Overcoming Chloroquine Resistance in Malaria: Design, Synthesis and Structure-Activity Relationships of Novel Hybrid Compounds

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Running Head: Synthesis and SAR of New Hybrid Antimalarial Compounds

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ABSTRACT

Resistance to antimalarial therapies, including artemisinin, has emerged as a significant challenge. Reversal of acquired resistance can be achieved using agents which resensitize resistant parasites to a previously efficacious therapy. Building on our initial work describing novel chemoreversal agents (CRAs) which resensitize resistant parasites to chloroquine (CQ), we herein report new hybrid single agents as an innovative strategy in the battle against resistant malaria. Synthetically linking a CRA scaffold to chloroquine produces hybrid compounds with restored potency towards a range of resistant malaria parasites. A preferred compound, **35**, showed broad activity and good potency against seven strains resistant to chloroquine and artemisinin. Assessment of aqueous solubility, membrane permeability and *in vitro* toxicity, in a hepatocyte and a cardiomyocyte cell line, indicates that **35** has a good therapeutic window and favourable drug-like properties. This study provides initial support for CQ-CRA hybrid compounds as a potential treatment for resistant malaria.

Antimicrobial Agents and Chemotherapy

INTRODUCTION

Malaria is a global infectious disease, caused by a parasitic protozoan of the genus *Plasmodium* (*P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*) which exhibits a complex life cycle involving an insect vector (mosquito) and a vertebrate host (human)(1). Following WHO estimates there were 214 million cases and 438,000 deaths in 2015 (2). Early diagnosis and treatment of malaria reduces disease burden, prevents death and contributes to reducing malaria transmission. Historically, chloroquine (CQ, **1**, Fig. 1) was a widely used drug due to its good efficacy, low toxicity and affordability. However *P. falciparum*, the predominant species infecting humans, has developed mechanisms to effectively neutralize the effects of **1** and as a result became resistant to the effects of **1**. The current best available treatment, particularly for *P. falciparum* malaria, is an artemisinin-based combination therapy (ACT). Unfortunately, ACT has already shown prolonged parasite clearance times in Thailand and Cambodia (3-5). The constant threat of developing resistance against antimalarial drugs has led to the search for innovative therapies, such as new compounds with novel mechanisms of action, or new combination therapies to prevent drug resistance (6, 7).

In our previous work we have described the reversal of chloroquine resistance with novel chemoreversal agents (CRAs) (8). Therein, we demonstrated the use of a fluorescent probe in screens for new chemosensitizing compounds (8, 9), the design of analogues of hit compounds as well as their activity and improved potential as CRAs (8).

Herein we report our results developing single agent hybrid compounds based on these CRAs. The concept of hybrid compounds, combining more than one biological activity in a single compound, is a relatively unexplored concept in antimalarial resistance related drug discovery (10). Hybrid compounds have several advantages, including a lower risk of drug-drug interactions, simpler pharmacokinetics and dosing regimens compared to combinations of single-mechanism drugs. Our aim

was to achieve the combined bioactive effects of each of the pharmacophores via linking, fusing or merging. One example of a combination strategy to create single molecule drugs is that of artemisinin and quinine (**2**) (11-13), another being the combination of antimalarial agents with other scaffolds such as **3** (Fig. 2) (14, 15). There are also a few reported examples of CRAs combined to **1**, aiming to have antimalarial activity of CQ and at the same time blocking the chloroquine resistance transporter (PfCRT) with the CRA (16-18).

With CRAs **4**-**6** (Fig. 3) at hand, we investigated their utility in hybrid compounds with **1** as the antimalarial component, seeking novel structures retaining good physicochemical properties with improved potency against resistant strains. Thus a series of hybrid compounds were designed, linking the scaffold from **1** *via* its terminal secondary or tertiary amine with CRAs **4**-**6** (Fig. 1 and Fig. 3). The 4-aminoquinoline scaffold has been shown to be essential for the binding of CQ to free heme (19), and both the terminal tertiary amino group and the basicity of the quinolyl nitrogen are key elements (20, 21). Ideally the length of the aliphatic side chain should be 2-4 carbons, however the methyl group has little influence on the activity and was thus not retained.

MATERIALS AND METHODS

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 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

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 ^{1}H NMR, ^{13}C NMR, and mass spectrometry. ^{1}H (400 MHz) and ^{13}C (101 MHz) NMR spectra were measured in $CDCl_3$ on a Bruker Avance III 400 (Ultrashield Plus) spectrometer. Chemical shifts are reported in parts per million (ppm) using the residual $CDCl_3$ peak at 7.26 (^{1}H) or 77.16 (^{13}C) as internal standard. ^{1}H 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 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.

Chemistry

Synthesis of precursors **13** to **17** is described in the Supporting Information.

*N1-(7-chloroquinolin-4-yl)-N4-(quinuclidin-3-yl)butane-1,4-diamine (**24**)*

To a stirred solution of quinuclidin-3-one hydrochloride (**23**) (154 mg, 0.95 mmol, 1.0 equiv.) in dry methanol (1.40 mL), under inert conditions, was added a 1 M solution of $ZnCl_2$ in Et_2O (0.19 mL, 0.19 mmol, 0.2 equiv.). After stirring at room temperature for 30 min was added N^1-(7-chloroquinolin-4-yl)butane-1,4-diamine (**19c**) (476 mg, 1.91 mmol, 2.0 equiv.). After stirring another hour at room temperature, solid sodium cyanoborohydride (120 mg, 1.91 mmol, 2.0 equiv.) was added in portions. The reaction was then stirred for 4 h at room temperature and quenched by addition of water (about 5 mL). The quenched reaction was partitioned between 5 M $NaOH_{(aq.)}$ and DCM. The aqueous layer

was extracted with DCM (3x), and the combined organic layers were dried with Na_2SO_4, filtered and concentrated. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 8/2), yielding 9.9 mg (3 %) of N^1-(7-chloroquinolin-4-yl)-N^4-(quinuclidin-3-yl)butane-1,4-diamine (**24**) as a yellow oil. 263 mg (55 %) of the starting material N^1-(7-chloroquinolin-4-yl)butane-1,4-diamine (**19c**) were also isolated in a second fraction.

R_f (DCM/MeOH = 95/5) = 0.20; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.50 (1 H, d, *J* = 5.3 Hz, H-3), 7.94 (1 H, d, *J* = 2.0 Hz, H-8), 7.74 (1 H, d, *J* = 9.0 Hz, H-5), 7.33 (1 H, dd, *J* = 9.0 Hz, *J* = 2.0 Hz, H-7), 6.40 (1 H, d, *J* = 5.3 Hz, H-2), 5.42 (1 H, bs, NH), 3.34 (2 H, m, H-10), 3.20 (2 H, m, H-13), 3.30 (4 H, m, H-16), 2.62 (3 H, m, H-14 and H-15), 1.82 (12 H, m, H-12, H-11, H-17, H-18 and NH); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 151.7 (C-3), 150.1 (C-1), 148.8 (C-4), 135.2 (C-6), 128.5 (C-5), 125.5 (C-8), 121.5 (C-7), 117.3 (C-9), 99.1 (C-2), 60.5 (C-15), 53.5 (C-14), 52.8 (C-10), 52.2 (C-13), 47.0 (C-16), 43.3 (C-16), 28.0 (C-11), 26.7 (C-12), 25.1 (C-18), 23.9 (C-17), 18.9 (C-17); **MS** (IT-TOF) m/z = 359.2 $[M+H]^+$.

*4-((2-((7-chloroquinolin-4-yl)amino)ethyl)(ethyl) amino)-2,2-diphenylbutanenitrile (**26**)*

To a solution of N^1-(7-chloroquinolin-4-yl)-N^2-ethylethane-1,2-diamine (**21a**) (426 mg, 1.71 mmol, 1.0 equiv.) and 4-bromo-2,2-diphenylbutanenitrile **11** (512 mg, 1.71 mmol, 1.0 equiv.) in dry MeCN (5.70 mL), under inert conditions, was added DIPEA (0.91 mL, 5.13 mmol, 3.0 equiv.). After stirring at reflux for 5 d, the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 9/1), yielding 321 mg (40 %) of 4-((2-((7-chloroquinolin-4-yl)amino)ethyl)(ethyl)amino)-2,2-diphenylbutanenitrile (**26**) as a yellow oil.

R_f (DCM/MeOH = 9/1) = 0.53; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.54 (1 H, d, *J* = 5.3 Hz, H-3), 7.98 (1 H, d, *J* = 2.0 Hz, H-8), 7.72 (1 H, d, *J* = 9.0 Hz, H-5), 7.39 (1 H, dd, *J* = 9.0 Hz, *J* = 2.0 Hz, H-7), 7.27 (10 H, m, H-Ph), 6.31 (1 H, d, *J* = 5.3 Hz, H-2), 5.89 (1 H, bs, NH), 3.14 (2 H,

q, J = 5.6 Hz, H-12), 2.79 (2 H, t, J = 5.7 Hz, H-10), 2.64 (4 H, m, H-14 and H-11), 2.55 (2 H, m, H-15), 1.06 (3 H, t, J = 7.0 Hz, H-13); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 151.2 (C-3), 150.0 (C-1), 148.8 (C-4), 139.8 (C-18), 135.1 (C-6), 129.1 (C-19), 128.9 (C-5), 128.2 (C-21), 126.7 (C-20), 125.5 (C-8), 122.4 (C-9), 121.4 (C-7), 117.6 (C-17), 99.61 (C-2), 51.7 (C-11), 50.0 (C-16), 49.4 (C-14), 47.5 (C-12), 40.1 (C-10), 37.3 (C-15), 12.0 (C-13); **MS** (ESI) m/z = 469.1 $[M+H]^+$; **HRMS** (ESI) m/z = 469.2168 $[M+H]^+$, calc.: 469.2154, Diff.: 3.1 ppm.

*N^1-(3-(2-chloro-10H-phenothiazin-10-yl)propyl)-N^3-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^3-ethylpropane-1,3-diamine (**42**)*

2-Chlorophenothiazine (**39**) (1.01 g, 4.30 mmol, 1.0 equiv.) was dissolved under nitrogen in 14.3 mL of dry THF with the aid of heat. This solution was added to a suspension of NaH (60 w% in oil, 172 mg, 4.30 mmol, 1.0 equiv.) under nitrogen in 7.41 mL of dry DMSO and 1.48 mL of dry THF. The mixture was stirred at 0 °C for 30 min and then added to a solution of 1-chloro-3-iodopropane (**33**) (0.48 mL, 4.52 mmol, 1.05 equiv.) under nitrogen in 1.48 mL of DMSO. The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was then poured into ice water and was extracted with DCM (3x). The combined extracts were washed with water, dried over Na_2SO_4, filtered and concentrated under reduced pressure to a pink oil. The crude was purified by flash chromatography (eluent Hexane/DCM = 97/3 to 8/2), yielding 615 mg (58 %) of 2-chloro-10-(3-chloropropyl)-10*H*-phenothiazine (**40**) as a colorless oil. 119 mg of the starting material **39** were also isolated in a second fraction (brsm yield 66 %).

R_f (Hexane/DCM = 9/1) = 0.33; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 7.17 (2 H, m, H-2 and H-3), 7.05 (1 H, d, J = 9.0 Hz, H-5), 6.92 (4 H, m, H-4, H-8, H-9 and H-11), 4.06 (2 H, t, J = 6.9 Hz, H-13), 3.67 (2 H, t, J = 6.0 Hz, H-15), 2.24 (2 H, m, H-14). The analytical data matched the published data (Harrold *et al., J. Med. Chem.* **1987**, *30*, 1631-1635).

