b. rhodesiense* are acute infections, lasting from a few weeks to several months, whereas *T. b. gambiense* infections which represent a large part of the infections, are chronic illnesses, generally lasting several years without any major signs or symptoms [5]. Sleeping sickness is fatal without treatment. Only a couple of drugs are available for the therapy of HAT: pentamidine, suramin, melarsoprol, eflornithine and nifurtimox. But serious side-effects are a problem with all of them, and resistance is

increasing [6]. The central phase of *T. b. rhodesiense* infections can only be cured with melarsoprol [5]. Unfortunately, melarsoprol causes a deadly encephalopathy in 3-10% of the treated patients [7]. Therefore, new drugs in the fight against Human African Trypanosomiasis are urgently needed [8].

Our current study deals with the derivatization of the 4-amino-7-chloroquinoline scaffold. Some of the newly synthesized compounds were already named by Krogstad [9], but no data concerning their synthesis and their characterization were given. The influence of different basic side-chains at ring position 4 of the 7-chloroquinoline core on the biological properties was investigated. The new compounds were tested in vitro for their activities against the sensitive strain NF54 and the multiresistant K_1 strain of *P. falciparum* as well as against *T. brucei rhodesiense*. Their cytotoxicity was determined using L6 rat cells. The results were compared to those of clinically used drugs.

Results and Discussion

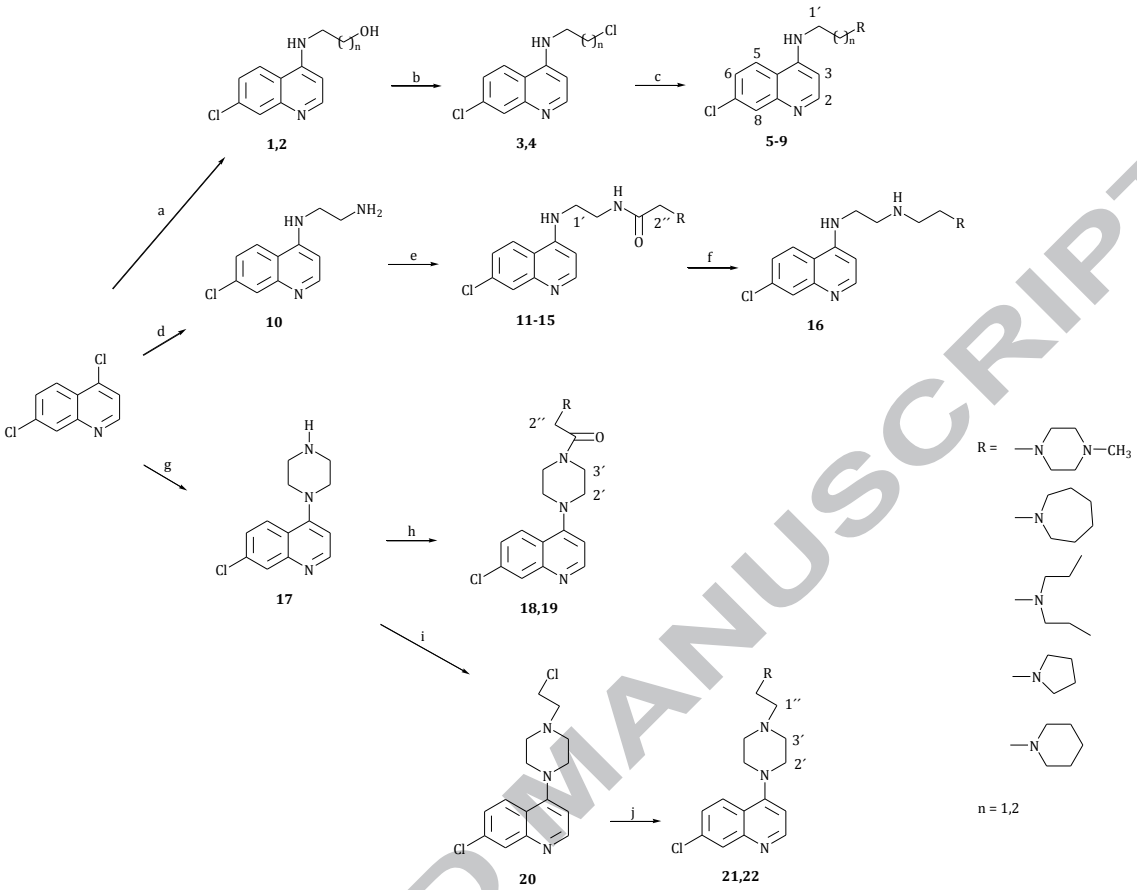
Chemistry

For all routes of synthesis 4,7-dichloroquinoline was used as starting material (Scheme 1). ω-(Quinolin-4-yl)alkanoles **1, 2** were prepared by reaction with ω-aminoalkanoles. Upon treatment with thionyl chloride the terminal hydroxy group was replaced by a chlorine substituent yielding

compounds **3, 4**. New derivatives **5-9** were obtained by reaction with secondary amines. Treatment of 4,7-dichloroquinoline with ethylendiamine gave compound **10** [10]. This intermediate reacted in a one-pot reaction [11] with 2-chloroacetyl chloride and subsequently with various secondary amines to the corresponding *N*-(2-aminoacyl) derivatives **11-15**. Their reduction with $LiAlH_4$ led to the loss of the chlorine atom in ring position 7. Therefore an alternative approach was applied using a borane-DMS-complex [12] yielding the corresponding *N*-(ω-aminoalkyl) analogue **16**.

In derivatives **18, 19, 21, 22** the cyclic secondary diamine piperazine was used as linkage between the quinoline core and the aminoalkyl side chain. The 4-(piperazin-1-yl)quinoline **17** was prepared from 4,7-dichloroquinoline with excess piperazine [13]. Compound **17** was reacted in a one-pot reaction with 2-chloroacetyl chloride and subsequently with different secondary amines yielding the 4-[4-(2-aminoacyl)piperazin-1-yl]quinolines **18, 19**.

Compound **17** was directly transformed into its 4-(2-chloroethyl)piperazine derivative **20** by reaction with a large excess of 1,2-dichloroethane. In a final step the reaction of intermediate **20** with secondary amines gave compounds **21, 22** (Scheme 1).



Scheme 1:

(a) ω-amino-alkanol, triethylamine, 130°C, 5 h; (b) $SOCl_2$, DMF, 24 h, 20°C; (c) method A: sec. amine, triethylamine, DMF, 10-20 h, 130°C; method B: sec. amine, KI, 20°C, 96 h; (d) ethylenediamine, 135°C, 3 h; (e) (1.) 2-chloroacetyl chloride, DIPEA or triethylamine, CH_2Cl_2, 0°C→20°C, 1 h; (2.) sec. amine, 20°C, 70-90 h; (f) borane-DMS-complex, dry THF, 95°C, 20 h; (g) piperazine, triethylamine, 130°C, 5 h; (h) (1.) 2-chloroacetyl chloride, DIPEA, CH_2Cl_2, 0°C→20°C, 1 h; (2.) sec. amine, 20°C, 108-168 h; (i) 1,2-dichloroethane, triethylamine, 110°C, 48 h; (j) sec. amine, triethylamine, MeOH, 85°C, 20 h;

Compounds	**R**	**n**
5	4-Methylpiperazin-1-yl	1
6	Dipropylamino	1
7	Azepan-1-yl	1
8	4-Methylpiperazin-1-yl	2
9	Azepan-1-yl	2
11, 18	4-Methylpiperazin-1-yl	-
12	Piperidino	-
13, 19, 21	Dipropylamino	-
14, 16, 22	Azepan-1-yl	-
15	Pyrrolidino	-

<Scheme 1>

The structures of all newly synthesized compounds were investigated by nmr spectroscopy. Evaluation of the below-mentioned typical chemical shifts provided structural evidence. Interpretation of the cross peaks in the 2D nmr spectra enabled the reliable assignment of signals. The replacement of the chlorine atom in ring position 4 by a nitrogen caused significant shifts of the signals in their ^{1}H and ^{13}C nmr spectra. In comparison to 4,7-dichloroquinoline the resonance of the proton in ring position 3 of compounds **1-22** is shifted ca. 1 ppm to lower frequencies in ^{1}H nmr spectra due to the mesomeric effect of the nitrogen substituent. Remarkable shifts were observed in their ^{13}C nmr spectra. For the alkylamino derivates **1-16** the resonances for C-3 and C-4a were shifted 23 and 7 ppm to lower frequencies, respectively. The corresponding shifts for their 4-piperazinyl analogues **17-22** were 13 ppm upfield for C-3 and 3 ppm upfield for C-4a.

