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Synthesis and antiplasmodial activity of new *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]carboxamides

Marcus Freitag,[a] Marcel Kaiser,[b] Tim Larsen,[a] Vida Zohrabi-Kalantari,[c] Philipp Heidler[c] and Andreas Link[a,*]

[a]*Institute of Pharmacy, Ernst-Moritz-Arndt-University, Friedrich-Ludwig-Jahn-Straße 17, 17487 Greifswald, Germany*
[b]*Swiss Tropical Institute, Socinstraße 57, 4002 Basel, Switzerland*
[c]*Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Marbacher Weg 6, 35032 Marburg, Germany*

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Abstract—The parallel acylation of *N*-{3-[4-(3-aminopropyl)piperazin-1-yl]propyl}-7-chloroquinolin-4-amine with polymer-bound carboxylic acids opened straightforward access to novel aminoquinolines with activity against *Plasmodium falciparum* strains in vitro. Using this amino scaffold prepared in solution and polymer-bound carboxylic, we have synthesized a series of 29 new compounds in good to excellent yield and purity. Biological evaluation included determination of the activity against a chloroquine (CQ) sensitive strain and a CQ resistant mutant. Most of the novel structures presented here displayed activity against both strains in the lower nanomolar range, four compounds showed an at least fourfold increase in the ratio of inhibition of CQ resistant to sensitive strains over CQ itself. These results suggest that this derivatization technique is a useful method to speed up structure–activity relationship studies on aminoquinolines toward improved activity versus CQ resistant strains of *P. falciparum* in vitro.

1. Introduction

Cheap and effective treatment for malaria for most countries in Africa will depend heavily on the identification of synergistic combinations of antimalarial drugs. Existing strategies to reach this therapeutic goal have recently been reviewed.[1] As a lesson learned from the successful anti-malaria program in KwaZulu–Natal province, South Africa, maybe the use of DDT treatment of bedroom walls to kill the vector is still more successful as the hitherto most effective ACT (artemisinin-based combination therapy) available.[2] Sad enough, attempted monotherapy with artemisinin has led to the occurrence of resistant strains meanwhile.[3]

Consequently, Medicines for Malaria Venture (MMV) added a new development project on drugs unlikely to be of value as single active ingredient but with potential as possible combination partner, namely AQ-13 (**2**), N^4-(7-chloroquinolin-4-yl)-N^1,N^1-dimethylpentane-1,4-diamine dihydrochloride trihydrate, a chloroquine (**1**) like aminoquinoline drug.[4] For many years, research in this direction was hampered by cross-resistance issues within this valuable class of antimalarials. However, AQ-13 (**2**) is fairly active in resistant variants, and may in contrast to artemisinins even be considered for use during pregnancy (see Fig. 1).[5]

This is of special importance, because pregnant women are the main adult risk group for malaria: Infection occurs more easily due to the altered immune system and may result in spontaneous abortion, and neonatal death.

In this context, synthesis and evaluation of novel aminoquinolines is reasonable. Inspired by recent studies of Ryckebusch et al. we selected cheap and easily accessible *N*-{3-[4-(3-aminopropyl)piperazin-1-yl]propyl}-7-chloroquinolin-4-amine **3** as a template (see Fig. 2).[6,7]

Rapid parallel derivatization of non-chiral **3** with polymer-bound acylation reagents **4a**–**d**′ unambiguously led to 29 novel amide derivatives **5a**–**d**′. While simple

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* Corresponding author. Tel.: +49 03834 86 4893; fax: +49 03834 86 4895; e-mail: link@pharmazie.uni-greifswald.de

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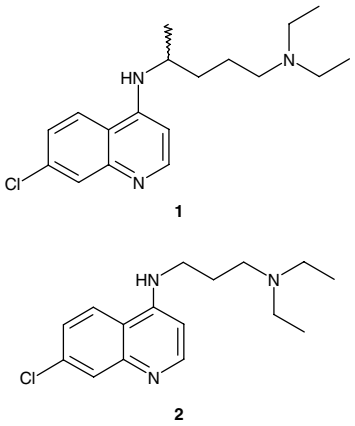


Figure 1. Chloroquine (CQ, **1**) and aminoquinolin AQ-13 (**2**).