Compound **41** was obtained using the same conditions as for **14**, employing N^1-(7-chloroquinolin-4-yl)-N^2-ethylethane-1,2-diamine (**21a**) (209 mg, 0.84 mmol, 1.0 equiv.), (9H-fluoren-9-yl)methyl (3-oxopropyl)carbamate (**13**) (297 mg, 1.01 mmol, 1.2 equiv.), dry DCM (21 mL) and $NaBH(OAc)_3$ (356 mg, 1.68 mmol, 2.0 equiv.). The reaction mixture was stirred for 5 h and the crude compound was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 9/1), yielding 363 mg (82 %) of desired Fmoc-protected amine as a white foam.

R_f (DCM/MeOH = 9/1) = 0.35; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.49 (1 H, d, *J* = 5.3 Hz, H-3), 7.95 (1 H, s, H-8), 7.72 (3 H, m, H-5 and H-Fluorene), 7.54 (2 H, m, H-Fluorene), 7.32 (5 H, m, H-Fluorene and H-Ar), 6.34 (1 H, d, *J* = 5.3 Hz, H-2), 5.98 (1 H, bs, NH), 5.20 (1 H, bs, NH), 4.39 (2 H, d, *J* = 6.7 Hz, H-18), 4.16 (1 H, t, *J* = 6.7 Hz, H-19), 3.32 (4 H, m, H-10 and H-16), 2.79 (2 H, m, H-12), 2.56 (4 H, m, H-11 and H-14), 1.69 (2 H, m, H-15), 1.04 (3 H, t, *J* = 6.9 Hz, H-13); **MS** (ESI) m/z = 529.2 $[M+H]^+$; **HRMS** (ESI) m/z = 529.2364 $[M+H]^+$, calc.: 529.2365, Diff.: 0.2 ppm.

To a stirred solution of the Fmoc-protected amine (880 mg, 1.66 mmol, 1.0 equiv.) in dry DCM (33 mL), under nitrogen and at room temperature, was then added piperidine (0.82 mL, 8.30 mmol, 5.0 equiv.). The reaction mixture was stirred for 36 h and the solvent was removed. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 9/1 to 8/2 + 0.1 % TEA), yielding 437 mg (86 %) of the desired free amine **41** as a colourless oil.

R_f (DCM/MeOH = 9/1) = 0.35; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.50 (1 H, d, *J* = 5.3 Hz, H-3), 7.93 (1 H, d, *J* = 2.1 Hz, H-8), 7.70 (3 H, d, *J* = 8.8 Hz, H-5), 7.33 (1 H, dd, *J* = 8.8 Hz, *J* = 2.2 Hz, H-7), 6.35 (1 H, d, *J* = 5.3 Hz, H-2), 6.14 (1 H, bs, NH), 3.22 (2 H, m, H-10), 2.78 (4 H, m, H-12 and H-16), 2.59 (4 H, m, H-11 and H-14), 2.26 (2 H, bs, NH_2), 1.63 (2 H, m, H-15), 1.06 (3 H, t, *J* = 7.2 Hz, H-13); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 152.2 (C-3), 150.1 (C-1), 149.2 (C-4), 134.9 (C-6), 128.8 (C-5), 125.4 (C-8), 121.4 (C-7), 117.6 (C-9), 99.4 (C-2), 51.5 (C-11), 51.1 (C-14),

47.2 (C-12), 40.7 (C-10), 40.1 (C-16), 31.0 (C-15), 12.0 (C-13); **MS** (ESI) m/z = 307.2 $[M+H]^+$; **HRMS** (ESI) m/z = 307.1688 $[M+H]^+$, calc.: 307.1684, Diff.: 1.4 ppm.

A mixture of free amine **41** (64 mg, 0.21 mmol, 1.0 equiv.), 2-chloro-10-(3-chloropropyl)-10*H*-phenothiazine (**40**) (65 mg, 0.21mmol, 1.0 equiv.) and K_2CO_3 (29 mg, 0.21 mmol, 1.0 equiv.) in 4.2 mL MeOH, under nitrogen, was heated and stirred at reflux for 4 d. After cooling, the solvent was removed. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 9/1 to 8/2 + 0.1 % TEA), yielding 53 mg of the desired compound with major impurities. This was further purified by HPLC, yielding 14 mg (12 %) of N^1-(3-(2-chloro-10H-phenothiazin-10-yl)propyl)-N^3-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^3-ethylpropane-1,3-diamine (**42**) as a yellowish oil.

R_f (DCM/MeOH = 8/2) = 0.14; **^{1}H NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.53 (1 H, d, *J* = 5.1 Hz, H-3), 7.95 (1 H, d, *J* = 2.1 Hz, H-8), 7.68 (3 H, d, *J* = 8.8 Hz, H-5), 7.33 (1 H, dd, *J* = 8.8 Hz, *J* = 2.1 Hz, H-7), 7.11 (2 H, m, H-21 and H-22), 7.00 (1 H, d, *J* = 7.9 Hz, H-24), 6.87 (4 H, m, H-23, H-27, H-28 and H-30), 6.36 (1 H, d, *J* = 5.1 Hz, H-2), 6.11 (1 H, bs, NH), 3.82 (2 H, t, *J* = 6.8 Hz, H-19), 3.24 (2 H, m, H-10), 2.77 (2 H, t, *J* = 5.2 Hz, H-14), 2.55 (8 H, m, H-11, H-12, H-16 and H-17), 1.85 (2 H, m, H-18), 1.62 (2 H, m, H-15), 1.06 (3 H, t, *J* = 6.8 Hz, H-13); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 152.3 (C-3), 150.0 (C-1), 149.3 (C-4), 146.7 (C-31), 144.7 (C-20), 134.9 (C-6), 144.7 (C-29), 128.9 (C-5), 128.1 (C-22), 127.7 (C-24), 127.6 (C-27), 125.4 (C-8), 125.2 (C-25), 123.9 (C-26), 123.1 (C-23), 122.5 (C-28), 121.5 (C-7), 117.6 (C-9), 116.0 (C-21), 115.9 (C-30), 99.5 (C-2), 51.5 (C-11), 51.3 (C-14), 48.6 (C-12), 47.5 (C-19), 47.3 (C-10), 45.5 (C-16), 40.1 (C-17), 27.7 (C-15), 27.1 (C-18), 12.1 (C-13); **MS** (ESI) m/z = 580.1 $[M+H]^+$; **HRMS** (ESI) m/z = 580.2059 $[M+H]^+$, calc.: 580.2063, Diff.: 0.8 ppm.

Method A for the synthesis of hybrid compounds

*tert-butyl 3-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)piperidine-1-carboxylate (**27a**)*

Under inert conditions, *tert*-butyl piperidin-4-ylcarbamate (199 mg, 0.99 mmol, 1.0 equiv.) and 2-((7-chloroquinolin-4-yl)amino)ethyl methanesulfonate (**22a**) (298 mg, 0.99 mmol, 1.0 equiv.) were suspended in 4.97 mL THF in a microwave tube, then TEA (0.28 mL, 1.98 mmol, 2.0 equiv.) was added. The tube was sealed and the reaction mixture was heated in a microwave reactor at 120 °C for 4 h. The reaction mixture was poured into saturated K_2CO_3 (aq.) and extracted with DCM (3x). The united organic phase was concentrated under reduced pressure and the crude residue was purified by flash chromatography (eluent DCM/MeOH = 99/1 to 9/1), yielding 136 mg (34 %) of *tert*-butyl 3-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)piperidine-1-carboxylate (**27a**) as a yellow oil.

R_f (DCM/MeOH = 9/1) = 0.48; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.49 (1 H, d, *J* = 5.2 Hz, H-3), 7.93 (1 H, d, *J* = 1.8 Hz, H-8), 7.72 (1 H, d, *J* = 8.8 Hz, H-5), 7.34 (1 H, dd, *J* = 8.8 Hz, *J* = 1.8 Hz, H-7), 6.36 (1 H, d, *J* = 5.2 Hz, H-2), 6.01 (1 H, bs, NH), 3.90 (1 H, m, H-13b), 3.66 (1 H, m, H-13b), 3.32 (2 H, m, H-10), 3.07 (3 H, m, H-11 and NH), 2.89 (1 H, m, NH), 2.64 (1 H, m, H-12), 2.15 (2 H, m, H-14), 1.92 (1 H, m, H-15a), 1.69 (1 H, m, H-15b), 1.46 (11 H, bs, H-19 and H-16); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 155.1 (C-17), 151.8 (C-3), 150.2 (C-1), 148.8 (C-4), 135.1 (C-6), 128.5 (C-5), 125.5 (C-8), 121.6 (C-7), 117.4 (C-9), 99.3 (C-2), 79.8 (C-17), 53.4 (C-12), 48.9 (C-13), 45.0 (C-10), 44.9 (C-14), 44.2 (C-11), 31.6 (C-15), 28.4 (C-19), 23.5 (C-16); **MS** (ESI) m/z = 405.1 $[M+H]^+$; **HRMS** (ESI) m/z = 405.2067 $[M+H]^+$, calc.: 405.2052, Diff.: 3.7 ppm.

*tert-butyl 3-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)piperidine-1-carboxylate (**27b**)*

Compound **27b** was obtained by Method A, using the same conditions as for **27a**: employing *tert*-butyl piperidin-4-ylcarbamate (**7** in Part I) (199 mg, 0.99 mmol, 1.05 equiv.), 3-((7-chloroquinolin-4-yl)amino)propyl methanesulfonate (**22b**) (298 mg, 0.94 mmol, 1.0 equiv.) and TEA (0.28 mL,

1.98 mmol, 2.1 equiv.) in 4.95 mL THF. The reaction mixture was heated in a microwave reactor at 120 °C for 5 h and the crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 8/2), yielding a 136 mg fraction of the desired compound **27b** together with some unreacted starting material **7** (yield **27b**: 28 %, based on 86 w%, calculated with the ^{1}H-NMR). This was further purified by HPLC, yielding 40 mg of *tert*-butyl 3-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)piperidine-1-carboxylate (**27b**) as a yellowish oil.

R_f (DCM/MeOH = 9/1) = 0.48; **^{1}H-NMR** (400 MHz, MeOH-D_4) δ (ppm) = 8.43 (2 H, m, H-3 and H-8), 7.90 (1 H, m, H-5), 7.69 (1 H, m, H-7), 6.93 (1 H, m, H-2), 4.09 (1 H, m, H-14a), 3.73 (3 H, m, H-14b and H-15), 3.58 (1 H, m, NH), 3.42-2.95 (5 H, m, H-10, H-12 and NH), 2.22 (4 H, m, H-13, H-11 and H-16a), 1.69 (3 H, m, H-16b and H-17), 1.46 (9 H, bs, H-20); **^{13}C NMR** (400 MHz, MeOH-D_4) δ (ppm) = 163.0 (C-18), 157.8 (C-1), 144.0 (C-3), 141.1 (C-4), 140.1 (C-6), 128.8 (C-5), 126.1 (C-8), 120.3 (C-7), 117.1 (C-9), 99.8 (C-2), 81.9 (C-19), 53.1 (C-13), 44.9 (C-14), 44.7 (C-10), 44.1 (C-15), 43.9 (C-12), 41.7 (C-17), 28.5 (C-20), 26.0 (C-11), 21.5 (C-16); **MS** (ESI) m/z = 419.2 $[M+H]^+$; **HRMS** (ESI) m/z = 419.2218 $[M+H]^+$, calc.: 419.2208, Diff.: 2.4 ppm.

*tert-butyl 3-((3-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)propyl) (2-iodobenzyl)amino)-piperidine-1-carboxylate (**28**)*

Compound **28** was obtained by Method A, using the same conditions as for **27a**: employing crude *tert*-butyl 3-((3-aminopropyl)(2-iodobenzyl)amino)piperidine-1-carboxylate (**15**) (79 mg, 0.11 mmol, 1.0 equiv.), 3-((7-chloroquinolin-4-yl)amino)ethyl methanesulfonate (**22a**) (33 mg, 0.11 mmol, 1.0 equiv.) and TEA (30 µL, 0.22 mmol, 2.0 equiv.) in 2.20 mL THF. The reaction mixture was heated in a microwave reactor at 120 °C for 5 h and the crude residue was purified by HPLC, yielding 15 mg (21 % over 2 steps) of *tert*-butyl 3-((3-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)propyl) (2-iodobenzyl)amino)-piperidine-1-carboxylate (**28**) as a yellowish oil.

