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Arylpiperazines displaying preferential potency against chloroquine-resistant strains of the malaria parasite *Plasmodium falciparum*

Carrie-Anne Molyneaux [a], *Miriam Krugliak* [b], *Hagai Ginsburg* [b], *Kelly Chibale* [a,*]

[a] *Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa*
[b] *Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, Edmund Safra Campus, Jerusalem 91904, Israel*

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ABSTRACT

Arylpiperazines in which the terminal secondary amino group is unsubstituted were found to display a mefloquine-type antimalarial behavior in being significantly more potent against the chloroquine-resistant (W2 and FCR3) strains of *Plasmodium falciparum* than against the chloroquine-sensitive (D10 and NF54) strains. Substitution of the aforementioned amino group led to a dramatic drop in activity across all strains as well as abolition of the preferential potency against resistant strains that was observed for the unsubstituted counterparts. The data suggest that unsubstituted arylpiperazines are not well-recognized by the chloroquine resistance mechanism and may imply that they act mechanistically differently from chloroquine. On the other hand, 4-aminoquinoline-based heteroarylpiperazines in which the terminal secondary amino group is also unsubstituted, were found to be equally active against the chloroquine-resistant and chloroquine-sensitive strains, suggesting that chloroquine cross-resistance is not observed with these two 4-aminoquinolines. In contrast, two 4-aminoquinoline-based heteroarylpiperazines are positively recognized by the chloroquine resistance mechanism. These studies provide structural features that determine the antimalarial activity of arylpiperazines for further development, particularly against chloroquine-resistant strains.

1. Introduction

Malaria is the leading infectious disease in the world's tropics, significantly in sub-Saharan Africa which accounts for >90% of the annual 515 million infections and is responsible for over 1 million deaths per year [1]. This is mostly due to the rapid spread of *Plasmodium falciparum* resistance to available antimalarial drugs. Thus, there is a constant need for developing new antimalarial compounds. Ethnic medicine has provided two of the most efficacious drugs, quinine and artemisinin (and its analogs) and the ongoing screening of medicinal plants yields new lead compounds [2]. In a previous study, totarol that has been isolated from a large variety of plants and shown to have a potent in vitro antibacterial activity was used as a scaffold to synthesize a series of β-amino alcohol derivatives [3]. As part of the antiplasmodial screening of target amino alcohol derivatives of totarol, the starting arylpiperazines, morpholine and piperidine amines were also tested. Among the amines tested, the arylpiperazines phenylpiperazine, 2-chlorophenylpiperazine and 2-ethoxyphenylpiperazine were found to be significantly more potent against a chloroquine-resistant (K1) strain than against a

* *Corresponding author*. Tel.: +27 21 650 2553; fax: +27 21 689 7499.
E-mail address: chibale@science.uct.ac.za (K. Chibale).
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chloroquine-sensitive (D10) strain. The presence of a chloro and ethoxy group in the *ortho* position of phenylpiperazine delivered a two-fold increase in potency against both D10 and K1. In the same assay the 7-chloro-4-aminoquinoline-based piperazine was found to be almost equipotent against both strains, a result noted to be in marked contrast to the aforementioned three arylpiperazines. These results prompted a further investigation into the antiplasmodial properties of a broader range of simple unsubstituted and substituted arylpiperazines against a broader range of chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*.

2. Materials and methods

2.1. Chemistry

All unsubstituted arylpiperazines were purchased from Sigma–Aldrich and used as received. **CMP10** is a known compound and was synthesized according to a reported procedure [4]. Heteroaryl (4-aminoquinoline) piperazines and unsubstituted arylpiperazines were synthesized by via nucleophilic substitution and reductive amination reactions.

