

1 **Overcoming Chloroquine Resistance in Malaria: Design, Synthesis and Structure-**
2 **Activity Relationships of Novel Chemoreversal Agents**

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11 Running Head: Synthesis and SAR of Novel Chemoreversal Agents

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15 Supplemental material for this article includes additional Tables S1-S9, table of known CRAs,
16 pharmacophore generation and validation, additional material describing difficulties in the synthesis of
17 the 4-series, structures and numbering of synthesised compounds, tables of probe 3 uptake, IC₅₀, RMI
18 values and statistics.

19

20 ABSTRACT

21 Malaria remains a significant infectious disease with even artemisinin-based therapies now facing
22 resistance in the field. Development of new therapies is urgently needed, either by finding new
23 compounds with unique modes of action, or by reversing resistance towards known drugs with
24 ‘chemosensitizers’ or ‘chemoreversal’ agents (CRA). Concerning the latter, we have focused on the
25 resistance mechanisms developed against chloroquine (CQ). We have synthesized a series of
26 compounds related to previously identified CRAs, and found promising novel compounds. These
27 compounds show encouraging results in a coumarin labeled chloroquine uptake assay, exhibiting a
28 dose response in resensitising parasites to the antimalarial effects of chloroquine. Selected compounds
29 show consistent potency across a panel of chloroquine and artemisinin sensitive and resistant parasites,
30 and a wide therapeutic window. This data supports further study of CRAs in the treatment of malaria
31 and, ultimately, their use in chloroquine-based combination therapies.

32 **KEYWORDS:** chemoreversal; chemosensitiser; antimalarial; chloroquine; chloroquine-resistant;
33 artemisinin-resistant

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35 1. Introduction

36 Malaria remains one of the most dangerous infectious diseases, with 214 million cases causing
37 438,000 deaths in 2015 by WHO estimates [1]. Particularly vulnerable groups include pregnant
38 women[2] and young children.[3] Carried by parasites of the genus *Plasmodium* (*P. falciparum*, *P.*
39 *vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*), the infection is transmitted by the bite of infected *Anopheles*
40 mosquitoes [4]. Chloroquine (CQ, **1**) has historically been the most commonly used antimalarial drug
41 due to its good efficacy, low toxicity, and affordability. However, resistance against **1** and now nearly
42 all other available treatments has become a major problem, necessitating the development of new

43 treatments on a regular basis [5, 6]. Even with artemisinin-based combination therapy (ACT), the
44 current standard treatment, prolonged parasite clearance times have been recently observed in Thailand
45 and Cambodia [7-9], including prominent reports of the failure of the dihydroartemisinin-piperaquine
46 combination [10]. The mechanisms of drug-resistance depend on the drug and are not yet completely
47 understood. Resistance to 1 (CQR) is known to be transporter-mediated, mostly due to mutations in
48 two genes: PfCRT K76T [11] and several mutations in pfMDR1 [12]. These modified transporters are
49 able to remove 1 from its site of action, the digestive vacuole (DV), where it interferes with heme
50 detoxification. Similar point mutations in PfCRT can be traced back to the geographical origin of the
51 *P. falciparum* isolates. For African and most South-east Asian resistant strains, mutation in amino
52 acids at positions 72-76 is a change from CVMNK to CVIET, while the South American resistant
53 strains encode SVMNT [13, 14].

54 Three approaches can lead to new malaria treatments [15]: searching for new compounds with new
55 mechanisms of action, addressing the development of drug resistance with combination therapies, or
56 oppose resistance by developing compounds that restore the drug-sensitivity of resistant parasites.
57 Compounds used for the latter concept are called chemoreversal agents (CRA) or chemosensitizers.
58 Multidrug resistance (MDR) in cancer chemotherapy *via* over-expression of drug efflux pumps or
59 increased DNA repair mechanisms is well established, and the concept of reversing this drug
60 resistance has been evaluated in several clinical trials [16, 17]. In the case of malaria drug resistance,
61 *in vitro* chemosensitization of resistant *P. falciparum* strains has been shown with, for example,
62 verapamil and promethazine [18-21]. Most of the published CRAs have poor *in vivo* pharmacokinetic
63 and pharmacodynamic properties [22, 23], or have a poor safety profile [24]. The only chemosensitizer
64 that we are aware of that has undergone clinical trials is chlorpheniramine [25], but the results were
65 disappointing. More recently reported novel analogues of chlorpheniramine may show greater promise

[26]. Clearly novel drug-like candidates with acceptable safety profiles and *in vivo* efficacy for CQ and ART-resistant strains are urgently required.

Potential CRAs can be detected using a fluorescent probe in a straightforward and rapid assay. We have previously published probe **3**, composed of a chloroquine (**1**) portion linked to a coumarin fluorophore (**2**) (Figure 1) [27].

A high throughput assay employing **3** to screen the LOPAC library (Sigma Aldrich), composed of 1280 drug-like compounds, was used in our program to find new CRA compounds. Four novel CRAs were identified: L-703,606 (**4**) [28], loperamide (**5**) [29], octoclotheperin (**6**) [30], and methiothepin (**7**) [31] (Figure 2).

Analogues of the hit compounds were designed based on a pharmacophore model, synthesized and screened for chemoreversal activity with probe **3**. The potential of the new compounds to sensitise CQR parasite strains to the effects of **1** was then assessed. Cytotoxicity and preliminary *in vitro* DMPK studies confirmed the drug-like potential of the new compounds.

2. Results and Discussion

2.1. *In silico* analysis and design of compounds

All of the seven hit compounds were observed to fit reasonably well into the pharmacophore model developed by Bhattacharjee *et al* [32]. This model describes a 3-point pharmacophore composed of two aromatic hydrophobic sites with a third site being a hydrogen bond acceptor. No protein crystal structure information is available for the mutant or wild type PfCRT, hence any new model must be ligand based and preferably simple to apply as in the case of a 3-point pharmacophore. To assess which parts of the scaffolds of our hit compounds are essential for their activity, and if these correspond to the previously published model, we created a 3-point ligand based pharmacophore model with LigandScout [33, 34]. Our model comprises of two aromatic hydrophobic interaction sites

89 and a hydrogen bond acceptor, preferably a basic nitrogen atom, similar to the Bhattacharjee model. A
90 database of over 100 compounds was compiled (see Table S1), all being proven chloroquine resistance
91 CRAs targeting the CVIET haplotype of PfCRT in *P. falciparum*. From this database, 77 were used for
92 generation and scoring of the pharmacophore (training set of 22 compounds and test set of 55
93 compounds, for entire list see Table S2). In order to avoid a predisposition of the pharmacophore
94 model in favor of the hit compounds, they were excluded in its generation. From this model the
95 predicted optimal pharmacophore consists of two hydrophobic regions and a positive-ionizable atom,
96 situated almost equidistant from one another (Figure 3).

97 The optimal pharmacophore features were situated about 6Å away from one another, and at an angle
98 of approximately 60°. In order to determine if the generated pharmacophore is reliable enough for
99 further virtual screening of compound libraries, a validation was carried out using the same library
100 previously screened in the assay, LOPAC1280™. After exclusion of obvious false positive hits and
101 duplicates, 189 hits were found (see Table S3). Within these 189 hits were included 17 of the 18
102 known CRA compounds present in the LOPAC library (see Table S4). The only CRA not found was
103 ketoconazole, probably due to its relatively complex structure. Furthermore, 18 hits from the previous
104 uptake screening were also found in this *in silico* study, including the seven new chemosensitizer hit
105 compounds intentionally omitted in the generation of the pharmacophore model. In conclusion,
106 identification of a number of CRA compounds and an understanding of their necessary features has
107 been achieved by application of a ligand-based pharmacophore model.

108 We then considered the complexity of the hit compounds and the options for simplification. By
109 reducing the scaffolds to the essential features for chemoreversal activity, shorter syntheses and
110 improved DMPK properties may be possible, through lowering of molecular weight and clogP.

Looking at the fit of **4** in the pharmacophore model, it appears that only two of the aromatic rings are required, the second phenyl ring of the *gem*-diphenyl group does not appear to be contributing (Figure 4).

For compound **5**, the two aromatic features of the model are satisfied by its *gem*-diphenyl group and the positive ionizable feature by the piperidine *N*-atom, questioning the need for both the *p*-Cl-phenyl and the amide (Figure 5).

With this information in mind, we designed a series of simplified analogues of four of the chemosensitizers, **4**, **5**, **6** and **7**. We excluded those having very challenging syntheses, e.g. mibefradil.

2.2. Chemistry

A 9-BBN induced reductive amination between ketone **8** and amine **9** has been reported for the preparation of compound **4**. In our hands this step proved to be problematic, so we opted for an alternative approach: *Cis* selective reductive amination of commercially available ketone **8** with ammonium formate in the presence of a catalytic amount of zinc chloride led to primary amine **13** (Figure 6). In the following reductive amination with aldehyde **15**, prepared by oxidation of 2-iodobenzyl alcohol (**14**), the minor *trans* diastereoisomer didn't react further and **4** was isolated in 84 % yield.

In order to test the necessity of the *gem*-diphenyl group of **4**, [42] compounds **18** and **19** were prepared. Hydrogenation of the double bond in **7** led to ketone **16**, which was successfully transformed to **18** using the previously problematic sequence with 9-BBN as reducing agent (Figure 7).

Employing again the *cis* selective reductive amination with ammonium formate/zinc chloride gave free amine **17**, which was transformed to **19** *via* reductive amination with aldehyde **15** in moderate yield. Compound **19** lacks one phenyl moiety compared to **4**.

We wanted to simplify these compounds even further, questioning the need for the C2-bridge in the piperidine core of **4**. Using commercially available Boc-protected piperidine **20**, we performed a reductive amination with aldehyde **15**, leading to amine **21**, devoid of both the *gem*-diphenyl and C2-bridge (Figure 8). With a similar idea in mind, 2,2-diphenylethylamine (**22**) was also reacted with aldehyde **15**, this time leading to compound **23**, possessing all three aromatic rings, but entirely lacking the piperidine core.

Synthesis of loperamide (**5**) is reported to be quite complex with the disadvantage that it leads directly to the tertiary amide *via* the intermediate ammonium bromide **28** (See Supporting Information for retrosynthesis) [43].

More recently, Lazarova *et al* described a straightforward method to access the loperamide scaffold from bromide **26** and piperidinol **27a**, via intermediate tertiary nitrile **25a** and primary amide **24a**, installing the two methyl groups on the amide only in the last step [44]. Following the more flexible latter approach, we obtained compounds **25** *via* the alkylation of **27** by bromide **26**. Hydrolysis of the nitrile gave amide **24**, in low to moderate yield (Figure 9).

In order to investigate the role of the amide, we reduced the nitrile group of **25** to the free amine **30** using LAH. In the case of the chlorophenyl substituted piperidine **25a**, solubility was poor in Et₂O, giving amine **30a** in poor yield. This could be improved simply using THF as the solvent giving a good yield of **30a**. Despite the improved conversion of **25a** to **30a**, application to piperidinol **25c** and unsubstituted piperidine **25d** resulted in isolation of the desired products but in poor yield. In the case of the Boc-protected amino-piperidine **25b**, partial reduction of the nitrile under basic conditions did not lead to the desired amide, but only to deprotected **25e** (Figure 10). Reduction of **25b** with LAH yielded only degradation products, however, nitrile **25e** was successfully reduced by LAH, leading to triamine **30e** in moderate yield.

Installation of an additional nitrogen atom to the piperidine ring of analogues of **5** was achieved by alkylation of *p*-fluoro-phenyl substituted piperazine (**31**) with bromide **26**. Although slow this reaction produced alkylated piperazine **32** in good yield (Fig. 11).

Partial reduction of the nitrile in **32** under basic conditions gave primary amide **33**, while total reduction with LAH led to the dibasic primary amine **34** but in very low yield. The additional nitrogen atom changes the vector of the aromatic substituent and also assesses the role of the tertiary alcohol in the piperidine series.

The last group of analogues selected for synthetic exploration is based on hit compounds **6** and **7**. Our initial aims were to verify the necessity of the chloro or methylthio groups. Construction of the 10,11-dihydro-dibenzo[*b,f*]thiepine scaffold was achieved in two steps, based on the method published by Kristensen *et al* [30]. Starting from suitable thiophenols (**35**), copper-mediated coupling with 2-iodophenylacetic acid (**36**) led to intermediates **37a-c** which were cyclized in phosphorus pentoxide at high temperature yielding ketones **38a-c** (Fig. 12).

Subsequently, a Lewis acid catalyzed two-step reductive amination process, with either methylpiperazine (**39a**) or Boc-protected piperazine (**39b**), gave access to piperazine analogues **40a-d** which were reduced with sodium borohydride in moderate to good yield. Deprotection of **41c-d** with TFA yielded free piperazines **41e-f**.

2.3. Uptake assay using probe **3** and SAR of analogues

For a first evaluation of the chemoreversal activity of the compounds, the same fluorescent-CQ **3** uptake method was used as described previously [27]. In short, the mutated PfCRT transporter in resistant parasites effluxes fluorescent **3** out of the digestive vacuole and reduced fluorescence is seen in the cell. However, if a reversal agent blocks the transporter, the probe accumulates inside the digestive vacuole and fluorescence can be measured in the cell; this is quantified using flow cytometry.

Beforehand, all compounds were tested for auto-fluorescence to exclude false positives (data not shown).

At first, we tested all compounds with a chloroquine resistant strain of CVIET haplotype, K1. In this assay compounds with values over 50% were statistically significant compared to controls. As shown in Figure 13 (and Table S5) for the series **4** analogues, the precursors **8**, **10** and **16** show no significant uptake over a PBS control. This indicates that one or two aromatic ring(s) and the basic quinuclidine *N*-atom are not enough to retain activity. Dibasic **17** maintains the amine adjacent to the quinuclidine core, but lacks one aromatic ring, and uptake of **3** is not improved. Compounds **13** and **18** possess all the required features as predicted by the model and show significant uptake; however, it seems that the distance of the two aromatic rings is more favorable in **18** than in the *gem*-diphenyl group of **13**. An isomeric mixture of **18** performs slightly poorer than the pure *cis* diastereomer and was thus not pursued further. The lack of activity of **19** appears to be due to the iodo substitution: according to the pharmacophore, two aromatic rings are necessary and the iodobenzyl group might be too voluminous in this position in the required binding conformation of **19**, creating repulsive interactions in the transporter. Much simpler ‘fragmented’ analogues **20-23** were weakly active in the assay indicating that the pharmacophoric features must all be present and arranged precisely in space.

For analogues of compound **5** (Fig. 14 and Table S6), primary amide **24a** maintained activity compared to **5** but suffered a reduction in activity with loss of the chlorophenyl (**24c**) and hydroxyl (**24d**). However nitriles **25a** suffered total loss of activity suggesting that the primary amide is important in this series. Boc-protected **25b** was also not active, however, deprotection to a primary amine (**25e**) restored activity. Simpler hydroxy-piperidine (**25c**) and unsubstituted piperidine (**25d**) retained activity. Fragmenting the molecule into two parts, **26** and **27a**, confirmed that all the pharmacophoric elements present in the molecule are required. Reducing nitrile to amine (**30c-d**) suggests that 4-piperidine substituents are not absolutely required for potency hence may be a useful

203 area of the molecule to tune DMPK properties. Taken together, the SAR in this series is complex
204 indicating that these compounds meet the required binding conformation potentially by adopting
205 different binding modes, all of which fit the pharmacophore. Further extensive studies will be required
206 to fully elucidate this hypothesis for chemosensitising compounds in series **5**.

207 Replacing the 4-*C* of the piperidine of **5** with a nitrogen led to piperazines which enabled study of a
208 more planar conformation of the ring. We were able to access 4-fluoro substituted piperazines with a
209 nitrile (**32**), primary amide (**33**) and primary amine (**34**), and compounds **33** and **34** were active in
210 uptake of probe **3** (Figure 14).

211 Finally, analogues of **6** series compounds show clearly that an amine is preferred for optimal
212 activity: the precursors **38a-c** showed only weak uptake (Figure 15 and Table S7). As expected the
213 non-basic carbamate, e.g. in Boc-protected **41d**, did not increase the uptake of **3** in parasites. On the
214 other hand, removal of the methyl substitution on the aromatic ring (compare **41e-f** with **6**) has little
215 influence on activity: both bearing a free piperazine, but one with chloro at R¹ (**41f**) and one without
216 (**41e**), show good activity similar to the parent compound. Unfortunately, **41a** and **41c** were not soluble
217 under the assay conditions.

218 **2.4. Dose Response IC₅₀ and EC₅₀ Assays**

219 In order to further characterise the observed chemoreversal activity, the best performing CRAs were
220 chosen for further study in an anti-parasite assay in combination with **1** (CQ IC₅₀ assay). The
221 compounds chosen were **13**, **18** (series **4**), **22** (fragment), **24a**, **25d** and **30c** (series **5**), as well as **41e**
222 and **41f** (series **6**). In order to exclude intrinsic anti-malarial activity of these compounds and to make
223 sure only chemoreversal activity is measured, the IC₅₀ of the CRAs against the parasites without **1**
224 were first measured (data not shown). In consequence, the concentrations of all analogues for the IC₅₀
225 assay that does not affect viability of parasites were fixed at a concentration of 500 nM. The selected

test compounds were studied against three strains: K1, the same CQ-resistant (CQR) strain as in the uptake assay of probe **3**; 3D7 as a typical CQ-sensitive (CQS) parasite strain as well as 7G8, a mildly CQR strain with the SVMNT haplotype. This last strain shows intermediate CQ resistance; however, it is notable as the known CRAs verapamil, chlorpromazine and desipramine have no or little effect on it [27]. An ideal CRA would have chemoreversal activity for a wide range of strains with different haplotypes and grades of resistance, but not influence the activity of **1** in sensitive strains. With some exceptions (**13**, **24a** and **6**), all thus tested CRA compounds lower the IC₅₀ of **1** significantly in strains resistant to **1**, compared to treatment with **1** only (Figure 16 and Table S8). Of the new compounds, **18**, **22**, **41e** and **41f** improved the IC₅₀ of **1** most significantly in the K1 strain. Compounds **18**, **22** and **41e** also reduced the IC₅₀ of **1** in the intermediate 7G8 strain, although to a lower extent than K1. Encouragingly, all compounds did not affect the potency of **1** in the CQS strain 3D7.