^{1}H NMR (400 MHz, MeOH-D_4) δ (ppm) = 8.49 (1 H, d, *J* = 6.9 Hz, H-3), 8.43 (1 H, d, *J* = 9.0 Hz, H-8), 7.93 (2 H, m, H-26 and H-5), 7.71 (1 H, m, H-7), 7.61 (1 H, m, H-28), 7.46 (1 H, m, H-27), 7.13 (1 H, m, H-29), 6.99 (1 H, d, *J* = 6.9 Hz, H-12), 4.31 (3 H, m, H-16 and H-17a), 3.99 (3 H, m, H-23 and H-17b), 3.44 (2 H, m, H-10), 3.14 (4 H, m, H-11 and H-12), 2.24 (1 H, m, H-15), 2.11 (2 H, m, H-19), 1.83 (2 H, m, H-18), 1.42 (11 H, bs, H-22 and H-13); **MS** (ESI) m/z = 678.1 $[M+H]^+$; **HRMS** (ESI) m/z = 678.2093 $[M+H]^+$, calc.: 678.2066, Diff.: 3.9 ppm.

*N^1-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^3-(2,2-diphenylethyl)-N^3-(2-iodobenzyl)propane-1,3-diamine (**29**)*

Compound **29** was obtained by Method A, using the same conditions as for **27a**: employing N^1-(2,2-diphenylethyl)-N^1-(2-iodobenzyl)propane-1,3-diamine (**17**) (48 mg, 0.11 mmol, 1.0 equiv.), 3-((7-chloroquinolin-4-yl)amino)ethyl methanesulfonate (**22a**) (50 mg, 0.16 mmol, 1.5 equiv.) and TEA (30 µL, 0.22 mmol, 2.0 equiv.) in 2.10 mL THF. The reaction mixture was heated in a microwave reactor at 120 °C for 10 h and the crude residue was purified by HPLC, yielding 24 mg (34 %) of N^1-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^3-(2,2-diphenylethyl)-N^3-(2-iodobenzyl)-propane-1,3-diamine (**29**) as a yellowish oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.53 (1 H, d, *J* = 5.4 Hz, H-3), 7.94 (1 H, d, *J* = 2.1 Hz, H-8), 7.71 (1 H, dd, *J* = 7.9 Hz, *J* = 1.0 Hz,H-26), 7.58 (1 H, d, *J* = 8.9 Hz, H-5), 7.34 (1 H, dd, *J* = 8.9 Hz, *J* = 2.1 Hz, H-7), 7.24 (4 H, m, H-Ar), 7.17 (7 H, m, H-Ar), 6.98 (2 H, m, H-Ar), 6.34 (1 H, d, *J* = 5.4 Hz, H-2), 5.79 (1 H, bs, NH), 4.16 (1 H, m, H-16), 3.64 (2 H, s, H-23), 3.15 (4 H, m, H-15 and H-10), 2.83 (2 H, t, *J* = 5.7 Hz, H-11), 2.62 (2 H, t, *J* = 6.5 Hz, H-14), 2.50 (2 H, t, *J* = 6.5 Hz, H-12), 1.63 (3 H, m, H-13 and NH); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 151.2 (C-3), 150.0 (C-1), 149.3 (C-4), 143.6 (C-17), 141.5 (C-24), 139.3 (C-26), 134.9 (C-6), 130.5 (C-28), 128.9 (C-5), 128.7 (C-27), 128.5 (C-19), 128.4 (C-18), 128.0 (C-8), 126.5 (C-20), 125.4 (C-29), 121.4 (C-7),

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117.5 (C-9), 100.3 (C-25), 99.3 (C-2), 63.7 (C-14), 60.3 (C-15), 52.8 (C-23), 49.8 (C-16), 47.9 (C-10), 47.5 (C-11), 41.9 (C-15), 27.4 (C-13); **MS** (ESI) m/z = 675.3 $[M+H]^+$; **HRMS** (ESI) m/z = 675.1760 $[M+H]^+$, calc.: 675.1746, Diff.: 2.2 ppm.

N^1-(7-chloroquinolin-4-yl)-N^2-(2,2-diphenylethyl)ethane-1,2-diamine ***(30)***

Compound **30** was obtained by Method A, using the same conditions as for **27a**: employing 2,2-diphenylethan-1-amine (**9** in Part I) (145 mg, 0.48 mmol, 1.0 equiv.), 3-((7-chloroquinolin-4-yl)amino)ethyl methanesulfonate (**22a**) (95 mg, 0.48 mmol, 1.0 equiv.) and TEA (0.13 mL, 0.96 mmol, 2.0 equiv.) in 4.80 mL THF. The reaction mixture was heated in a microwave reactor at 120 °C for 7 h and the crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 8/2), yielding a 80 mg (42 %) of N^1-(7-chloroquinolin-4-yl)-N^2-(2,2-diphenylethyl)ethane-1,2-diamine (**30**) as a yellowish oil.

R_f (DCM/MeOH = 9/1) = 0.44; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.49 (1 H, d, *J* = 5.1 Hz, H-3), 7.93 (1 H, d, *J* = 1.7 Hz, H-8), 7.24 (10 H, m, H-Ar), 6.31 (1 H, d, *J* = 5.1 Hz, H-2), 5.76 (1 H, bs, NH), 4.17 (1 H, t, *J* = 8.1 Hz, H-16), 3.32 (2 H, d, *J* = 8.1 Hz, H-12), 3.26 (2 H, m, H-10), 3.05 (2 H, m, H-11), 1.58 (1 H, bs, NH); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 151.8 (C-3), 150.1 (C-1), 148.8 (C-4), 142.8 (C-14), 135.1 (C-6), 128.8 (C-16), 128.4 (C-5), 128.1 (C-15), 126.8 (C-17), 125.4 (C-8), 117.4 (C-9), 99.2 (C-2), 53.8 (C-12), 51.5 (C-13), 47.3 (C-10), 47.8 (C-11); **MS** (ESI) m/z = 402.2 $[M+H]^+$; **HRMS** (ESI) m/z = 402.1746 $[M+H]^+$, calc.: 402.1732, Diff.: 3.6 ppm.

7-chloro-N-(2-(4-(10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazin-1-yl)ethyl)quinolin-4-amine ***(31)***

Compound **31** was obtained by Method A, using the same conditions as for **27a**: employing 1-(10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine (56 mg, 0.19 mmol, 1.0 equiv.), 2-((7-chloroquinolin-4-yl)amino)ethyl methanesulfonate (**22a**) (57 mg, 0.19 mmol, 1.0 equiv.) and TEA

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(53 µL, 0.38 mmol, 2.0 equiv.) in 1.90 mL THF; the mixture was heated in a microwave reactor at 120 °C for 7 h and the crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 9/1). Thus were obtained 21 mg of the hybrid compound **31** as a fluffy yellow solid as well as 26 mg of a second impure fraction (**31**/impurity ~ 4/1, overall yield ~44 %).

R_f (DCM/MeOH = 9/1) = 0.17; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.53 (1 H, d, *J* = 5.3 Hz, H-3), 7.96 (1 H, d, *J* = 2.1 Hz, H-8), 7.72 (1 H, d, *J* = 8.5 Hz, H-5), 7.62 (1 H, dd, *J* = 7.9 Hz, *J* = 1.4 Hz, H-23), 7.52 (1 H, dd, *J* = 7.7 Hz, *J* = 1.2 Hz, H-7), 7.40 (2 H, m, H-17 and H-18), 7.22 (3 H, m, H-25, H-24 and H-22), 7.10 (2 H, m, H-16 and H-19), 6.37 (1 H, d, *J* = 5.3 Hz, H-2), 5.98 (1 H, bs, NH), 4.05 (1 H, m, H-14), 3.91 (1 H, m, H-27a), 3.30 (2 H, m, H-10), 3.21 (1 H, m, H-27b), 2.73 (6 H, m, H-13 and H-11), 2.54 (4 H, m, H-12); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 152.2 (C-3), 150.0 (C-1), 149.2 (C-4), 142.3 (C-20), 140.3 (C-21), 137.1 (C-26), 135.4 (C-6), 137.1 (C-15), 132.6 (C-23), 131.6 (C-18), 131.4 (C-25), 129.7 (C-24), 128.9 (C-16), 128.6 (C-17), 127.2 (C-5), 126.9 (C-19), 126.5 (C-22), 125.5 (C-8), 121.3 (C-7), 117.5 (C-9), 99.4 (C-2), 65.9 (C-14), 55.7 (C-11), 53.4 (C-12), 50.0 (C-13), 39.1 (C-10), 33.6 (C-27); **MS** (ESI) m/z = 501.1 $[M+H]^+$; **HRMS** (ESI) m/z = 501.1885 $[M+H]^+$, calc.: 501.1874, Diff.: 2.1 ppm.

*7-chloro-N-(2-(4-(8-chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazin-1-yl)ethyl)quinolin-4-amine (**32**)*

Compound **32** was obtained by Method A, using the same conditions as for **27a**: employing 1-(8-chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine (36 mg, 0.12 mmol, 1.0 equiv.), 2-((7-chloroquinolin-4-yl)amino)ethyl methanesulfonate (**22a**) (36 mg, 0.12 mmol, 1.0 equiv.) and TEA (33 µL, 0.24 mmol, 2.0 equiv.) in 2.40 mL THF; the mixture was heated in a microwave reactor at 120 °C for 7 h and the crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 9/1), yielding a 37 mg (58 %). of hybrid compound **32** as a colourless oil.

R_f (DCM/MeOH = 9/1) = 0.55; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.53 (1 H, d, *J* = 5.4 Hz, H-3), 7.97 (1 H, d, *J* = 2.1 Hz, H-8), 7.69 (2 H, m, H-22 and H-5), 7.51 (1 H, d, *J* = 7.3 Hz, H-23), 7.52 (1 H, dd, *J* = 8.9 Hz, *J* = 2.1 Hz, H-7), 7.35 (1 H, d, *J* = 7.9 Hz, H-18), 7.27 (2 H, m, H-25 and H-24), 7.09 (2 H, m, H-16 and H-19), 6.38 (1 H, d, *J* = 5.3 Hz, H-2), 5.99 (1 H, bs, NH), 3.92 (2 H, m, H-14 and H-27a), 3.32 (2 H, m, H-10), 3.18 (1 H, m, H-27b), 2.77 (6 H, m, H-13 and H-11), 2.77 (4 H, m, H-12); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 152.1 (C-3), 150.0 (C-1), 149.1 (C-4), 142.3 (C-20), 142.2 (C-21), 136.4 (C-26), 135.1 (C-6), 133.9 (C-15), 133.2 (C-17), 132.9 (C-23), 132.2 (C-18), 131.5 (C-25), 129.7 (C-24), 129.0 (C-16), 128.8 (C-5), 127.0 (C-19), 126.7 (C-22), 125.5 (C-8), 121.3 (C-7), 117.5 (C-9), 99.4 (C-2), 65.6 (C-14), 55.6 (C-11), 53.3 (C-12), 48.9 (C-13), 39.2 (C-10), 32.8 (C-27); **MS** (ESI) m/z = 535.1 $[M+H]^+$; **HRMS** (ESI) m/z = 535.1484 $[M+H]^+$, calc.: 535.1484, Diff.: 0.2 ppm.