2.2. Synthesis of heteroaryl (4-aminoquinoline) piperazines and unsubstituted arylpiperazines

All the reactions were monitored by thin layer chromatography using aluminum-backed silica gel $60F_{254}$ plates (Merck). Ultraviolet light was used to visualise the plates. The column chromatography was carried out on silica gel (Merck Kieselgel 60: 70–230 mesh for gravity). ^{1}H NMR were recorded on a Varian Mercury (300 MHz) or a Varian Unity Spectrophotometer (400 MHz) and were recorded in parts per million (ppm) with respect to tetramethylsilane. ^{13}C NMR were recorded on the same machines but at 75 or 100 MHz. The infra red spectra were recorded on a Perkin-Elmer spectrum one FT-IR Spectrometer. Melting points (mp) were determined on a Reichert–Jung Thermovar and a Fischer–Johns hot stage microscope and are uncorrected. The masses were determined by the Department of Pharmacology (University of Cape Town) on an API2000 from Applied Biosystems. Elemental analysis was determined on a Fisons EA 110 CHN elemental analyzer.

2.3. 2,8-Bis(trifluoromethyl)-4-piperazin-1-yl-quinoline (CMP15)

This compound was prepared from 4-bromo-2,8-bis(trifluoromethyl)quinoline (0.5 g, 1.5 mmol), piperazine (0.63 g, 7.3 mmol), potassium carbonate (0.006 g, 0.04 mmol) and triethylamine (0.06 ml, 0.44 mmol) by the same method as **CMP10** to give **CMP15** (0.47 g, 92%) as yellow-cream crystals; mp 128–131 °C (from EtOH); R_f 0.52 (MeOH:DCM, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1589 (C=C and C=N), 1423 (CF), 1306 (CF), 1264 (CN); ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 1.77 (1H, broad s, N*H*, H12), 3.19 (4H, broad s, N–C*H*$_2$, H10, H14), 3.26 (4H, broad s, N–C*H*$_2$, H11, H13), 7.21 (1H, s, Ar–*H*, H3), 7.61 (1H, t, *J* = 7.94 Hz, Ar–*H*, H6), 8.09 (1H, d, *J* = 7.32 Hz, Ar–*H*, H5), 8.35 (1H, d, *J* = 7.63 Hz, Ar–*H*, H7); δ_C ($CDCl_3$) 45.85 (2C), 53.58 (2C), 105.24, 125.43 (2C), 128.09 (2C), 128.61, 128.68 (2C), 148.00, 159.22; anal. calc. for $C_{15}H_{13}N_3F_6$: C, 51.59; H, 3.75; N, 12.03; *m/z* 349.10136. Found C, 51.84; H, 3.97; N, 11.69; *m/z* 349.10042 $(M)^+$.

2.4. General procedure for the synthesis of CMP1–CMP9, CMP19

A mixture of piperazine (1 eq.) and aldehyde (1.1 eq.) was stirred in anhydrous methanol (10 ml) for 4 h at room temperature under nitrogen. Sodium cyanoborohydride (2.1 eq.) was added and the mixture stirred for a further 2 h at room temperature under nitrogen. The solvent was removed under reduced pressure. The residue was dissolved in 1N HCl (20 ml), the mixture washed with diethyl ether (2× 20 ml) to remove any excess aldehyde. The organic fraction was discarded and the aqueous layer was neutralized with anhydrous sodium carbonate (white precipitate forms). The organic layer was extracted with dichloromethane (3× 20 ml), dried over anhydrous sodium sulphate and concentrated to give the target compounds.

2.5. 7-Chloro-4-(4-cyclohexylmethyl-piperazin-1-yl)-quinoline (CMP1)