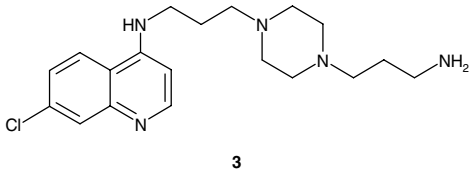


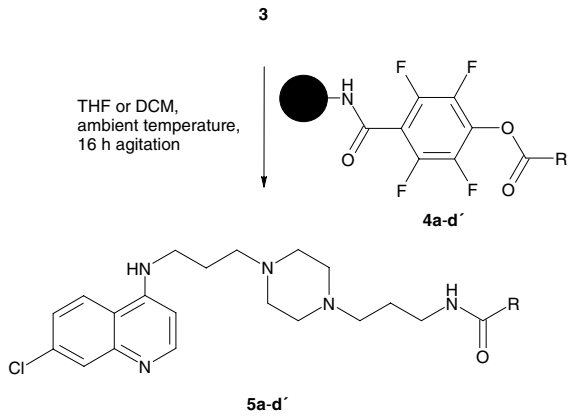
Figure 2. Aminoquinoline template **3** proposed by Ryckebusch et al.

synthetic transformations such as amide bond formation can be performed swiftly, work-up procedures often have to be optimized for every individual new molecule. This time-consuming step hampers the generation of arrays of lead structure analogs in the search of structure activity relationships. Here we suggest to use polymer-bound active carboxylic acid equivalents for the fast parallel synthesis of *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]carboxamide (**5a**–**d**′) fulfilling the structural requirements for antimalarial activity.

2. Results

Throughout the last years, the use of polymer-supported acylating agents in solution-phase synthesis in medicinal chemistry projects was extensively studied. The selective quantitative acylation of primary amino groups, the straightforward product separation by filtration, and the absence of impurities of residual coupling reagents being the main concerns of this approach. In continuation of our efforts to contribute to the identification of biologically active compounds via parallel synthesis, a set of *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}-piperazin-1-yl)propyl]carboxamide was envisioned.

At the outset, 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid was attached onto aminomethylated polystyrene to yield a phenolic couple and release linker as described.[8] Using *N*,*N*′-diisopropylcarbodiimide as activating agent in the absence of a base, addition of bromophenol blue allowed for a naked-eye control of the crucial linker anchorage.[9,10] The initially deep blue suspension turned green and slowly faded to yellow. The alteration of the color indicated the quantitative disappearance of free amino groups and assured a complete loading of the starting polymer.



Scheme 1. Parallel derivatization of template **3**. R in compounds **4a**–**d**′ equals R in **5a**–**d**′ and can be deduced from Table 1.

Subsequently, commercially available carboxylic acids were immobilized on this couple and release linker yielding reagents **4a**–**d**′. After exhaustive washing to remove residual *N*,*N*′-diisopropylcarbodiimide, *N*,*N*′-diisopropylurea, and base impurities from the polymer beads, the active polymer-bound acids were used as acylation reagents **4a**–**d**′. Due to the electron-withdrawing tetrafluoro substitution of the linker, a labile ester bond was formed. By parallel incubation with limiting amounts of amine **3** as a free base in freshly distilled THF, the target amides **5a**–**d**′ could thus be obtained under very mild conditions (Scheme 1). The reaction mixtures were separated by filtration; the resulting pure solutions were subjected to evaporation of the freely volatile solvent under reduced pressure. Hence, compounds **5a**–**d**′ were obtained as colorless solids in good to high relative purity. Growth inhibition of the clinically relevant blood stream form of the parasites cultured in red blood cells, caused by addition of candidates **5a**–**d**′, was determined using an established protocol.[11,12] For comparative purposes, the activity of chloroquine (**1**) was determined in the same experiment and is also reported (Table 1).