Method B for the synthesis of hybrid compounds

*N^1-(3-chloropropyl)-N^2-(7-chloroquinolin-4-yl)-N^1-ethylethane-1,2-diamine (**34**)*

To a solution of N^1-(7-chloroquinolin-4-yl)-N^2-ethylethane-1,2-diamine (**21a**) (580 mg, 2.32 mmol, 1.0 equiv.) and K_2CO_3 (962 mg, 6.96 mmol, 3.0 equiv.) in dry MeCN (42 mL), under inert conditions, was added 1-chloro-3-iodopropane (**33**) (0.50 mL, 4.64 mmol, 2.0 equiv.). After stirring at room temperature for 4 d, the reaction was quenched with water and extracted with DCM (3x). The combined organic phase was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (eluent DCM/MeOH = 97/3 to 9/1), yielding 383 mg (51 %) of N^1-(3-chloropropyl)-N^2-(7-chloroquinolin-4-yl)-N^1-ethylethane-1,2-diamine (**34**) as an off-white solid.

R_f (DCM/MeOH = 9/1) = 0.38; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.53 (1 H, d, *J* = 5.3 Hz, H-3), 7.96 (1 H, d, *J* = 2.1 Hz, H-8), 7.65 (1 H, d, *J* = 9.0 Hz, H-5), 7.38 (1 H, dd, *J* = 9.0 Hz,

J = 2.1 Hz, H-7), 6.38 (1 H, d, J = 5.3 Hz, H-2), 5.90 (1 H, bs, NH), 3.61 (2 H, t, J = 6.2 Hz, H-10), 3.29 (2 H, q, J = 5.4 Hz, H-12), 2.84 (2 H, t, J = 5.7 Hz, H-11), 2.70 (2 H, t, J = 7.0 Hz, H-12), 2.61 (2 H, q, J = 7.0 Hz, H-16), 1.95 (2 H, m, H-15), 1.08 (3 H, t, J = 7.1 Hz, H-13); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 152.2 (C-3), 150.0 (C-1), 149.2 (C-4), 135.0 (C-6), 128.9 (C-5), 125.5 (C-8), 121.1 (C-9), 117.5 (C-7), 99.5 (C-2), 51.6 (C-11), 50.0 (C-14), 46.9 (C-12), 43.0 (C-10), 40.0 (C-16), 30.2 (C-15), 11.9 (C-13); **MS** (ESI) m/z = 325.9 $[M+H]^+$.

*N^1-benzyl-N^1-((2R,3R)-2-benzylquinuclidin-3-yl)-N^3-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^3-ethylpropane-1,3-diamine (**35**)*

Method B: Under inert conditions, N^1-(3-chloropropyl)-N^2-(7-chloroquinolin-4-yl)-N^1-ethylethane-1,2-diamine (**34**) (45 mg, 0.14 mmol, 1.0 equiv.) and **4** (42 mg, 0.14 mmol, 1.0 equiv.) were suspended in 1.40 mL MeCN in a microwave tube, then K_2CO_3 (39 mg, 0.28 mmol, 2.0 equiv.) and NaI (4 mg, 0.03 mmol, 0.2 equiv.) were added. The tube was sealed and the reaction mixture was heated in a microwave reactor at 100 °C for 90 min. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography (eluent DCM/MeOH = 95/5 to 85/15 + 0.1 % TEA), yielding 35 mg of a fraction with major TEA impurities. This was dissolved in DCM and stirred with Amberlyst 21 for 1 H, filtered and the solvent was removed under reduced pressure. Thus were obtained 19 mg (22 %) of hybrid compound **35** as a colourless oil (with minor impurities).

R_f (DCM/MeOH = 9/1) = 0.11; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.52 (1 H, d, J = 5.2 Hz, H-3), 7.94 (1 H, d, J = 2.1 Hz, H-8), 7.85 (1 H, d, J = 8.8 Hz, H-5), 7.37 (1 H, dd, J = 8.8 Hz, J = 2.1 Hz, H-7), 7.25 (8 H, m, H-Ph), 7.00 (2 H, m, H-Ph), 6.37 (1 H, d, J = 5.2 Hz, H-2), 5.91 (1 H, bs, NH), 3.86 (2 H, t, J = 5.4 Hz, H-11), 3.73 (1 H, d, J = 13.0 Hz, H-27a), 3.43 (4 H, m, H-17, H27b and H-16), 3.22 (2H, m, H-10), 3.01 (3 H, m, H-22a and H-21a), 2.84 (4 H, m, H-18, H-22b and H-21b), 2.74 (2 H, t, J = 6.0 Hz, H-14), 2.62 (2 H, q, J = 7.1 Hz, H-12), 1.74 (3 H, m, H-15 and H-19),

1.56 (2 H, m, H-20a), 1.40 (2 H, m, H-20b), 1.07 (3 H, t, J = 7.1 Hz, H-13); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 151.9 (C-3), 149.8 (C-1), 149.1 (C-4), 140.0 (C-28), 139.2 (C-23), 134.9 (C-6), 128.7 (C-24), 128.6 (C-25), 128.3 (C-30), 127.8 (C-29), 126.9 (C-31), 126.2 (C-26), 125.4 (C-7), 125.4 (C-8), 121.6 (C-5), 117.5 (C-9), 98.9 (C-2), 63.9 (C-22), 60.2 (C-17), 54.8 (C-18), 53.4 (C-11), 52.1 (C-14), 51.9 (C-16), 48.9 (C-12), 47.9 (C-10), 41.9 (C-21), 40.6 (C-21), 33.3 (C-22), 28.3 (C-20), 24.6 (C-20), 24.5 (C-19), 19.2 (C-15), 11.7 (C-13); **MS** (ESI) m/z = 596.4 $[M+H]^+$; **HRMS** (ESI) m/z = 596.3527 $[M+H]^+$, calc.: 596.3515, Diff.: 2.1 ppm.

*tert-butyl 3-((3-((2-((7-chloroquinolin-4-yl)amino)ethyl)(ethyl)amino)propyl)amino)piperidine-1-carboxylate (**36**)*

Compound **36** was obtained by Method B, using the same conditions as for **35**: employing N^1-(3-chloropropyl)-N^2-(7-chloroquinolin-4-yl)-N^1-ethylethane-1,2-diamine (**34**) (102 mg, 0.31 mmol, 1.0 equiv.), *tert*-butyl piperidin-4-ylcarbamate (63 mg, 0.31 mmol, 1.0 equiv.), K_2CO_3 (86 mg, 0.63 mmol, 2.0 equiv.) and NaI (9 mg, 0.06 mmol, 0.2 equiv.) in 3.13 mL MeCN. The mixture was heated in a microwave reactor at 100 °C for 90 min. After flash chromatography (eluent DCM/MeOH = 95/5 to 8/2 + 0.1 % TEA) and treatment with Amberlyst 21 were thus obtained 81 mg (44 %) of the hybrid compound **36** as a fluffy yellowish solid with some minor impurities.

R_f (DCM/MeOH = 9/1) = 0.36; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.52 (1 H, d, J = 5.3 Hz, H-3), 7.94 (1 H, d, J = 2.1 Hz, H-8), 7.70 (1 H, d, J = 8.8 Hz, H-5), 7.36 (1 H, dd, J = 8.8 Hz, J = 2.1 Hz, H-7), 6.36 (1 H, d, J = 5.3 Hz, H-2), 6.08 (1 H, bs, NH), 3.88 (3 H, m, H-18 and NH), 3.26 (2 H, m, H-10), 2.64 (11 H, m, H-21, H-17, H-11, H-12, H-14 and H-16), 1.65 (6 H, m, H-19, H-20 and H-15), 1.43 (9 H, s, H-24), 1.07 (3 H, t, J = 13.8 Hz, H-13); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 154.0 (C-22), 151.6 (C-3), 150.5 (C-1), 148.7 (C-4), 135.3 (C-6), 128.2 (C-7), 125.6 (C-8), 122.2 (C-5), 117.6 (C-9), 99.2 (C-2), 80.0 (C-23), 54.0 (C-17), 52.3 (C-11), 51.3 (C-18), 47.8 (C-21),

47.5 (C-14), 46.1 (C-10), 46.0 (C-16), 40.3 (C-12), 28.6 (C-15), 28.6 (C-24), 26.4 (C-19), 23.5 (C-20), 9.3 (C-13); **MS** (ESI) m/z = 490.2 $[M+H]^+$; **HRMS** (ESI) m/z = 490.2939 $[M+H]^+$, calc.: 490.2943, Diff.: 0.9 ppm.

*N^1-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^3-(2,2-diphenylethyl)-N^1-ethylpropane-1,3-diamine (**37**)*

Under inert conditions, N^1-(3-chloropropyl)-N^2-(7-chloroquinolin-4-yl)-N^1-ethylethane-1,2-diamine (**34**) (58 mg, 0.18 mmol, 1.0 equiv.) and 2,2-diphenylethylamine (**9**) (35 mg, 0.18 mmol, 1.0 equiv.) were suspended in 1.80 mL MeCN in a microwave tube, then K_2CO_3 (50 mg, 0.36 mmol, 2.0 equiv.) was added. The tube was sealed and the reaction mixture was heated in a microwave reactor at 100 °C for 90 min. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography (solid deposit, eluent DCM/MeOH = 95/5 to 85/15), yielding 40 mg (46 %) of N^1-(2-((7-chloroquinolin-4-yl)amino)ethyl)-N^3-(2,2-diphenylethyl)-N^1-ethylpropane-1,3-diamine (**37**) as a colourless oil. Two additional fractions were also obtained: 15 mg (26 %) of chloride **34** as well as 24 mg of a mixture of **34** and 2,2-diphenylethylamine (**9**).

R_f (DCM/MeOH = 9/1) = 0.28; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.53 (1 H, d, *J* = 5.7 Hz, H-3), 7.95 (1 H, d, *J* = 2.1 Hz, H-8), 7.76 (1 H, d, *J* = 8.8 Hz, H-5), 7.32 (1 H, dd, *J* = 8.8 Hz, *J* = 2.1 Hz, H-7), 7.26 (4 H, m, H-Ph), 7.16 (6 H, m, H-Ph), 6.35 (1 H, d, *J* = 5.7 Hz, H-2), 6.25 (1 H, bs, NH), 4.10 (1 H, t, *J* = 7.7 Hz, H-18), 3.18 (4 H, m, H-14 and H-10), 2.74 (4 H, m, H-17 and H-12), 2.48 (4 H, m, H-16 and H-11), 2.03 (1 H, bs, NH), 1.66 (2 H, m, H-15), 0.96 (3 H, t, *J* = 7.1 Hz, H-13); **MS** (ESI) m/z = 487.2 $[M+H]^+$; **HRMS** (ESI) m/z = 487.2644 $[M+H]^+$, calc.: 487.2623, Diff.: 4.2 ppm.

*N^1-(3-(4-(8-chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazin-1-yl)propyl)-N^2-(7-chloroquinolin-4-yl)-N^1-ethylethane-1,2-diamine (**38a**)*

Compound **38a** was obtained by Method B, using the same conditions as for **35**: employing N^1-(3-chloropropyl)-N^2-(7-chloroquinolin-4-yl)-N^1-ethylethane-1,2-diamine (**34**) (32 mg, 0.10 mmol, 1.0 equiv.), 1-(10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine (30 mg, 0.10 mmol, 1.0 equiv.), K_2CO_3 (27 mg, 0.20 mmol, 2.0 equiv.) and NaI (3 mg, 0.02 mmol, 0.2 equiv.) in 1.00 mL MeCN. The mixture was heated in a microwave reactor at 100 °C for 3 h. After flash chromatography (eluent DCM/MeOH = 95/5 to 8/2 + 0.1 % TEA) were thus obtained 36 mg (62 %) of the hybrid compound **38a** as a fluffy yellowish solid with some TEA impurities.