Further characterization of the effect of the selected CRAs were carried out on six additional field and laboratory isolates with a range of resistance profiles including artemisinin-resistant strains (ArtR, see Figure 17 and Table S8). Broadly, similar activity patterns were seen for these compounds in all the resistant strains. Compounds with the most significant broad activity include **18**, **22**, **25d**, **41e** and **41f**. This is even more apparent when illustrating the *relative* effects of chemosensitization for the CRA analogues using the response modification index (RMI, ratio of each compound's IC₅₀ to the IC₅₀ of **1** alone) as in Figure 18 (Table S9). A desirable profile is an RMI of significantly less than 1 for the resistant strains (CQR, ArtR) and around 1 for the CQS strains. Key compounds **18**, **22**, **25d**, **41e** and **41f** have RMI values well below 1 for all strains except the two CQ sensitive ones. The highest efficacy of the new analogues can be seen for **18** and the very simple fragment diphenylethylamine (**22**) lowering the IC₅₀ of **1** by approximately 50% or more at 500 nM concentration in all resistant strains (RMI ≤ 0.5) except 7G8.

Overall, the best performing CRA compounds were **18**, **22**, **25d**, **41e** and **41f**; their CQ-IC₅₀ values are summarized in Table 1, along with the ratios of their CQ-IC₅₀ on K1 or 7G8 over the CQ-IC₅₀ of sensitive 3D7. The lower this ‘resistance ratio’, the greater is the reduction in resistance to that strain towards **1**. Of the simpler synthesized analogues, **18** shows the best overall decrease in this ratio to 6.4 from 20 for CQ only (K1 strain) and down to 3.9 for the 7G8 strain. Other promising compounds are the fragment **22** and the analogue of **6**, compound **41e**.

Subsequently, the dose dependent effect on the CQ IC₅₀, the EC₅₀, was determined for **18**, **22**, **24a**, **25d** and **41e**. More precisely, EC₅₀ is the concentration of CRA that reduces by 50% the required concentration of **1** (CQ) to inhibit the survival of half the parasites. The CQR strain K1 was chosen for this assay, but for the most promising new compound, **18**, the EC₅₀ values were also measured for the CQS strain 3D7 and for the SVMNT haplotype strain 7G8 (Table 2).

In each series, the best performing analogue compound has EC₅₀ values in the nanomolar range for CQR K1: **25d** has a lower EC₅₀ than **5** and the EC₅₀ of **41e** is considerably improved compared to **6**. The most effective new compound proves to be **18**, with a slightly higher EC₅₀ than the more complex and higher molecule weight parent compound **4**. Its activity was validated as a pure chemosensitizer for chloroquine resistance, as it has no effect on the CQ IC₅₀ of the CQ-sensitive strain 3D7. The concentration of **18** needed to reduce the CQ IC₅₀ in 7G8 by half is two-fold higher. Nevertheless a dose dependent effect can clearly be seen even for this SVMNT haplotype strain. Overall, of the new compounds, **18** shows the best and consistent results against various strains resistant to **1**. However EC₅₀s for **22**, **25d** and **41e** are within two-fold of **18** indicating that all these 3 compounds show promise as chemosensitizing agents.

2.5. Cytotoxicity tests

The cytotoxicity of the analogues and parent compounds were investigated with two normal cell lines characterized for toxicity against a wide range of drugs in the laboratories of the NUS Drug Development Unit [45]. Firstly, a TGF α -transfected mouse hepatocyte (TAMH) cell line was used to assess toxicity of the new compounds [46]. This cell line was selected due to the liver being a major organ of drug metabolism. The IC₅₀ values of **1** alone and **1** in combination with **18** and **4** were determined using an ATP luminescence assay. **1** was itself only weakly toxic with an IC₅₀ of 85.26 μ M. With addition of 0.1, 1 and 10 μ M of **4** and **18** no significant increase in cytotoxicity was observed with any combination in TAMH cells (Table 3).

The IC₅₀s of **4** and **18** in TAMH alone were determined to be 17.2 and >100 μ M, respectively, suggesting that the parent hit compound **4** was about 3-fold more toxic than **18** in TAMH hepatocytes (Table 4). However these are high IC₅₀ values and good therapeutic windows of several orders of magnitude are seen with both compounds. One example from the promising series **5** compounds, **24a**, was also selected for study and had a clear therapeutic window of over 200. A further normal cell line, the cardiomyocyte AC10, was studied with selected CRAs [47] **18** and **24a**. Although more toxic than against TAMH, these compounds were still only weakly toxic against AC10 cells with IC₅₀'s of 71.47 and 45.19 μ M, giving therapeutic windows of 420 and 94, for **18** and **24a**, respectively.

To further explore the toxicity of the most promising compound, **18**, we carried out an AMES test to assess if **18** could be potentially mutagenic [48]. Strains of *Salmonella typhimurium* TA98 and TA100 were studied with and without S9 fraction using 2-aminoanthracene as a positive control and **18** at 100 and 10 μ g per plate. At both concentrations tested of **18** there was no detectable differences with or without S9 and levels observed were lower than DMSO vehicle controls.

The very good therapeutic windows in two relevant normal cell lines coupled with the negative AMES test of **18** underline the *in vitro* safety of this compound.

2.6. *In Vitro* Physicochemical Properties

295 Assessment of *in vitro* physicochemical properties provides important data to help predict cellular and *in vivo* performance of a new molecule. Selected CRAs were profiled for molecular weight, cLogP, aqueous kinetic solubility and permeability (Table 5).

Compound **4**, although having good permeability and acceptable molecular weight (MWt), exceeded the normally desirable cLogP of 5, which has led to a rather low solubility of 31 µg/mL. Most likely
300 the presence of the iodide and two phenyl rings are responsible. On the other hand the much smaller **18** has a cLogP two orders of magnitude lower, much improved solubility and a higher permeability as well. Although **24a** has a lower MWt, cLogP and higher solubility than parent compound **5**, the de-dimethyl primary amide analogue, **24a**, has a significantly lower permeability probably due to the introduction of two additional hydrogen bond donors. Compound **6** has a rather high cLogP and low
305 solubility but the de-methylated and de-chlorinated analogue, **41e**, has a much reduced cLogP with a concomitant increase in solubility.

3. Conclusions

Focusing on the resistance mechanisms developed against chloroquine (CQ, **1**), we have designed and synthesized several new chloroquine chemosensitizers based on hit compounds **4**, **5**, **6** and **7**.
310 These compounds were tested in a coumarin labeled CQ uptake assay using probe **3**, and subsequently IC₅₀ and EC₅₀ studies were carried out. Selected CRAs **18**, **22**, **25d** and **41e** showed potency to resensitize parasites to the antimalarial effects of **1** (EC₅₀) at concentrations less than 500 nM, and were equally effective across a panel of parasites both sensitive and resistant to **1** and, importantly, artemisinin. Hit compound **4** and new compounds **18** and **24a** were further tested in a hepatocyte and a
315 cardiomyocyte cell line and found to have very good therapeutic windows ranging from 94 to over 500. Compound **18** also had high aqueous solubility (*c.* 0.3 mg/mL) and high permeability in a PAMPA

assay [49-51]. These compounds represent starting points for further studies towards revitalized chloroquine-based malaria therapies.

320 4. Experimental Section

4.1. General

All reagents purchased from commercial sources were of the highest purity grade available and were used without further purification. Commercially available AR grade solvents or anhydrous solvents packed in resealable bottles were used as received. All reaction temperatures stated in the procedures
325 are external bath temperatures. Non-aqueous reactions were performed under a positive pressure of nitrogen in oven-dried glassware. Yields refer to chromatographically and spectroscopically homogeneous materials, unless otherwise stated. Reaction progress was monitored by analytical thin layer chromatography (TLC) with 0.25 mm Merck pre-coated silica gel plates (60F-254) using UV light (254 nm) as visualizing agent, and ceric ammonium molybdate or potassium permanganate
330 solutions as developing stains. Flash chromatography was performed on silica gel 60 (0.040 – 0.063 mm) purchased from SiliCycle or Merck. The structures of synthesized compounds were verified by ^1H NMR, ^{13}C NMR, and mass spectrometry. ^1H (400 MHz) and ^{13}C (101 MHz) NMR spectra were measured in CDCl_3 on a Bruker Avance III 400 (Ultraschield Plus) spectrometer. Chemical shifts are reported in parts per million (ppm) using the residual CDCl_3 peak at 7.26 (^1H) or
335 77.16 (^{13}C) as internal standard. ^1H NMR coupling constants (J) are reported in Hertz (Hz), and multiplicities are presented as follows: s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Mass spectra were obtained on a Bruker amaZonX for the nominal mass or on a Bruker micrOTOFQII spectrometer for the high resolution mass analysis. Purity of the compounds was assessed by high pressure liquid chromatography detecting at 254 nm using an Agilent 1200 series

340 HPLC system with a Zorbax SB-C18 5 micron 4.6 x 250 mm column using a gradient elution starting from a 5:95 solution of acetonitrile/water, with 1% trifluoroacetic acid (TFA) to 100% acetonitrile and 1% TFA with flowrate 0.5 mL per minute over 15 mins. HPLC purity is greater than 95% unless stated. All compounds synthesized were stored in a -20 °C freezer.

4.2. Synthesis

345 4.2.1. (2*R*,3*R*)-2-Benzhydryl-*N*-(2-iodobenzyl)quinuclidin-3-amine (L 703,606, **4**)

To a stirred solution of 2-benzhydrylquinuclidin-3-amine (**13**) (10.0 mg, 34 μmol, 1.0 equiv.) and 2-iodobenzaldehyde (**15**) (15.9 mg, 68 μmol, 2.0 equiv.) in dry DCM (1.10 mL), under nitrogen and at 0 °C, was added NaBH(OAc)₃ (14.4 mg, 68 μmol, 2.0 equiv.). The reaction mixture was allowed to warm to room temperature, stirred for 5 h and quenched with water and saturated aqueous NaHCO₃.
350 The aqueous layer was extracted with DCM (3x), and combined the organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5), yielding 12 mg (84 %) of desired compound **4**.

R_f (DCM/MeOH = 95/5) = 0.14; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.81-7.83 (1 H, m, Ar-H), 7.73-7.76 (1 H, m, Ar-H), 6.94-7.37 (12 H, m, Ar-H), 4.06 (1 H, d, *J* = 11.6 Hz), 3.75 (1 H, dd, *J* = 11.6 Hz, 5.1 Hz), 3.13-3.22 (2 H, m), 2.97-3.10 (2 H, m), 2.62-2.72 (1 H, m), 2.09-2.19 (1 H, m), 1.72 (1 H, bs, NH), 1.55-1.68 (3 H, m), 1.30-1.40 (1 H, m), 1.18-1.30 (1 H, m); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 144.0 (C-12), 142.0 and 137.4 (2 x C-7), 139.6 (C-14), 131.5 (C-17), 129.4 and 128.6 (2 x C-9), 128.9 (C-15), 128.4 and 127.8 (2 x C-8), 128.1 (C-16), 127.0 and 126.3 (2 x C-10), 99.8 (C-13), 73.5 (C-2), 69.5 (C-11), 65.6 (C-1), 55.5 (C-6), 50.4 and 41.0 (C-5), 31.6 (C-3), 26.1 and 20.9 (C-5); **MS**(ESI) *m/z* = 507.2[M+H]⁺; **HRMS**(ESI) *m/z* = 507.1294 [M+H]⁺, calc.: 507.1292, Diff.: 0.4 ppm.

360

4.2.2. 1-(8-Chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)-4-methylpiperazine (**6**)

Compound **6** was obtained using the same conditions as for **41a**, employing **38a** (136 mg, 0.52 mmol, 1.0 equiv.), 1-methylpiperazine (**39a**) (0.28 mL, 2.56 mmol, 4.9 equiv.) and Ti(*i*-OPr)₄ (0.12 mL, 0.39 mmol, 0.75 equiv.) and the mixture was refluxed for 30 h. Thus were obtained 205 mg (100 % plus impurities) of the intermediate enamine **40b** as a dark orange oil; the crude was used in the next step without further purification.

In the second step, the intermediate enamine **40b** and NaBH₄ (295 mg, 7.80 mmol, 15 equiv.) were stirred in 3.7 mL of glacial acetic acid. The crude residue was purified by flash chromatography (DCM/MeOH = 99/1 to 8/2), yielding 143 mg (80 %) of 1-(8-chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)-4-methylpiperazine (**6**) as a light-yellow oil.

R_f (DCM/MeOH = 9/1) = 0.27; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.63 (1 H, d, H-6, *J* = 2.5 Hz), 7.49 (1 H, d, H-11, *J* = 7.6 Hz), 7.34 (1 H, d, H-4, *J* = 7.6 Hz), 7.20-7.32 (2 H, m, H-5 and H-7), 7.08-7.17 (1 H, m, H-10), 7.02-7.09 (1 H, m, H-13), 3.84-4.01 (2 H, m, H-1, H-2a), 3.17 (1 H, dd, *J* = 12.9, 3.3 Hz, H-2b), 2.80-2.97 and 2.57-2.80 (8 H, m, H-15 and H-16), 2.47 (3 H, s, H-17); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 142.0 (C-9), 141.9 (C-12), 136.3 (C-8), 133.9 (C-3), 133.2 (C-14), 133.0 (C-11), 132.2 (C-6), 131.4 (C-4), 129.7 (C-13), 129.1 (C-5), 127.2 (C-7), 126.7 (C-10), 65.6 (C-1), 55.0 (C-15), 47.3 (C-16), 44.9 (C-17), 32.7 (C-2). The analytical data matched the published data [30].

4.2.3. 2-Benzhydrylquinuclidin-3-one (**8**)

A solution of **10** (124 mg, 0.58 mmol, 1.0 equiv.) in 5.8 mL of benzene was added drop wise to a solution of phenylmagnesium chloride (2 M in THF, 0.58 mL, 1.16 mmol, 2.0 equiv.) and CuI (124 mg, 0.58 mmol, 1.0 equiv.) in 5.8 mL of benzene at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched by adding DCM and

385 some drops of water, the resulting suspension was filtered through celite and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (eluent Hexanes/EtOAc = 9/1 to 8/2), yielding **8** together with major amounts of side products and impurities. HPLC purification allowed for the isolation of a pure fraction of **8** (yield ~ 3 %) and comparison to the commercial compound.

390 R_f (Hexane/EtOAc = 8/2) = 0.26; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.36-7.42 (2 H, m, Ar-H), 7.10-7.30 (8 H, m, Ar-H), 4.53 (1 H, d, H-2, J = 8.0 Hz), 3.98 (1 H, d, H-6, J = 8.0 Hz), 3.03-3.12 (2 H, m, H-5), 2.49-2.64 (2 H, m, H-5), 2.38-2.44 (1 H, m, H-3), 1.85-2.06 (4 H, m, H-4) 1.63 (1 H, bs, OH); HRMS(ESI) m/z = 292.1700 $[\text{M}+\text{H}]^+$, calc.: 292.1696, Diff.: 1.3 ppm.

4.2.4. *(Z)*-2-Benzylidenequinuclidin-3-one (**10**)

395 A solution of 3-quinuclidinone **11** (537 mg, 3.32 mmol, 1.0 equiv.) and benzaldehyde (0.34 mL, 3.32 mmol, 1.0 equiv.) in 33 mL of EtOH was treated with two pellets of sodium hydroxide and refluxed for 16 h. The reaction mixture was evaporated to reduce the volume of EtOH and placed in the fridge for 1 h. The orange solid which formed was filtered, washed with EtOH and dried to yield 619 mg (87 %) of the desired *(Z)*-2-benzylidenequinuclidin-3-one (**10**) as a yellow solid.

400 R_f (Hexane/EtOAc = 8/2) = 0.55; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 8.01-8.06 (2 H, m, Ar-H), 7.39-7.40 (3 H, m, Ar-H), 7.02 (1 H, s, H-6), 3.10-3.21 (2 H, m, H-5), 2.95-3.05 (2 H, m, H-5), 2.63 (1 H, q, H-3, J = 3.0 Hz), 1.99-2.08 (4 H, m, H-4); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 205.5 (C-1), 144.9 (C-7), 134.1 (C-2), 132.3 (C-9), 129.7 (C-10), 128.6 (C-8), 125.3 (C-6), 47.6 (C-5), 40.4 (C-3), 26.1 (C-4); MS(ESI) m/z = 214.1 $[\text{M}+\text{H}]^+$; HRMS(ESI) m/z = 214.1230 $[\text{M}+\text{H}]^+$, calc.:
405 214.1226, Diff.: 1.7 ppm.

4.2.5. (*Z*)-2-Benzylidene-3-phenylquinuclidin-3-ol (**12**)

A solution of **10** (100 mg, 0.47 mmol, 1.0 equiv.) in 5 mL of benzene was added drop wise to a solution of phenylmagnesium chloride (2 M in THF, 0.35 mL, 0.70 mmol, 1.5 equiv.) in 0.35 mL of benzene at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight.

410 The reaction was quenched by adding DCM and some drops of water, the resulting suspension was filtered through celite and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (eluent Hexanes/EtOAc = 9/1 to 8/2), yielding 67.6 mg (49 %) of **12**, the undesired 1,2-addition product together with some impurities.

R_f (Hexane/EtOAc = 9/1) = 0.24; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.88 (2 H, d, Ar-H, J = 7.2 Hz), 7.63 (2 H, d, Ar-H, J = 8.4 Hz), 7.18-7.48 (6 H, m, Ar-H), 6.23 (1 H, s, H-6), 3.05-3.24 (2 H, m, H-5), 2.95-3.05 (1 H, m, H-5), 2.83-2.95 (1 H, m, H-5), 2.19-2.49 (3 H, m, H-3 and OH), 1.22-1.68 (4 H, m, H-4); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 155.0 (C-2), 145.6 (C-7), 136.0 (C-7), 129.8 (C-8), 128.3 (C-9), 128.1 (C-9'), 127.5 (C-10), 127.4 (C-8'), 127.4 (C-10'), 124.4 (C-6), 77.3 (C-1), 48.8 (C-5), 45.62 (C-5), 37.4 (C-3), 23.8 (C-4), 21.9 (C-4).