3. Discussion

The length and nature of the basic sidechain of 4-aminoquinoline antimalarials is currently believed to be the primary modulator of activity against CQ resistant parasite strains.[13] Counterintuitively, minor modifications of the side-chain with retained heterocyclic heme binding motif can lead to compounds with a dramatically altered resistance profile in vitro and in vivo. The data presented by De et al. and Ridley et al. underline the highly specific nature of the chloroquine resistance mechanism in this context.[14,15] Thus, increasing lipophilicity of the chain terminus clearly has a major impact on the antimalarial activities versus resistant parasites. Therefore, 29 amide residues were selected from the universe of available carboxylic acids to cover a reasonable range of small isopropyl to relatively large 4-phenoxyphenyl residues. Saturated and

Table 1. Growth inhibition (nM), selectivity ratio, cytotoxicity, and purity of derivatives **5a**–**d**′ produced via route outlined in Scheme 1

Entry	Residue R	IC_{50} (μM) strain (rounded)		Ratio (NF54/K1)	IC_{50} (μM) L6 (tox.)	Purity[a] (%)	From entry
		NF54	K1				
1		0.013	0.089	0.15	7.7	89.4	
5a	2-Methylpropyl	0.013	0.078	0.17	n.d.	91.9	**4a**
5b	Pentyl	0.338	0.900	0.38	n.d.	82.7	**4b**
5c	4-Acetamidophenyl	0.083	0.249	0.33	n.d.	83.6	**4c**
5d	3-Trifluoromethylphenyl	0.037	0.053	0.68	30.1	94.9	**4d**
5e	Diphenylmethyl	0.025	0.106	0.24	9.3	93.4	**4e**
5f	Naphth-1-ylmethyl	0.013	0.041	0.32	6.2	89.4	**4f**
5g	3,4-Dimethoxyphenyl	0.036	0.447	0.08	n.d.	96.6	**4g**
5h	3-(1*H*-Indol-3-yl)propyl	0.015	0.069	0.22	6.5	99.8	**4h**
5i	3-Oxobutyl	0.059	0.380	0.15	11.0	99.9	**4i**
5j	2,4-Dichlorobenzyl	0.008	0.024	0.34	15.1	87.5	**4j**
5k	3,4-Difluorophenyl	0.007	0.013	0.54	10.0	84.5	**4k**
5l	2-Benzoylphenyl	0.010	0.017	0.59	3.2	90.9	**4l**
5m	2-Methylprop-1-enyl	0.023	0.065	0.36	30.0	95.6	**4m**
5n	2,6-Dichlorobenzyl	0.011	0.036	0.32	5.3	94.2	**4n**
5o	3-Phenoxypropyl	0.012	0.073	0.16	9.3	92.3	**4o**
5p	4-Ethoxyphenyl	0.008	0.012	0.67	5.3	97.3	**4p**
5q	3-Phenylpropyl	0.017	0.061	0.28	11.0	80.0	**4q**
5r	Propyl	0.019	0.159	0.12	84.7	81.0	**4r**
5s	3,5-Difluorophenyl	0.010	0.026	0.39	11.6	84.1	**4s**
5t	Cyclohexylmethyl	0.010	0.027	0.37	7.2	94.0	**4t**
5u	2-Methylphenyl	0.010	0.034	0.29	n.d.	96.4	**4u**
5v	3-Methoxyphenyl	0.010	0.028	0.36	9.3	89.6	**4v**
5w	4-Phenylbutyl	0.012	0.054	0.22	9.4	84.7	**4w**
5x	3,5-Dichlorophenyl	0.007	0.012	0.58	3.0	89.1	**4x**
5y	4-Benzoylphenyl	1.545	3.069	0.50	n.d.	89.0	**4y**
5z	4-Chloro-3-nitrophenyl	0.020	0.043	0.47	11.3	87.9	**4z**
5a′	4-Phenoxyphenyl	0.009	0.015	0.60	1.4	87.5	**4a**′
5b′	4-Ethylphenyl	0.008	0.013	0.62	3.9	89.4	**4b**′
5c′	3-Cyclohexylpropyl	0.156	0.272	0.57	n.d.	86.0	**4c**′
5d′	3-Trifluoromethoxy-phenyl	0.012	0.015	0.80	8.5	89.4	**4d**′

[a] Relative purity as determined by HPLC analysis using the 100% method with UV-detection at 250 nm.