R_f (DCM/MeOH = 8/2) = 0.69; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.52 (1 H, d, *J* = 5.4 Hz, H-3), 7.94 (1 H, d, *J* = 2.1 Hz, H-8), 7.67 (1 H, d, *J* = 8.8 Hz, H-5), 7.58 (1 H, dd, *J* = 7.6 Hz, *J* = 0.9 Hz, H-28), 7.58 (1 H, dd, *J* = 7.6 Hz, *J* = 1.1 Hz, H-23), 7.37 (2 H, m, H-7 and H-30), 7.22 (2 H, m, H-29 and H-27), 7.09 (3 H, m, H-21, H-22 and H-24), 6.37 (1 H, d, *J* = 5.4 Hz, H-2), 6.06 (1 H, bs, NH), 3.94 (1 H, m, H-19), 3.84 (1 H, m, H-32a), 3.23 (2 H, m, H-10), 3.13 (1 H, m, H-32b), 2.81 (6 H, m, H-12, H-17 and H-18), 2.61 (4 H, m, H-17 and H-18), 2.53 (2 H, m, H-11), 2.34 (4 H, m, H-14 and H-16), 1.67 (2 H, m, H-15), 1.06 (1 H, t, *J* = 7.1 Hz, H-13); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 152.2 (C-3), 150.0 (C-1), 149.2 (C-4), 142.6 (C-25), 140.3 (C-26), 137.0 (C-31), 135.2 (C-6), 134.9 (C-20), 132.6 (C-28), 131.5 (C-23), 131.3 (C-30), 129.6 (C-29), 128.8 (C-21), 128.6 (C-22), 127.1 (C-5), 126.8 (C-24), 126.3 (C-27), 125.4 (C-8), 121.3 (C-7), 117.5 (C-9), 99.4 (C-2), 65.8 (C-19), 56.7 (C-10), 54.0 (C-11), 51.4 (C-14), 51.0 (C-16), 48.5 (C-17), 47.1 (C-12), 46.1 (C-18), 39.1 (C-10), 33.3 (C-32), 24.7 (C-15), 10.0 (C-13); **MS** (ESI) m/z = 586.2 $[M+H]^+$; **HRMS** (ESI) m/z = 586.2777 $[M+H]^+$, calc.: 586.2766, Diff.: 1.9 ppm.

*N^1-(7-chloroquinolin-4-yl)-N^2-(3-(4-(10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazin-1-yl)propyl)-N^2-ethylethane-1,2-diamine (**38b**)*

Compound **38b** was obtained by Method B, using the same conditions as for **35**: employing N^1-(3-chloropropyl)-N^2-(7-chloroquinolin-4-yl)-N^1-ethylethane-1,2-diamine (**34**) (36 mg, 0.11 mmol, 1.0 equiv.), 1-(8-chloro-10,11-dihydrodibenzo[b,f]thiepin-10-yl)piperazine (34 mg, 0.11 mmol, 1.0 equiv.), K_2CO_3 (30 mg, 0.22 mmol, 2.0 equiv.) and NaI (3 mg, 0.02 mmol, 0.2 equiv.) in 1.10 mL MeCN. The mixture was heated in a microwave reactor at 100 °C for 90 min. After flash chromatography (eluent DCM/MeOH = 95/5 to 8/2 + 0.1 % TEA) and treatment with Amberlyst 21 were thus obtained 52 mg (75 %) of the hybrid compound **38b** as a yellowish oil.

R_f (DCM/MeOH = 8/2 + 0.1 % TEA) = 0.57; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) = 8.54 (1 H, d, *J* = 5.2 Hz, H-3), 7.96 (1 H, d, *J* = 2.1 Hz, H-8), 7.64 (2 H, m, H-5 and H-28), 7.49 (1 H, m, H-23), 7.37 (1 H, dd, *J* = 8.8 Hz, *J* = 2.1 Hz, H-7), 7.33 (1 H, d, *J* = 8.3 Hz, H-30), 7.22 (2 H, m, H-29 and H-27), 7.10 (1 H, m, H-21), 7.03 (1 H, m, H-24), 6.38 (1 H, d, *J* = 5.2 Hz, H-2), 6.03 (1 H, bs, NH), 3.87 (2 H, m, H-19 and H-32a), 3.27 (2 H, m, H-10), 3.12 (1 H, m, H-32b), 2.88 (2 H, t, *J* = 5.5 Hz, H-11), 2.89 (8 H, m, H-12, H-14, H-17 and H-18), 2.37 (4 H, m, H-16, H-17 and H-18), 1.68 (2 H, m, H-15), 1.08 (1 H, t, *J* = 14.2 Hz, H-13); **^{13}C NMR** (400 MHz, $CDCl_3$) δ (ppm) = 152.3 (C-3), 150.0 (C-1), 149.3 (C-4), 142.5 (C-25), 142.3 (C-22), 136.4 (C-26), 154.0 (C-31), 133.8 (C-6), 133.2 (C-20), 132.8 (C-28), 132.2 (C-23), 131.4 (C-30), 129.7 (C-29), 129.0 (C-21), 129.0 (C-5), 127.0 (C-24), 126.6 (C-27), 125.4 (C-8), 121.3 (C-7), 117.6 (C-9), 99.5 (C-2), 65.6 (C-19), 56.7 (C-10), 53.9 (C-11), 51.4 (C-14), 51.0 (C-16), 48.7 (C-17), 47.1 (C-12), 46.1 (C-18), 39.9 (C-10), 32.8 (C-32), 24.8 (C-15), 12.1 (C-13); **MS** (ESI) m/z = 620.2 $[M+H]^+$; **HRMS** (ESI) m/z = 620.2377 $[M+H]^+$, calc.: 620.2376, Diff.: 0.2 ppm.

Biology

Compound preparation

Synthesized compounds for biological testing were diluted in dimethylsulfoxide (DMSO) to create stock solutions that were stored in aliquots in the dark at −20 ºC. All other drugs were purchased from Sigma-Aldrich including chloroquine diphosphate, desipramine hydrochloride, loperamide hydrochloride and L703-606 oxalate salt hydrate. 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.

Inhibitory concentration (IC_{50}) determination for hybrid compounds

Parasitized cultures, synchronized to rings stage and diluted with fresh erythrocytes and MCM to 1% parasitemia and 1.25% haematocrit, were incubated with various concentrations of hybrid compounds for 48 h. Appropriate controls consist of vehicle control (PBS or DMSO), CQ and CQ in combination with known chemosensitizer. To determine parasitemia, cultures were stained with Hoechst 33342 and analyzed by flow cytometry. Data obtained from flow analysis were used to plot the sigmoidal dose response curve (i.e. % parasitemia against concentration). The IC_{50} values, which were the concentrations required to inhibit 50% growth could then be determined from the plot.

Hoechst staining

Hoechst 33342 stain (Hoechst, Life Technologies) is a DNA-binding fluorescent stain that has an excitation wavelength of 350 nm (ultraviolet range) and an emission wavelength of 461 nm (blue fluorescence). After drug treatment, 1 µg/mL of Hoechst stain was added per well for 30 min at 37 °C. Cells were then washed twice and re-suspended in PBS before flow cytometry analysis.

Flow cytometric analyses

Flow cytometry (DAKO Cytomation Cyan ADP, Fort Collins, CO, USA) was used for Hoechst-stained cells. Both were excited with a 405 nm violet laser prior to 450/50BP(±25) filter. For the determination of the proportion of CM-CQ (9) 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. At least three independent experiments were performed, unless otherwise stated, to take into account any inter-assay variability. All results are saved in FCS 2.0 format and analyzed using the Flowjo version X (Tree Star) software.

Determination of the digestive vacuole disruption potency of the hybrid compounds

Parasitized cultures, synchronized to trophozoite stage and diluted with fresh erythrocytes and MCM to 8-10% parasitemia and 2.5% haematocrit, were incubated with either 10 µM or 1 µM of hybrid compounds for 4 h. Appropriate controls consist of vehicle control (PBS with 0.1% DMSO) and 3 µM of CQ with 0.1% DMSO. After drug treatment, 1 µg/mL of Hoechst stain and 1 µM of Flu0-4 AM (Molecular Probes) diluted in MCM were added per well for 30 min at 37 °C. Cells were then washed twice and re-suspended in PBS to 5% hematocrit before Imagestream X MkII (AMNIS) analysis. Four independent experiments were performed to take into account any inter-assay variability.

Graphical data plots

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

Quantitative analysis

All assays were done independently at least three times to account for inter-assay variability. After which, statistical analyses were conducted on the data obtained before making conclusive remarks.

Statistical analysis

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

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 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 the NUS was in accordance with the NUS IRB (reference code 12-369E).

RESULTS AND DISCUSSION

Synthesis of hybrid compounds

Simplified analogs of CRA hit compounds **4**-**6** were used as precursor starting materials for hybrid compound synthesis (compound **7**-**12**, Fig. 4). For compounds **8** and **10**, a linker was attached in order to study the influence of the distance of the two attached scaffolds (Fig. 5). After optimization to

identify the most suitable protecting group,[1] the Fmoc-protected C3 linker **13** could be installed onto CRAs **8** and **10** *via* reductive amination in good yield. Fmoc deprotection with piperidine yielded the free amines **15** and **17**.

Preserving only the key elements of the 4-amino-quinoline scaffold, analogues of **1**, **19** and **20**, were obtained by refluxing of the neat amines with 4,7-dichloroquinoline (**18**, Fig. 6) (16-18). Alcohols **21** were transformed to mesylates **22**, possessing better leaving groups for subsequent substitution (16-18). New hybrid compounds were obtained by reacting amines **6**, **7**, **9**, **12**, **15** and **17** with **22** *via* a microwave mediated[2] displacement reaction (Fig. 7). This afforded access to hybrid compounds with features of various CRAs in moderate yields: **27a**, **27b**, **28**, **29**, and **30** from analogues of compound **4**, as well as **31** and **32** derived from **6**.

In order to explore the structural features necessary to retain chemoreversal activity in the hybrid compounds, some analogues linking relatively simple precursors of the CRAs were synthesized. Joining quinuclidinone **23** *via* reductive amination to amine **19c**, the hybrid **24** - with only the quinuclidine core of **4** attached to the CQ scaffold - was obtained albeit in very poor yield (Fig. 8). Substitution of bromide **25** with amine **21a** gave hybrid **26**, possessing the *gem*-diphenylbutanenitrile feature of compound **5** (Fig. 9).

A further series of hybrid compounds retaining the tertiary ethylamine from **1** was prepared from intermediate **21a**. It has been shown previously that a tertiary amine in this position impacts the activity of CQ analogues as it influences the diffusion of the compounds in the digestive vacuole (20, 21). Ethyl amine **21a** was first connected to a C3 linker, using chloro-iodo-propane (**33**), leading to

[1] Boc and Cbz groups were tested for C2 and C3 linkers, but failed due to difficult deprotection.

[2] Reaction times with thermal heating were 7 days or more, this could be significantly reduced to 4- 10 hours by microwave induced heating.

chloro-propylamine **34** (Fig. 10). Chloride **34** was then attached to various amines *via* a microwave mediated Finkelstein reaction, yielding hybrid compounds in moderate to good yields: **35**, **36**, and **37** with features of **4**, as well as **38a** and **38b** with features of CRA **6**.

Additionally, a hybrid compound combining **3** with the scaffold of known chemosensitizer chlorpromazine was synthesized. Phenothiazine (**39**) was transformed to chloride **40** using chloro-iodopropane (**33**) in moderate yield (22). Chloroquine fragment **21a** was attached to a C3 linker with the same method used previously, yielding primary amine **41**. Chloride **40** then underwent a substitution with amine **41**, giving hybrid compound **42** in moderate yield (Fig. 11).