This compound was prepared from **CMP10** (0.5 g, 1.46 mmol), cyclohexanecarboxaldehyde (0.28 g, 2.2 mmol) and sodium cyanoborohydride (0.19 g, 3 mmol) by the above method to give **CMP1** (0.27 g, 54%) as cream crystals; mp 91–92 °C (from EtOH); R_f 0.46 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1576 (C=C and C=N), 1264 (CN); ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 0.93 (2H, q, *J* = 11.85 Hz, C*H*$_2$, H6′), 1.22–1.30 (3H, m, C*H*$_2$, H2′, H3′a), 1.54 (1H, m, C*H*, H1′), 1.72–1.83 (5H, m, C*H*$_2$, H5′, H4′, H3′b), 2.26 (2H, d, *J* = 7.15 Hz, C*H*$_2$, Hα), 2.69 (4H, broad s, N–C*H*$_2$, H11, H13), 3.25 (4H, broad s, N–C*H*$_2$, H10, H14), 6.81 (1H, d, *J* = 5.054 Hz, Ar–*H*, H3), 7.41 (1H, dd, *J* = 8.89, 2.18 Hz, Ar–*H*, H6), 7.96 (1H, d, *J* = 9.06 Hz, Ar–*H*, H5), 8.07 (1H, d, *J* = 2.09 Hz, Ar–*H*, H8), 8.74 (1H, d, *J* = 5.05 Hz, Ar–*H*, H2); ^{13}C NMR δ_C ($CDCl_3$) 26.14 (2C), 26.79, 31.89 (2C), 35.09, 52.23 (2C), 53.55 (2C), 65.61, 108.88, 121.98, 125.30, 125.99, 128.84, 134.81, 150.18, 151.92, 157.13; anal. calc. for $C_{20}H_{25}N_3Cl$: C, 70.06; H, 7.35; N, 12.25; Cl, 10.34; *m/z* 343.18152. Found C, 69.74; H, 7.44; N, 12.20; *m/z* 343.18214 (M + H).

2.6. 4-(4-Benzyl-piperazin-1-yl)-7-chloro-quinoline (CMP2)

This compound was prepared from **CMP10** (0.5 g, 1.46 mmol), benzaldehyde (0.24 g, 2.2 mmol) and sodium cyanoborohydride (0.19 g, 3.1 mmol) by the above method to give **CMP2** (0.32 g, 64%) as cream crystals; mp 119–122 °C (from EtOH); R_f 0.27 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1576 (C=C and C=N), 1264 (CN); ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.75 (4H, t, *J* = 4.71 Hz, N–C*H*$_2$, H11, H13), 3.26 (4H, t, *J* = 4.79 Hz, N–C*H*$_2$, H10, H14), 3.62 (2H, s, C*H*$_2$, Hα), 6.85 (1H, d, *J* = 5.05 Hz, Ar–*H*, H3), 7.28–7.33 (3H, m, Ar–*H*, H3′, H4′, H5′), 7.35 (2H, d, *J* = 6.80 Hz, Ar–*H*, H2′, H6′), 7.41 (1H, dd, *J* = 8.98, 2.18 Hz, Ar–*H*, H6), 7.95 (1H, d, *J* = 8.89 Hz, Ar–*H*, H5), 8.05 (1H, d, *J* = 2.09 Hz, Ar–*H*, H8), 8.73 (1H, d, *J* = 5.05 Hz, Ar–*H*, H2); ^{13}C NMR δ_C ($CDCl_3$) 52.19 (2C), 52.95 (2C), 62.79, 108.63, 122.50, 124.90, 126.15, 127.25, 128.33 (2C), 129.07, 129.17 (2C), 135.64,

138.32, 150.26, 151.87, 157.09; anal. calc. for $C_{20}H_{20}ClN_3$: C, 71.10; H, 5.97; N, 12.44; Cl, 10.49; *m/z* 337.13457. Found C, 70.92; H, 5.34; N, 12.04; *m/z* 337.13438 $(M)^+$.

2.7. 4-[4-(2′-Bromo-benzyl)-piperazin-1-yl]-7-chloro-quinoline (CMP3)