unsaturated branched and linear aliphatic as well as aromatic acids were selected, hydrogen bond donors were included as well as hydrogen bond acceptors, the main focus however was put on lipophilicity enhancing substituents. Prior to synthesis, the initially envisioned compounds were filtered manually using the web-based OSIRIS property explorer to estimate parameters like high druglikeness, reasonable drug-scores, and low toxicity risk (available at http://www.actelion.com; © 2001 Thomas Sander) of the compounds based on fragment data sets. Only those entries for which favorable properties were predicted were prepared by the above-demonstrated synthesis using polymer-bound carboxylic acid equivalents. This parallel approach permits the fast preparation of milligram quantities of test samples for biological evaluation. On the one hand, the number of compounds achievable by this procedure is considerably lower than in combinatorial solid-phase synthesis approaches or multi-component reactions used in the field of 4-aminoquinoline-SAR-generation.[16] But on the other hand, the quality of the compounds in terms of reliable data sets obtained at least equals the typical purity of compounds from classical organic synthesis projects. Like in solid-phase synthesis, the polymer-assisted solution-phase (PASP) synthesis performed enables parallel product isolation because the excess of reagent that is applied to drive reactions to completion can be removed by filtration. In contrary to solid-phase synthesis, the reagents are polymer-bound and not the product, saving a final cleavage reaction that might lead to product contamination, for example, by TFA salt formation. Therefore, this intermediate technique is well suited for the fast and convenient access of compounds intended for the determination of quantitative structure activity relationships in drug research.

AQ-13-analogs are generally suited to circumvent the *N*-dealkylation-associated inactivation reported for many CQ-derivatives. Although the reason for this observation could not be clarified in detail, the inter-nitrogen separation has a role to play.[13] A shorter chain between the two exocyclic nitrogen atoms seems to be of great consequence for this effect. Thus, the AQ-13 derivatives **5a-d**′ should not as easily be converted to hydrophilic dealkylated metabolites as CQ and accumulate in higher concentration due to their enhanced lipophilicity with maintained basic centers for weak base ion-trapping inside intracellular compartments, theoretically.

Making use of an already highly active lead, the compounds **5a**–**d**′ obtained in this study display the desired activity against *Plasmodium falciparum* in the low nanomolar concentration range. The broadest range of activity was found within the subset of compounds with direct connection of a benzoic acid derivative to the

3-[4-(3-amino-propyl)-piperazin-1-yl]-propyl-spacer. The most active compounds **5j**, **k**, **p**, **x**, **a′**, and **5b′** belong to this subset as well as the only compound with poor activity, the 4-benzoylphenyl derivative **5y**. Aliphatic acids without aromatic substituents display a slightly inferior activity versus the NF54 strain but a significant decrease in activity when tested on the resistant K1 strain. As an exception from this rule, the branched aliphatic 2-methylpropyl residue leading to **5a** displayed practically the same activity as the 3-phenoxypropyl derivative **5o**, a derivative of mediocre activity versus both strains. The same mediocre activity was generally determined for arylacetic acid derivatives. The 3-cyclohexylpropyl derivative **5c′** exhibited the lowest activity within the subset of aliphatic carboxylic acid derived entries **5**. Direct comparison with **5o** goes to show that a flexible chain with additional rotatable bonds decorated with a three dimensionally space filling hydrocarbon is inferior to a planar lipophilic end group. Omitting additional rotatable bonds the even more rigid system of substituted benzoic acid amides turned out to be the modification of choice. The most active benzoic acid derived compounds bear a 3,4-difluoro- or 3,5-dichloro-substitution pattern, 4-ethyl- or 4-ethoxy-substitution or a 4-phenoxy-substituent. No preference for a certain halogen substituent or substitution pattern can be deduced from the data set. This result supports the assumption, that the increased lipophilicity is more important for binding to the putative drug target Ferriprotoporphyrin IX than occupation of a defined molecular recognition area. Similarly, the closely related 4-ethyl or 4-ethoxy-substitution leads to practically the same activity in **5b′** and **5b**, respectively. Both the trifluoromethoxy analog **5d′** and its trifluoromethylsubstituted counterpart **5d** are active against both strains, however **5d′** shows a threefold increase in activity versus both strains and the best ratio of NF54 versus K1 inhibition of all compounds discussed in this paper. Because this ratio is suited to indicate the degree of possible cross-resistance, the most interesting compounds can be identified by IC_{50}-values in the low nanomolar range in both assays and a high NF54/K1 ratio. The optimum of 1 was not found in either of the compounds but whereas for CQ this ratio is 0.15, for example, for compounds **5d**, **p**, **b′**, and **d′** it was 0.6 or higher with 0.8 for **5d′**. Even though all compounds were more active against the strain NF54, these results demonstrate that subtle changes in the physicochemical properties of the amide moiety can be used to circumvent resistance phenomena, experimentally.