Anti-parasitic testing in CQ/Art sensitive and resistant strains

Mechanisms of drug-resistance in malaria depend on the specific drug and are not yet completely understood. In the case of chloroquine (**1**) resistance (CQR) it originates in mutations in the PfCRT (*P. falciparum* chloroquine resistance transporter) (23, 24). The anti-malarial mechanism of action of **1** is to hinder heme detoxification of the parasite in the digestive vacuole. In resistant strains the modified transporters are able to remove **1** from this vacuole. The mutated PfCRT have altered amino acids in positions 72-76 (CVMNK in the wild type), depending on the geographical origin: CVIET for African and most South-east Asian resistant strains versus SVMNT for the South American resistant strains (25, 26). The hybrid compounds were initially tested in an IC_{50} assay, determining the concentration required to inhibit the survival of 50% of parasites. Chloroquine sensitive (CQS) parasite strain 3D7, and resistant (CQR) strain of CVIET haplotype, K1, were used for initial studies (Fig. 12 and Table 1). Compound **1** was used as a reference compound in all assays, with an IC_{50} of 28.6 nM for 3D7 and a higher IC_{50} of 514 nM for resistant strain K1 (18-fold resistance), both in line with reported data for these strains (8, 9).

Hybrids **42** and **29** showed the weakest effects of the tested compounds but similarly active for both strains. All other hybrid compounds had IC_{50}s below 500 nM for both strains. Gratifyingly, approximately half of the compounds eradicated any effects of resistance, having very similar IC_{50}s against both 3D7 and K1. Compounds with IC_{50} values of about 200 nM or lower for both strains are **27a**, **27b**, **31**, **35**, **37**, and **38b**. Hybrid **35**, linking CQ with the most active CRA analogue **4**, shows an IC_{50} of 190 nM for K1, more potent than **1**, and retains an excellent anti-malarial effect on 3D7 with an IC_{50} of 32.4 nM. However, although exhibiting reduced resistance of about 6-fold, hybrid **35** still has a similar pattern to **1** and significant resistance. More in line with our goal to identify compounds with minimal resistance, **27a** and **31** both stand out as they show similarly good effects on both strains, albeit less potent against 3D7: **27a**, combining the chloroquine pharmacophore with model compound **7**, possessing the major features of the CRA pharmacophore, shows IC_{50} values of 98.6 nM and 110 nM for 3D7 and K1, respectively; **31** has IC_{50}s 114 nM for 3D7 and 127 nM for K1, the best activity for compounds linking the chloroquine pharmacophore with a CRA analogue of series **6** compounds based on octoclothepin. Compounds **27b**, **37** and **38b** were similar but less potent.

Based on these results, three hybrid compounds representing the most potent hybrids without any resistance to K1 (**27a** and **31**) and the improved compound with good potency against 3D7 (**35**) were chosen for further testing on multiple lab strains and field isolates. Strains selected include chloroquine sensitive (CQS) or resistant (CQR) strains as well as artemisinin resistant strains (ArtR) and the intermediate chloroquine resistant strain with SVMNT haplotype, 7G8 (Fig. 13 with data presented in Table 2). Compound **31** shows a slightly lower IC_{50} than **1** for three strains, K1, ARS-272 and 7G8. However, **27a** and **35** proved to be efficient on all tested strains. This indicates that aromatic rings, present in **35** and **31**, but not **27a**, are not strictly required for broad activity.

These trends can be seen clearly in Fig. 14 (data presented in Table 3), illustrating the Response Modification Index (RMI – the ratio of IC_{50} for new compounds divided by the IC_{50} for **1**) for CQR

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and CQS strains. **31** is 4-5 times more effective against K1 and 7G8 CQR strains (RMI 0.25 and 0.19, respectively) whereas it is 4-10 fold less effective than **1** against both CQS strains. On the other hand, **27a** and **35** show RMIs of 0.2-0.4 for most CQR strains while retaining acceptable activity for CQS strains (≤100 nM). Hybrid **35** was particularly significant given its consistent broad spectrum activity for all the resistant strains studied. These strains include not only CQR strains but also artemisinin resistant strains (ArtR), further evidence that the chemosensitising approach has general applicability in tackling some of the latest most concerning field isolates. Furthermore, this conclusion is supported by the fact that the CRA component of compound **35**, i.e. compound **4**, is the most promising CRA from our earlier studies of combinations of CRAs with **1**. We next carried out preliminary ADME and toxicity profiling of both of the most promising molecules **27a** and **35**.

Digestive vacuole disruption efficacy of hybrid compounds

The mechanisms of action of chloroquine have been previously elucidated. Apart from heme polymerization (27), recent studies have shown that chloroquine at micromolar concentrations, disrupts the digestive vacuole of the parasite, triggering a cascade of programmed cell death (28, 29). We performed a high content phenotypic assay using the imaging flow cytometer (Imagestream X MkII) to detect for digestive vacuole disruption after drug treatment. We observed that there was disruption of the parasite's digestive vacuole upon treatment with all the hybrid compounds, and the highest amount of disruption occurred after treatment with **35** (Supplementary Fig S1). The digestive vacuole disrupting activity of all compounds tested were not as potent as chloroquine, suggesting that they probably work in a similar manner to chloroquine but the potency was reduced possibly due to an increase in the size of the compound.

Cytotoxicity

589 The cytotoxicity of **1** and selected hybrids were investigated with a TGF α-transfected mouse
590 hepatocyte (TAMH) cell line, determining the IC_{50} *via* an ATP luminescence assay (30). **27a** and **1** had
591 little effect on cell viability requiring high concentrations, hence very high therapeutic windows. **35**
592 was only weakly toxic with an IC_{50} of 19.4 µM in TAMH cells representing a therapeutic window of
593 over 100 fold. However **37**, selected for testing due to it being a biaryl pharmacophore analogue of **35**
594 but without the quinuclidine, was more toxic with an IC_{50} of 2.84 µM and lower therapeutic window of
595 16 (Table 4).

596 In addition to TAMH cells, the cardiomyocyte cell line, AC10, was selected to study the potential
597 cardiotoxic properties of the hybrid compounds (31). Interestingly, in AC10s, **37** had the same toxicity
598 as in TAMH cells, confirming its lower therapeutic window. **27a** was about 10-fold less toxic than **37**
599 with a therapeutic window of over 200. Encouragingly, **35** was only weakly toxic with an IC_{50}
600 of 71.7 µM equating to a high therapeutic window of 378 in AC10 cells.

601 **Solubility and permeability**

602 In a kinetic solubility assay with HPLC quantification, **35** had solubility of 108 µg/mL at pH 7.4
603 following 24 h incubation, similar to the data at 3 h. In a parallel artificial membrane permeability
604 assay (PAMPA)(32-34) **35** was permeable with an effective permeability (P_e) of 1.10 x 10^{-6} cms^{-1} after
605 a 16 hour equilibration (Table 5). Molecular weight is below 600 and the calculated LogD (pH 7.4)
606 value of 2.0 is suitable for an orally administered drug.

607 **Concluding Remarks**

608 In this study we have designed and synthesized new hybrid compounds merging the antimalarial
609 activity of chloroquine (**1**) with the chemoreversal activity of selected chemoreversal agents (CRAs)
610 into a single agent. Linkers of 3 carbons were effective in joining the CRA to a minimum
611 pharmacophore chloroquine moiety. Hybrids **27a**, **31** and **35** were tested in dose response studies

against a panel of malaria parasites both sensitive and resistant to **1** and artemisinin. The most promising hybrid, **35**, presents a good solubility, permeability and *in vitro* toxicity profile. *In vivo* safety studies will be conducted before nominating **35** as a clinical candidate. GLP toxicology studies would involve dosing **35** to two species at high, medium and low doses to establish a no-effect level dose and to identify the starting dose in humans. This preliminary study provides support for potent hybrid CQ-CRA compounds as promising potential therapy for the treatment of CQR malaria.

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726 parallel artificial membrane permeation assay in the description of passive absorption
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728 34. **Kerns EH, Di L, Petusky S, Farris M, Ley R, Jupp P.** 2004. Combined application of
729 parallel artificial membrane permeability assay and Caco-2 permeability assays in drug
730 discovery. J Pharm Sci **93:**1440-1453.

731

732

733 **FIGURE LEGENDS**

734

735 **FIG 1** SAR summary of chloroquine antimalarial activity and application to the design of chloroquine-
736 CRA hybrids

737

738 **FIG 2** Examples of hybrid compounds linking two pharmacophores: linking two antimalarial agents
739 artemisinin (grey line) and quinine (grey dashed line) to give **2**, and combining the heme-binding 4-
740 amino-quinoline scaffold (grey dashed line) with a dihydropteroate synthase inhibiting sulfonamide
741 group (grey line) to give **3**

742

743 **FIG 3** Reported CRA compounds from our earlier studies: compound **4** derived from L,703,606,
744 compound **5** derived from loperamide and compound **6** derived from octoclothepin

745

746 **FIG 4** Additional preferred CRA components used for hybrid compound synthesis

747

748 **FIG 5** Synthesis of compounds **14** to **17**

749

750 **FIG 6.** Synthesis of intermediates **19** to **22**

751

752 **FIG 7** Preparation of hybrids **27-31**

753

754 **FIG 8** Synthesis of hybrid compound **24**

FIG 9 Synthesis of hybrid compound **26**

FIG 10 Synthesis of chloride **34** and preparation of hybrid compounds **35** to **38**

FIG 11 Synthesis of chlorpromazine related hybrid compound **42**

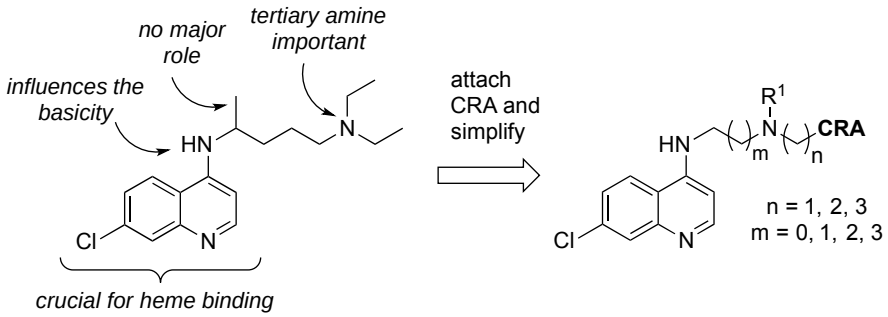
FIG 12 Results for the IC_{50} assay for the synthesized hybrid compounds, performed on chloroquine sensitive (CQS) strain 3D7 and chloroquine resistant (CQR) strain K1 (see Table S1 for statistics). Figure shows mean ± SEM of 3 or more repeats and 2-tailed P-values in comparison to CQ (***, $p<0.001$; **, $p<0.01$; *, $p<0.05$, no indication = not significant).

FIG 13 Results for the IC_{50} assay for **1 (**CQ**)**, **27a**, **31** and **35**, performed on various strains, including chloroquine sensitive (CQS), chloroquine resistant (CQR) and artemisinin resistant (ArtR) strains as well as 7G8 showing intermediate chloroquine resistance (see Table S2 for SEM and statistics). Figure shows mean ± SEM of 3 or more repeats and 2-tailed P-values in comparison to CQ (***, $p<0.001$; **, $p<0.01$; *, $p<0.05$, no indication = not significant).

FIG 14 Response Modification Index (RMI) for **27a**, **31** and **35** compared to **1 (**CQ**)** tested on A. CQR and B. CQS strains. RMI = potency of compound in each strain divided by potency of **1** and error bars = SEM of compound in each strain divided by potency of **1**.