The signals of the C-4 atoms were shifted 7 ppm to higher frequencies for compounds **1-16** and 13 ppm downfield for compounds **17-22**. The resonances of the other ring atoms changed marginally compared to 4,7-dichloroquinoline. The nmr data of compounds **1-4, 10, 17** were in accordance with published data [13, 14, 15]. *N*-substitution of compounds **3, 4** caused a significant 1 ppm upfield shift of the resonances for the adjacent methylene protons in the ^{1}H spectra of compounds **5-9** due to the replacement of a chlorine by a nitrogen atom. In addition, the *N*-alkylation was confirmed by a 3.5 ppm downfield shift of the $N(CH_2)_2$ signal in the ^{13}C nmr spectrum of compound **6**. The $N(CH_2)_2$ resonances of the corresponding cyclic amino groups of compounds **5, 7-9** were shifted 7 ppm to higher frequencies. The published NMR-data for compound **8** [16] turned out wrong. The resonances of all NCH_2 protons are between 2.4-2.8 ppm, a typical value for tertiary amines. Reference [16] gives signals at 4.37-4.40 ppm for eight of these protons instead. The $ArNCH_2$ carbon of **8** appears at ca. 44 ppm, a common shift in case of a propyl chain. In comparison reference [16] describes a signal at 34 ppm. Obviously the authors picked wrong data sets. The correct nmr data of **8** are presented in the experimental part. The *N*-acylation of compounds **10** and **17** was indicated by the appearance of an additional signal for a carbonyl resonance at ca. 174 ppm in the ^{13}C nmr spectra of the amides **11-15** and

18, 19. The resonances of their NCH_2 protons were shifted 0.4-0.7 ppm downfield due to acylation, whereas the corresponding carbon signals were shifted to lower frequencies. The vicinal $N(CH_2)_2$ signals of the piperazinyl moiety of compound **17** were shifted 7 ppm downfield due to *N*-alkylation. The reduction of the amide **14** to compound **16** caused the replacement of the carbonyl resonance at 175 ppm by an additional signal for a methylene group at 47 ppm. Moreover, the signal of the adjacent α-methylene group was shifted 5 ppm to higher field. The success of all *N*-alkylation reactions was evident from the appearance of $N(CH_2)_2$ resonances for all tertiary amino groups of compounds **5-9** and **11- 22** at 51-62 ppm in their ^{13}C nmr spectra, which is a common value.

Antiprotozoal activity

One goal of this study was the investigation of the influence of the different aminoalkyl side-chains of the new compounds on the antiprotozoal activity. They were tested in vitro for their activities against the K_1 strain (resistant to chloroquine and pyrimethamine) as well as against the chloroquine sensitive strain NF54 (sensitive to all known anti-malarial drugs) of *Plasmodium falciparum.* Furthermore, their activity against *Trypanosoma brucei rhodesiense* was determined using microplate

assays. Their cytotoxicity was examined with rat skeletal myoblasts (L-6 cells). Chloroquine, melarsoprol and podophyllotoxin served as standards.

The antitrypanosomal activity and selectivity of all new compounds was only moderate. The 4-[2-(dipropylamino)ethyl]amino quinoline **6** showed the highest antitrypanosomal activity (IC_{50} = 0.14 µM). It also had the best selectivity among the new compounds (SI: 106.7).

Concerning the antiplasmodial activity, most of the newly synthesized compounds exhibited very persuading activities. Representatives of the *N*-[ω-(dialkylamino)alkyl]quinolin-4-amine series showed compared to chloroquine (*P.f.*NF54: 0.0039 µM) similar (*P.f.*NF54: **6, 7, 8**: 0.0031-0.0038 µM) or slightly decreased (*P.f.*NF54: **5, 9**: 0.0063-0.0066 µM) activity against the sensitive NF54 strain of *Plasmodium falciparum*. The linker between the terminal amino group and the quinoline moiety is an alkylamino chain of 3-4 atoms. An elongation of the linker by an additional alkylamino or an acetamido group leads to a decrease of antiplasmodial activity (*P.f.*NF54: **11-16**: 0.011-0.036 µM), but the compounds still possess antiplasmodial activity in low nanomolar concentrations. The reduction the amido group in the linker of compound **14** to a secondary amino group in **16** decreased the cytotoxicity. However, the antiplasmodial activity remained almost unaffected indicating that the influence of the amido group is negligible. The larger distance between the

quinoline ring and the terminal amino group could also explain the comparably low activity of the 4-piperazinyl quinolines (*P.f.*NF54: **18, 19, 21, 22**: 0.070-0.230 µM) (Table 1).

Table 1

Activities of compounds **5-22** against *T.brucei rhodesiense*, *P.falciparum* NF54, *P.falciparum* K_1 and L-6 cells, expressed as IC_{50} (µM).[a]

Compounds	*T.brucei rhodesiense* IC_{50} (µM)	S.I.= IC_{50} (Cyt.)/IC_{50} (*T.b.r.*)	*P.f. NF54* [b] IC_{50} (µM)	S.I.= IC_{50} (Cyt.)/IC_{50} (*P.f.NF54)*	*P.f.* K_1 [c] IC_{50} (µM)	S.I.= IC_{50} (Cyt.)/IC_{50} (*P.f.*K_1)	Cytotoxicity L-6 cells IC_{50} (µM)
5	0.69	24.25	0.0066	2535	0.036	464.7	16.73
6	0.14	106.7	0.0033	4527	0.0098	1524	14.94
7	0.59	22.42	0.0033	4009	0.016	826.9	13.23
8	1.14	15.93	0.0031	5858	0.031	585.8	18.16
9	0.78	25.58	0.0063	3167	0.038	525.0	19.95
11	18.32	2.08	0.036	1057	2.87	13.26	38.05
12	1.28	18.54	0.011	2157	0.092	257.9	23.73
13	0.94	23.36	0.014	1568	0.030	732.0	21.96
14	0.28	50.78	0.011	1293	0.055	258.5	14.22
15	0.74	62.69	0.018	2577	0.075	618.5	46.39
16	1.28	59.48	0.011	6921	0.066	1153	76.13
18	2.02	39.52	0.14	570.3	0.28	285.1	79.84
19	1.46	48.27	0.23	306.4	0.31	227.3	70.47
21	1.07	53.11	0.11	516.6	0.088	645.8	56.83
22	1.61	15.76	0.070	362.4	0.075	338.3	25.37
CQ			0.0039	23313	0.15	606.1	90.92
MEL	0.01	778.0					7.78
POD							0.012