Taking a look at the physicochemical properties of the compound set, all of the entries **5** deserve to be called druglike. Especially the entries **5a** and **b** bearing smaller substituents fulfill the established predictive requirements for oral availability.[17] Most of the more active compounds reach the upper limit of typically recommended molecular size expressed as molecular mass of 500 but still fit into the scheme of Lipinski's rule of five.[18]

In relation to the standard CQ (**1**), compounds **5a**, **d**, **f**, **h**, **k**–**q**, **s**–**x**, **z**–**b′**, and **d′** show improved activity versus the CQ-resistant K1 strain. These compounds were selected in order to evaluate the cytotoxicity of these new 4-aminoquinolines. While the observed cytotoxicity of the derivatives is generally not neglectable, it is comparable to the safe and well tolerated drug CQ (**1**).

4. Conclusion

Acylation of a suitable amino scaffold by use of polymer-bound activated carboxylic acid equivalents yielded adequately pure carboxamides without the need of further purification steps. Exploiting the easily accessible diversity site incorporated in derivative **3** of the highly active lead structure AQ-13 (**2**) made it possible to synthesize 29 new antiplasmodial compounds. The biological results of this screen correspond to the anticipated gain in knowledge and will contribute to a deeper understanding of SAR in this field.

5. Experimental

5.1. General experimental

Due to the use of a synthesizer with a 96-well reactor block, the presented set of 29 structures was prepared in parallel with chemically related carboxamides with different pharmacophores. All library members thus obtained were subjected to HPLC purity analysis. According to the commonly accepted "80/80-Standard" criteria only 20 randomly selected sample structures, nine out of the subset described here, were analyzed in detail (https://paragon.acs.org/paragon/ShowDocServlet?contentId=paragon/menu_content/authorchecklist/cc_authguide.pdf). ^{1}H NMR spectra were recorded on a JEOL ECLIPSE+ 500 spectrometer, using tetramethylsilane as internal standard. The purity of the latter compounds was deduced from ^{1}H NMR data as well as evaluated by HPLC, using a Dionex Summit HPLC-system with a DAD detector and CC 125/4 Nucleodur™ 100-5 C18 ec columns, supplied by Macherey-Nagel. For analytical evaluation the following eluent systems were used: A (H_2O/TFA, 100:0.05) and B (methanol/TFA, 100:0.05). Compounds were dissolved in methanol/water and injected at a flow rate of 1 mL/min. The applied gradient system started with a ratio A/B of 9:1 and was run to 1:9 one minute after injection over the following 30 min. Purity was calculated using the UV data at 250 and 220 nm. The lower frequency was included in order to be able to identify impurities with low absorption coefficients at the standard wavelengths 250 or 254 nm, notoriously present in small molecule libraries. High resolution MS data were obtained on a Micromass Autospec instrument (ESI, methanol (1/1, v/v) infusion at 10 μL/min with polyethylene glycol as reference). TLC reaction control was performed on Macherey-Nagel Polygram™ Sil G/UV_{254} precoated microplates, and spots were visualized under UV-illumination at 254 nm. IR-spectra were recorded as KBr tablets on a Nicolet 510P FT-IR spectrometer.

5.2. General procedure for the attachment of the linker onto aminomethylated polystyrene

6.8 g of aminomethylated polystyrene (Novabiochem batch A25711, loading level = 1.40 mmol/g) was

suspended in 78 mL DMF and 10 mg bromophenol blue was added. The suspension turned purple due to the presence of basic amino functions. In a second flask 3.7 g of 2,3,5,6-tetrafluoro-4-hydroxy-benzoic acid hydrate (Sigma–Aldrich 36, 385-5; 16.2 mmol, 1.7 equiv) was dissolved in 14 mL DMF. Next, 2.25 mL DIC (14.4 mmol, 1.5 equiv) was added and the mixture was shaken for 10 min. After that, a solution of 1.94 g of 1-hydroxybenzotriazole (14.4 mmol, 1.5 equiv) dissolved in 3.6 mL DMF was added and shaken for 3 h. The resulting solution was then poured into the suspension of the resin and shaken for 16 h. The color of the suspended beads slowly turned to yellow, which indicated the absence of amino functionalities. The resin was washed three times with 100 mL DMF. IR control experiments (resin sample washed with DMF, THF, and dichloromethane) showed the expected amido band at 1652 cm^{-1}, but also a signal at 1763 cm^{-1} due to unwanted, but expected, formation of the ester. Therefore, the resin was suspended in 78 mL DMF and 1.0 mL piperidine was added slowly. The mixture was shaken for 90 min and the resin was washed three times with 100 mL DMF. To remove previously formed piperidine salts, the resin was swollen in 78 mL DMF and 9 mL HCl (c = 2 mol/L) was added slowly. After shaking the suspension for another 90 minutes, the solvent was removed via filtration and the remaining resin was washed thoroughly (3× 80 mL DMF, 3× 80 mL THF, and 3× 80 mL dichloromethane) and dried under reduced pressure. IR control showed the complete disappearance of the unwanted ester band at 1763 cm^{-1}. The obtained resin displayed a loading level of 1.11 mmol/g (yield 102.5%).