420 4.2.6. 2-Benzhydrylquinuclidin-3-amine (**13**)

To a stirred solution of 2-benzhydrylquinuclidin-3-one (**8**) (52.0 mg, 0.18 mmol, 1.0 equiv.) in dry methanol (52 μL), under nitrogen, was added a 1 M solution of ZnCl_2 in Et_2O (36.0 μL , 2.78 mmol, 0.2 equiv.). After stirring at ambient temperature for 30 min, this mixture was treated with solid ammonium formate (135 mg, 2.14 mmol, 12 equiv.). After stirring another hour at ambient
425 temperature, solid sodium cyanoborohydride (22.6 mg, 0.36 mmol, 2.0 equiv.) was added. The reaction was then stirred at ambient temperature overnight and terminated by addition of water. The quenched reaction was partitioned between 5 M NaOH and DCM. The aqueous layer was extracted with DCM, and combined organic layers were dried over Na_2SO_4 , filtered and concentrated under

reduced pressure. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 8/2), yielding 8.5 mg (16 %) of unreacted starting material **8**, 14.8 mg (28 %) of desired amine **13**, as a 9/1 mixture of *cis/trans*. A further fraction of 12 mg of a mixture of **13** with an unidentified secondary product was also isolated.

R_f (DCM/MeOH = 9/1) = 0.27; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.08-7.48 (10 H, m, Ar-H), 4.53 (0.1 H, d, H-6_{trans}, J = 11.7 Hz), 3.96 (0.9 H, d, H-6_{cis}, J = 11.7 Hz), 3.39-3.48 (1 H, m, H-2), 3.02-3.13 (1 H, m, H-1), 2.73-3.00 (3 H, m, H-5), 2.57-2.69 (1 H, m, H-5), 1.51-2.02 (5 H, m, H-3,4), 1.30-1.42 (1 H, m); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 143.5 and 143.0 (C-7 and C-7'), 129.1 and 128.9 (C-9 and C-9'), 128.6 and 128.1 (C-10 and C-10'), 126.9 and 126.4 (C-8 and C-8'), 69.6 (C-2), 55.6 (C-6), 54.1 (C-1), 49.9 and 40.8 (C-5), 30.3 (C-3), 27.3 and 19.7 (C-5); **MS**(ESI) m/z = 293.1 $[\text{M}+\text{H}]^+$; **HRMS**(ESI) m/z = 293.2021 $[\text{M}+\text{H}]^+$, calc.: 293.2012, Diff.: 2.9 ppm.

4.2.7. 2-Iodobenzaldehyde (**15**)

PCC (415 mg, 1.93 mmol, 1.2 equiv.) and celite (1.75 g) were dried under vacuum in 2-neck-flask and then flushed with nitrogen. After suspension in 19.3 mL of dry DCM, a solution of *o*-iodobenzyl alcohol (**14**) (390 mg, 1.60 mmol, 1.0 equiv.) in dry DCM (5.33 mL) was added drop wise at room temperature. The reaction mixture turns from red to brown and then black, and is stirred at ambient temperature overnight. After filtration through celite, the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (short column, eluent Hexanes/EtOAc = 9/1), yielding 367 mg (98 %) of aldehyde **15**.

R_f (Hexanes/EtOAc = 9/1) = 0.63; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 10.1 (1 H, s, H-7), 7.97 (1 H, dd, H-3, J = 8.0, 1.0 Hz), 7.87 (1 H, dd, H-6, J = 8.0, 1.8 Hz), 7.42-7.48 (1 H, m, H-4), 7.25-7.31 (1 H, m, H-5); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 195.9 (C-7), 140.8 (C-4), 135.6 (C-3), 135.3 (C-1), 130.4 (C-5), 128.9 (C-6), 100.9 (C-2).

4.2.8. 2-Benzylquinuclidin-3-one (**16**)

To a solution of (*Z*)-2-benzylidenequinuclidin-3-one **10** (1.11 g, 5.20 mmol, 1.0 equiv.) in 26 mL of MeOH in a Parr flask was added palladium on charcoal, 5 w% (111 mg, 0.1 g/1 g starting material).
455 The air was removed by flushing with H₂, then the reaction was shaken in a Parr apparatus for 6 h at room temperature under 25-30 psi of H₂. The reaction mixture was filtered through celite to remove the catalyst, and the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography (eluent Hexane/EtOAc = 8/2 to 7/3), yielding 991 g (88 %) of 2-benzylquinuclidin-3-one **16** as a white solid.

460 **R_f** (Hexane/EtOAc = 8/2) = 0.11; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.18-7.32 (5 H, m, Ar-H), 3.38 (1 H, dd, H-2, *J* = 10.8, 4.0 Hz), 3.07-3.28 (3 H, m, H-5a and H-6a), 2.75-2.93 (3 H, m, H-5b and H-6a), 2.47 (1 H, q, H-3, *J* = 3.0 Hz), 1.92-2.08 (4 H, m, H-4); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 221.3 (C-1), 139.2 (C-7), 128.9 (C-9), 128.6 (C-8), 126.5 (C-10), 71.7 (C-2), 49.1 (C-5), 41.2 (C-5), 40.2 (C-3), 33.9 (C-6), 26.9 (C-4), 25.3 (C-4); **MS**(ESI) *m/z* = 216.1 [M+H]⁺; **HRMS**(ESI) *m/z* =
465 216.1381 [M+H]⁺, calc.: 216.1383, Diff.: 0.8 ppm.

4.2.9. 2-Benzylquinuclidin-3-amine (**17**)

To a stirred solution of 2-benzylquinuclidin-3-one (**16**) (316 mg, 1.47 mmol, 1.0 equiv.) in dry methanol (2.1 mL), under nitrogen, was added a 1 M solution of ZnCl₂ in Et₂O (0.29 mL, 0.29mmol, 0.2 equiv.). After stirring at ambient temperature for 30 min, this mixture was treated with solid
470 ammonium formate (1.11 g, 17.6 mmol, 12 equiv.). After stirring another hour at ambient temperature, solid sodium cyanoborohydride (185 mg, 2.94 mmol, 2.0 equiv.) was added. The reaction was then stirred at ambient temperature overnight and terminated by addition of water. The quenched reaction was partitioned between 5 M NaOH and DCM. The aqueous layer was extracted with DCM, and combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure.

475 The crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 7/3 + 0.1% TEA), yielding 228.5 mg (71 %) of the desired amine **17**, as a *cis/trans* mixture.

R_f (DCM/MeOH = 9/1) = 0.16; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.15-7.35 (5 H, m, Ar-H), 3.12-3.25 (1 H, m, H-6), 2.80-3.01 (3 H, m, H-6, H-2 and H-1), 2.52-2.80 (3 H, m, H-5), 1.96-0.77 (6 H, m, H-4, H-3 and NH_2); MS(ESI) m/z = 218.2 $[\text{M}+2\text{H}]^+$.

480 4.2.10. *N*,2-Dibenzylquinuclidin-3-amine (**18**)

A solution of 2-benzylquinuclidin-3-one **16** (488 mg, 2.27 mmol, 1.0 equiv.), benzylamine (0.37 mL, 3.40 mmol, 1.5 equiv.) and *p*-TsOH (172 mg, 0.90 mmol, 0.04 equiv.) in 23 mL of PhMe was prepared in a round-bottom-flask fitted with a Dean-Stark distilling trap. The mixture was refluxed until the TLC showed the consumption of the starting material. The solvent was removed under reduced pressure. The residue was dried *in vacuo*, set under inert gas (N_2) and was dissolved in dry THF. 9-BBN ($c = 0.5$ M in THF, 9.08 mL, 4.54 mmol, 2.0 equiv.) was added and the mixture was stirred at room temperature for 24 h. The reaction was quenched with HCl_{aq} ($c = 1$ M) and the aqueous phase was washed with DCM. Then, the aqueous phase was adjusted to pH = 13-14 with NaOH_{aq} ($c = 1$ M) and extracted with DCM (3x). The organic phases were united, dried over Na_2SO_4 , filtered and the solvent was removed under reduced pressure. The crude residue (650 mg) was purified by HPLC (A: water + 0.1 % TFA, B: MeCN + 0.1 % TFA, $t = 20$ min, 10-95 % B), and two fractions were collected. In order to isolate the free amines, a basic work up was conducted with both fractions. The MeCN was removed under reduced pressure, the aqueous residue was dissolved in sat. $\text{NaHCO}_{3\text{aq}}$ and extracted with DCM (3x). The solvent was removed under reduced pressure and the residues were dried *in vacuo*, yielding two fractions of the diastereoisomers: 231 mg of pure *cis*-*N*,2-dibenzylquinuclidin-3-amine **18** as a white solid and 99 mg of **18(mix)**, a mixture of both diastereoisomers (**18(mix)**, *cis/trans* = 4/6) as a yellow oil. Total yields: *cis* = 39 %, *trans* = 9 %.

$R_f(\text{DCM/MeOH} = 9/1) = 0.15$.

cis-N,2-dibenzylquinuclidin-3-amine: $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.32-7.15 (8 H, m, Ar-H), 7.02-7.08 (2H, m, Ar-H), 3.60 (2 H, AB_q system, $\Delta d_{\text{AB}} = 0.28$, $J_{\text{AB}} = 12.7$ Hz, H-11), 3.36-3.40 (1 H, m, H-6a), 3.27-3.12 (2 H, m, H-2 and H-6b), 2.84-2.99 (4 H, m, H-5), 2.77 (1 H, bt, H-1, $J = 12.4$ Hz), 2.00-2.05 (1 H, m, H-3), 1.87-1.98 (1 H, m, H-4a), 1.64-1.76 (1 H, m, H-4b), 1.45-1.58 (1 H, m, H-4c), 1.29-1.41 (1 H, m, H-4d); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 140.5 (C-12), 140.1 (C-7), 128.8 (C-14), 128.7 (C-9), 128.4 (C-13), 128.0 (C-8), 127.0 (C-15), 126.2 (C-10), 60.4 (C-2), 55.5 (C-1), 52.4 (C-11), 49.4 (C-5), 42.2 (C-5), 34.0 (C-6), 25.4 (C-4), 24.8 (C-3), 19.7 (C-4); $\text{MS}(\text{ESI})$ $m/z = 307.2$ $[\text{M}+\text{H}]^+$; $\text{HRMS}(\text{ESI})$ $m/z = 307.2180$ $[\text{M}+\text{H}]^+$, calc.: 307.2169, Diff.: 3.8 ppm.

4.2.11. 2-Benzyl-N-(2-iodobenzyl)quinuclidin-3-amine (**19**)

To a stirred solution of 2-benzylquinuclidin-3-amine (**17**) (110 mg, 0.51 mmol, 1.0 equiv.) and 2-iodobenzaldehyde (**15**) (177 mg, 0.76 mmol, 1.5 equiv.) in dry DCM (12.8 mL), under nitrogen and at 0 °C, was added $\text{NaBH}(\text{OAc})_3$ (214 mg, 1.01 mmol, 2.0 equiv.). The reaction mixture was allowed to warm to room temperature, stirred for 3 h and quenched with water and saturated aqueous NaHCO_3 . The aqueous layer was extracted with DCM (3x), and combined the organic layers were dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography (eluent $\text{DCM/MeOH} = 95/5$ to $9/1$), yielding 29 mg of desired compound **19** and a 2nd fraction (29 mg) of **19** with its diastereoisomer (overall: yield 26 %, *cis/trans* = ~ 95/5).

$R_f(\text{DCM/MeOH} = 9/1) = 0.30$; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.88 (1 H, s, NH), 7.77-7.82 (2 H, m, Ar-H), 7.31 (1 H, t, Ar-H, $J = 7.2$ Hz), 7.18-7.22 (4 H, m, Ar-H), 7.02-7.11 (2 H, m, Ar-H), 3.07-3.39 (6 H, m, H-7, H-6 and H-5), 2.87-2.98 (1 H, m, H-1), 2.76 (1 H, dd, H-2, $J = 12.6$, 8.4 Hz), 2.19-2.29 (1 H, m, H-3), 1.68-1.82 (3 H, m, H-4), 1.44-1.55 (1 H, m, H-4); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 139.7 (C-14), 137.9 (C-7), 137.1 (C-12), 131.9 (C-17), 129.5 (C-9),

129.0 (C-15), 128.6 (C-8), 128.3 (C-16), 126.6 (C-10), 99.9 (C-13), 73.6 (C-2), 64.6 (C-1), 50.1 (C-11), 41.4 (C-6), 38.7 (C-5), 30.4 (C-3), 25.2 and 20.2 (C-5); **MS**(ESI) $m/z = 431.2[M+H]^+$; **HRMS**(ESI) $m/z = 431.0983 [M+H]^+$, calc.: 431.0979, Diff.: 1.1 ppm.

4.2.12. *tert*-Butyl 3-((2-iodobenzyl)amino)piperidine-1-carboxylate (**21**)

525 To a stirred solution of *tert*-butyl 3-aminopiperidine-1-carboxylate (**20**) (330 mg, 1.65 mmol, 1.0 equiv.) and 2-iodobenzaldehyde (**15**) (573 mg, 2.47 mmol, 1.5 equiv.) in dry DCM (41.3 mL), under nitrogen and at 0 °C, was added NaBH(OAc)₃ (1.05 g, 4.95 mmol, 2.0 equiv.). The reaction mixture was allowed to warm to room temperature, stirred for 5 h and quenched with water and saturated aqueous NaHCO₃. The aqueous layer was extracted with DCM (3x), and combined the
530 organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 98/2 to 95/5), yielding 637 mg (70 %) of desired compound **21** and a second fraction containing the unreduced imine (271 mg).

R_f (DCM/MeOH = 95/5) = 0.38; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.81 (1 H, d, H-12, $J = 8.0$ Hz), 7.39 (1 H, d, H-14, $J = 9.6$ Hz), 7.31 (1 H, t, H-13, $J = 7.6$ Hz), 6.95 (1 H, t, H-15, $J = 7.6$ Hz), 3.73-3.91 (3 H, m, H-1 and H-9), 2.55-2.99 (3 H, m, H-2, H-3 and NH), 1.89-1.98 (1 H, m, H-2), 1.65-1.75 (2 H, m, H-3 and H-4), 1.25-1.65 (13 H, m, H-8, H-3); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 155.1 (C-6), 142.5 (C-10), 139.7 (C-12), 129.9 (C-15), 129.0 (C-13), 128.5 (C-14), 99.7 (C-11), 79.6 (C-7), 55.7 (C-10), 49.3 (C-2), 44.2 (C-5), 31.7 (C-4), 28.6 (C-8), 22.8 (C-3); **MS**(ESI) $m/z = 417.0[M+H]^+$; **HRMS**(ESI) $m/z = 417.1039 [M+H]^+$, calc.: 417.1033, Diff.: 1.4 ppm.

540 4.2.13. *N*-(2-Iodobenzyl)-2,2-diphenylethanamine (**23**)

To a stirred solution of 2,2-diphenylethan-1-amine (**22**) (235 mg, 1.19 mmol, 1.0 equiv.) and 2-iodobenzaldehyde (**15**) (332 mg, 1.43 mmol, 1.2 equiv.) in dry DCM (30.0 mL), under nitrogen and

at 0 °C, was added NaBH(OAc)₃ (504 mg, 2.38 mmol, 2.0 equiv.). The reaction mixture was allowed to warm to room temperature, stirred for 5 h and quenched with water and saturated aqueous NaHCO₃.

545 The aqueous layer was extracted with DCM (3x), and combined the organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 99/1 to 98/2), yielding 392 mg (80 %) of desired compound **23**.

R_f (DCM/MeOH = 95/5) = 0.38; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.80 (1 H, d, H-12, *J* = 7.6 Hz), 7.17-7.35 (12 H, m, Ar-H), 6.90-6.98 (1 H, m, H-15), 4.25 (1 H, t, H-2, *J* = 7.6 Hz), 3.84 (2 H, s, H-7), 3.26 (2 H, d, H-1, *J* = 7.6 Hz), 1.73 (2 H, bs, NH); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 142.9 (C-3), 142.0 (C-8), 139.5 (C-10), 129.9 (C-13), 128.9 (C-11), 128.8 (C-4), 128.4 (C-12), 128.2 (C-5), 126.7 (C-6), 99.7 (C-9), 58.3 (C-1), 53.8 (C-7), 51.4 (C-2); **MS**(ESI) *m/z* = 414.0[M+H]⁺; **HRMS**(ESI) *m/z* = 414.0712 [M+H]⁺, calc.: 414.0713, Diff.: 0.4 ppm.

555 4.2.14. 4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylbutanamide (**24a**)

Diphenylbutanenitrile **25a** (640 mg, 1.48 mmol, 1.0 equiv.) was dissolved in 4.90 mL *t*-BuOH and potassium hydroxide (292 mg, 5.20 mmol, 3.5 equiv.) was added. The reaction mixture was stirred at reflux for 4 d. After concentration under reduced pressure, the crude residue was purified by flash chromatography (eluent DCM/MeOH = 98/2 to 9/1 + 0.1 %TEA), yielding 298 mg (44 %) of 4-(4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylbutanamide (**24a**) as a white solid. A second
560 fraction with remaining starting material was also isolated (200 mg, 31 %).

R_f (DCM/MeOH = 8/2) = 0.40; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.38-7.44 (2 H, m, Ar-H), 7.22-7.38 (12 H, m, Ar-H), 6.44 (1 H, bs, NH), 5.29 (1 H, bs, NH), 2.78-2.96, 2.63-2.77, 2.34-2.60 (2 H, 2H, 4H, br m x3, H-2, H-3 and H-4), 1.85-2.29 (3 H, m, H-5a, OH), 1.72 (2 H, d, H-5b, *J* =
565 13.0 Hz); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 176.69 (C-15), 143.21 (C-11), 133.03 (C-7),

128.79, 128.70, 128.61, 128.58, 127.30, 126.25 (C8-14), 70.77 (C-6), 59.95 (C-1), 55.09 (C-3), 49.58 (C-4), 38.00, 35.59 (C-2, C-5). The analytical data matched the published data [44].

4.2.15. 4-(4-Hydroxy-1-piperidyl)-2,2-diphenyl-butanamide (**24c**)

Compound **24c** was obtained using the same conditions as for **24a**, employing diphenylbutyronitrile
570 **25c** (146 mg, 0.46 mmol, 1.0 equiv.), potassium hydroxide (90 mg, 1.60 mmol, 3.5 equiv.) and refluxing for 7 d. The crude was purified by flash chromatography (eluent DCM/MeOH = 99/1 to 9/1, SiO₂ pretreated with 1 % TEA) yielding 23 mg (15 %) of amide **24c** as a light-yellow oil with triethylamine as impurity.