This compound was prepared from **CMP10** (0.5 g, 1.46 mmol), 2-bromobenzaldehyde (0.29 g, 1.61 mmol) and sodium cyanoborohydride (0.19 g, 3.1 mmol) by the above method to give **CMP3** (1.16 g, 100%) as cream-yellow crystals; mp 90–93 °C (from EtOH); R_f 0.52 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1576 (C═C and C═N), 1264 (CN); ^1H NMR δ_H (400 MHz, $CDCl_3$) 2.83 (4H, t, *J* = 4.79 Hz, N–C*H*$_2$, H11, H13), 3.25 (4H, t, *J* = 4.71 Hz, N–C*H*$_2$, H10, H14), 3.77 (2H, s, C*H*$_2$, Hα), 6.84 (1H, d, *J* = 5.05 Hz, Ar–*H*, H3), 7.16 (1H, t, *J* = 7.67 Hz, Ar–*H*, H5′), 7.33 (1H, t, *J* = 7.49 Hz, Ar–*H*, H4′), 7.43 (1H, dd, *J* = 8.89, 2.18 Hz, Ar–*H*, H6), 7.50 (1H, d, *J* = 7.67, Ar–*H*, H6′), 7.59 (1H, d, *J* = 7.84 Hz, Ar–*H*, H3′), 7.97 (1H, d, *J* = 9.06 Hz, Ar–*H*, H5), 8.07 (1H, d, *J* = 2.27 Hz, Ar–*H*, H8), 8.73 (1H, d, *J* = 5.05 Hz, Ar–*H*, H2); ^{13}C NMR δ_C ($CDCl_3$) 52.24 (2C), 52.91 (2C), 61.74, 108.94, 121.94, 124.79, 125.24, 126.09, 127.27, 128.64, 128.83, 130.86, 132.89, 134.90, 137.19, 150.12, 151.84, 157.04; anal. calc. for $C_{20}H_{19}BrClN_3$: C, 57.64; H, 4.60; N, 10.08; Br, 19.17; Cl, 8.51; *m/z* 415.04509. Found C, 57.52; H, 4.59; N, 9.60; *m/z* 415.04329 $(M)^+$.

2.8. 7-Chloro-4-[4-(2′-iodo-benzyl)-piperzine-1-yl]-quinoline (CMP4)

This compound was prepared from **CMP10** (0.5 g, 1.46 mmol), 2-iodobenzaldehyde (0.38 g, 1.61 mmol) and sodium cyanoborohydride (0.19 g, 3.1 mmol) by the above method to give **CMP4** (0.18 g, 27%) as a yellow oil; R_f 0.57 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1576 (C═C and C═N), 1264 (C–N); ^1H NMR δ_H (400 MHz, $CDCl_3$) 2.83 (4H, t, *J* = 4.79 Hz, N–C*H*$_2$, H11, H13), 3.25 (4H, t, *J* = 4.71 Hz, N–C*H*$_2$, H10, H14), 3.67 (2H, s, C*H*$_2$, Hα), 6.82 (1H, d, *J* = 5.05 Hz, Ar–*H*, H3), 7.14 (1H, t, *J* = 7.67 Hz, Ar–*H*, H5′), 7.34 (1H, t, *J* = 7.49 Hz, Ar–*H*, H4′), 7.43 (1H, dd, *J* = 8.98, 2.09 Hz, Ar–*H*, H6), 7.51 (1H, d, *J* = 7.67 Hz, Ar–*H*, H6′), 7.86 (1H, d, *J* = 6.71 Hz, Ar–*H*, H3′), 7.95 (1H, d, *J* = 9.06 Hz, Ar–*H*, H5), 8.03 (1H, d, *J* = 2.21 Hz, Ar–*H*, H8), 8.71 (1H, d, *J* = 5.05 Hz, Ar–*H*, H2); ^{13}C NMR δ_C ($CDCl_3$) 52.22 (2C), 52.76 (2C), 66.34, 100.64, 108.92, 121.91, 125.24, 126.04, 128.02, 128.76, 128.88, 130.39, 134.85, 139.62, 140.15, 150.07, 151.80, 157.05; anal. calc. for $C_{20}H_{19}ClIN_3$: C, 51.80; H, 4.13; N, 9.06; Cl, 7.64; I, 27.36; *m/z* 463.03122. Found C, 51.31; H, 4.05; N, 8.80; *m/z* 463.02897 $(M)^+$.

2.9. 4-[4-(2′-Bromo-benzyl)-piperazin-1-yl]-2,8-bis(trifluoromethyl)quinoline (CMP5)