5.3. General procedure for the preparation of the polymer-bound carboxylic acid residues 4a–d′

500 mg of the previously described resin was swollen in 10 mL DMF and 2 equiv (1.1 mmol) of the particular carboxylic acid were added to the suspension, followed by 13.5 mg DMAP (0.11 mmol, 0.2 equiv). The mixture was shaken for five minutes and 171 μl DIC (1.1 mmol, 2 equiv) was added. The reaction vials were sealed and shaken for 16 h. After extensive washing (3× 30 mL DMF, 3× 30 mL THF, and 3× 30 mL dichloromethane), the resin was dried in vacuo. IR control showed a characteristic band in the range of 1750–1790 cm^{-1}. The obtained yields were in between 83 and 98% (loading level 0.73–0.98 mmol/g).

5.4. General procedure for the transfer of the polymer-bound carboxylic acid residues 4a–d′ onto 3

200 mg of the appropriate resin **4a**–**d**′ (approx. 0.16 mmol) was splitted and put into the reaction vessels of the ares-block of the ACT Vanguard synthesizer and pre-swollen in 1.5 mL per vessel of freshly distilled THF. After the preparation of a stock solution of **3** (concentration of 6 mg of **3** in 1 mL THF), 0.84 mL (5 mg of **3**, 0.017 mmol, approx. 0.2 equiv) of this stock solution was added to the suspension of the resin in each vessel. The block was then flushed with argon and sealed. After 16 h of shaking, TLC indicated complete conversion of the amine **3** and the resin was filtered off and washed 10 times with 1.5 mL THF. All fractions were collected in appropriate glass tubes put into the cleavage block. The organic fractions were combined, evaporated, and dried in vacuo. All yields were in between 70% and 96%.

5.5. Compounds 5a–d′

5.5.1. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]-3-methylbutanamide (5a). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.39 (d, 1H, J = 5.4 Hz, quinolyl), 8.23 (d, 1H, J = 9.0 Hz, quinolyl), 7.78 (m, 1H, amino), 7.71 (t, 1H, J = 5.2 Hz, amido), 7.43 (m, 1H, quinolyl), 7.38 (t, 1H, J = 5.1 Hz, quinolyl), 6.48 (d, 1H, J = 5.5 Hz, quinolyl), 3.29 (m, 4H, propylene overlapping $COCH_2$), 3.05 (m, 2H, propylene), 2.46–2.31 (m, 8H, piperazinyl), 2.28 (t, 2H, J = 7.1 Hz, propylene), 1.99–1.96 (m, 1H, CH), 1.92 (m, 2H, propylene), 1.81 (m, 2H, propylene), 1.54 (m, 2H, propylene), 0.86 (d, 6H, J = 6.3 Hz, CH_3). HRESI-MS; found: 446.2691; requires 446.2687 [M+H]. HPLC = 91.9% (250 nm); 80.1% (220 nm).

5.5.2. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]-3,4-dimethoxybenzamide (5g). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.39 (d, 1H, J = 5.4 Hz, quinolyl), 8.30 (t, 1H, J = 5.4 Hz, Amido), 8.23 (d, 1H, J = 9.0 Hz, quinolyl), 7.77 (m, 1H, amino), 7.47–7.40 (m, 3H, phenyl overlapping quinolyl), 7.38 (m, 1H, quinolyl), 6.99 (d, 2H, J = 8.4 Hz, phenyl), 6.47 (d, 1H, J = 5.4 Hz, quinolyl), 3.80 (s, 6H, methoxy), 3.34–3.23 (m, 8H, propylenel overlapping benzyl and water signal), 2.46–2.31 (m, 10H, piperazinyl overlapping propylene), 1.85 (m, 2H, propylene), 1.68 (m, 2H, propylene). HRESI-MS; found: 526.2615; requires 526.2585 [M+H]. HPLC = 96.6% (250 nm); 85.0% (220 nm).