R_f (DCM/MeOH = 9/1) = 0.22; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.15-7.40 (10 H, m, Ar-H),
575 6.94 (1 H, bs, NH), 5.70 (1 H, bs, NH), 3.62-3.73 (2 H, m, OH and H-6), 3.45 (2 H, s, H-3), 2.65 (2 H, t, H-4a, *J* = 6.8 Hz), 2.32 (2 H, t, H-4b, *J* = 6.8 Hz), 2.18-2.29 (2 H, m, H-2), 1.85-1.96 (2 H, m, H-5a), 1.51-1.64 (2 H, m, H-5b); **MS**(ESI) *m/z* = 499.0 [M+H]⁺; **HRMS**(ESI) *m/z* = 339.2076 [M+H]⁺, calc.: 339.2067, Diff.: 2.8 ppm.

4.2.16. 2,2-diphenyl-4-(1-piperidyl)butanamide (**24d**)

580 Compound **24d** was obtained using the same conditions as for **24a**, employing diphenylbutyronitrile **25d** (250 mg, 0.82 mmol, 1.0 equiv.), potassium hydroxide (322 mg, 5.74 mmol, 7.0 equiv.) and refluxing for 4 d. The crude was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 8/2 + 0.1 %TEA), yielding 190 mg (72 %) of amide **24d** as a white solid as well as a second fraction of remaining starting material **25d** (53 mg, 21 %).

585 **R_f** (DCM/MeOH = 8/2) = 0.33; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.79 (1 H, bs, NH), 7.15-7.35 (10 H, m, Ar-H), 5.55 (1 H, bs, NH), 2.62 (2 H, t, *J* = 6.0 Hz, H-3), 2.30-2.50 (4 H, m, H-4), 2.24 (2 H, t, *J* = 6.0 Hz, H-2), 1.48-1.60 (4 H, m, H-5), 1.30-1.48 (2 H, m, H-5); **¹³C NMR** (101 MHz,

CDCl_3) δ (ppm) = 176.9 (C-15), 144.0 (C-11), 128.9 (C-13), 128.3 (C-12), 60.5 (C-1), 55.5 (C-3), 54.8 (C-4), 36.0 (C-2), 26.0 (C-5), 24.3 (C-6); **MS**(ESI) m/z = 324.20 $[\text{M}+2\text{H}]^+$ and 323.20 $[\text{M}+\text{H}]^+$.

590 4.2.17. 4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylbutanenitrile (**25a**)

Under inert conditions, 4-(4-Chlorophenyl)-4-hydroxypiperidine (**27a**) (1.78 g, 8.41 mmol, 1.0 equiv.) was suspended in 28 mL MeCN and DIPEA (4.30 mL, 23.2 mmol, 3.0 equiv.) was added. 4-bromo-2,2-diphenylbutyronitrile (**26**) (2.52 g, 8.41 mmol, 1.0 equiv.) was then added and the reaction mixture was stirred at reflux for 40 h. After concentration under reduced pressure, the crude
595 residue was purified by flash chromatography (eluent DCM/MeOH = 98/2 to 9/1), yielding 2.78 g (77 %) of diphenylbutanenitrile (**25a**) as a white solid.

R_f (DCM/MeOH = 9/1) = 0.38; **^1H NMR** (400 MHz, CDCl_3) δ (ppm) = 7.28-7.46 (14 H, m, Ar-H), 2.40-2.90 (8 H, m, H-2, H-3 and H-4), 2.00-2.21 (2 H, m, H-5a), 1.71 (2 H, d, H-5b, J = 12.9 Hz), 1.57 (1 H, bs, OH). The analytical data matched the published data [44].

600 4.2.18. *tert*-Butyl (1-(3-cyano-3,3-diphenylpropyl)piperidin-4-yl)carbamate (**25b**)

Compound **25b** was obtained using the same conditions as for **25a**, employing *tert*-butyl piperidin-4-ylcarbamate (**27b**) (1.08 g, 3.60 mmol, 1.0 equiv.), DIPEA (1.84 mL, 10.8 mmol, 3.0 equiv.) and 4-bromo-2,2-diphenylbutyronitrile (**26**) (721 mg, 3.60 mmol, 1.0 equiv.). The crude was purified by flash chromatography (eluent DCM/MeOH = 97/3 to 9/1), yielding 1.12 g (74 %) of
605 diphenylbutanenitrile (**25b**) as a light-yellow solid.

R_f (DCM/MeOH = 9/1) = 0.55; **^1H NMR** (400 MHz, CDCl_3) δ (ppm) = 7.23-7.42 (10 H, m, Ar-H), 4.43 (1 H, bs, NH), 3.45 (1 H, bs, H-6), 2.72-2.89 (2 H, m, H-2), 2.55-2.68 (2 H, m, H-3), 2.39-2.51 (2 H, m, H-4a), 2.01-2.18 (2 H, m, H-4b), 1.84-1.99 (2 H, m, H-5a), 1.46 (11 H, bs, H-9 and H-5b); **^{13}C NMR** (101 MHz, CDCl_3) δ (ppm) = 155.3 (C-7), 140.1 (C-10), 129.1 (C-11), 128.1 (C-13), 126.9

610 (C-12), 122.1 (C-10), 79.5 (C-8), 54.8 (C-4), 52.7 (C-3), 50.2 (C-1), 47.6 (C-6), 36.9 (C-2), 32.4 (C-5), 28.6 (C-9); **MS**(ESI) $m/z = 420.1 [M+H]^+$; **HRMS**(ESI) $m/z = 420.2661 [M+H]^+$, calc.: 420.2646, Diff.: 3.6 ppm.

4.2.19. 4-(4-Hydroxy-1-piperidyl)-2,2-diphenyl-butanenitrile (**25c**)

Compound **25c** was obtained using the same conditions as for **25a**, employing piperidin-4-ol (**27c**)
615 (331 mg, 3.27 mmol, 1.0 equiv.), DIPEA (1.67 mL, 9.81 mmol, 3.0 equiv.) and 4-bromo-2,2-diphenylbutyronitrile (**26**) (983 mg, 3.27 mmol, 1.0 equiv.). The crude was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 9/1), yielding 904 mg (86 %) of diphenylbutanenitrile **25c** as a light-yellow solid.

R_f(DCM/MeOH = 9/1) = 0.39; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.18-7.46 (10 H, m, Ar-H),
620 3.75 (1 H, bs, H-6), 2.78-2.90 (2 H, m, H-2), 2.62-2.78 (2 H, m, H-3), 2.45-2.62 (2 H, m, H-4a), 2.18-2.42 (2 H, m, H-4b), 1.87-2.05 (2 H, m, H-5a), 1.52-1.59 (2 H, m, H-5b); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 139.8 (C-11), 129.1 (C-12), 128.1 (C-14), 126.9 (C-13), 122.0 (C-15), 66.8 (C-6), 54.7 (C-4), 50.9 (C-3), 50.2 (C-1), 36.4 (C-2), 33.7 (C-5); **MS**(ESI) $m/z = 321.0 [M+H]^+$; **HRMS**(ESI) $m/z = 321.1974 [M+H]^+$, calc.: 321.1961, Diff.: 4.0 ppm.

625 4.2.20. 2,2-Diphenyl-4-(1-piperidyl)butanenitrile (**25d**)

Compound **25d** was obtained using the same conditions as for **25a**, employing piperidine (**27d**)
(340 mg, 4.00 mmol, 1.0 equiv.), DIPEA (2.04 mL, 12.0 mmol, 3.0 equiv.) and 4-bromo-2,2-diphenylbutyronitrile (**26**) (1.20 g, 4.00 mmol, 1.0 equiv.). The crude was purified by flash chromatography (eluent DCM/MeOH = 95/5), yielding 1.14 g (94 %) diphenylbutanenitrile **R12** as a
630 yellow oil.

R_f (DCM/MeOH = 95/5) = 0.42; ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 7.20-7.45 (10 H, m, Ar-H), 2.58-2.67 (2 H, m, H-2), 2.22-2.48 (6 H, m, H-3 and H-4), 1.48-1.62 (4 H, m, H-5), 1.33-1.47 (2 H, m, H-6); ^{13}C NMR (101 MHz, CDCl_3) δ (ppm) = 140.3 (C-7), 129.0 (C-8), 128.0 (C-10), 126.9 (C-9), 122.2 (C-11), 55.6 (C-3), 55.0 (C-4), 50.2 (C-1), 36.8 (C-2), 26.1 (C-5), 24.4 (C-6); MS(ESI) m/z = 305.3 $[\text{M}+\text{H}]^+$; HRMS(ESI) m/z = 305.2022 $[\text{M}+\text{H}]^+$, calc.: 305.2012, Diff.: 3.2 ppm.

4.2.21. 1-(4-Amino-3,3-diphenylbutyl)-4-(4-chlorophenyl)piperidin-4-ol (**30a**)

Under inert conditions, diphenylbutanenitrile **25a** (364 mg, 0.84 mmol, 1.0 equiv.) was dissolved in 2.80 mL dry THF and LiAlH_4 (128 mg, 3.38 mmol, 4.0 equiv.) was added. The reaction mixture was stirred at room temperature for 24 h, then quenched with NaOH_{aq} ($c = 1 \text{ M}$). Then, the aqueous phase was extracted with DCM (3x). The organic phases were united, dried over Na_2SO_4 , filtered and the solvent was removed under reduced pressure. The crude residue was purified by elution through a patch (height 6 cm) of silica gel (eluent DCM/MeOH = 9/1 to 9/1 + 0.1 %TEA), yielding 251 mg (68 %) of 1-(4-amino-3,3-diphenylbutyl)-4-(4-chlorophenyl)piperidin-4-ol (**30a**) as a white solid.

R_f (DCM/MeOH = 9/1 + 0.1 %TEA) = 0.25; ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 7.15-7.45 (14 H, m, Ar-H), 3.35 (2 H, s, H-15), 2.97 (3 H, bs, OH and H-4a), 2.85-2.98 (2 H, m, H-4a), 2.40-2.60 (4 H, m, H-3 and H-4b), 2.20-2.36 (4 H, m, H-3 and H-5a), 1.71 (2 H, d, H-5b, $J = 12.4 \text{ Hz}$); ^{13}C NMR (101 MHz, CDCl_3) δ (ppm) = 146.4 (C-7), 146.0 (C-11), 128.6 (C-14), 128.4 (C-12), 128.2 (C-13), 126.5 (C-8), 126.3 (C-9), 70.8 (C-6), 54.1 (C-3), 51.0 (C-1), 49.5 (C-4), 49.0 (C-15), 45.6 (C-2), 37.7 (C-5); MS(ESI) m/z = 435.1 $[\text{M}+\text{H}]^+$; HRMS (ESI) m/z = 435.2195 $[\text{M}+\text{H}]^+$, calc.: 435.2198, Diff.: 0.7 ppm.

4.2.22. 1-(4-Amino-3,3-diphenyl-butyl)piperidin-4-ol (**30c**)

Compound **30c** was obtained using the same conditions as for **30a**, employing diphenylbutanenitrile **25c** (310 mg, 0.97 mmol, 1.0 equiv.) and LiAlH_4 (147 mg, 3.87 mmol, 4.0 equiv.) and was stirred at

room temperature for 40 h. The crude compound was purified by flash chromatography (eluent
655 DCM/MeOH = 9/1 to 8/2 + 0.1 %TEA), yielding 58 mg (19 %) of amine **30c** as a colorless oil.

R_f (DCM/MeOH = 9/1) = 0.10; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.40-7.51 (4 H, m, Ar-H),
7.23-7.36 (4 H, m, Ar-H), 7.12-7.22 (2 H, m, Ar-H), 3.68 (2 H, bs, 6), 2.67-2.82 (2 H, m, H-4a), 2.43
(4 H, bs, H-15 and H-4b), 2.00-2.22 (2 H, m, H-3), 1.82-1.95 (4 H, m, H-5), 1.51-1.68 (2 H, m,
H-2); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 148.0 (C-11), 128.2 (C-13), 126.5 (C-14), 125.9 (C-12),
660 79.0 (C-1), 67.2 (C-6), 54.8 (C-4), 50.9 (C-3), 45.9 (C-15), 35.5 (C-5), 34.4 (C-2).

4.2.23. 2,2-Diphenyl-4-(1-piperidyl)butan-1-amine (**30d**)

Compound **30d** was obtained using the same conditions as for **30a**, employing diphenylbutanenitrile
25d (303 mg, 1.00 mmol, 1.0 equiv.) and LiAlH₄ (151 mg, 3.98 mmol, 4.0 equiv.) and was stirred at
room temperature for 20 h. The crude compound was purified by flash chromatography (eluent
665 DCM/MeOH = 97/3 to 8/2 + 0.1 %TEA), yielding three fractions: 90 mg (29 %) of amine **30d** as a
colorless oil, 74 mg (25 %) of starting material **25d** as well as 129 mg of a secondary, unidentified
product.

¹H NMR (400 MHz, CDCl₃) δ (ppm) = 7.22-7.32 (4 H, m, Ar-H), 7.05-7.22 (6 H, m, Ar-H), 3.33
(2 H, s, H-15), 2.20-2.48 (6 H, m, NH₂ and H-4), 2.00-2.05 (2 H, m, H-3), 1.50-1.68 (6 H, m, H-5 and
670 H-2), 1.35-1.45 (2 H, m, H-6); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 146.6 (C-11), 128.4 (C-13),
128.3 (C-12), 126.3 (C-14), 55.1 (C-4), 51.3 (C-1), 49.3 (C-3), 33.1 (C-15), 26.1 (C-5), 24.5 (C-6);
MS(ESI) m/z = 309.2 [M+H]⁺; **HRMS**(ESI) m/z = 309.2321 [M+H]⁺, calc.: 309.2325, Diff.: 1.3 ppm.

4.2.24. 1-(4-Amino-3,3-diphenylbutyl)piperidin-4-amine (**30e**)

Under inert conditions, 4-(4-aminopiperidin-1-yl)-2,2-diphenylbutanenitrile (**25e**) (178 mg,
675 0.56 mmol, 1.0 equiv.) was dissolved in 1.90 mL dry THF and LiAlH₄ (85.0 mg, 2.23 mmol,

4.0 equiv.) was added. The reaction mixture was stirred at room temperature for 24 h, then quenched with NaOH_{aq.} (c = 1 M). Then, the aqueous phase was extracted with DCM (3x). The organic phases were united, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude residue was purified by elution through a patch (height 6 cm) of silica gel (eluent DCM/MeOH = 9/1 to 8/2 + 0.1 %TEA), yielding 99 mg (55 %) of 1-(4-amino-3,3-diphenylbutyl)piperidin-4-amine (**30e**) as a colorless oil.

R_f (DCM/MeOH = 8/2) = 0.15; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.20-7.40 (10 H, m, Ar-H), 4.00-4.10 (1 H, m, H-6), 2.85-3.00 (2 H, m, H-7), 2.67-2.78 (2 H, m, H-3), 2.36 (4 H, s, H-4), 2.05 (2 H, dt, H-2, *J* = 12.8, 1.2 Hz), 1.89 (2 H, d, H-5a, *J* = 12.8 Hz), 1.35-1.60 (4 H, m, NH₂ and H-5b); **¹³C NMR** (101 MHz, CDCl₃) δ(ppm) = 145.0 (C-7), 128.5 (C-8), 128.0 (C-9), 126.2 (C-10), 57.0 (C-4), 52.7 (C-3), 49.4 (C-11), 48.9 (C-6), 46.3 (C-1), 36.2 (C-5), 33.2 (C-2).

4.2.25. 4-(4-(4-Fluorophenyl)-piperidin-1-yl)-2,2-diphenylbutanenitrile (**32**)

Compound **32** was obtained using the same conditions as for **25a**, employing 1-(4-fluorophenyl)piperazine (**31**) (580 mg, 3.22 mmol, 1.0 equiv.), DIPEA (1.64 mL, 9.66 mmol, 3.0 equiv.) and 4-bromo-2,2-diphenylbutyronitrile (**26**) (966 mg, 3.22 mmol, 1.0 equiv.). The crude was purified by flash chromatography (eluent DCM/MeOH = 99/1 to 9/1), yielding 904 mg (74 %) of diphenylbutanenitrile **32** as a light-yellow oil.

R_f (DCM/MeOH = 9/1) = 0.44; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.28-7.45 (10 H, m, Ar-H), 6.90-7.00 (2 H, m, H-9), 6.82-6.89 (2 H, m, H-10), 3.05-3.15 (4 H, m, H-5), 2.48-2.70 (8 H, m, H-2,3,4); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 157.3 (d, C-10, *J*_{C,F} = 235 Hz), 148.1 (C-7), 140.1 (C-11), 129.1 (C-13), 128.1 (C-14), 126.9 (C-12), 122.2 (C-15), 117.9 (d, C-8, *J*_{C,F} = 7.7 Hz), 115.6 (d, C-9, *J*_{C,F} = 21.8 Hz), 54.8 (C-3), 53.5 (C-4), 50.2 (C-5), 50.2 (C-1), 36.8 (C-2); **MS**(ESI) *m/z* = 400.3 [M+H]⁺; **HRMS**(ESI) *m/z* = 400.2196 [M+H]⁺, calc.: 400.2184, Diff.: 3.0 ppm.

4.2.26. 4-[4-(4-Fluorophenyl)-1-piperidyl]-2,2-diphenyl-butanamide (**33**)

700 Compound **33** was obtained using the same conditions as for **24a**, employing diphenylbutyronitrile **32** (650 mg, 1.63 mmol, 1.0 equiv.), potassium hydroxide (320 mg, 5.70 mmol, 3.5 equiv.) and refluxing for 4 d. The crude was purified by flash chromatography (eluent DCM/MeOH = 9/1 to 8/2), yielding 550 mg (80 %) of amide **33** as an orange solid.