This compound was prepared from **CMP15** (0.5 g, 1.43 mmol), 2-bromobenzaldehyde (0.29 g, 1.61 mmol) and sodium cyanoborohydride (0.19 g, 3.1 mmol) by the above method to give **CMP5** (0.39 g, 59%) as cream crystals; mp 131–133 °C (from EtOH); R_f 0.83 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1589 (C═C and C═N), 1309 (C–F), 1264 (C–N); ^1H NMR δ_H (400 MHz, $CDCl_3$) 2.83 (4H, t, *J* = 4.73 Hz, N–C*H*$_2$, H11, H13), 3.32 (4H, t, *J* = 4.88 Hz, N–C*H*$_2$, H10, H14), 3.76 (2H, s, C*H*$_2$, Hα), 7.15 (1H, t, *J* = 7.78 Hz, Ar–*H*, H5′), 7.22 (1H, s, Ar–*H*, H3), 7.32 (1H, t, *J* = 7.48 Hz, Ar–*H*, H4′), 7.50 (1H, d, *J* = 7.63 Hz, Ar–*H*, H6′), 7.64–7.57 (2H, m, Ar–*H*, H6, H3′), 8.07 (1H, d, *J* = 7.32 Hz, Ar–*H*, H5), 8.26 (1H, d, *J* = 7.63 Hz, Ar–*H*, H7); ^{13}C NMR δ_C ($CDCl_3$) 52.34 (2C), 52.59 (2C), 61.22, 105.11, 122.38, 124.09, 124.33, 125.06, 127.18, 127.99, 128.70 (2C), 130.90, 133.10, 134.09, 136.98, 144.79, 148.71, 148.94, 158.94; anal. calc. for $C_{22}H_{18}BrF_6N_3$: C, 50.99; H, 3.50; N, 8.10; Br, 15.42; F, 21.99; *m/z* 517.05883. Found C, 50.93; H, 3.52; N, 8.15; *m/z* 517.05931 $(M)^+$.

2.10. 1-(2′-Bromo-benzyl)-4-phenyl-piperazine (CMP6)

This compound was prepared from phenylpiperazine (0.5 g, 0.47 ml, 3.1 mmol), 2-bromobenzaldehyde (0.63 g, 3.4 mmol) and sodium cyanoborohydride (0.41 g, 6.5 mmol) by the above method to give **CMP6** (0.437 g, 42%) as cream crystals; mp 109–113 °C (from EtOH); R_f 0.76 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1599 (C═C Ar), 1264 (CN); ^1H NMR δ_H (400 MHz, $CDCl_3$) 2.70 (4H, t, *J* = 4.88 Hz, N–C*H*$_2$, H2, H5), 3.23 (4H, t, *J* = 4.88 Hz, N–C*H*$_2$, H3, H6), 3.68 (2H, s, C*H*$_2$, Hα), 6.85 (1H, t, *J* = 7.32 Hz, Ar–*H*, H10), 6.92 (2H, d, *J* = 7.94 Hz, Ar–*H*, H12, H8), 7.12 (1H, t, *J* = 7.63 Hz, Ar–*H*, H5′), 7.26 (2H, t, *J* = 8.09, Ar–*H*, H9, H11), 7.33 (1H, t, *J* = 7.32 Hz, Ar–*H*, H4′), 7.55 (1H, d, *J* = 7.94 Hz, Ar–*H*, H6′), 7.57 (1H, d, *J* = 7.94 Hz, Ar–*H*, H3′); ^{13}C NMR δ_C ($CDCl_3$) 49.20 (2C), 53.11 (2C), 61.74, 116.04, 116.05 (2C), 116.41, 119.60, 127.23, 128.47, 129.07, 129.12, 130.77, 132.78, 151.39; anal. calc. for $C_{17}H_{19}BrN_2$: C, 61.64; H, 5.78; N, 8.45; Br, 24.12; *m/z* 330.07316. Found C, 61.79; H, 5.84; N, 8.33; *m/z* 330.07242 $(M)^+$.

2.11. 1-(2′-Bromo-benzyl)-4-(10-fluoro-phenyl)-piperazine (CMP7)