CQ = chloroquine; MEL = melarsoprol; POD = podophyllotoxine
[a] Values represent the average of four determinations (two determinations of two independent experiments)
[b] active against all known anti-malarial drugs
[c] Resistant to chloroquine and pyrimethamine

<Table 1>

In resistant strains of *Plasmodium falciparum* the activity of chloroquine is seriously decreased by resistance mechanisms in the vacuoles of the parasites [17]. This is the reason why the usage of chloroquine is extremely

restricted nowadays. The majority of the new compounds **5-22** possessed much higher activity (*P.f.*K_1: 0.0098-0.092 µM) against the multiresistant K_1 strain than chloroquine (*P.f.*K_1: 0.15 µM). They show good selectivity indices (SI: 227.3-1524). The *N*-[ω-(dipropylamino)ethyl]quinolin-4-amine **6** is the most promising in this series. It retains activity in low nanomolar concentration (*P.f.*K_1: 0.0098 µM) and exhibits the highest selectivity index (SI: 1524). The resistance index (RI) is defined as the ratio of the IC_{50} of a resistant line to that of a sensitive strain. Thus, the higher the RI, the higher the level of resistance. The RI of chloroquine (RI: 38.5) in *P.f.*K_1 versus *P.f.*NF54 demonstrates a significant inactivation of the drug. The newly prepared compounds had far better RI-values than chloroquine with the exception of compound **11** (RI: 79.7). The antiplasmodial activities of the 4-piperazinylquinolines **18, 19, 21, 22** as well as of compounds with a terminal dipropylamino group **6, 13** differed only slightly between the sensitive and the resistant strain, which is obvious from their RIs (RI: 0.8-2.97). All remaining compounds showed a distinct decrease of activity in the resistant strain (RI: 4.17-10.0). The most promising compound is *N*-[ω-(dipropylamino)ethyl]quinolin-4-amine **6**. It exhibited higher activity than chloroquine in both strains (*P.f.*NF54: 0.0033 µM vs. 0.0039 µM; *P.f.*K_1: 0.0098 µM vs. 0.15 µM). Its far better RI (2.97 vs. 38.5) indicates that the

antiplasmodial activity of compound **6** is only slightly influenced by the reaction mechanisms that inactivate chloroquine in *P.f.*K_1.

Therefore the emphasis for future studies will be placed on the synthesis of further modified 4-amino-7-chloroquinolines with low RI to circumvent resistance (Table 2).

Table 2

resistance index RI = ratio of the IC_{50} (µM) for the resistant versus the sensitive strain

Compounds	*P.f. K1* IC_{50} (µM)	*P.f. NF54* IC_{50} (µM)	***resistance index RI***
CQ	0.15	0.0039	**38.5**
5	0.036	0.0066	**5.45**
6	0.0098	0.0033	**2.97**
7	0.016	0.0033	**4.85**
8	0.031	0.0031	**10.0**
9	0.038	0.0063	**6.03**
11	2.87	0.036	**79.7**
12	0.092	0.011	**8.36**
13	0.030	0.014	**2.14**
14	0.055	0.011	**5.00**
15	0.075	0.018	**4.17**
16	0.066	0.011	**6.00**
18	0.28	0.14	**2.00**
19	0.31	0.23	**1.35**
21	0.088	0.11	**0.80**
22	0.075	0.070	**1.07**

<Table 2>

Conclusion

This paper dealt with the syntheses and the antitrypanosomal and antiplasmodial activities of new ω-aminoacyl and ω-aminoalkyl substituted derivatives of 7-chloroquinolin-4-amine. The biological activities of the new compounds are heterogeneous. All newly synthesized compounds

showed only weak to moderate activity against *Trypanosoma brucei rhodesiense*. However, most of the compounds demonstrate good activity against the sensitive NF54 strain and the multiresistant K_1 strain of *Plasmodium falciparum*.

Compounds with a short alkylamino linker of 3-4 atoms between the terminal amino group and the quinoline core exhibited significantly higher activities in the chloroquine-sensitive strain than analogues with longer side chains. Some of these compounds were as active as chloroquine. An insertion of an alkylamino or an acetamido group in the linker decreases the antiplasmodial activity significantly. Compounds with a 4-piperazinylquinoline moiety were the least active. This could be caused by sterical reasons and by the larger distance between quinoline ring and terminal amino group. Unlike chloroquine, the majority of new compounds had small resistance indices and therefore retained activity in the resistant strain in low nanomolar concentration. A single compound possessing a terminal dipropylamino group and an ethylamino linker showed antiplasmodial activity in low nanomolar concentration against a sensitive and a multiresistant strain. Its selectivity indices as well as its resistance index are very promising.

Experimental

6.1 Instrumentation and chemicals

Melting points were obtained on a digital melting point apparatus Electrothermal IA 9200. IR spectra were recorded using a Bruker Alpha Platinum ATR FTIR spectrometer (KBr discs). The purity (>95%) of compounds is checked with HPLC using UV detection as a matter of routine. NMR spectra: Varian Unity Inova 400 (298 K) 5 mm tubes, TMS as internal standard. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz), ^{1}H- and ^{13}C-resonances were assigned using ^{1}H,^{1}H- and ^{1}H,^{13}C-correlation spectra and are numbered as given in the formulas. The resonances of the terminal ω-dialkylamino group of compounds **5-9** are marked with a double quote, the resonances of compounds **11-16, 18, 19, 21, 22** are marked with a triple quote. Signal multiplicities are abbreviated as follows: br broad, d doublet, dd double doublet, m multiplet, s singlet, t triplet, td triple doublet, quin quintet, sex sextet. HRMS: GCT-Premier, Waters (EI, 70eV). Materials: column chromatography (CC): silica gel 60 (Merck 70 - 230 mesh, pore-diameter 60 Å), thin-layer chromatography (TLC): TLC plates silica gel 60 F254 (Merck).

6.2 Syntheses

The routes of syntheses of compounds **1-4, 10, 17** have already been reported elsewhere [10, 13, 16].

General procedures for the synthesis of 4-[(ω-aminoalkyl)amino]-7-chloroquinolines (5-9).

Method A: 7-Chloro-*N*-(ω-chloroalkyl)quinolin-4-amine **3, 4**, equimolar quantities of triethylamine and an excess of the corresponding secondary amine were refluxed at 130°C-150°C in dry dimethylformamide (DMF) for 20 hours in an atmosphere of Ar. Subsequently the reaction batch was coevaporated with benzene. Afterwards the residue was dissolved in CH_2Cl_2 and was washed with aqua dest. The organic phase was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in vacuo yielding compounds **5-7**, **9**.

Method B: 7-Chloro-*N*-(ω-chloroalkyl)quinolin-4-amine **4** was dissolved in an excess of secondary amine. A catalytic amount of KI was added. The mixture was stirred for 96 h at ambient temperature in an atmosphere of Ar. Subsequently, benzene was added and the solvents were evaporated. The residue was dissolved in CH_2Cl_2, washed with water until the aqueous phase reacted neutral, dried over anhydrous sodium sulfate, filtered and finally the solvent was removed in vacuo giving compound **8**.

7-Chloro-*N*-[2-(4-methylpiperazin-1-yl)ethyl]quinolin-4-amine (**5**).