R_f (DCM/MeOH = 8/2) = 0.48; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.30-7.40 (8 H, m, Ar-H),
705 7.25-7.30 (2 H, m, Ar-H), 6.90-6.99 (2 H, m, H-9), 6.80-6.89 (2 H, m, H-8), 6.42 (1 H, bs, NH), 5.57 (1 H, bs, NH), 3.03-3.17 (4 H, m, H-5), 2.64-2.80 (2 H, m, H-3), 2.50-2.64 (4 H, m, H-4), 2.28-2.43 (2 H, m, H-2); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 176.6 (C-15), 157.3 (d, C-10, $J_{\text{C,F}}$ = 235 Hz), 148.0 (C-7), 143.4 (C-11), 128.8 (C-13), 126.5 (C-12), 127.2 (C-14), 117.9 (d, C-8, $J_{\text{C,F}}$ = 8.3 Hz), 115.6 (d, C-9, $J_{\text{C,F}}$ = 22.8 Hz), 60.1 (C-1), 55.0 (C-3), 53.4 (C-4), 50.2 (C-5), 36.0 (C-2); **MS**(ESI) m/z
710 = 418.2 $[\text{M}+\text{H}]^+$; **HRMS**(ESI) m/z = 418.2308 $[\text{M}+\text{H}]^+$, calc.: 418.2289, Diff.: 4.5 ppm.

4.2.27. 4-[4-(4-Fluorophenyl)-1-piperidyl]-2,2-diphenyl-butan-1-amine (**34**)

Compound **34** was obtained using the same conditions as for **30a**, employing diphenylbutanenitrile **32** (274 mg, 0.69 mmol, 1.0 equiv.) and LiAlH_4 (104 mg, 2.74 mmol, 4.0 equiv.) and was stirred at room temperature for 20 h. The crude compound was purified by flash
715 chromatography (eluent DCM/MeOH = 95/5 to 8/2), yielding 17 mg (6 %) of amine **34** as a yellow oil and a second fraction of 153 mg (56 %) of starting material **32**. In a second attempt, employing a longer reaction time of 3 d, the reaction didn't evolve further than the same ratio of starting material **32** and product **34**.

R_f (DCM/MeOH = 8/2) = 0.15; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.15-7.33 (10 H, m, Ar-H),
720 6.88-6.99 (2 H, m, H-9), 6.79-6.88 (2 H, m, H-8), 3.75 (4 H, bs, NH), 3.42 (2 H, bs, H-15), 3.10-3.19 (4 H, m, H-4), 2.59-2.71 (4 H, m, H-5), 2.47-2.53 (2 H, m, H-2), 2.25-2.32 (2 H, m, H-2); $^{13}\text{C NMR}$

(101 MHz, CDCl₃) δ (ppm) = 157.5 (d, C-10, $J_{C,F}$ = 244 Hz), 147.7 (C-7), 145.4 (C-11), 128.6 (C-13), 128.0 (C-12), 126.7 (C-14), 118.3 (d, C-8, $J_{C,F}$ = 7.6 Hz), 115.7 (d, C-9, $J_{C,F}$ = 22.9 Hz), 146.4 (C-7), 146.0 (C-11), 60.5 (C-1), 55.0 (C-3), 54.1 (C-15), 53.3 (C-4), 49.8 (C-5), 33.0 (C-2); **MS**(ESI) m/z = 404.1 [M+H]⁺; **HRMS**(ESI) m/z = 404.2511 [M+H]⁺, calc.: 404.2497, Diff.: 3.5 ppm.

4.2.28. Dibenzo[b,f]thiepin-10(11H)-one (**38a**)

KOH (722 mg, 12.9 mmol, 3.3 equiv.) was dissolved in 7.8 mL of water, benzenethiol (**35a**) (0.40 mL, 3.90 mmol, 1.0 equiv.) was added and the mixture was heated to 50 °C. Copper powder (74 mg, 1.17 mmol, 0.3 equiv.) and 2-(2-iodophenyl)acetic acid (**36**) (980 mg, 3.74 mmol, 0.96 equiv.) was added. The reaction mixture was stirred at reflux for 24 h, then cooled to room temperature. The yellow precipitate was filtered off, and the filtrate was acidified with HCl_{aq.} (1 M). The aqueous phase was extracted with EtOAc (5x). The organic phases were united, washed with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. Thus were obtained 1.20 g (100 % plus impurities) of 2-(2-(phenylthio)phenyl)acetic acid (**37a**) as a yellow solid; the crude was used in the next step without further purification.

4 g of oily P₂O₅ was heated to 150 °C under stirring. The crude 2-(2-(phenylthio)phenyl)acetic acid (**37a**) was added in little portions and the mixture was stirred at 150 °C for 4 h, then cooled to room temperature. Ice and water were carefully added, and the resulting aqueous solution was extracted with EtOAc (5x). The organic phases were united, washed with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (eluent Hexane/EtOAc = 97/3 to 9/1), yielding 403 mg (46 %) of dibenzo[b,f]thiepin-10(11H)-one (**38a**) as a green-yellow solid.

R_f (Hexane/EtOAc = 8/2) = 0.15; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 8.20 (1 H, dd, H-13, J = 8.0 Hz, J = 1.7 Hz), 7.65 (1 H, dd, H-7, J = 7.7 Hz, J = 1.2 Hz), 7.61 (1 H, dd, H-11, J = 7.8 Hz, J =

745 1.1 Hz), 7.28-7.49 (4 H, m, H-10, H-6, H-12 and H-4), 7.20 (1 H, dt, H-5, $J = 7.6$ Hz, $J = 1.4$ Hz), 4.38 (2 H, s, H-2); ^{13}C NMR (101 MHz, CDCl_3) δ (ppm) = 191.5 (C-1), 140.4 (C-3), 137.8 (C-8), 136.3 (C-9), 134.7 (C-14), 132.6 (C-11), 131.7 (C-6), 131.4 (C-4), 131.0 (C-13), 130.1 (C-5), 129.6 (C-7), 127.3 (C-12), 127.3 (C-10), 51.2 (C-2).

4.2.29. 8-Chlorodibenzo[b,f]thiepin-10(11H)-one (**38b**)

750 Compound **38b** was obtained using the same conditions as for **38a**, employing KOH (1.29 g, 23.0 mmol, 3.3 equiv.), 4-chlorobenzenethiol (**35b**) (1.01 g, 6.96 mmol, 1.0 equiv.). Copper powder (133 mg, 2.09 mmol, 0.3 equiv.) and 2-(2-iodophenyl)acetic acid (**36**) (1.95 g, 6.68 mmol, 0.96 equiv.) Thus were obtained 2.14 g (100 % plus impurities) of 2-(2-((4-chlorophenyl)thio)phenyl)acetic acid (**37b**) as a light yellow solid; the crude was used in the next step without further purification.

755 In the second step, 4 g of oily P_2O_5 was heated with ((4-chlorophenyl)thio)phenyl)acetic acid (**37b**) (750 mg, 2.69 mmol, 1.0 equiv.) The crude residue was purified by flash chromatography (eluent Hexane/EtOAc= 95/5 to 9/1), yielding 541 mg (50 %) of 8-chlorodibenzo[b,f]thiepin-10(11H)-one (**38b**) as a light-yellow oil.

R_f (Hexane/EtOAc= 8/2) = 0.15; ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 8.17 (1 H, d, H-13, $J =$
760 2.7 Hz), 7.63 (1 H, d, H-7, $J = 8.0$ Hz), 7.54 (1 H, d, H-11, $J = 8.4$ Hz), 7.45 (1 H, d, H-10, $J =$
7.7 Hz), 7.36-7.40 (2 H, m, H-6 and H-4), 7.21 (1 H, dt, H-5, $J = 7.6$ Hz, $J = 1.5$ Hz), 4.36 (2 H, s, H-2); ^{13}C NMR (101 MHz, CDCl_3) δ (ppm) = 191.2 (C-1), 138.3 (C-3), 137.5 (C-8), 136.5 (C-9), 136.4 (C-12), 134.7 (C-14), 131.5 (C-11), 131.3 (C-6), 130.6 (C-4), 130.0 (C-13), 129.5 (C-5), 128.3 (C-7), 127.3 (C-10), 51.2 (C-2), 15.7 (C-15). The analytical data matched the published data [30].

765 4.2.30. 8-(Methylthio)dibenzo[b,f]thiepin-10(11H)-one (**38c**)

Compound **38c** was obtained using the same conditions as for **38a**, employing KOH (539 mg, 9.60 mmol, 3.3 equiv.), 4-(methylthio)benzenethiol (**35c**) (454 mg, 2.91 mmol, 1.0 equiv.). Copper powder (56 mg, 0.87 mmol, 0.3 equiv.) and 2-(2-iodophenyl)acetic acid (**36**) (732 mg, 2.79 mmol, 0.96 equiv.) Thus were obtained 1.40 g (100 % plus impurities) of 2-(2-(phenylthio)phenyl)acetic acid
770 (**37c**) as a light yellow solid; the crude was used in the next step without further purification.

In the second step, 6 g of oily P₂O₅ were heated with **37c**. The crude residue was purified by flash chromatography (eluent Hexane/EtOAc= 95/5 to 8/2), yielding 94 mg (12 %) of 8-(methylthio)dibenzo[b,f]thiepin-10(11H)-one (**38c**) as a light-yellow oil.

R_f (Hexane/EtOAc = 9/1) = 0.42; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 8.04 (1 H, d, H-13, *J* =
775 2.3 Hz), 7.62 (1 H, d, H-7, *J* = 7.8 Hz), 7.49 (1 H, d, H-11, *J* = 8.4 Hz), 7.43 (1 H, d, H-10, *J* =
7.2 Hz), 7.35 (1 H, td, H-6, *J* = 7.6 Hz, *J* = 1.3 Hz), 7.28 (1 H, dd, H-4, *J* = 8.2 Hz, *J* = 2.4 Hz), 7.19
(1 H, dt, H-5, *J* = 7.6 Hz, *J* = 1.4 Hz), 4.36 (2 H, s, H-2), 2.47 (3 H, s, H-15); **MS**(ESI) *m/z* = 273.1
[*M*+*H*]⁺; **HRMS**(ESI) *m/z* = 271.0270 [*M*+*H*]⁺, calc.: 271.0257, Diff.: 4.9 ppm.

4.2.31. 1-(10,11-Dihydrodibenzo[b,f]thiepin-10-yl)-4-methylpiperazine (**41a**)

780 Under nitrogen, **38a** (132 mg, 0.58 mmol, 1.0 equiv.) was dissolved in 5.8 mL of toluene.
1-Methylpiperazine (**39a**) (0.31 mL, 2.86 mmol, 4.9 equiv.) was added, then Ti(*i*-OPr)₄ (0.13 mL,
0.44 mmol, 0.75 equiv.) and the mixture was refluxed for 20 h, then cooled to room temperature. Ice
and water were carefully added, and the resulting aqueous solution was extracted with EtOAc (5x).
The organic phases were united, washed with brine, dried over Na₂SO₄, filtered and the solvent was
785 removed under reduced pressure. Thus was obtained 200 mg (100 % plus impurities) of the
intermediate enamine **40a** as a yellow oil; the crude was used in the next step without further
purification.

The crude intermediate **40a** was dissolved in 4.1 mL of glacial acetic acid and NaBH₄ (329 mg, 8.70 mmol, 15 equiv.) was added in portions. The mixture was stirred at room temperature overnight, then EtOAc was added. The pH of the mixture was adjusted to 6-7 using first NaOH pellets and then NaHCO₃, and the resulting aqueous solution was extracted with EtOAc (3x). The organic phases were united, washed with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 9/1), yielding 99 mg (55 %) of 1-(10,11-dihydrodibenzo[b,f]thiepin-10-yl)-4-methylpiperazine (**41a**) as an orange solid.

R_f (DCM/MeOH = 9/1) = 0.31; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.63 (1 H, d, H-6, *J* = 7.8 Hz), 7.51 (1 H, d, H-11, *J* = 7.8 Hz), 7.41 (1 H, dd, H-12, *J* = 7.8 Hz, *J* = 1.4 Hz), 7.18-7.30 (2 H, m, H-4 and H-5), 7.02-7.18 (3 H, m, H-7, H-10 and H-13), 4.01 (1 H, dd, *J* = 11.2, 3.6 Hz, H-1), 3.89 (1 H, dd, *J* = 13.2, 11.6 Hz, H-2a), 3.18 (1 H, dd, *J* = 12.8, 3.6 Hz, H-2b), 2.62-2.80 (4 H, m, H-15), 2.28-2.62 (4 H, m, H-16), 2.31 (3 H, s, H-17); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 142.6 (C-9), 140.3 (C-8), 137.0 (C-3), 135.3 (C-14), 132.6 (C-11), 131.6 (C-6), 131.3 (C-4), 129.7 (C-13), 128.7 (C-5), 127.1 (C-7), 126.9 (C-12), 126.4 (C-10), 65.8 (C-1), 55.6 (C-15), 48.3 (C-16), 45.9 (C-17), 33.4 (C-2); **MS**(ESI) *m/z* = 311.0 [M+H]⁺; **HRMS**(ESI) *m/z* = 311.1581 [M+H]⁺, calc.: 311.1576, Diff.: 1.4 ppm.

4.2.32. *tert*-Butyl 4-(10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine-1-carboxylate (**41c**)

Compound **41c** was obtained using the same conditions as for **41a**, employing **28a** (180 mg, 0.80 mmol, 1.0 equiv.), 1-Boc-piperazine (**39b**) (726 mg, 3.90 mmol, 4.9 equiv.) and Ti(*i*-OPr)₄ (0.18 mL, 0.60 mmol, 0.75 equiv.) and the mixture was refluxed for 40 h. Thus were obtained 800 mg (100% plus impurities) of the intermediate enamine **40c** as a yellow oil; the crude was used in the next step without further purification.

In the second step, the intermediate enamine **40c** and NaBH₄ (451 mg, 11.9 mmol, 15 equiv.) were stirred in 5.7 mL of glacial acetic acid. The crude residue was purified by flash chromatography (Hexane/EtOAc = 9/1 to 8/2), yielding 236 mg (74 %) of tert-butyl 4-(10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine-1-carboxylate (**41c**) as a light-yellow oil.

815 **R_f** (Hexane/EtOAc = 8/2) = 0.43; **¹H NMR** (400 MHz, CDCl₃) δ (ppm) = 7.62 (1 H, d, H-6, *J* = 8.0 Hz), 7.51 (1 H, d, H-11, *J* = 8.0 Hz), 7.41 (1 H, d, H-12, *J* = 8.0 Hz), 6.45-7.38 (5 H, m, H-4, H-5, H-7, H-10, H-13), 3.97-4.07 (1 H, m, H-1), 3.70-3.95 (1 H, m, H-2a), 3.34-3.50 (4 H, m, H-16), 3.05-3.20 (1 H, m, H-2b), 2.38-2.75 (4 H, m, H-15), 1.45 (9 H, s, H-19); **¹³C NMR** (101 MHz, CDCl₃) δ (ppm) = 155.0 (C-17), 142.4 (C-9), 140.0 (C-8), 137.1 (C-3), 135.4 (C-14), 132.8 (C-11), 131.7 (C-6),
820 131.4 (C-4), 129.6 (C-13), 128.7 (C-5), 127.2 (C-7), 126.9 (C-12), 126.5 (C-10), 79.6 (C-18), 66.2 (C-1), 48.8 (C-16), 44.5 (C-15), 33.5 (C-2), 28.6 (C-19); **MS**(ESI) *m/z* = 397.0 [M+H]⁺; **HRMS**(ESI) *m/z* = 397.1955 [M+H]⁺, calc.: 397.1944, Diff.: 2.6 ppm.

4.2.33. tert-Butyl 4-(8-chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine-1-carboxylate (**41d**)

825 Compound **41d** was obtained using the same conditions as for **41a**, employing **38b** (246 mg, 0.94 mmol, 1.0 equiv.), 1-Boc-piperazine (**39b**) (858 mg, 4.61 mmol, 4.9 equiv.) and Ti(*i*-OPr)₄ (0.21 mL, 0.71 mmol, 0.75 equiv.) and the mixture was refluxed for 32 h. Thus were obtained 700 mg (100% plus impurities) of the intermediate enamine **40d** as a yellow oil; the crude was used in the next step without further purification.

830 In the second step, the intermediate enamine **40d** and NaBH₄ (533 mg, 14.1 mmol, 15 equiv.) were stirred in 6.7 mL of glacial acetic acid. The crude residue was purified by flash chromatography (Hexane/EtOAc = 95/5 to 8/2), yielding 264 mg (65 %) of tert-butyl 4-(8-chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine-1-carboxylate (**41d**) as a light-yellow oil.

R_f (Hexane/EtOAc= 8/2) = 0.32; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.69 (1 H, d, H-6, J = 2.2 Hz), 7.53 (1 H, d, H-11, J = 7.8 Hz), 7.37 (1 H, d, H-4, J = 8.0 Hz), 7.22-7.32 (2 H, m, H-5 and H-7), 7.10-7.18 (1 H, m, H-10), 7.04-7.10 (1 H, m, H-13), 3.84-4.03 (2 H, m, H-1 and H-2a), 3.30-3.54 (4 H, m, H-16), 3.13 (1 H, dd, H-2b, J = 12.8, 3.0 Hz), 2.49-2.74 (4 H, m, H-15), 1.46 (9 H, s, H-19); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 155.0 (C-17), 142.3 (C-9), 142.0 (C-12), 136.5 (C-8), 133.9 (C-3), 133.2 (C-14), 132.9 (C-11), 132.4 (C-6), 131.5 (C-4), 129.6 (C-13), 129.0 (C-5), 127.1 (C-7), 126.7 (C-10), 79.7 (C-18), 66.0 (C-1), 48.6 (C-16), 44.3 (C-15), 32.8 (C-2), 28.6 (C-19); MS(ESI) m/z = 431.0 $[\text{M}+\text{H}]^+$; HRMS(ESI) m/z = 431.1549 $[\text{M}+\text{H}]^+$, calc.: 431.1555, Diff.: 1.2 ppm.

4.2.34. 1-(10,11-Dihydrodibenzo[*b,f*]thiepin-10-yl)piperazine (**41e**)

41c (45 mg, 0.11 mmol, 1.0 equiv.) was dissolved in 1.1 mL of DCM and THF (0.86 mL, 1.10 mmol, 10 equiv.) was added at room temperature. The mixture was stirred for 6 h, then quenched with saturated aqueous NaHCO_3 and the resulting aqueous solution was extracted with DCM (3x). The organic phases were united, dried over Na_2SO_4 , filtered and the solvent was removed under reduced pressure. Thus was obtained 34 mg (100%) of the free amine **41e** as a light-yellow oil; the crude shows only minor impurities and was used without further purification.