This compound was prepared from 4-fluorophenylpiperazine (0.5 g, 2.7 mmol), 2-bromobenzaldehyde (0.57 g, 3.4 mmol) and sodium cyanoborohydride (0.37 g, 5.8 mmol) by the above method to give **CMP7** (0.34 g, 35%) as cream crystals; mp 76–78 °C (from EtOH); R_f 0.76 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1508 (C═C Ar), 1264 (CN); ^1H NMR δ_H (400 MHz, $CDCl_3$) 2.70 (4H, t, *J* = 4.88 Hz, N–C*H*$_2$, H2, H5), 3.14 (4H, t, *J* = 5.04 Hz, N–C*H*$_2$, H3, H6), 3.68 (2H, s, C*H*$_2$, Hα), 6.85–6.99 (4H, m, Ar–*H*, H8, H9, H11, H12), 7.12 (1H, t, *J* = 7.63 Hz, Ar–*H*, H5′), 7.30 (1H, t, *J* = 7.62 Hz, Ar–*H*, H4′), 7.51 (1H, d, *J* = 7.63 Hz, Ar–*H*, H6′), 7.56 (1H, d, *J* = 7.96 Hz, Ar–*H*, H3′); ^{13}C NMR δ_C ($CDCl_3$) 50.15 (2C), 53.04 (2C), 61.97, 115.18, 115.43, 117.62, 117.76, 124.69, 126.87, 128.34, 130.73, 132.97, 145.82, 155.64, 158.81; anal. calc. for $C_{17}H_{18}BrFN_2 \cdot 0.1H_2O$, C, 58.17; H, 5.17; N, 7.98; Br, 22.88; F, 5.44; *m/z* 348.06374. Found C, 58.26; H, 5.11; N, 7.85; *m/z* 348.06045 $(M)^+$.

2.12. 1-(2′-Bromo-benzyl)-4-(8-ethoxy-phenyl)-piperazine (CMP8)

1-(2-Ethoxyphenyl)piperazine monohydrochloride (1 g, 4.12 mmol) and polymer-supported tetraalkylammonium carbonate macroporous triethylammonium methylpolystyrene carbonate (MP-carbonate) 2.74 mmol/g loading (3.01 g, 8.24 mmol) were shaken in methanol for 3 h at room temperature, the resin was removed by filtration and washing with methanol, the filtrate was concentrated under reduced pressure to give the free base (0.84 g, 98%).

This target compound was prepared from 1-(2-ethoxyphenyl)piperazine (0.5 g, 2.1 mmol), 2-bromobenzaldehyde (0.42 g, 2.3 mmol) and sodium cyanoborohydride (0.27 g, 4.3 mmol) by the above method to give **CMP8** (0.49 g, 64%) as a cream oil. R_f 0.84 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3052 (CH Ar), 1593 (C=C Ar), 1238 (CN), 1143 (C–O), 1042 (C–O); ^1H NMR δ_H (400 MHz, $CDCl_3$) 1.45 (3H, t, J = 7.02 Hz, CH_3, H14), 2.74 (4H, t, J = 4.73 Hz, N–CH_2, H2, H5), 3.14 (4H, t, J = 4.58 Hz, N–CH_2, H3, H6), 3.69 (2H, s, CH_2, Hα), 4.06 (2H, q, J = 7.02 Hz, CH_2, H13), 6.93–6.98 (4H, m, Ar–*H*, H9, H10, H11, H12), 7.12 (1H, t, J = 7.78 Hz, Ar–*H*, H5′), 7.30 (1H, t, J = 7.63 Hz, Ar–*H*, H4′), 7.53 (1H, d, J = 5.19 Hz, Ar–*H*, H6′), 7.57 (1H, d, J = 7.94 Hz, Ar–*H*, H3′); ^{13}C NMR δ_C ($CDCl_3$) 15.27, 50.83 (2C), 53.42 (2C), 61.55, 63.04, 112.70, 117.86, 121.02, 122.47, 124.68, 127.63, 128.58, 131.01, 132.96, 137.59, 141.76, 151.72; *m*/*z* 374.09937. Found *m*/*z* 374.10063 $(M)^+$.

2.13. 1-(2-Bromo-benzyl)-4-(8-chloro-phenyl)-piperazine (CMP9)

1-(2-Chlorophenyl)piperazine monohydrochloride (1 g, 4.29 mmol) and polymer-supported tetraalkylammonium carbonate macroporous triethylammonium methylpolystyrene carbonate 2.74 mmol/g loading (3.13 g, 8.58 mmol) were shaken in methanol at room temperature for 3 h, the resin was removed by filtration and washing with methanol, the filtrate was concentrated under reduced pressure to give the free base (0.76 g, 90%).