777 # FIGURES AND TABLES

778

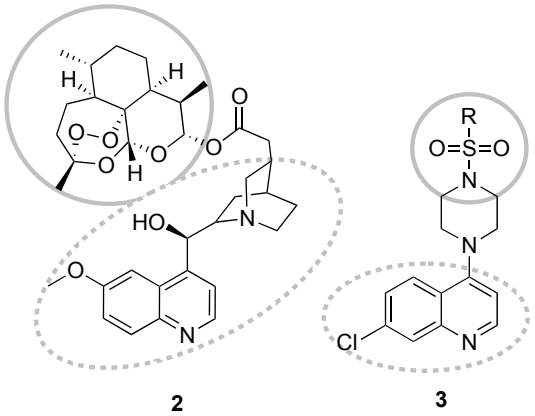


779 **3** (chloroquine) hybrid compound design

780 **FIG 1** SAR summary of chloroquine antimalarial activity and application to the design of

781 chloroquine-CRA hybrids

782



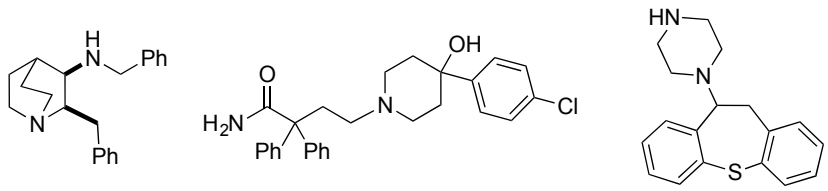
783 **2** **3**

784 **FIG 2** Examples of hybrid compounds linking two pharmacophores: linking two antimalarial agents

785 artemisinin (grey line) and quinine (grey dashed line) to give **2**, and combining the heme-binding 4-

786 amino-quinoline scaffold (grey dashed line) with a dihydropteroate synthase inhibiting sulfonamide

787 group (grey line) to give **3**.



4 **5** **6**

788

789 **FIG 3** Reported CRA compounds from our earlier studies: compound **4** derived from L,703,606,

790 compound **5** derived from loperamide and compound **6** derived from octoclothepin

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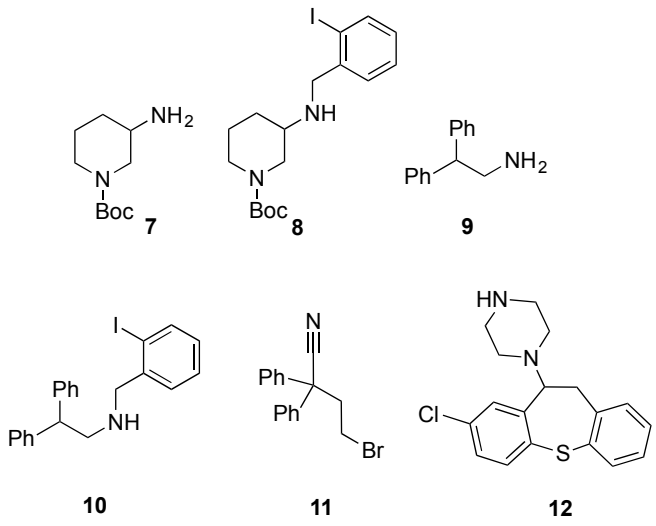
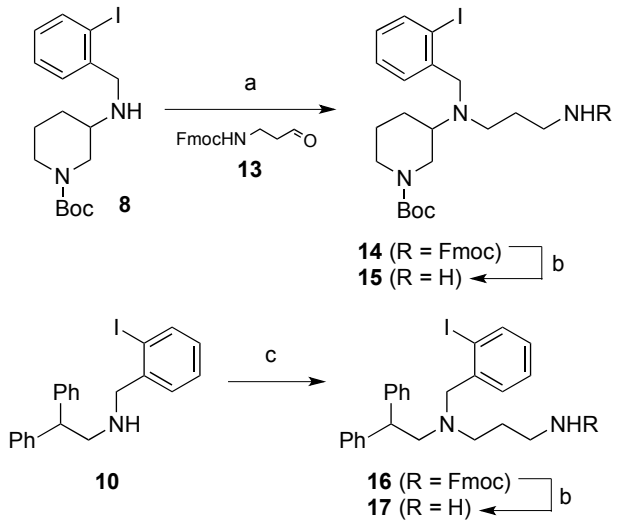


7 **8** **9**

10 **11** **12**

792

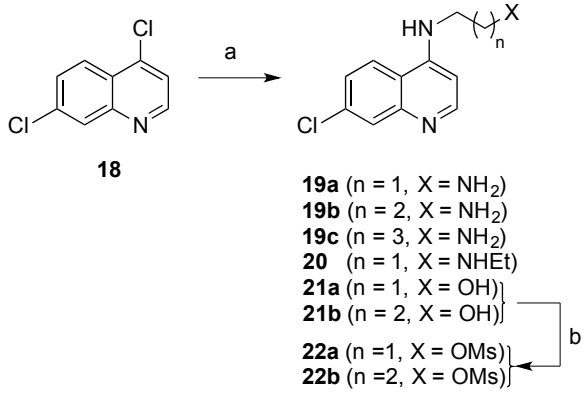
793 **FIG 4** Additional preferred CRA components used for hybrid compound synthesis



794

795 Reagents and Conditions: (a) **9**, $NaHB(OAc)_3$, DCM, 0 °C to rt, 5 h, 76%; (b) piperidine,
796 DCM, rt, used crude (**15**); 85% (**17**) ; (c) **9**, $NaHB(OAc)_3$, DCM, 0 °C to rt, 3 h, 98%.
797
798 **FIG 5** Synthesis of compounds **14** to **17**

799

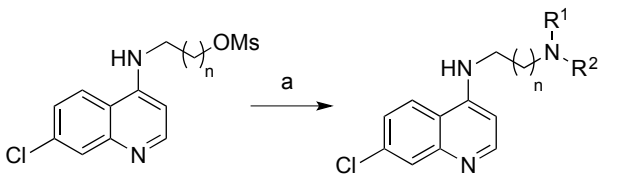


800

801 Reagents and Conditions: (a) neat, reflux, 6-20 h, 74-100%; (b) MsCl, Et_3N, DCM, 0 °C, 3 h, 87-
802 100%.

803 **FIG 6.** Synthesis of intermediates **19** to **22**

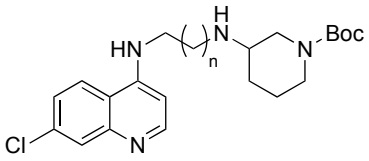
804



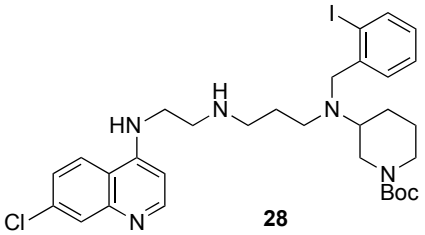
22a (n =1, X = OMs)
22b (n =2, X = OMs)

27a (n = 1, R^1NH_2 = **7**)
27b (n = 2, R^1NH_2 = **7**)
28 (n = 1, R^1NH_2 = **15**)
29 (n = 1, R^1NH_2 = **17**)
30 (n = 1, R^1NH_2 = **9**)
31a (n = 1, R^1R_2NH = **6**)
31b (n = 1, R^1R_2NH = **12**)

805



27a (n = 1)
27b (n = 2)



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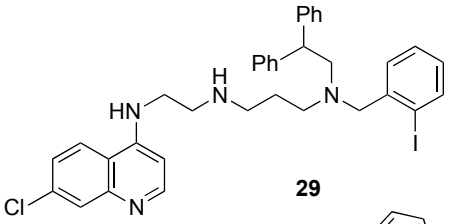

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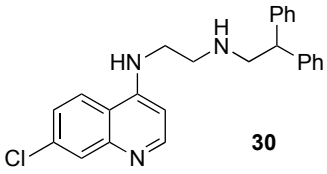

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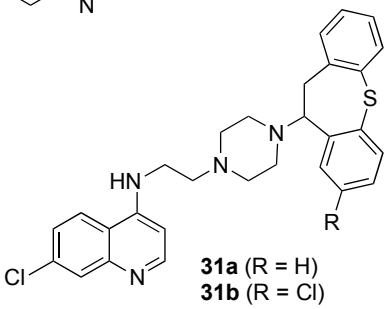


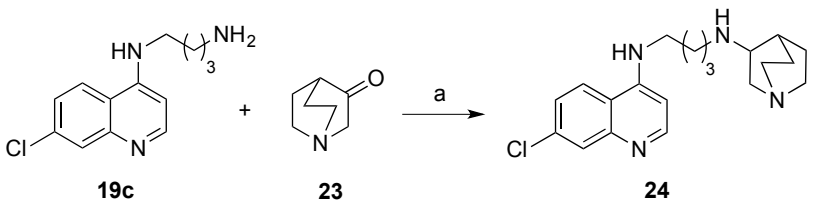
31a (R = H)
31b (R = Cl)

806

807 Reagents and Conditions: (a) R^1NH_2 or R^1R^2NH, NEt_3, THF, microwave, 28-58%.

808 **FIG 7** Preparation of hybrids **27-31**

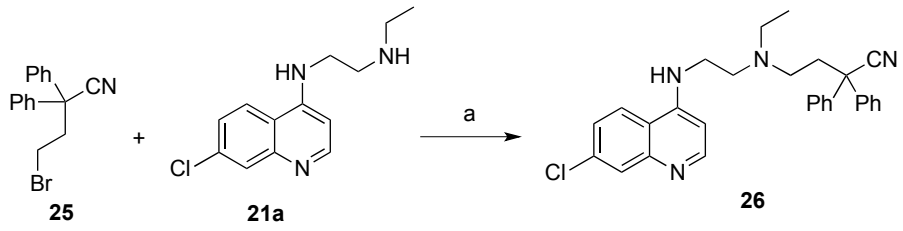
809



19c **23** **24**

Reagents and Conditions: (a) $ZnCl_2$, $NaBH_3CN$, MeOH, rt, 20 h, 3 %.

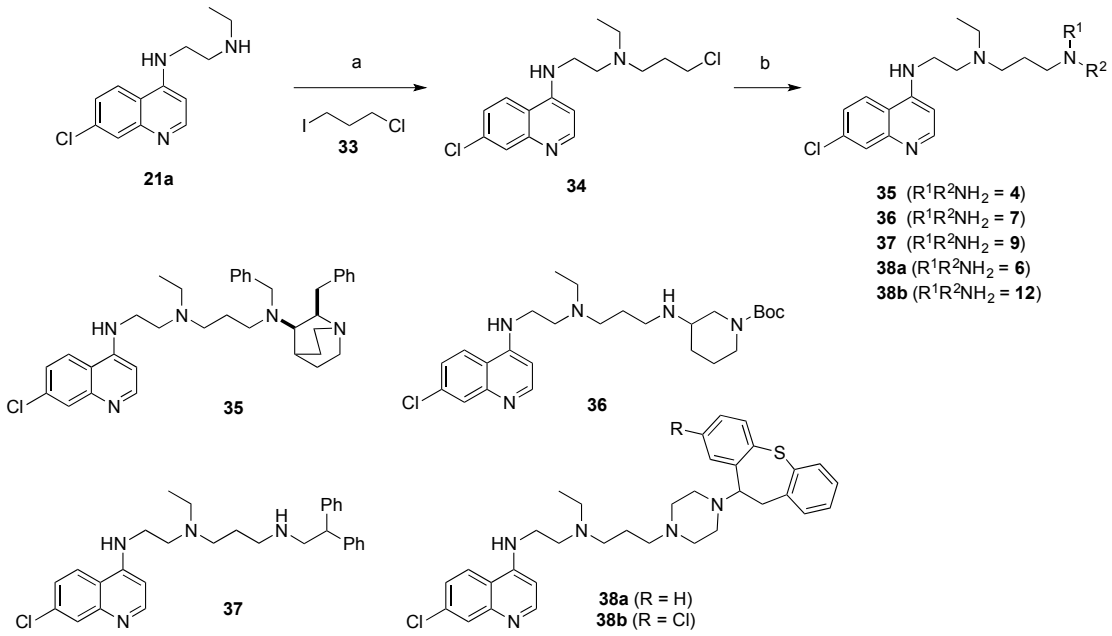
FIG 8 Synthesis of hybrid compound **24**



25 **21a** **26**

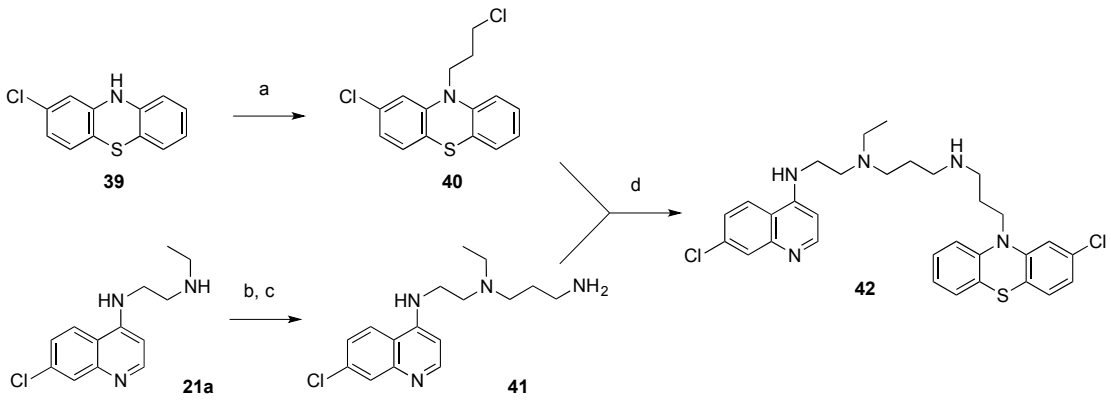
Reagents and Conditions: (a) MeCN, DIPEA, reflux, 5 d, 40%.