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) = 7.66 (1 H, d, H-6, J = 7.7 Hz), 7.51 (1 H, d, H-11, J = 7.7 Hz), 7.41 (1 H, dd, H-12, J = 7.7 Hz, J = 1.5 Hz), 7.20-7.32 (2 H, m, H-4 and H-5), 7.12-7.20 (1 H, m, H-7), 7.03-7.12 (2 H, m, H-10 and H-13), 4.01 (1 H, br s, NH), 3.85-4.02 (2 H, m, H-1 and H-2a), 3.17 (1 H, dd, H-2b, J = 12.8, 3.0 Hz), 2.83-3.04 (4 H, m, H-15), 2.65-2.79 (4 H, m, H-16); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) = 142.7 (C-9), 140.4 (C-8), 137.1 (C-3), 135.3 (C-14), 132.8 (C-11), 131.6 (C-6), 131.4 (C-4), 129.6 (C-13), 128.7 (C-5), 127.2 (C-7), 126.8 (C-12), 126.4 (C-10), 66.5 (C-1), 50.1 (C-15), 46.6 (C-16), 33.2 (C-2); MS(ESI) m/z = 297.0 $[\text{M}+\text{H}]^+$; HRMS(ESI) m/z = 297.1421 $[\text{M}+\text{H}]^+$, calc.: 297.1420, Diff.: 0.3 ppm.

4.2.35. 1-(8-Chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine (**41f**)

Compound **41f** was obtained using the same conditions as for **41e**, employing **41d** (180 mg, 0.42 mmol, 1.0 equiv.) and TFA (0.32 mL, 4.18 mmol, 10 equiv.) and Ti(*i*-OPr)₄ (0.21 mL, 0.71 mmol, 0.75 equiv.) and the mixture was stirred for 4 h. Thus were obtained 126 mg (100%) of the free amine **41f** as a yellow oil; the crude shows only minor impurities and was used without further purification.

¹H NMR (400 MHz, CDCl₃) δ (ppm) = 7.70 (1 H, d, H-6, *J* = 2.5 Hz), 7.50 (1 H, d, H-11, *J* = 7.5 Hz), 7.33 (1 H, d, H-4, *J* = 8.4 Hz), 7.22-7.30 (2 H, m, H-5 and H-7), 7.11 (1 H, td, H-10, *J* = 7.3 Hz, *J* = 1.7 Hz), 7.05 (1 H, dd, H-13, *J* = 8.2 Hz, *J* = 2.4 Hz), 3.85-3.95 (2 H, m, H-1 and H-2a), 3.10-3.21 (1 H, m, H-2b), 2.80-3.03 (4 H, m, H-15), 2.57-2.72 (4 H, m, H-16), 2.17 (2 H, bs, NH₂); ¹³C NMR (101 MHz, CDCl₃) δ (ppm) = 142.6 (C-9), 142.3 (C-12), 136.4 (C-8), 133.8 (C-3), 133.2 (C-14), 132.8 (C-11), 132.4 (C-6), 131.4 (C-4), 129.6 (C-13), 129.0 (C-5), 127.0 (C-7), 126.6 (C-10), 66.3 (C-1), 49.8 (C-15), 46.6 (C-16), 32.6 (C-2); MS(ESI) *m/z* = 331.0 [M+H]⁺; HRMS(ESI) *m/z* = 331.1031 [M+H]⁺, calc.: 331.1030, Diff.: 0.3 ppm.

4.3. Compound preparation

Synthesized compounds for biological testing were diluted in dimethylsulfoxide (DMSO) to create stock solutions that are stored in aliquots in the dark at -20 °C. All other drugs were purchased from Sigma-Aldrich including chloroquine diphosphate, desipramine hydrochloride, loperamide hydrochloride, L703-606 oxalate salt hydrate and octoclothepepin maleate. Chloroquine and desipramine were dissolved in PBS and filter-sterilized and stored at -20 °C for up to a month. All individually purchased compounds were dissolved in DMSO, stored in aliquots at -20 °C and diluted to working concentrations with PBS. All compounds were protected from light.

4.4. Uptake Assay with probe **3** to Investigate The Chemosensitizing Activity

880 Probe **3** was synthesized, stored and prepared as described previously [27]. Parasite cultures at 2.5% hematocrit and between 7–12% parasitemia (synchronized to late-rings and early trophozoites stage) were incubated in a 96-well plate at a staining concentration of 6 μ M of **3** and 10 μ M of various chemoreversal agents for 10 hours at 37 °C. After which, cultures were washed and finally re-suspended in fresh PBS for analysis with a flow cytometer. PBS was used as a negative control while
885 desipramine (DSP) was used as positive control along with the respective parent compound of the analogues. The percentage uptake of **3** referred to the proportion of total infected erythrocytes with detectable fluorescence.

4.5. Hoechst Staining

Hoechst 33342 stain (Life technologies) is a DNA-binding fluorescent stain that has an excitation of
890 350 nm (ultraviolet range) and an emission wavelength of 461 nm (blue fluorescence). After drug treatment, 2 μ g/ml of hoechst stain was added per well for 30 minutes at 37 °C. Cells were then washed twice and re-suspended in PBS before flow cytometry analysis.

4.6. Flow Cytometric Analyses

Flow cytometry (DAKO Cytomation Cyan ADP, Fort Collins, CO, USA) was used for probe **3** and
895 Hoechst-stained cells. Both were excited with a 405nm violet laser prior to 450/50BP (\pm 25) filter. For the determination of the proportion of **3**-positive parasites, Hoechst-stained duplicate wells were used to determine parasitemia. To detect both infected erythrocytes and liberated parasites, forward and side scattering adjustments were made. All results are saved in FCS 2.0 format and analyzed using the Flowjo version X (Tree Star) software.

900 4.7. Graphical Data Plots

All histograms were generated using Microsoft Excel Starter 2010. All other regression plots were plotted using Graphpad Prism version 5.0.

4.8. Quantitative Analysis

At least three independent experiments are performed for all assays to take into account any inter-
905 assay variability. After which, statistical analysis were conducted on the data obtained before making conclusive remarks.

4.9. Statistical Analysis

All data were presented as means \pm SEM. Statistical differences were measured using univariate two-tailed t-test. Conclusive remarks were made based on no difference between independent runs (i.e.
910 p-value more than 0.05) whereas significant results were indicated as p-value less than 0.05.

4.10. Ethics Statement

The blood collection protocol used for *in vitro* malaria parasite culture was approved by the National University of Singapore Institutional Review Board (NUS IRB; reference code 11-383, approval number NUS-1475). Written informed consent was obtained from all of the participants involved in
915 this study. The clinical isolates used in this study were collected in accordance with the ethical guidelines in the approved protocols (OXTREC reference number 29-09; Center for Clinical Vaccinology and Tropical Medicine, University of Oxford, Oxford, United Kingdom). The use of field isolates for work done at NUS was in accordance with the NUS IRB (reference code 12-369E).

ACKNOWLEDGMENTS

920 Research from KT and BD laboratories has been generously supported by grants from the National
 Medical Research Council (NMRC/1310/2011) and National University of Singapore Faculty of
 Science start-up grant (R-148-000-169-133), respectively. The authors thank all of the patients and
 staff of the SMRU for their contribution to this study. SMRU is sponsored by the Wellcome Trust of
 Great Britain, as part of the Oxford Tropical Medicine Research Program of Wellcome Trust–Mahidol
 925 University. The authors are also thankful for the following reagents which were obtained through the
 MR4 as part of the BEI Resources Repository, NIAID, NIH: *Plasmodium falciparum* 3D7, MRA-102,
 deposited by DJ Carucci; *P. falciparum* HB3, MRA-155, deposited by TE Wellems; *P. falciparum*
 7G8, MRA-154, deposited by DE Kyle; *P. falciparum* K1, MRA-159, deposited by DE Kyle; *P.*
falciparum Dd2, MRA-156, deposited by TE Wellems. The authors thank the NUS Drug Development
 930 Unit (<http://ddu.nus.edu.sg/>) for technical support with solubility, permeability and toxicity assays. We
 also want to warmly thank Dr. Martin Lear for helpful discussions.

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CAPTIONS

1080 **Figure 1** Chloroquine (CQ, **1**), the fluorescent tag **2** and probe **3**.

Figure 2 Structures of the four CRA hit compounds.

Figure 3 Optimal chemoreversal agent pharmacophore with distances and angles, consisting of two
1085 hydrophobic regions (yellow spheres) and a positive-ionizable atom (blue). Predicted by LigandScout
3.1.

Figure 4 Fit of **4** in the pharmacophore model.

1090 **Figure 5** Fit of **5** in the pharmacophore model.

Figure 6 Synthesis of compound **4** from commercially available ketone **8**.

Figure 7 Access to simplified derivatives **16-19**.

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Figure 8 Synthesis of iodo-benzylamine analogues **21** and **23**.

Figure 9 Synthesis of simplified analogues of **5**.

1100 **Figure 10** Synthesis of amino-piperidine **30e**.

Figure 11 Synthesis of piperazine analogs of loperamide (**5**).

Figure 12 Synthesis of analogues of octoclotheptin (**6**) and methiothepin (**7**).

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Figure 13 Results for the uptake assay of probe **3** for analogues of **4** (10 μ M), performed on the K1 strain (CQR). Values 50% and above were regarded as active. White bar = PBS blank (negative control). Black bars = positive controls. Shaded bars = test compounds. 18(mix) = mixture of cis/trans. Figure shows mean \pm SEM, $n \geq 3$, two-tailed p-values are in comparison to negative control PBS (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

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Figure 14 Results for the uptake assay of probe **3** for analogues of **5** (10 μ M), performed on the K1 strain (CQR). White bar = PBS blank (negative control). Black bars = positive controls. Shaded bars = test compounds. Figure shows mean \pm SEM, $n \geq 3$, two-tailed p-values are in comparison to negative control PBS (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

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Figure 15 Results for the uptake assay of probe **3** for analogues of **6** and **7** (10 μ M), performed on the K1 strain (CQR). White bar = PBS blank (negative control). Black bars = positive controls. Shaded

bars = test compounds. Figure shows mean \pm SEM, $n \geq 3$, two-tailed p-values are in comparison to negative control PBS (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

Figure 16 Results for the CQ IC₅₀ assay for the selected analogues, performed on three strains at a fixed concentration of 500 nM of each CRA. Chloroquine sensitive (CQS) 3D7, 7G8 showing intermediate chloroquine resistance as well as chloroquine resistant (CQR) K1. Reference standards (**1**, **4**, **5** and **6**) are in solid color. Figure shows mean \pm SEM, two-tailed t test p-values are in comparison to CQ with no CRA (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

Figure 17 Results for the CQ IC₅₀ assay for the selected analogues on additional strains: chloroquine sensitive (CQS) Hb3, chloroquine resistant (CQR) Dd2 and NHP4559 as well as both chloroquine and artemisinin resistant (CQR, ArtR) ARS233, ARS272 and NHP4773. Figure shows mean \pm SEM, two-tailed t test p-values are in comparison to CQ with no CRA (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

Figure 18 Relative effects of chemosensitization for the selected CRA analogues in the CQ IC₅₀ assay for selected analogues, compared to CQ (**1**) only, performed on various chloroquine sensitive (CQS), chloroquine resistant (CQR) and artemisinin-resistant (ArtR) strains. The RMI is defined as the ratio of each compound's IC₅₀ to the IC₅₀ of CQ alone. The dotted line at RMI=1 indicates no change in IC₅₀ of **1** with the indicated CRA. Bars under the dotted line indicate compounds, which lower the IC₅₀ of **1** in the indicated strain. Figure shows mean \pm SEM.

Table 1 Compilation of IC₅₀ values for the best performing compounds^a

Table 2 Compilation of EC₅₀ values for the best performing compounds^a

Table 3 Toxicity of **1** with increasing levels of CRAs **4** and **18** in TAMH cells^a

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Table 4 Toxicity of three CRAs in two normal cell lines with their therapeutic windows. All TAMH and AC10 data are the means of 4 independent determinations.

Table 5 Various measured properties for parent compounds and their best performing analogues

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Table 1 Compilation of IC₅₀ values for the best performing compounds^a

Compound	CQ IC₅₀ 3D7 (nM)	CQ IC₅₀K1 (nM)	K1/3D7 (resistance ratio)	CQ IC₅₀ 7G8 (nM)	7G8/3D7 (resistance ratio)
1 (CQ)^b	35.9	737.0	20.5	220.3	6.1
4	38.2	191.7	5.0	130.8	3.4
13	39.3	684.6	17.4	204.2	5.2
18	45.4	288.2	6.4	176.3	3.9
22	39.7	333.4	8.4	151.6	3.8
5	38.8	542.5	14.0	130.0	3.4
24a	44.1	654.7	14.8	274.7	6.2
25d	43.0	524.4	12.2	219.6	5.1
30c	40.5	544.7	13.4	215.5	5.3
6	38.2	692.6	18.1	245.7	6.4
41e	43.5	404.2	9.3	166.6	3.8
41f	40.8	506.3	12.4	194.8	4.8

^a Table shows mean values, n = 3; Concentration of CRAs: 500 nM. ^b Chloroquine (**1**) tested on its own, all other data are IC₅₀s of **1** with 500 nM of CRAs.

Table 2 Compilation of EC₅₀ values for the best performing compounds^a

Compound	EC ₅₀ 3D7	EC ₅₀ K1 (nM)	EC ₅₀ 7G8 (nM)
4	-	98 ^b	-
18	No effect	170	380 nM
22	-	200	-
5	-	685 ^b	-
24a	-	482	-
25d	-	295	-
6	-	1267 ^b	-
41e	-	343	-

^a Table shows mean values, n = 3. EC₅₀ is defined as the concentration of the CRA required to reduce the IC₅₀ of **1** by 50%. ^b Values published previously (23).

Table 3 Toxicity of **1** with increasing levels of CRAs **4** and **18** in TAMH cells^a

	1 + 4				1 + 18			
	1	1 + 100nM	1 + 1μM	1 + 10μM	1	1 + 100nM	1 + 1μM	1 + 10μM
TAMH IC ₅₀ (μM)	85.26	80.90	78.19	64.28	85.26	74.12	83.68	77.76
SEM	3.01	8.27	7.46	9.77	3.01	3.52	6.61	2.28

^a Data is presented as mean of n = 4 independent determinations with standard error of the mean (SEM).

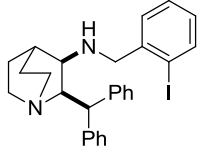
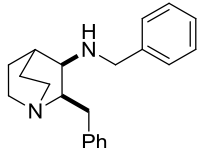
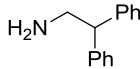
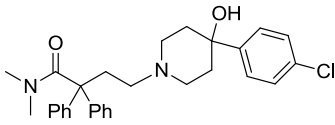
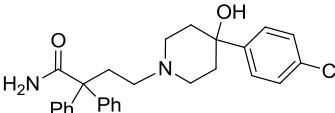
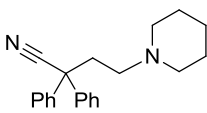
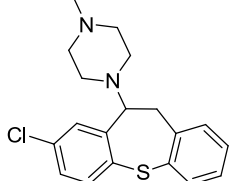
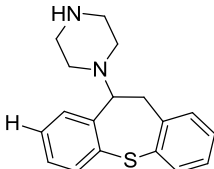
Table 4 Toxicity of three CRAs in two normal cell lines with their therapeutic windows. All TAMH and AC10 data are the means of 4 independent determinations.

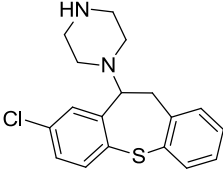
Compound	EC ₅₀ K1 (nM) ^a	TAMH IC ₅₀ (nM) ^b	AC10 IC ₅₀ (nM) ^b	Therapeutic window	
				TAMH/K1	AC10/K1
4	98	17,200	ND	175	ND
18	170	>100,000	71,470	>588	420
24a	482	101,100	45,190	210	94

^a EC₅₀ defined as the concentration of CRA that reduces by 50% the required concentration of **1** to inhibit the survival of half the parasites. ^b IC₅₀ determined by CellTiter-Glo® Cell Viability Assay (Promega Corporation).

ND = not determined.

Table 5 Various measured properties for parent compounds and their best performing analogues

Compound	Name	MW (g/mol)	cLog P ^a	Aqueous Solubility ($\mu\text{g/mL}$, pH 7.4, 24h) ^b	PAMPA Permeability (P_e) ^c (10^{-6} cm/s, pH 7.4)
	4 (L-703,606)	508.44	6.48	31.0 ± 8.7	10.36 ± 1.76 (6h) 8.24 ± 1.12 (16h)
	18	306.44	4.21	>310	19.18 ± 1.09 (6h) 14.52 ± 2.08 (16h)
	22	292.42	3.44	ND	ND
	5 (Loperamide)	477.04	4.66	410 ± 50	15.99 ± 0.79 (6h) 12.49 ± 3.10 (16h)
	24a	448.98	4.00	> 450	0.65 ± 0.17 (6h) 1.78 ± 0.32 (16h)
	25d	304.43	4.46	ND	ND
	6 (OctoclothePIN)	344.90	5.59	34.93 ± 3.83	ND
	41e	296.43	3.10	87.1 ± 0.7	ND

Compound	Name	MW (g/mol)	cLog P ^a	Aqueous Solubility (μg/mL, pH 7.4, 24h) ^b	PAMPA Permeability (P _e) ^c (10 ⁻⁶ cm/s, pH 7.4)
	41f	330.87	5.02	38.1 ± 3.5	ND

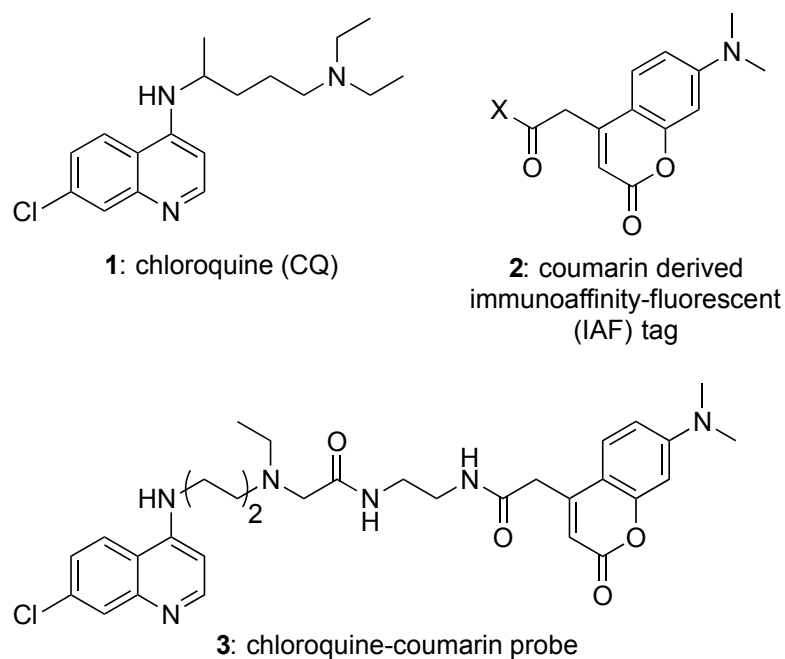


Figure 1 Chloroquine (CQ, **1**), the fluorescent tag **2** and probe **3**.