5.5.3. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]-4-(1*H*-indol-3-yl)butanamide (5h). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 10.71 (br s, 1H, indole-NH), 8.39 (d, 1H, J = 5.4 Hz, quinolyl), 8.21 (d, 1H, J = 9.0 Hz, quinolyl), 7.77 (m, 1H, amino), 7.73 (t, 1H, J = 5.6 Hz, amido), 7.48 (d, 1H, J = 7.8 Hz, indolyl), 7.43 (m, 1H, quinolyl), 7.37 (m, 1H, quinolyl), 7.32 (d, 1H, J = 8.1 Hz, indolyl), 7.08 (m, 1H, indolyl), 7.04 (t, 1H, J = 7.4 Hz, indolyl), 6.95 (t, 1H, J = 7.4 Hz, indolyl), 6.47 (d, 1H, J = 5.4 Hz, quinolyl), 3.33–3.23 (m, 6H, propylene overlapping water signal), 3.06 (m, 2H, butyryl), 2.66 (t, 2H, J = 7.6 Hz, propenyl), 2.43–2.24 (m, 10H, piperazinyl overlapping propylene), 2.11 (t, 2H, J = 7.4 Hz, butyryl), 1.86 (m, 2H, propylene), 1.80 (m, 2H, butyryl), 1.54 (m, 2H, propylene). HRESI-MS; found: 527.2957; requires 547.2952 [M+H]. HPLC = 99.8% (250 nm); 97.5% (220 nm).

5.5.4. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]-4-oxopentanamide (5i). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.39 (d, 1H, J = 5.4 Hz, quinolyl), 8.23 (d, 1H, J = 9.0 Hz, quinolyl), 8.10 (m, 1H, amido), 7.77 (m, 1H, amino), 7.43 (m, 1H, quinolyl), 7.38 (m, 1H, quinolyl), 6.48 (d, 1H, J = 5.4 Hz, quinolyl), 3.34–3.20 (m, 6H, propenyl over-

lapping water signal), 2.95 (s, 3H, CH_3), 2.94 (m, 2H, propylene), 2.44–2.25 (m, 12H, piperazinyl overlapping propylene), 1.81 (m, 2H, propylene), 1.62 (m, 2H, propylene). HRESI-MS; found: 460.2482; requires 460.2479 [M+H]. HPLC = 100.0% (250 nm); 96.9% (220 nm).

5.5.5. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]-3-methylbut-2-enamide (5m). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.39 (d, 1H, J = 5.4 Hz, quinolyl), 8.23 (d, 1H, J = 9.0 Hz, quinolyl), 7.78 (m, 1H, amino). 7.67 (t, 1H, J = 5.0 Hz, amido), 7.43 (m, 1H, quinolyl), 7.38 (m, 1H, quinolyl), 6.48 (d, 1H, J = 5.4 Hz, quinolyl), 5.61 (s, 1H, CH), 3.34–3.23 (m, 4H, propenyl overlapping water signal), 3.08 (m, 2H, propylene), 2.45–2.25 (m, 10H, piperazinyl overlapping propylene), 2.06 (s, 3H, CH_3), 1.81 (m, 2H, propylene), 1.76 (s, 3H, CH_3), 1.54 (m, 2H, propylene). HRESI-MS; found: 444.2537; requires 444.2530 [M+H]. HPLC = 95.6% (250 nm); 86.9% (220 nm).