This target compound was prepared from 1-(2-chlorophenyl)piperazine (0.5 g, 2.1 mmol), 2-bromobenzaldehyde (0.43 g, 2.4 mmol) and sodium cyanoborohydride (0.28 g, 4.5 mmol) by the above method to give **CMP9** (0.35 g, 38%) as cream crystals; mp 82–84 °C (from EtOH); R_f 0.84 (hexane:ethyl acetate, 1:9); IR ($CHCl_3$): ν_{max} (cm^{-1}) 3059 (CH Ar), 1586 (C=C Ar), 1264 (CN); ^1H NMR δ_H (400 MHz, $CDCl_3$) 2.74 (4H, t, J = 4.58 Hz, N–CH_2, H2, H5), 3.10 (4H, t, J = 4.58 Hz, N–CH_2, H3, H6), 3.70 (2H, s, CH_2, Hα), 6.96 (1H, t, J = 7.63 Hz, Ar–*H*, H11), 7.04 (1H, d, J = 8.24 Hz, Ar–*H*, H12), 7.12 (1H, t, J = 7.78 Hz, Ar–*H*, H5′), 7.21 (1H, t, J = 7.63 Hz, Ar–*H*, H10), 7.30 (1H, t, J = 7.58 Hz, Ar–*H*, H4′), 7.34 (1H, d, J = 7.94 Hz, Ar–*H*, H11), 7.36 (1H, d, J = 7.63 Hz, Ar–*H*, H6′), 7.57 (1H, d, J = 7.94 Hz, Ar–*H*, H3′); ^{13}C NMR δ_C ($CDCl_3$) 51.29 (2C), 53.28 (2C), 61.81, 120.31, 122.98, 124.91, 127.17, 127.84, 128.33, 128.56, 130.52, 131.01, 133.21, 137.57, 149.04; anal. calc. for $C_{17}H_{18}BrClN_2$: C, 55.83; H, 4.96; N, 7.66; Br, 21.85; Cl, 9.69; *m*/*z* 364.03419. Found C, 55.60; H, 2.73; N, 7.47; *m*/*z* 364.0328 $(M)^+$.

2.14. Ferrocene benzylpiperazine (CMP19)

Orange-yellow (0.38 g, 53%) crystals; R_f 0.1 (methanol:dichloromethane, 1:9); ν_{max} (cm^{-1}) 3688 (NH), 3015 (C–H Ar), 1603 (C=C Ar), 1216 (CN); ^1H NMR δ_H (400 MHz, $CDCl_3$) 1.24 (1H, broad s, N–*H*, H4), 2.56 (4H, t, J = 4.76 Hz, N–CH_2, H3, H5), 3.01 (4H, t, J = 4.85 Hz, N–CH_2, H2, H6), 3.39 (2H, s, CH_2, H7), 4.09 (7H, s, C–*H*, H10, H11, H13, H14, H15, H16, H17), 4.14 (2H, s, C–*H*, H9, H12); ^{13}C NMR δ_C ($CDCl_3$) 44.34 (2C), 51.01 (2C), 58.29, 68.27 (2C), 68.54 (5C), 70.20 (2C), 81.66; calc. for $C_{15}H_{20}N_2Fe$; *m*/*z* 284.09828. Found: *m*/*z* 284.09795 $(M)^+$.

2.14.1. Parasite cultivation

The chloroquine-sensitive NF54 and D10 strains and the chloroquine-resistant W2 and FCR3 strains of *P. falciparum* were cultivated as described using human red blood cells. Cultures were synchronized by the sorbitol method [5].