FIG 9 Synthesis of hybrid compound **26**



Reagents and Conditions: (a) MeCN, rt, 4 d, 51 %; (b) R_1R_2NH, K_2CO_3, NaI, MeCN, µW (100 °C, 90 min), 22-75%.

FIG 10 Synthesis of chloride **34** and preparation of hybrid compounds **35** to **38**



826

827 Reagents and Conditions: (a) **33**, NaH, THF, DMSO, 0 °C to rt, 5 h, 58%; (b) **13**, $NaHB(OAc)_3$, DCM, 0 °C to
828 rt, 3 h, 82%; (c) Piperidine, DCM, rt, 86%; (d) K_2CO_3, MeOH, reflux, 4 d, 10-12%.

829 **FIG 11** Synthesis of chlorpromazine related hybrid compound **42**

830

831

TABLE 1. IC_{50} assay data for the synthesized hybrid compounds, performed on chloroquine sensitive (CQS) strain 3D7 and chloroquine resistant (CQR) strain K1.

Cpd	3D7		K1	
	Mean IC_{50} (nM)	SEM	Mean IC_{50} (nM)	SEM
1 (CQ)	28.6	5.37	513.6	29.7
24	129.1	13.8	362.7	52.7
26	262.4	37.2	408.1	91.8
27a	98.6	13.1	110.3	11.6
27b	199.6	51.4	196.9	28.0
28	456.8	5.55	221.5	28.3
29	513.3	73.2	508.6	107.6
30	351.8	36.3	303.9	75.0
31	113.6	41.9	127.5	23.3
32	282.4	63.7	280.1	33.4
35	32.4	2.08	189.5	6.53
36	101.9	3.07	337.3	28.9
37	150.9	18.0	172.3	21.3
38a	289.0	77.5	433.5	69.1
38b	149.7	59.8	229.1	49.8
42	603.3	40.4	492.7	54.1

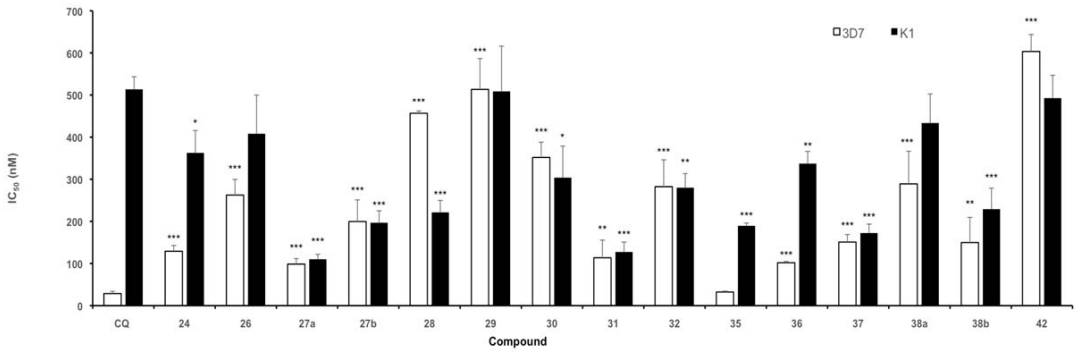
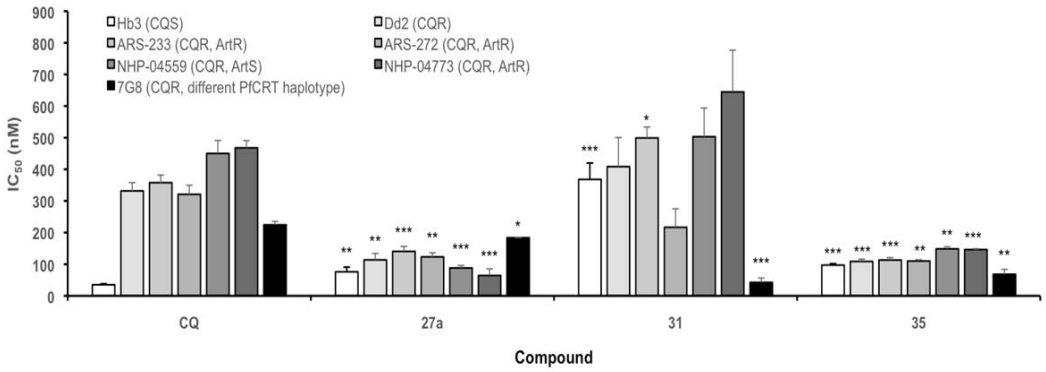


FIG 12 Results for the IC_{50} assay for the synthesized hybrid compounds, performed on chloroquine sensitive (CQS) strain 3D7 and chloroquine resistant (CQR) strain K1 (see Table S1 for statistics). Figure shows mean ± SEM of 3 or more repeats and 2-tailed P-values in comparison to CQ (***, $p<0.001$; **, $p<0.01$; *, $p<0.05$, no indication = not significant)

841 **TABLE 2.** IC_{50} assay data for selected hybrid compounds, performed on CQS, CQR and ArtR strains.

Strain	Mean IC_{50} (nM)			
	1 (CQ)	**27a**	**31**	**35**
Hb3 (CQS)	35.0	75.9	368.2	97.3
Dd2 (CQR)	331.9	113.8	408.4	108.8
ARS-233 (CQR, ArtR)	357.8	140.6	499.2	113.4
ARS-272 (CQR, ArtR)	321.1	123.2	216.7	110.0
NHP-04559 (CQR)	450.1	88.3	503.3	148.5
NHP-04773 (CQR, ArtR)	468.0	64.4	644.8	146.5
7G8 (CQR, diff *PfCRT* haplotype)	224.7	183.8	42.6	68.2

842



843
844

845 **FIG 13** Results for the IC_{50} assay for **1** (CQ), **27a**, **31** and **35**, performed on various strains, including
846 chloroquine sensitive (CQS), chloroquine resistant (CQR) and artemisinin resistant (ArtR) strains as
847 well as 7G8 showing intermediate chloroquine resistance (see Table S2 for SEM and statistics). Figure
848 shows mean ± SEM of 3 or more repeats and 2-tailed P-values in comparison to CQ (***, $p<0.001$; **,
849 $p<0.01$; *, $p<0.05$, no indication = not significant)

850

TABLE 3. Response Modification Index (RMI) for **27a**, **31** and **35** compared to **1 (**CQ**)** tested on CQS and CQR strains

Strain	**27a**	**31**	**35**
3D7 (CQS)	3.44	3.97	1.13
Hb3 (CQS)	2.17	10.52	2.78
7G8 (CQR, diff *PfCRT* haplotype)	0.82	0.19	0.30
K1 (CQR)	0.21	0.25	0.37
Dd2 (CQR)	0.34	1.23	0.33
ARS-233 (CQR, ArtR)	0.39	1.40	0.32
ARS-272 (CQR, ArtR)	0.38	0.68	0.34
NHP-04559 (CQR, ArtS)	0.20	1.12	0.33
NHP-04773 (CQR, ArtR)	0.14	1.38	0.31

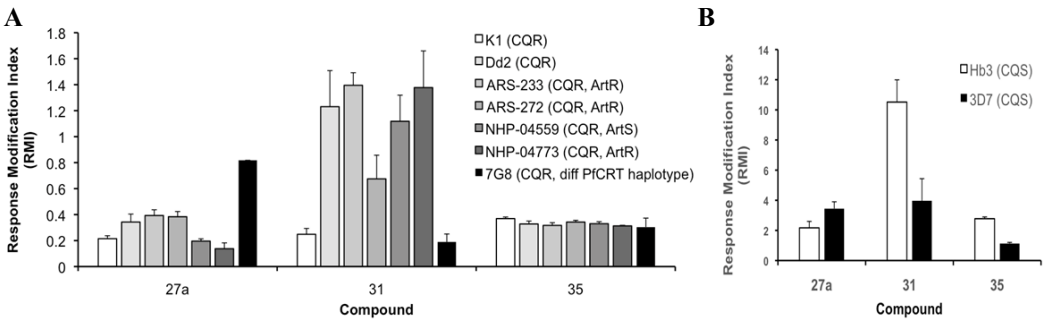


FIG 14 Response Modification Index (RMI) for **27a**, **31** and **35** compared to **1 (**CQ**)** tested on **A.** CQR strains and **B.** CQS strains. RMI = potency of compound in each strain divided by potency of **1** and error bars = SEM of compound in each strain divided by potency of **1**

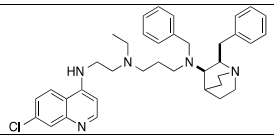
860 **TABLE 4.** Toxicity of three hybrid compounds in two normal cell lines with their therapeutic
861 windows.

Cpd	K1 IC_{50} (nM)	TAMH IC_{50} (μM)[a]	AC10 IC_{50} (μM)[a]	Therapeutic window	
				TAMH/K1	AC10/K1
1	514	85.3±3.0	ND	166	ND
27a	110	72.0±3.5	24.1±1.0	655	219
35	190	19.4±1.3	71.7±3.3	102	378
37	172	2.84±0.03	2.84±0.27	16	16

862 [a] TAMH and AC10 IC_{50} determined by CellTiter-Glo® Cell Viability Assay (Promega Corporation) and are the means of
863 3-4 independent determinations. ND = not determined.
864

865

866 **TABLE 5.** Solubility and permeability properties for the best performing hybrid compound **35**

Cpd	Structure	MW (g/mol)	cLogD (pH7.4)[a]	Aqueous Solubility (µg/mL, pH 7.4, 24h)[b]	PAMPA Permeability (P_e)[c] (10^{-6} cm/s, pH 7.4)
35		596	2.0	3 h: 115 ± 2 (192± 3 µM) 24 h: 108 ± 3 (181 ± 5 µM)	6h: 1.28 ± 0.06 16h: 1.10 ± 0.04

867 [a] Calculated LogD using the properties viewer by Chemaxon (www.chemicalize.org). [b] Aqueous solubility: mean ± SD (n = 3), solubilities at 3 h time point were also
868 recorded and were in good agreement with the reported values at 24 h; [c] P_e = Effective Permeability, determined by the Parallel Artificial Membrane Permeation
869 Assay (PAMPA), mean ± SD (n = 3).