5.5.6. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]-2,6-dichlorophenylacetamide (5n). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.39 (d, 1H, J = 5.4 Hz, quinolyl), 8.23 (d, 1H, J = 9.0 Hz, quinolyl). 8.00 (t, 1H, J = 5.6 Hz, Amido). 7.78 (m, 1H, Amino), 7.46–7.41 (m, 3H, quinolyl overlapping Benzen), 7.38 (m, 1H, quinolyl), 7.30 (t, 1H, J = 8.0 Hz, Benzen), 6.48 (d, 1H, J = 5.5 Hz, quinolyl), 3.77 (s, 2H, Benzyl), 3.34–3.22 (m, 4H, Propenyl overlapping Wassersignal), 3.08 (m, 2H, propylenel), 2.44–2.27 (m, 10H, Piperazinyl overlapping propylene), 1.81 (m, 2H, propylene). 1.58 (m, 2H, propylene). HRESI-MS; found: 548.1779; requires 548.1751 [M+H]. HPLC = 94.2% (250 nm); 83.2% (220 nm).

5.5.7. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]-4-ethoxybenzamide (5p). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.39 (d, 1H, J = 5.4 Hz, quinolyl), 8.28 (t, 1H, J = 5.4 Hz, amido), 8.23 (d, 1H, J = 9.0 Hz, quinolyl), 7.78 (m, 3H, amino overlapping phenyl), 7.42 (m, 1H, quinolyl), 7.38 (m, 1H, quinolyl), 6.94 (d, 2H, J = 8.8 Hz, phenyl), 6.48 (d, 1H, J = 5.4 Hz, quinolyl), 4.08 (q, 2H, J = 7.0 Hz, 7.0 Hz, ethoxy). 3.34–3.23 (m, 6H, propylene overlapping water signal), 2.46–2.31 (m, 10H, piperazinyl overlapping propylene), 1.81 (m, 2H, propylene), 1.67 (m, 2H, propylene), 1.33 (t, 3H, J = 7.0 Hz, ethoxy). HRESI-MS; found: 510.2670; requires 510.2636 [M+H]. HPLC = 97.3% (250 nm); 80.9% (220 nm).

5.5.8. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]-2-methylbenzamide (5u). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.38 (d, 1H, J = 5.4 Hz, quinolyl). 8.24–8.17 (m, 2H, quinolyl overlapping amido), 7.78 (m, 1H, amino), 7.42 (m, 1H, quinolyl), 7.34 (m, 1H, quinolyl), 7.32–7.36 (m, 2H, phenyl), 7.24–7.18 (m, 2H, phenyl), 6.47 (d, 1H, J = 5.5 Hz, quinolyl), 3.34–3.22 (m, 6H, propylene overlapping water signal), 2.44–2.33 (m, 10H, piperazinyl overlapping propylene), 2.32 (s, 3H, CH_3), 1.80 (m, 2H, propylene), 1.66 (m, 2H, propylene). HRESI-MS; found: 480.2552; requires 480.2530 [M+H]. HPLC = 96.4% (250 nm); 86.1% (220 nm).

5.5.9. *N*-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}-piperazin-1-yl)propyl]-3,5-dichlorobenzamide (5x). ^{1}H NMR δH (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.68 (t, 1H, J = 5.3 Hz, amido), 8.40 (d, 1H, J = 5.4 Hz, quinolyl), 8.22 (d, 1H, J = 9.1 Hz, quinolyl), 7.84 (m, 1H, phenyl), 7.78 (m, 2H, amino overlapping phenyl), 7.45–7.38 (m, 2H, phenyl overlapping quinolyl), 7.40–7.32 (m, 2H, quinolyl overlapping phenyl), 6.48 (d, 1H, J = 5.5 Hz, quinolyl), 3.32–3.23 (m, 6H, propylene overlapping water signal), 2.46–2.33 (m, 10H, piperazinyl overlapping propylene), 1.81 (m, 2H, propylene), 1.69 (m, 2H, propylene). HRESI-MS; found: 534.1628; requires 534.1594 [M+H]. HPLC = 89.1% (250 nm); 72.1% (220 nm).

5.6. Growth of *P. falciparum* parasites

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a [^{3}H]hypoxanthine incorporation assay, using the chloroquine- and pyrimethamine-resistant K1 strain and the chloroquine-sensitive strain NF54.[19] Compounds, including chloroquine (**1**) (Sigma C6628) as standard, 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% hematocrit (0.3% parasitemia). Serial doubling dilutions of each drug were prepared in 96-well microtiter plates and incubated in a humidified atmosphere at 37 °C; 4% CO_2, 3% O_2, 93% N_2. After 48 h, 50 μL of [^{3}H]hypoxanthine (=0.5 μCi) in medium 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 were transferred onto a glass fiber filter and 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, Zürich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves using Microsoft Excel.

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