Method A: The reaction of 7-chloro-*N*-(2-chloroethyl)quinolin-4-amine **3** (0.300 g, 1.24 mmol), triethylamine (0.13 g, 1.24 mmol) and *N*-methylpiperazine (0.62 g, 6.2 mmol) in 1 mL DMF yielded compound **5** (0.284 g, 76%). A purification by use of column chromatography was done (silica, CH_2Cl_2+MeOH=19+1). IR (KBr): $\tilde{\nu}$ = 3065, 2961, 2933, 2787, 1610, 1580, 1549, 1453, 1429, 1365, 1334, 1283, 1250, 1166, 1150, 1082, 1016, 875, 838, 803 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 2.33 (s, 3H, NCH_3), 2.42-2.64 (m, 8H, 2´´-H, 3´´-H, 5´´-H, 6´´-H), 2.79 (t, *J* = 5.9 Hz, 2H, 2´-H), 3.32 (td, *J* = 5.9 , 4.1 Hz, 2H, 1´-H), 5.98 (br s, 1H, NH), 6.38 (d, *J* = 5.3 Hz, 1H, 3-H), 7.40 (dd, *J* = 9.0, 2.0 Hz, 1H, 6-H), 7.68 (d, *J* = 9.0 Hz, 1H, 5-H), 7.96 (d, *J* = 2.0 Hz, 1H, 8-H), 8.54 (d, *J* = 5.3 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 38.86 (C-1´), 46.03 (NCH_3), 52.57 (C-2´´, C-6´´), 55.27 (C-3´´, C-5´´), 55.37 (C-2´), 99.29 (C-3), 117.33 (C-4a), 121.05 (C-5), 125.34 (C-6), 128.79 (C-8), 134.81 (C-7), 149.09 (C-8a), 149.73 (C-4), 152.12 (C-2) ppm; HRMS (EI+) calcd for $C_{16}H_{21}ClN_4$: 304.1455; found: 304.1471.

7-Chloro-*N*-[2-(dipropylamino)ethyl]quinolin-4-amine (**6**).

Method A: The reaction of 7-chloro-*N*-(2-chloroethyl)quinolin-4-amine **3** (0.300 g, 1.24 mmol), triethylamine (0.13 g, 1.24 mmol) and dipropylamine (1.25 g, 12.4 mmol) in 1 mL DMF yielded compound **6**

(0.265 g, 70%). IR (KBr): $\bar{\nu}$ = 3065, 2957, 2929, 1613, 1579, 1549, 1453, 1430, 1377, 1339, 1282, 1232, 1201, 1167, 1139, 1081, 1029, 899, 874, 847, 820, 799 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.92 (t, *J* = 7.4 Hz, 6H, 3´´-H), 1.52 (sex, *J* = 7.4 Hz, 4H, 2´´-H), 2.46 (t, *J* = 7.4 Hz, 4H, 1´´-H), 2.82 (t, *J* = 5.9 Hz, 2H, 2´-H), 3.25 (td, *J* = 5.9, 4.0 Hz, 2H, 1´-H), 6.13 (br s, 1H, NH), 6.37 (d, *J* = 5.4 Hz, 1H, 3-H), 7.37 (dd, *J* = 9.0, 2.1 Hz, 1H, 6-H), 7.65 (d, *J* = 9.0 Hz, 1H, 5-H), 7.95 (d, *J* = 2.1 Hz, 1H, 8-H), 8.53 (d, *J* = 5.3 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.89 (C-3´´), 20.35 (C-2´´), 39.69 (C-1´), 51.83 (C-2´), 55.45 (C-1´´), 99.30 (C-3), 117.44 (C-4a), 121.07 (C-5), 125.25 (C-6), 128.73 (C-8), 134.74 (C-7), 149.12 (C-8a), 149.84 (C-4), 152.11 (C-2) ppm; HRMS (EI+) calcd for $C_{17}H_{24}ClN_3$: 305.1659; found: 305.1671.

***N*-[2-(Azepan-1-yl)ethyl]-7-chloroquinolin-4-amine** (**7**).

Method A: The reaction of 7-chloro-*N*-(2-chloroethyl)quinolin-4-amine **3** (0.300 g, 1.24 mmol), triethylamine (0.13 g, 1.24 mmol) and azepane (0.600 g, 6.4 mmol) in 1 mL DMF yielded compound **7** (0.244 g, 65%).

IR (KBr): $\bar{\nu}$ = 3064, 2926, 1610, 1580, 1550, 1453, 1429, 1365, 1333, 1278, 1200, 1161, 1145, 1081, 1031, 912, 873, 805 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.60-1.74 (m, 8H, 3´´-H, 4´´-H, 5´´-H, 6´´-H), 2.68-2.75 (m, 4H, 2´´-H, 7´´-H), 2.91 (t, *J* = 5.8 Hz, 2H, 2´-H), 3.22-3.28 (m, 2H, 1´-

H), 6.32 (br s, 1H, NH), 6.36 (d, *J* = 5.3 Hz, 1H, 3-H), 7.38 (dd, *J* = 9.0, 2.0 Hz, 1H, 6-H), 7.72 (d, *J* = 9.0 Hz, 1H, 5-H), 7.95 (d, *J* = 2.0 Hz, 1H, 8-H), 8.52 (d, *J* = 5.3 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.96 (C-4´´, C-5´´), 28.47 (C-3´´, C-6´´), 39.71 (C-1´), 54.90 (C-2´´, C-7´´), 55.32 (C-2´), 99.23 (C-3), 117.38 (C-4a), 121.21 (C-5), 125.28 (C-6), 128.55 (C-8), 134.79 (C-7), 148.94 (C-8a), 149.88 (C-4), 151.93 (C-2) ppm; HRMS (EI+) calcd for $C_{17}H_{22}ClN_3$: 303.1502; found: 303.1518.

7-Chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]quinolin-4-amine (**8**).

Method B: The reaction of 7-chloro-*N*-(3-chloropropyl)quinolin-4-amine **4** (0.300 g, 1.24 mmol), KI and *N*-methylpiperazine (1.20 g, 11.8 mmol) yielded after 96 h compound **8** (0.335 g, 85%). The structure was clarified by one- and two-dimensional NMR spectroscopy. The published NMR-data [16] are incorrect. IR (KBr): $\tilde{\nu}$ = 3041, 2938, 2788, 1611, 1584, 1537, 1447, 1369, 1331, 1284, 1239, 1155, 1097, 1081, 1013, 987, 895, 834, 798, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.95 (quin, *J* = 5.3 Hz, 2H, 2´-H), 2.40 (s, 3H, NCH_3), 2.48-2.75 (m, 10H, 2´´-H, 3´-H, 3´´-H, 5´´-H, 6´´-H), 3.35-3.40 (m, 2H, 1´-H), 6.32 (d, *J* = 5.4 Hz, 1H, 3-H), 7.33 (dd, *J* = 9.0, 2.0 Hz, 1H, 6-H), 7.59 (br s, 1H, NH), 7.89 (d, *J* = 9.0 Hz, 1H, 5-H), 7.94 (d, *J* = 2.0 Hz, 1H, 8-H), 8.51 (d, *J* = 5.4 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.32 (C-2´), 44.48 (C-1´), 46.33 (NCH_3),

53.55 (C-2´´, C-6´´), 55.24 (C-3´´, C-5´´), 58.77 (C-3´), 98.45 (C-3), 117.46 (C-4a), 122.45 (C-5), 124.56 (C-6), 128.65 (C-8), 134.57 (C-7), 149.13 (C-8a), 150.53 (C-4), 152.23 (C-2) ppm; HRMS (EI+) calcd for $C_{17}H_{23}ClN_4$: 318.1611; found: 318.1617.