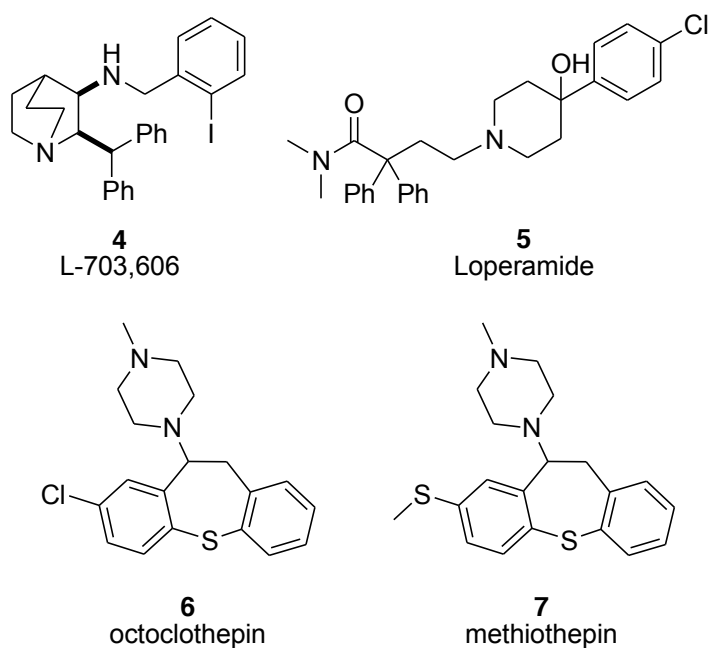


Figure 2 Structures of the four CRA hit compounds.

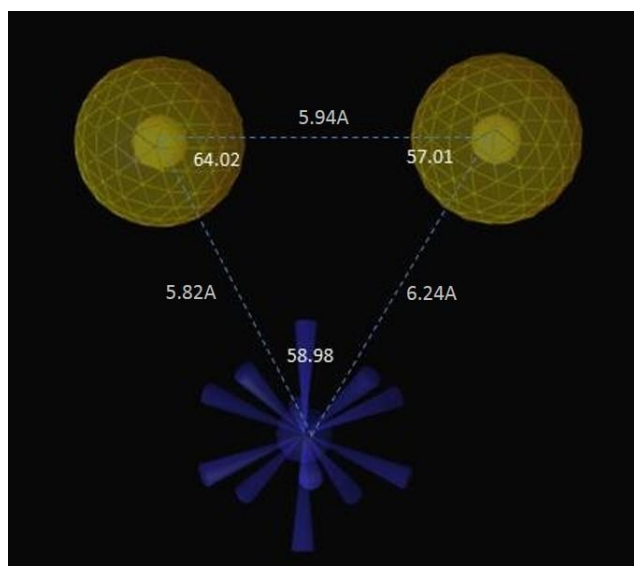


Figure 3 Optimal chemoreversal agent pharmacophore with distances and angles, consisting of two hydrophobic regions (yellow spheres) and a positive-ionizable atom (blue). Predicted by LigandScout 3.1.

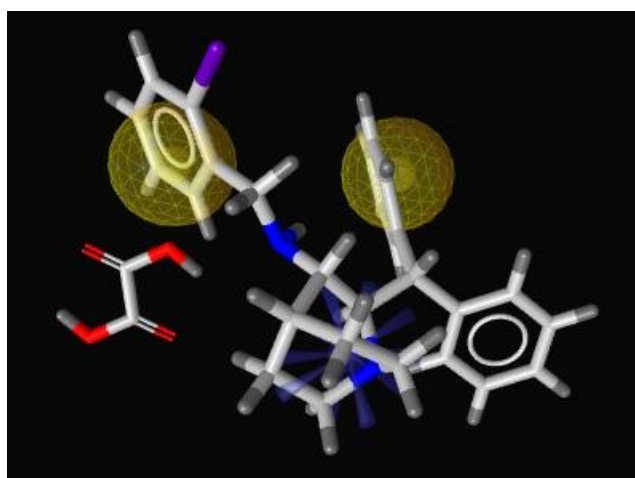


Figure 4 Fit of 4 in the pharmacophore model.

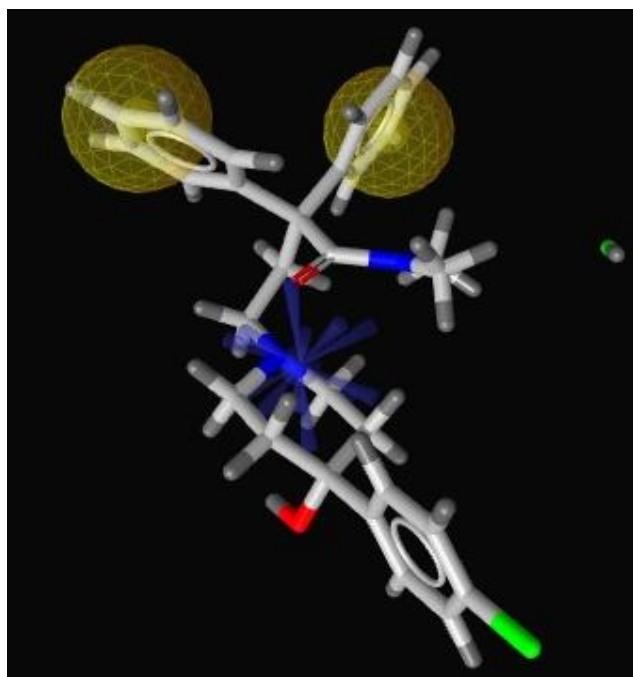
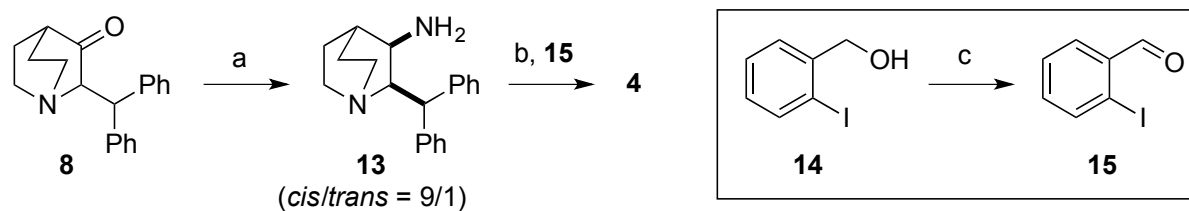
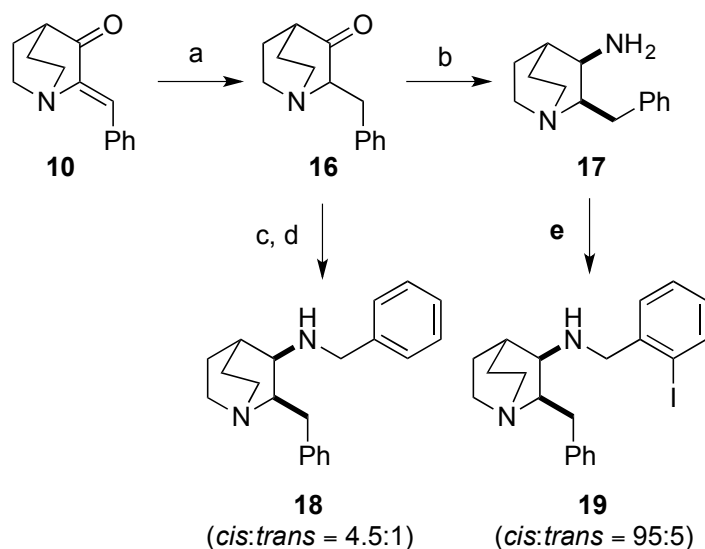


Figure 5 Fit of **5** in the pharmacophore model.



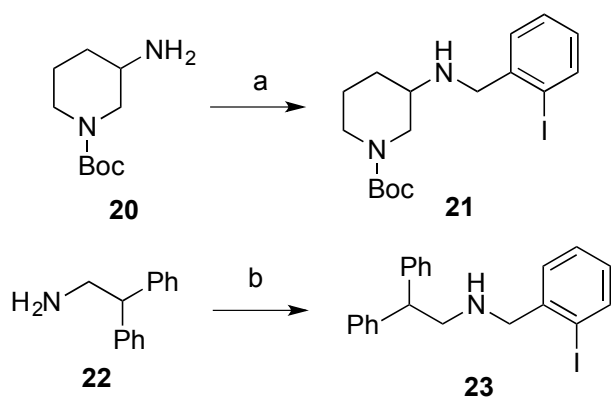
Reagents and Conditions: (a) $\text{NH}_4\text{CO}_2\text{H}$, ZnCl_2 (1 M in Et_2O), NaBH_3CN , MeOH, 20 h, 28%;
 (b) $\text{NaHB}(\text{OAc})_3$, DCM, RT, 5 h, 84%; (c) PCC, Celite, DCM, RT, 16 h, 98%.

Figure 6 Synthesis of compound **4** from commercially available ketone **8**.



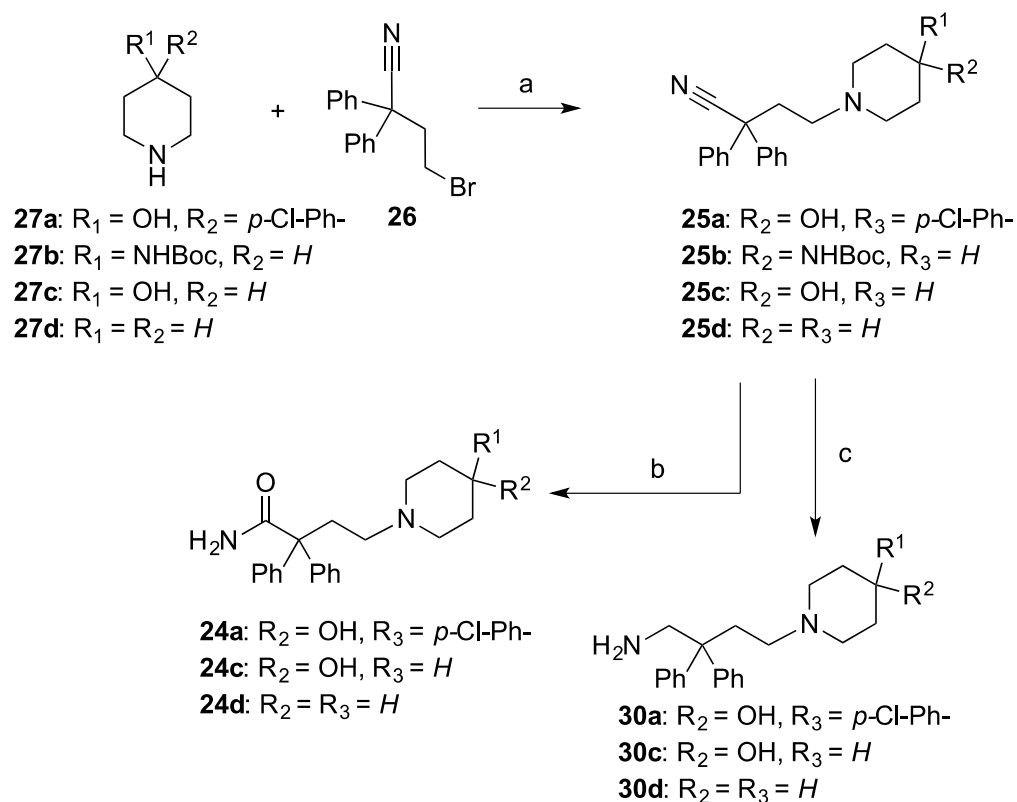
Reagents and Conditions: (a) H_2 (25 psi), Pd/C, MeOH, RT, 7 h, 88%; (b) ZnCl_2 , $\text{NH}_4\text{CO}_2\text{H}$, NaBH_3CN , MeOH, 20 h, 71% (*cis:trans* 9:1); (c) BnNH_2 , *p*-TsOH, PhMe, reflux (Dean-Stark), 24 h; (d) 9-BBN, THF, RT, 24 h, 48% over 2 steps; (e) **15**, $\text{NaHB}(\text{OAc})_3$, DCM, 0 °C to RT, 2 h, 26%.

Figure 7 Access to simplified derivatives **16-19**.



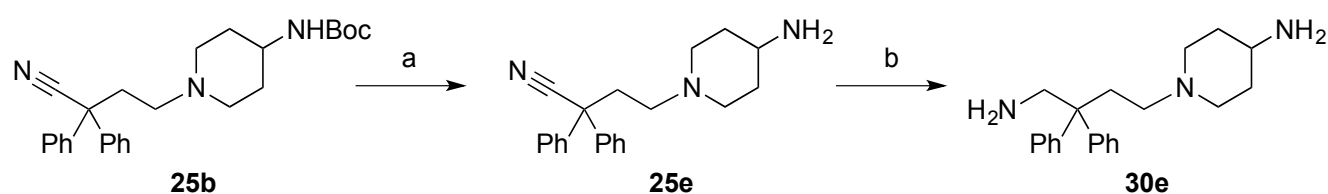
Reagents and Conditions: (a) **15**, $\text{NaHB}(\text{OAc})_3$, DCM, 0 °C to RT, 2 h, 70%; (b) **15**, $\text{NaHB}(\text{OAc})_3$, DCM, 0 °C to RT, 5 h, 80%.

Figure 8 Synthesis of iodo-benzylamine analogues **21** and **23**.



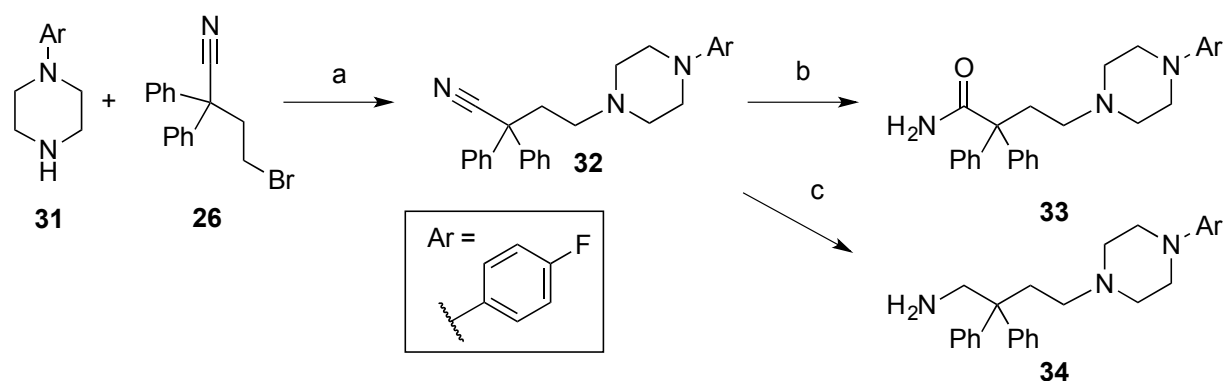
Reagents and Conditions: (a) DIPEA, MeCN, reflux, 40 h, 74-94%; (b) KOH, *t*-BuOH, 100 °C, 15-72%; (c) LiAlH₄, THF, 0 °C to RT, 19-68%.

Figure 9 Synthesis of simplified analogues of **5**.



Reagents and Conditions: (a) KOH, *t*-BuOH, 100 °C, 62%; (b) LiAlH₄, THF, 0 °C to RT, 55%.

Figure 10 Synthesis of amino-piperidine **30e**.



Reagents and Conditions: (a) DIPEA, MeCN, reflux, 40 h, 74%; (b) KOH, *t*-BuOH, 100 °C, 80%; (c) LiAlH₄, THF, 0 °C to RT, 6%.

Figure 11 Synthesis of piperazine analogs of looperamide (5).

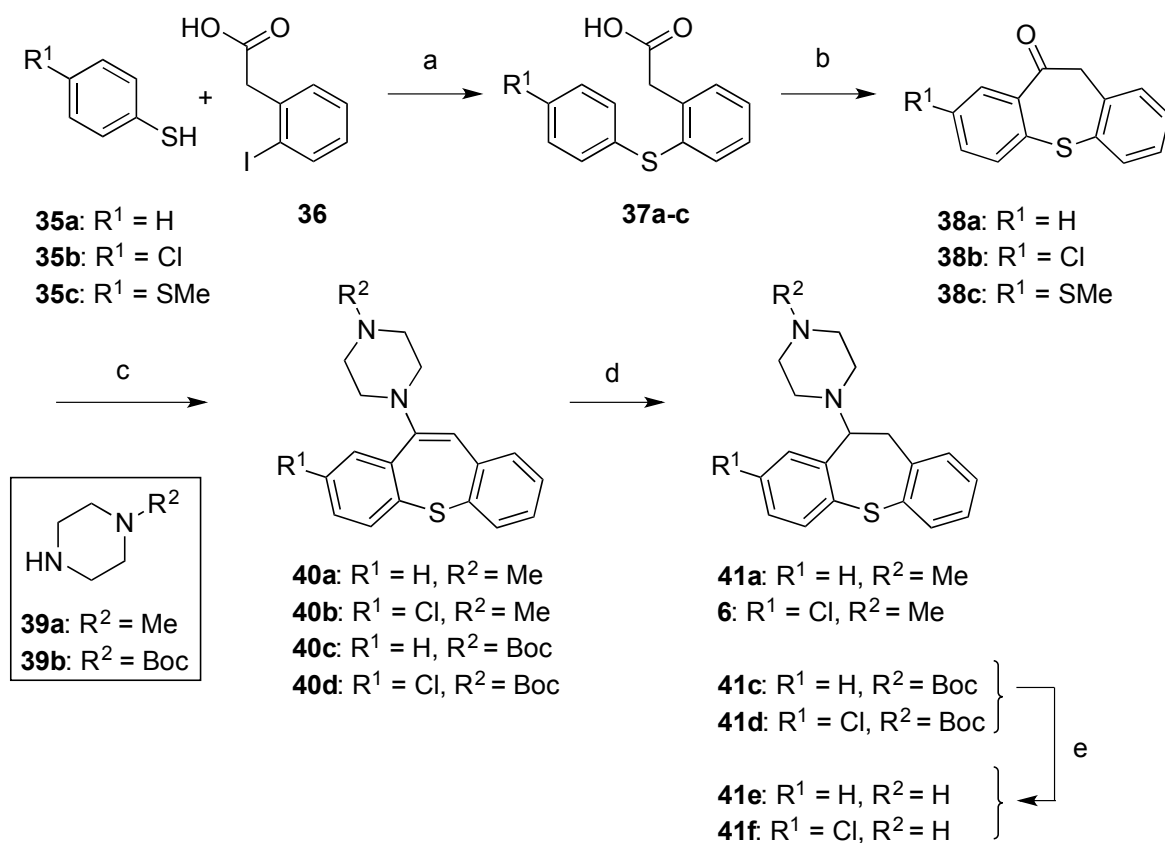


Figure 12 Synthesis of analogues of octoclotheptin (6) and methiotheptin (7).