***N*-[3-(Azepan-1-yl)propyl]-7-chloroquinolin-4-amine (9).**

Method A: The reaction of 7-chloro-*N*-(3-chloropropyl)quinolin-4-amine **4** (0.400 g, 1.60 mmol), triethylamine (0.16 g, 1.6 mmol) and azepane (1.6 g, 16.0 mmol) in 1 mL DMF yielded compound **9** (0.431 g, 85%). IR (KBr): $\bar{\nu}$ = 3065, 2961, 2933, 2787, 1610, 1580, 1549, 1452, 1365, 1334, 1283, 1250, 1166, 1150, 1082, 1017, 876, 838, 803 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.66-1.78 (m, 8H, 3´´-H, 4´´-H, 5´´-H, 6´´-H), 1.87-1.94 (m, 2H, 2´-H), 2.72 (t, *J* = 5.3 Hz, 2H, 3´-H), 2.72-2.77 (m, 4H, 2´´-H, 7´´-H), 3.36-3.41 (m, 2H, 1´-H), 6.31 (d, *J* = 5.4 Hz, 1H, 3-H), 7.33 (dd, *J* = 9.0, 1.9 Hz, 1H, 6-H), 7.75 (d, *J* = 9.0 Hz, 1H, 5-H), 7.81 (br s, 1H, NH), 7.93 (d, *J* = 1.9 Hz, 1H, 8-H), 8.50 (d, *J* = 5.4 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.44 (C-2´), 26.96 (C-4´´, C-5´´), 27.31 (C-3´´, C-6´´), 44.63 (C-1´), 56.04 (C-2´´, C-7´´), 57.85 (C-3´), 98.34 (C-3), 117.58 (C-4a), 122.07 (C-5), 124.60 (C-6), 128.52 (C-8), 134.54 (C-7), 149.12 (C-8a), 150.66 (C-4), 152.14 (C-2) ppm; HRMS (EI+) calcd for $C_{18}H_{24}ClN_3$: 317.1659; found: 317.1663.

General procedure for the synthesis of *N*-[2-(7-chloroquinolin-4-ylamino)ethyl]-2-(dialkylamino)acetamides (11-15) and *N*-[4-(7-chloroquinolin-4-yl)piperazin-1-yl]-2-(dialkylamino)acetamides (18, 19)

N-(2-Aminoethyl)-7-chloroquinolin-4-amine **10**, respectively 7-chloro-4-(piperazin-1-yl)quinoline **17**, and *N*,*N*-diisopropylethylamine (DIPEA) or triethylamine were suspended in dry dichloromethane and cooled on an ice bath in an atmosphere of argon. Chloroacetyl chloride was diluted with dry dichloromethane and added dropwise. Then the ice bath was removed and the mixture was stirred for 1 hour at room temperature. Afterwards the corresponding secondary amine was slowly added dropwise and the reaction mixture was stirred for another 70-160 hours at room temperature. The mixture was coevaporated with benzene or toluene. The residue was taken up in dichloromethane, washed with water, dried over anhydrous sodium sulfate and the solvent was evaporated in vacuo. The crude product was purified by crystallization or by column chromatography using silica gel as stationary phase to yield compounds **11-15, 18, 19**.

***N*-[2-(7-Chloroquinolin-4-ylamino)ethyl]-2-(4-methylpiperazin-1-yl)acetamide** (**11**).

Compound **10** (0.920 g, 4.15 mmol) and DIPEA (1.35 g, 10.5 mmol) were reacted with chloroacetyl chloride (0.600 g, 5.31 mmol) in 10 mL of dry dichloromethane. After addition of *N*-methylpiperazine (4.20 g, 42.0 mmol) the reaction mixture was stirred for 90 hours. Subsequent workup and crystallization from acetone at -18°C yielded compound **11** (0.520 g, 35%) as a white solid. M.p. (decomp.): 187°C; IR (KBr): $\tilde{\nu}$ = 2938, 2814, 1643, 1610, 1576, 1520, 1451, 1427, 1369, 1338, 1282, 1213, 1169, 1141, 1076, 1012, 896, 873, 830, 770 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ = 2.24 (s, 3H, NCH_3), 2.34-2.52 (m, 8H, 2´´´-H, 3´´´-H, 5´´´-H, 6´´´-H), 3.01 (s, 2H, 2´´-H), 3.51-3.56 (m, 2H, 1´-H), 3.57-3.63 (m, 2H, 2´-H), 6.64 (d, *J* = 5.7 Hz, 1H, 3-H), 7.44 (dd, *J* = 8.9, 1.9 Hz, 1H, 6-H), 7.79 (d, *J* = 1.9 Hz, 1H, 8-H), 8.05 (d, *J* = 8.9 Hz, 1H, 5-H), 8.37 (d, *J* = 5.7 Hz, 1H, 2-H) ppm; ^{13}C NMR (CD_3OD, 100 MHz): δ = 39.07 (C-2´), 44.04 (C-1´), 45.97 (NCH_3), 53.87 (C-2´´´, C-6´´´), 55.78 (C-3´´´, C-5´´´), 62.21 (C-2´´), 99.82 (C-3), 118.76 (C-4a), 124.44 (C-5), 126.50 (C-6), 127.47 (C-8), 136.86 (C-7), 149.35 (C-8a), 152.25 (C-2), 153.19 (C-4), 173.89 (C-1´´) ppm; HRMS (EI+) calcd for $C_{18}H_{24}ClN_5O$: 361.1669; found: 361.1683.

***N*-[2-(7-Chloroquinolin-4-ylamino)ethyl]-2-piperidinoacetamide** (**12**).

Compound **10** (0.450 g, 2.03 mmol) and DIPEA (0.630 g, 4.87 mmol) were reacted with chloroacetyl chloride (0.340 g, 3.01 mmol) in 10 mL of dry

dichloromethane. After addition of piperidine (1.75 g, 20.6 mmol) the reaction mixture was stirred for 85 hours. Subsequent workup and crystallization from acetone at -18°C yielded compound **12** (0.270 g, 38%) as a white solid. M.p. (decomp.): 205°C; IR (KBr): $\tilde{\nu}$ = 2935, 2856, 2789, 1647, 1611, 1580, 1521, 1452, 1429, 1369, 1336, 1305, 1282, 1215, 1163, 1141, 897, 874, 850, 810, 768 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.38-1.48 (m, 2H, 4´´´-H), 1.51-1.59 (m, 4H, 3´´´-H, 5´´´-H), 2.39-2.47 (m, 4H, 2´´´-H, 6´´´-H), 3.01 (s, 2H, 2´´-H), 3.37-3.43 (m, 2H, 1´-H), 3.71-3.77 (m, 2H, 2´-H), 6.28 (d, *J* = 5.2 Hz, 1H, 3-H), 6.84 (br s, 1H, NH), 7.38 (dd, *J* = 8.9, 2.2 Hz, 1H, 6-H), 7.80 (d, *J* = 8.9 Hz, 1H, 5-H), 7.84 (br s, 1H, NH), 7.92 (d, *J* = 2.2 Hz, 1H, 8-H), 8.50 (d, *J* = 5.2 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.54 (C-4´´´), 26.13 (C-3´´´, C-5´´´), 38.47 (C-2´), 46.04 (C-1´), 54.99 (C-2´´´, C-6´´´), 62.06 (C-2´´), 98.05 (C-3), 117.24 (C-4a), 122.06 (C-5), 125.47 (C-6), 128.35 (C-8), 134.86 (C-7), 148.98 (C-8a), 150.13 (C-4), 151.85 (C-2), 174.15 (C-1´´) ppm; HRMS (EI+) calcd for $C_{18}H_{23}ClN_4O$: 346.1560; found: 346.1570.