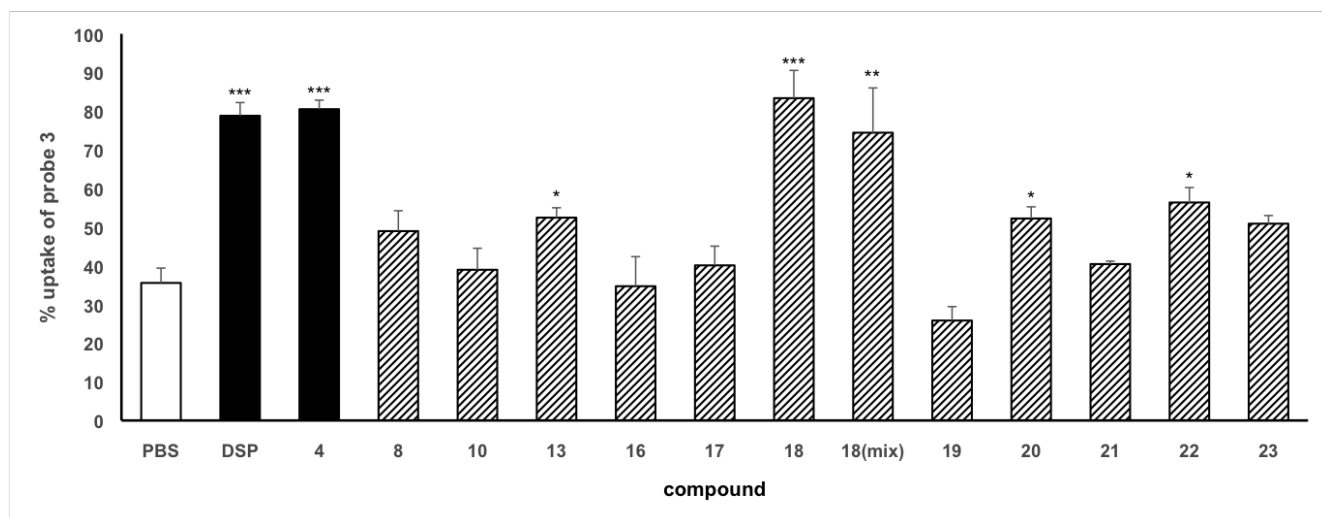


Figure 13 Results for the uptake assay of probe **3** for analogues of **4** (10 μ M), performed on the K1 strain (CQR). Values 50% and above were regarded as active. White bar = PBS blank (negative control). Black bars = positive controls. Shaded bars = test compounds. 18(mix) = mixture of cis/trans. Figure shows mean \pm SEM, $n \geq 3$, two-tailed p-values are in comparison to negative control PBS (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

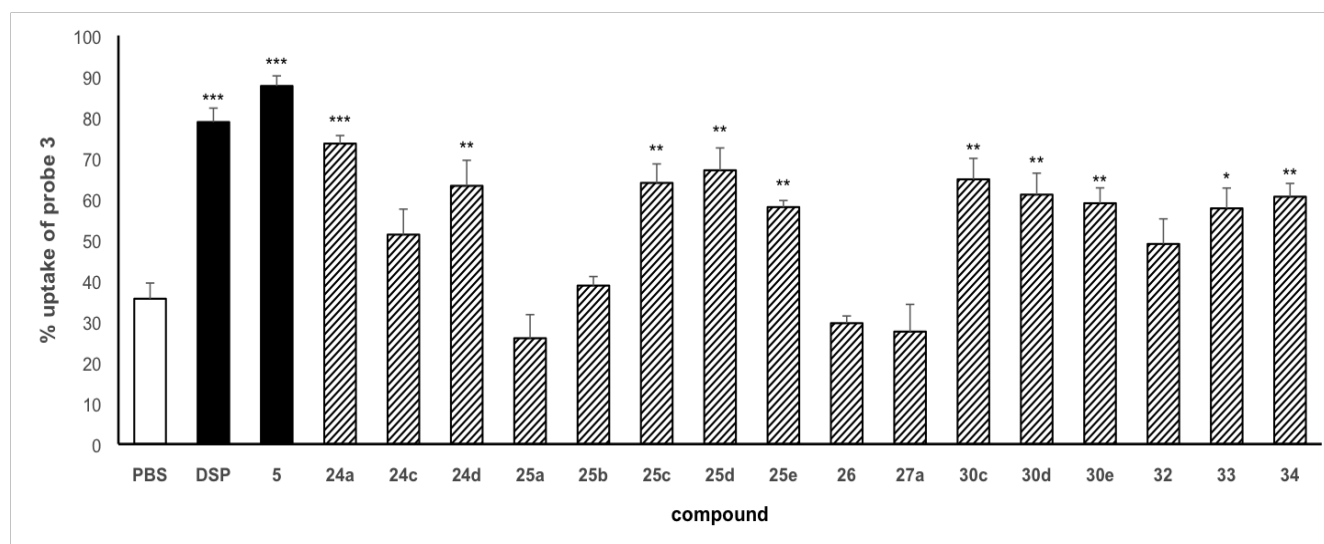


Figure 14 Results for the uptake assay of probe **3** for analogues of **5** (10 μ M), performed on the K1 strain (CQR). White bar = PBS blank (negative control). Black bars = positive controls. Shaded bars = test compounds. Figure shows mean \pm SEM, $n \geq 3$, two-tailed p-values are in comparison to negative control PBS (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

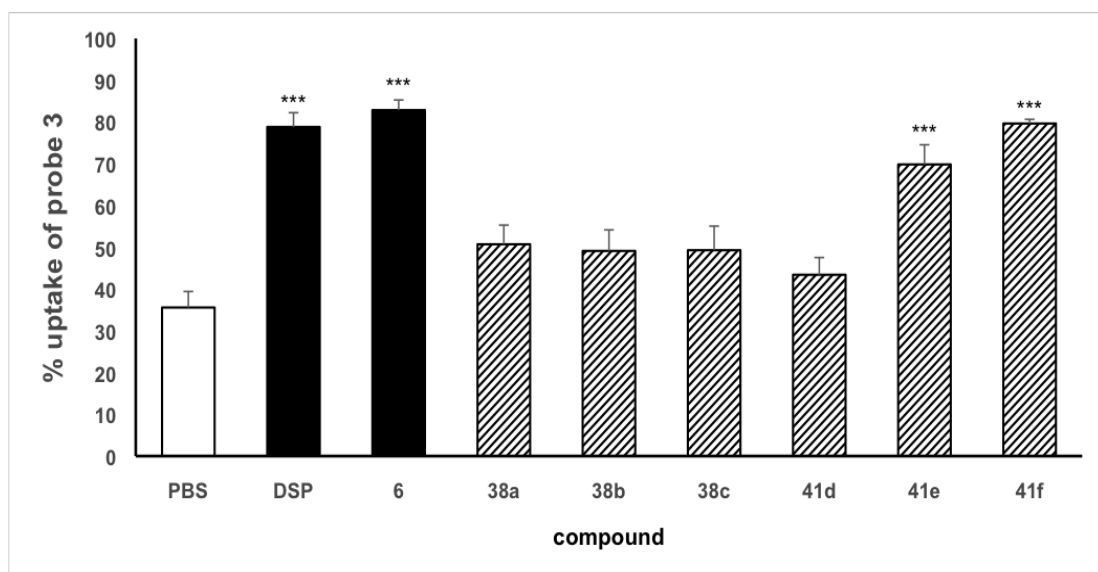


Figure 15 Results for the uptake assay of probe **3** for analogues of **6** and **7** (10 μ M), performed on the K1 strain (CQR). White bar = PBS blank (negative control). Black bars = positive controls. Shaded bars = test compounds. Figure shows mean \pm SEM, $n \geq 3$, two-tailed p-values are in comparison to negative control PBS (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

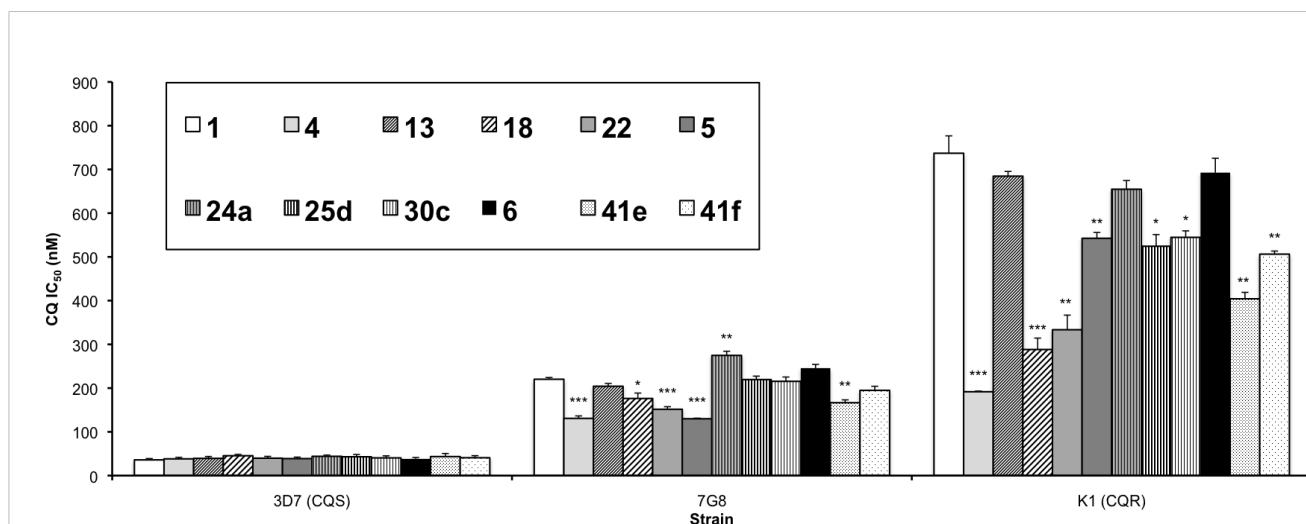


Figure 16 Results for the CQ IC₅₀ assay for the selected analogues, performed on three strains at a fixed concentration of 500 nM of each CRA. Chloroquine sensitive (CQS) 3D7, 7G8 showing intermediate chloroquine resistance as well as chloroquine resistant (CQR) K1.

Reference standards (**1**, **4**, **5** and **6**) are in solid color. Figure shows mean \pm SEM, two-tailed t test p-values are in comparison to CQ with no CRA (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

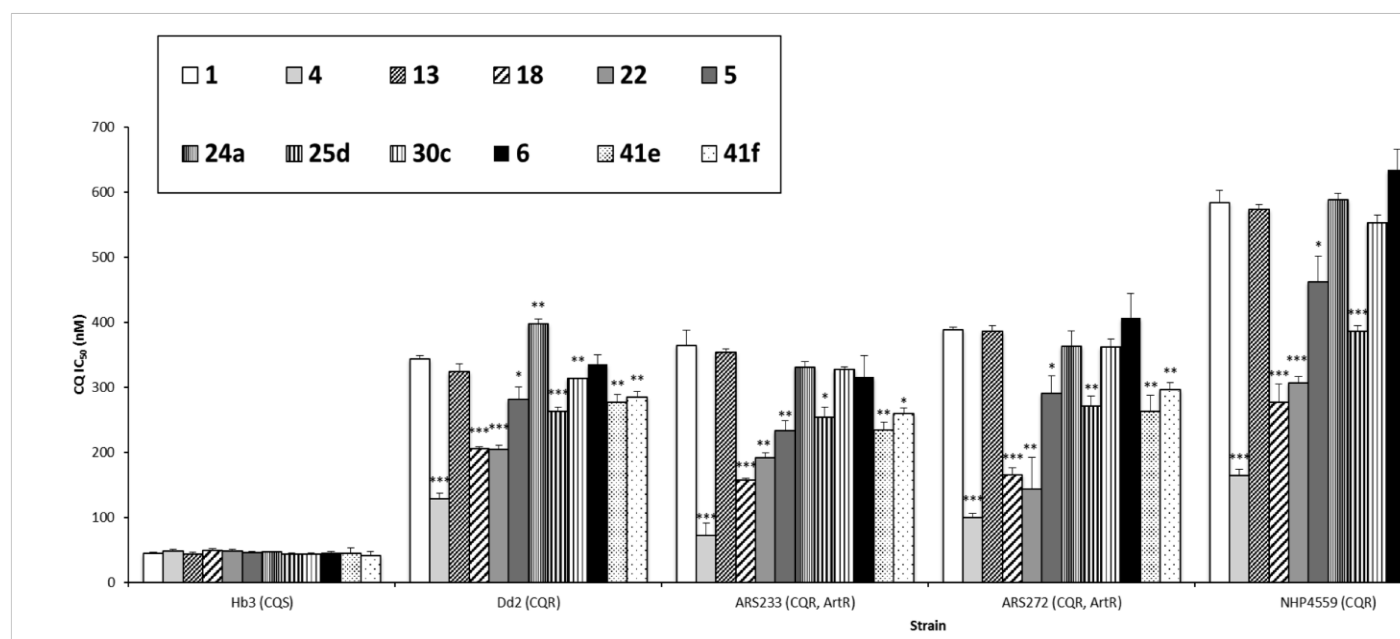


Figure 17 Results for the CQ IC_{50} assay for the selected analogues on additional strains: chloroquine sensitive (CQS) Hb3, chloroquine resistant (CQR) Dd2 and NHP4559 as well as both chloroquine and artemisinin resistant (CQR, ArtR) ARS233, ARS272 and NHP4773. Figure shows mean \pm SEM, two-tailed t test p-values are in comparison to CQ with no CRA (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$).

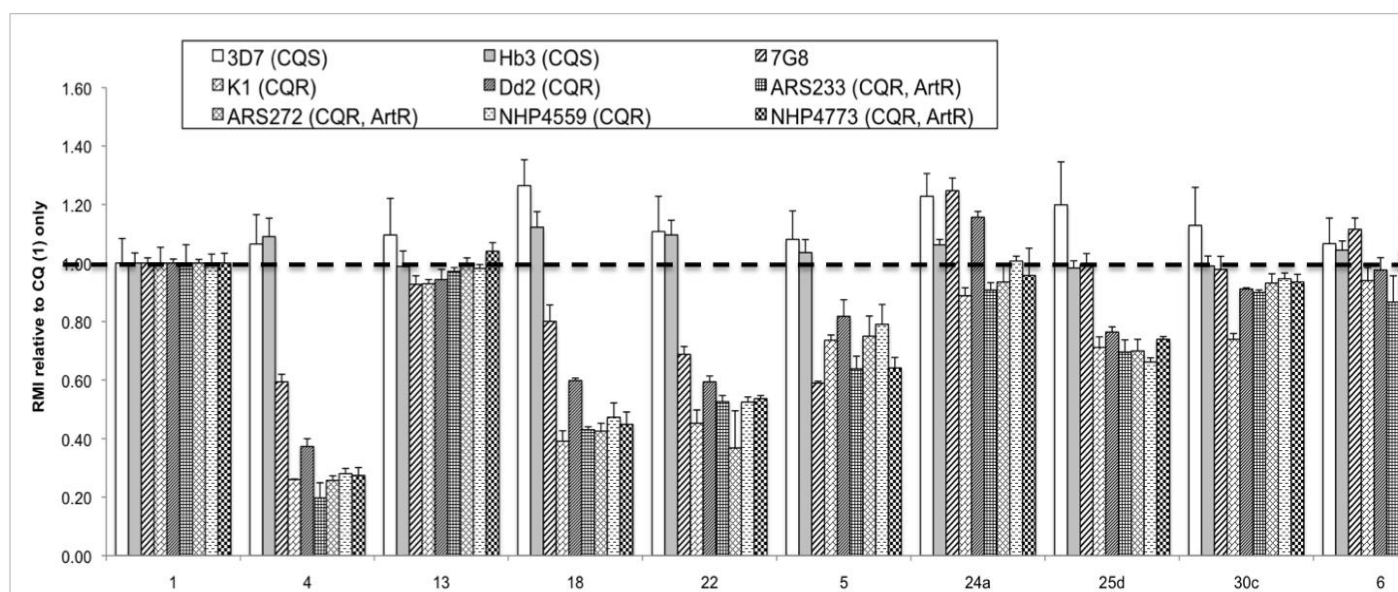


Figure 18 Relative effects of chemosensitization for the selected CRA analogues in the CQ IC_{50} assay for selected analogues, compared to CQ (**1**) only, performed on various chloroquine sensitive (CQS), chloroquine resistant (CQR) and artemisinin-resistant (ArtR) strains. The RMI is defined as the ratio of each compound's IC_{50} to the IC_{50} of CQ alone. The dotted line at RMI=1 indicates no change in IC_{50} of **1** with the indicated CRA. Bars under the dotted line indicate compounds, which lower the IC_{50} of **1** in the indicated strain. Figure shows mean \pm SEM.