***N*-[2-(7-Chloroquinolin-4-ylamino)ethyl]-2-(dipropylamino)acetamide (13).**

Compound **10** (1.09 g, 4.9 mmol) and triethylamine (1.04 g, 10.3 mmol) were reacted with chloroacetyl chloride (0.634 g, 5.61 mmol) in 15 mL of

dry dichloromethane. After addition of dipropylamine (5.13 g, 50.7 mmol) the reaction mixture was stirred for 70 hours. Subsequent workup and crystallization from acetone at -18°C yielded compound **13** (0.530 g, 30%) as a white solid. M.p.: 149°C; IR (KBr): $\bar{\nu}$ = 2961, 2873, 2808, 1650, 1610, 1579, 1519, 1451, 1427, 1370, 1337, 1306, 1248, 1215, 1140, 1078, 897, 877, 848, 813, 771 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.82 (t, *J* = 7.3 Hz, 6H, 3´´´-H), 1.40 (sex, *J* = 7.3 Hz, 4H, 2´´´-H), 2.40 (t, *J* = 7.3 Hz, 4H, 1´´´-H), 3.09 (s, 2H, 2´´-H), 3.36-3.41 (m, 2H, 1´-H), 3.71-3.76 (m, 2H, 2´-H), 6.28 (d, *J* = 5.2 Hz, 1H, 3-H), 6.77 (br s, 1H, NH-Ar), 7.38 (dd, *J* = 9.0, 2.0 Hz, 1H, 6-H), 7.80 (d, *J* = 9.0 Hz, 1H, 5-H), 7.90-7.94 (m, 2H, 8H, NH-CO), 8.50 (d, *J* = 5.2 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.73 (C-3´´´), 20.38 (C-2´´´), 38.41 (C-2´), 46.04 (C-1´), 57.39 (C-1´´´), 58.52 (C-2´´), 98.05 (C-3), 117.24 (C-4a), 122.01 (C-5), 125.43 (C-6), 128.44 (C-8), 134.79 (C-7), 149.09 (C-8a), 150.06 (C-4), 151.97 (C-2), 175.36 (C-1´´) ppm; HRMS (EI+) calcd for $C_{19}H_{27}ClN_4O$: 362.1873; found: 362.1889.

***N*-[2-(7-Chloroquinolin-4-ylamino)ethyl]-2-(azepan-1-yl)acetamide (14).**

Compound **10** (0.419 g, 1.89 mmol) and DIPEA (0.562 g, 4.35 mmol) were reacted with chloroacetyl chloride (0.272 g, 2.41 mmol) in 10 mL of dry

dichloromethane. After addition of azepane (1.88 g, 18.9 mmol) the reaction mixture was stirred for 72 hours. Subsequent workup and crystallization from acetone at -18°C yielded compound **14** (0.300 g, 38%) as a white solid. M.p. (decomp.): 188°C; IR (KBr): $\overline{\nu}$ = 2922, 2852, 1654, 1610, 1580, 1522, 1450, 1427, 1369, 1335, 1305, 1215, 1140, 1077, 876, 848, 812, 768 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ =1.59 (br s, 8H, 3´´´-H, 4´´´-H, 5´´´-H, 6´´´-H), 2.62-2.68 (m, 4H, 2´´´-H, 7´´´-H), 3.19 (s, 2H, 2´´-H), 3.38-3.78 (m, 2H, 1´-H), 3.72-3.78 (m, 2H, 2´-H), 6.28 (d, *J* = 5.4 Hz, 1H, 3-H), 6.89 (br s, 1H, NH), 7.38 (dd, *J* = 9.0, 2.0 Hz, 1H, 6-H), 7.81 (d, *J* = 9.0 Hz, 1H, 5-H), 7.91 (br s, 1H, NH), 7.93 (d, *J* = 2.0 Hz, 1H, 8-H), 8.49 (d, *J* = 5.4 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.75 (C-4´´´, C-5´´´), 28.26 (C-3´´´, C-6´´´), 38.46 (C-2´), 46.06 (C-1´), 56.53 (C-2´´´, C-7´´´), 61.47 (C-2´´), 98.04 (C-3), 117.19 (C-4a), 122.11 (C-5), 125.49 (C-6), 128.18 (C-8), 134.92 (C-7), 148.78 (C-8a), 150.22 (C-4), 151.67 (C-2), 174.72 (C-1´´) ppm; HRMS (EI+) calcd for $C_{19}H_{25}ClN_4O$: 360.1717; found: 360.1731.

***N*-[2-(7-Chloroquinolin-4-ylamino)ethyl]-2-pyrrolidinoacetamide** (**15**).

Compound **10** (0.855 g, 3.86 mmol) and DIPEA (1.18 g, 9.2 mmol) were reacted with chloroacetyl chloride (0.566 g, 5.01 mmol) in 10 mL of dry dichloromethane. After addition of pyrrolidine (2.79 g, 38.7 mmol) the

reaction mixture was stirred for 85 hours. Subsequent workup and crystallization from acetone at -18°C yielded compound **15** (0.300 g, 38%) as a white solid. M.p. (decomp.): 195°C; IR (KBr): $\bar{\nu}$ = 2960, 2795, 2360, 1655, 1611, 1579, 1525, 1427, 1370, 1217, 1140, 876, 849 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ =1.76-1.81 (m, 4H, 3´´´-H, 4´´´-H), 2.57-2.61 (m, 4H, 2´´´-H, 5´´´-H), 3.22 (s, 2H, 2´´-H), 3.37-3.41 (m, 2H, 1´-H), 3.72-3.76 (m, 2H, 2´-H), 6.28 (d, *J* = 5.4 Hz, 1H, 3-H), 6.80-6.84 (br, 1H, NH-Ar), 7.37 (dd, *J* = 9.1, 2.1 Hz, 1H, 6-H), 7.11-7.17 (br, 1H, NHCO), 7.80 (d, *J* = 9.1 Hz, 1H, 5-H), 7.92 (d, *J* = 2.1 Hz, 1H, 8-H), 8.49 (d, *J* = 5.4 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.97 (C-3´´´, C-4´´´), 38.46 (C-2´), 46.00 (C-1´), 54.62 (C-2´´´, C-5´´´), 58.99 (C-2´´), 98.08 (C-3), 117.28 (C-4a), 122.08 (C-5), 125.45 (C-6), 128.38 (C-8), 134.84 (C-7), 149.06 (C-8a), 150.13 (C-4), 151.90 (C-2), 174.28 (C-1´´) ppm; HRMS (EI+) calcd for $C_{17}H_{21}ClN_4O$: 332.1404; found: 332.1414.

***N*-[4-(7-Chloroquinolin-4-yl)piperazin-1-yl]-2-(4-methylpiperazin-1-yl) acetamide** (**18**).

Compound **17** (0.499 g, 2.01 mmol) and DIPEA (0.650 g, 5.03 mmol) were reacted with chloroacetyl chloride (0.309 g, 2.74 mmol) in 10 mL of dry dichloromethane. After addition of *N*-methylpiperazine (1.65 g, 20.5 mmol) the reaction mixture was stirred for 160 hours. Subsequent workup

and purification by column chromatography (silica, dichloromethane+methanol=29+1) yielded compound **18** (0.180 g, 23%) as a colorless oil. IR (KBr): $\bar{\nu}$ = 2924, 1642, 1423, 1379, 1281, 1012, 867 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.29 (s, 3H, NCH_3), 2.37-2.50 (m, 4H, 2´´´-H, 6´´´-H), 2.50-2.61 (m, 4H, 3´´´-H, 5´´´-H), 3.15-3.25 (m, 4H, 2´-H, 6´-H), 3.26 (s, 2H, 2´´-H), 3.88-3.95 (m, 4H, 3´-H, 5´-H), 6.85 (d, *J* = 5.0 Hz, 1H, 3-H), 7.47 (dd, *J* = 9.0, 2.0 Hz, 1H, 6-H), 7.96 (d, *J* = 9.0 Hz, 1H, 5-H), 8.07 (d, *J* = 2.0 Hz, 1H, 8-H), 8.76 (d, *J* = 5.0 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 41.72 (C-5´), 45.69 (C-3´), 46.01 (NCH_3), 52.11 (C-2´), 52.83 (C-6´), 53.10 (C-3´´´, C-5´´´), 55.08 (C-2´´´, C-6´´´), 61.65 (C-2´´), 109.27 (C-3), 121.85 (C-4a), 124.79 (C-5), 126.56 (C-6), 129.11 (C-8), 135.15 (C-7), 150.23 (C-8a), 151.97 (C-2), 156.47 (C-4), 168.30 (C-1´´) ppm; HRMS (EI+) calcd for $C_{20}H_{26}ClN_5O$: 387.1826; found: 387.1831.

***N*-[4-(7-Chloroquinolin-4-yl)piperazin-1-yl]-2-(dipropylamino) acetamide** (**19**).

Compound **17** (0.497 g, 2.01 mmol) and DIPEA (0.668 g, 5.17 mmol) were reacted with chloroacetyl chloride (0.290 g, 2.57 mmol) in 10 mL of dry dichloromethane. After addition of dipropylamine (2.11 g, 20.8 mmol) the reaction mixture was stirred for 108 hours. Subsequent workup and

purification by column chromatography (silica, dichloromethane+methanol=29+1 and then 19+1) yielded compound **19** (0.240 g, 31%) as a colorless oil. IR (KBr): $\bar{\nu}$ = 2926, 1647, 1577, 1422, 1380, 1229, 1014, 868, 824 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, *J* = 7.3 Hz, 6H, 3´´´-H), 1.49 (sex, *J* = 7.3 Hz, 4H, 2´´´-H), 2.46 (t, *J* = 7.3 Hz, 4H, 1´´´-H), 3.16-3.23 (m, 4H, 2´-H, 6´-H), 3.33 (s, 2H, 2´´-H), 3.86-3.91 (m, 2H, 5´-H), 3.95-4.00 (m, 2H, 3´-H), 6.84 (d, *J* = 5.1 Hz, 1H, 3-H), 7.46 (dd, *J* = 8.9 Hz, 2.0 Hz, 1H, 6-H), 7.96 (d, *J* = 8.9 Hz, 1H, 5-H), 8.06 (d, *J* = 2.0 Hz, 1H, 8-H), 8.74 (d, *J* = 5.1 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.94 (C-3´´´), 19.92 (C-2´´´), 41.66, 45.42 (C-3´, C-5´), 52.09, 52.59 (C-2´, C-6´), 56.32 (C-1´´´), 58.97 (C-2´´), 109.21 (C-3), 121.83 (C-4a), 124.81 (C-5), 126.51 (C-6), 129.02 (C-8), 135.08 (C-7), 150.16 (C-8a), 151.93 (C-2), 156.51 (C-4), 169.89 (C-1´´) ppm; HRMS (EI+) calcd for $C_{21}H_{29}ClN_4O$: 388.2030; found: 388.2027.

***N*-(2-{[2-(Azepan-1-yl)ethyl]amino}ethyl)-7-chloroquinolin-4-ylamine (16).**

The reaction of compound **14** (0.720 g, 2.00 mmol) with 5.0 mL borane-dimethyl sulfide complex (borane-DMS-complex 2 M in THF; 10.0 mmol) in 30 mL of dry tetrahydrofuran (THF) was carried out in the reported way

[12]. The workup gave a yellow resin which was taken up in water, acidified with conc. HCl and extracted with 150 mL *tert*-butylmethyl ether (MTBE). The aqueous phase was alkalized with 2 N NaOH and extracted with 250 mL MTBE. The combined organic phases were washed with water, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in vacuo. Crystallization from a minimum amount of hot acetone yielded compound **16** (0.385 g, 56%) as colorless crystals. M.p.: 88°C; IR (KBr): $\tilde{\nu}$ = 2923, 2830, 1580, 1543, 1423, 1355, 1325, 1280, 1206, 1144, 1117, 1073, 1004, 967, 877, 837, 800, 764, 636 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.50-1.62 (m, 8H, 3´´´-H, 4´´´-H, 5´´´-H, 6´´´-H), 2.57-2.64 (m, 6H, 2´´-H, 2´´´-H, 7´´´-H), 2.68 (t, *J* = 5.5 Hz, 2H, 1´´-H), 3.01 (t, *J* = 5.4 Hz, 2H, 2´-H), 3.33 (q, *J* = 5.4 Hz, 2H, 1´-H), 6.05 (br t, *J* = 5.0 Hz, 1H, NH), 6.38 (d, *J* = 5.4 Hz, 1H, 3-H), 7.32 (dd, *J* = 9.0, 2.1 Hz, 1H, 6-H), 7.73 (d, *J* = 9.0 Hz, 1H, 5-H), 7.93 (d, *J* = 2.1 Hz, 1H, 8-H), 8.51 (d, *J* = 5.4 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.91 (C-4´´´, C-5´´´), 28.21 (C-3´´´, C-6´´´), 41.91 (C-1´), 46.83 (C-1´´), 47.43 (C-2´), 55.33 (C-2´´´, C-7´´´), 56.91 (C-2´´), 99.05 (C-3), 117.33 (C-4a), 121.39 (C-5), 124.94 (C-6), 128.51 (C-8), 134.57 (C-7), 149.06 (C-8a), 149.84 (C-4), 151.96 (C-2) ppm; HRMS (EI+) calcd for $C_{19}H_{27}ClN_4$: 345.1846; found: 345.1817.

7-Chloro-4-[4-(2-chloroethyl)piperazin-1-yl]quinoline (**20**).

Compound **17** (1.39 g, 5.6 mmol) and triethylamine (0.708 g, 7.00 mmol) were heated to 110 °C in 15 mL 1,2-dichloroethane (18.8 g, 0.19 mol) in an anhydrous atmosphere for 48 hours. After cooling to room temperature, the solution was diluted with dichloromethane and washed with water. The aqueous phase was reextracted once with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered and the solvent evaporated in vacuo. The residue was furthermore coevaporated with toluene to obtain compound **20** as a brown solid. M.p.: 99°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.80-2.83 (m, 4H, 3´-H, 5´-H), 2.87 (t, *J* = 6.9 Hz, 2H, 1´´-H), 3.24-3.27 (m, 4H, 2´-H, 6´-H), 3.66 (t, *J* = 6.9 Hz, 2H, 2´´-H), 6.83 (d, *J* = 5.0 Hz, 1H, 3-H), 7.42 (dd, *J* = 9.1, 1.9 Hz, 1H, 6-H), 7.93 (d, *J* = 9.1 Hz, 1H, 5-H), 8.04 (d, *J* = 1.9 Hz, 1H, 8-H), 8.72 (d, *J* = 5.0 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 40.90 (C-2´´), 52.04 (C-2´, C-6´), 52.99 (C-3´, C-5´), 59.69 (C-1´´), 109.01 (C-3), 121.90 (C-4a), 125.10 (C-5), 126.15 (C-6), 128.91 (C-8), 134.87 (C-7), 150.15 (C-8a), 151.94 (C-2), 156.82 (C-4) ppm.

General procedure for the synthesis of 7-chloro-4-{4-[2-(dialkylamino)ethyl]piperazin-1-yl}quinoline (21, 22).

7-Chloro-4-[4-(2-chloroethyl)piperazin-1-yl]quinoline **20**, triethylamine and an excess of the corresponding secondary amine were refluxed at 80°C

in dry methanol for 20 hours in an anhydrous atmosphere. The reaction progress was monitored by TLC. After cooling to room temperature the reaction mixture was diluted with dichloromethane and washed with water, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in vacuo. The residue was purified by column chromatography using silica gel as stationary phase yielding compounds **21, 22**.

7-Chloro-4-{4-[2-(dipropylamino)ethyl]piperazin-1-yl}quinoline (**21**).

The reaction of compound **20** (0.641 g, 2.07 mmol), triethylamine (0.262 g, 2.59 mmol) and dipropylamine (2.19 g, 21.7 mmol) in 5 mL of dry methanol and subsequent purification by column chromatography (silica, dichloromethane+methanol=49+1) yielded compound **21** (0.402 g, 52%) as a colorless oil. IR (KBr): $\overline{\nu}$ = 2957, 1577, 1424, 1381, 1136, 872, 824 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, J = 7.5 Hz, 6H, 3´´´-H), 1.49 (sex, J = 7.5 Hz, 4H, 2´´´-H), 2.44 (t, J = 7.5 Hz, 4H, 1´´´-H), 2.57-2.62 (m, 2H, 1´´-H), 2.63-2.68 (m, 2H, 2´´-H), 2.78 (br, 4H, 3´-H, 5´-H), 3.24 (br, 4H, 2´-H, 6´-H), 6.82 (d, J = 5.1 Hz, 1H, 3-H), 7.40 (dd, J = 9.0, 2.1 Hz, 1H, 6-H), 7.94 (d, J = 9.0 Hz, 1H, 5-H), 8.03 (d, J = 2.1 Hz, 1H, 8-H), 8.70 (d, J = 5.1 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.90 (C-3´´´), 20.23 (C-2´´´), 51.60 (C-2´´), 52.12 (C-2´, C-6´), 53.53 (C-3´, C-5´), 56.69 (C-1´´), 56.77 (C-1´´´), 108.89 (C-3), 121.89 (C-4a), 125.19 (C-5),

126.01 (C-6), 128.82 (C-8), 134.79 (C-7), 150.11 (C-8a), 151.89 (C-2), 156.94 (C-4) ppm; HRMS (EI+) calcd for $C_{21}H_{31}ClN_4$: 374.2237; found: 374.2230.

4-{4-[2-(Azepan-1-yl)ethyl]piperazin-1-yl}-7-chloroquinoline (22).

The reaction of compound **20** (0.600 g, 1.93 mmol), triethylamine (0.280 g, 2.77 mmol) and azepane (1.96 g, 19.8 mmol) in 5 mL of dry methanol and subsequent purification by column chromatography (silica, dichloromethane+methanol=29+1) yielded compound **22** (40 mg, 6%) as a colorless oil. IR (KBr): $\bar{\nu}$ = 2927, 2823, 1607, 1576, 1497, 1456, 1423, 1380, 1298, 1138, 1007, 928, 871, 822, 630 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ =1.58-1.71 (m, 8H, 3´´-H, 4´´-H, 5´´-H, 6´´-H), 2.61-2.65 (m, 2H, 1´´-H), 2.69-2.75 (m, 6H, 2´´-H, 2´´´-H, 7´´´-H), 2.77-2.81 (m, 4H, 3´-H, 5´-H), 3.23-3.27 (m, 4H, 2´-H, 6´-H), 6.83 (d, J = 5.1 Hz, 1H, 3-H), 7.41 (dd, J = 9.0, 2.2 Hz, 1H, 6-H), 7.95 (d, J = 9.0 Hz, 1H, 5-H), 8.03 (d, J = 2.2 Hz, 1H, 8-H), 8.71 (d, J = 5.1 Hz, 1H, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.95 (C-4´´´, C-5´´´), 27.86 (C-3´´´, C-6´´´), 52.10 (C-2´, C-6´), 53.48 (C-3´, C-5´), 55.22 (C-2´´), 55.83 (C-2´´´, C-7´´´), 56.55 (C-1´´), 108.85 (C-3), 121.84 (C-4a), 125.16 (C-5), 125.96 (C-6), 128.78 (C-8), 134.73 (C-7), 150.07 (C-8a), 151.85 (C-2), 156.89 (C-4) ppm; HRMS (EI+) calcd for $C_{21}H_{29}ClN_4$: 372.2081; found: 372.2091.

6.3 Biological tests

6.3.1 In vitro microplate assay against P. falciparum

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay [18, 19], using the drug sensitive NF54 strain [20] or the chloroquine and pyrimethamine resistant K_1 strain that originate from Thailand [21] and the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), Albumax® (5 g/L) and washed human red cells A^+ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37°C; 4% CO_2, 3% O_2, 93% N_2. After 48 h 0.05 mL of ^{3}H-hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fibre filter then washed with distilled water. The dried filters were inserted into a

plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression [22] using Microsoft Excel. Chloroquine was used as control.

6.3.2 In vitro microplate assay against T. brucei rhodesiense

Activity against *Trypanosoma brucei rhodesiense* STIB900. This stock was isolated in 1982 from a human patient in Tanzania and after several mouse passages cloned and adapted to axenic culture conditions [23]. Minimum Essential Medium (0.05 mL) supplemented with 25 mM HEPES, 1g/L additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. Then 4×10^3 bloodstream forms of *T. b. rhodesiense* STIB 900 in 0.05 mL medium was added to each well and the plate incubated at 37°C under a 5 % CO_2 atmosphere for 70 h. 0.01 mL Alamar Blue (resazurin, 12.5 mg in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2-4 h [24]. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an

excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression [22] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA). Melarsoprol (Arsobal Sanofi-Aventis, received from WHO) was used as control.

6.3.3 In vitro cytotoxicity with L-6 cells

Assays were performed in 96-well microtiter plates, each well containing 0.1 mL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L-6 cells (a primary cell line derived from rat skeletal myoblasts) [25, 26]. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. After 70 hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 0.01 mL of Alamar Blue was then added to each well and the plates incubated for another 2 hours. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression [22] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation,

Sunnyvale, CA, USA). Podophyllotoxin (Sigma P4405) was used as control.

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Graphical abstract

New derivatives of 7-chloroquinolin-4-amine with antiprotozoal activity

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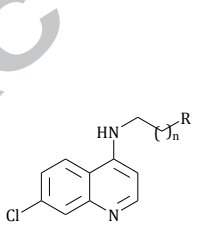
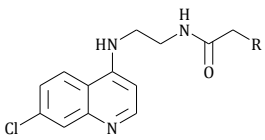


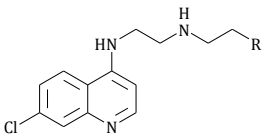
5-9

T.b.r. 0.14-1.14 µM
P.f.K$_1$ 0.0098-0.038 µM
P.f.NF54 <0.0066 µM



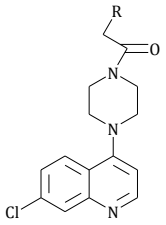
11-15

T.b.r. 0.28-18.32 µM
P.f.K$_1$ 0.03-2.87 µM
P.f.NF54 0.011-0.036 µM



16

T.b.r. 1.28 µM
P.f.K$_1$ 0.066 µM
P.f.NF54 0.011 µM



18,19

T.b.r. 1.46-2.02 µM
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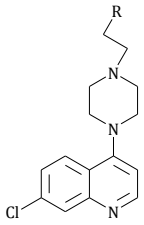
P.f.NF54 0.14-0.23 µM



21,22

T.b.r. 1.07-1.61 µM
P.f.K$_1$ 0.075-0.088 µM
P.f.NF54 0.07-0.11 µM