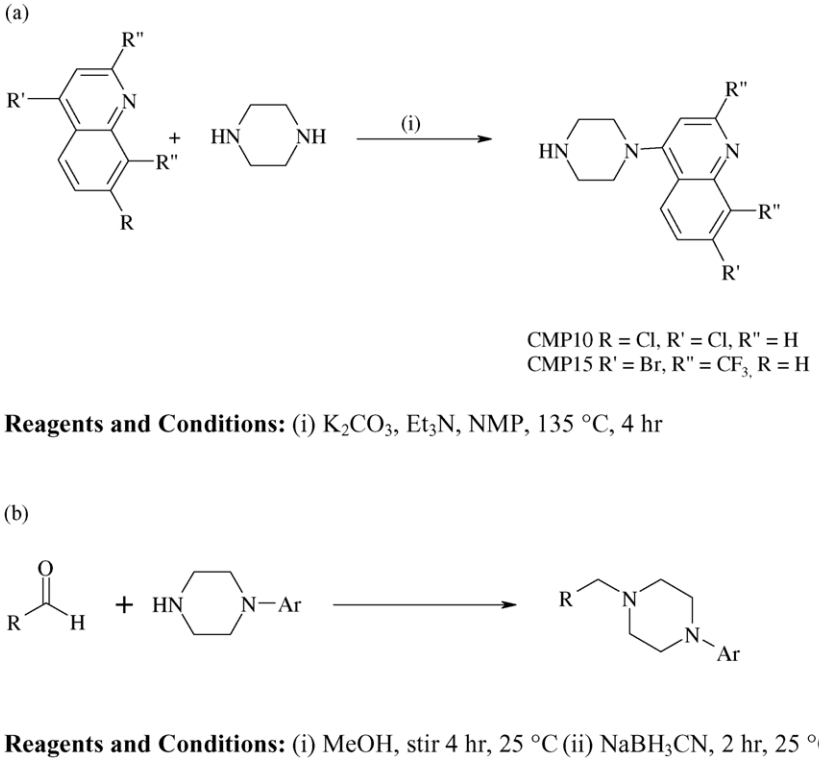
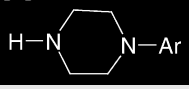
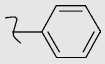
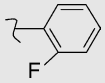
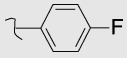
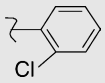
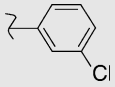
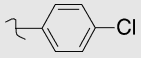
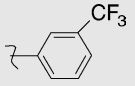
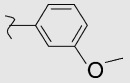
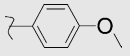
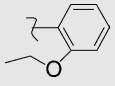
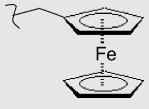
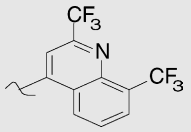
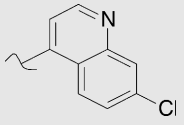


Fig. 1 – Synthetic schemes for (a) 4-aminoquinoline-based unsubstituted piperazines and (b) substituted arylpiperazines.

2.14.2. Determination of inhibitory concentration (IC_{50})
Synchronized cultures at the ring stage were cultured at 1% hematocrit and 2% parasitemia in the presence of increasing concentrations of totarol derivatives. After 18 h of cultivation, parasite viability was determined by [^{3}H]hypoxanthine uptake (final concentration was 2 μCi/ml) during 6 h and compared to controls (drug). Thereafter, parasite-associated radioactivity was determined using the Filtermate/Matrix 96 Direct Beta counter. Data were analyzed to determine the 50% inhibitory concentration by nonlinear regression fitting of the data using Sigmaplot®. Each drug was tested in each strain twice. For the sake of consistency, as many compounds as possible were measured on the same cultures and cultures of different strains were done with the same blood and pooled plasma batches. Chloroquine was always included in order to verify that drug sensitivity remained unaltered throughout the investigation.

Table 1 – Antiplasmodial activities of unsubstituted piperazine derivatives



Compound	Ar	W2 IC_{50} (μM)	FCR3 IC_{50} (μM)	NF54 IC_{50} (μM)	D10 IC_{50} (μM)
CMP 11		24.8 ± 4.3	13.8 ± 2.07	92.5 ± 8.6	152.7 ± 24.4
CMP 16		68.7 ± 11.6	44.61 ± 15.8	103.2 ± 17.5	105.6 ± 29
CMP 12		24.9 ± 4.5	19.8 ± 1.05	95.2 ± 23.8	135.0 ± 17.1
CMP 14		18.1 ± 4.5	11.8 ± 1.5	78.7 ± 42.3	152.2 ± 47.5
CMP 17		4.67 ± 0.39	4.69 ± 0.68	59.1 ± 43.6	67.7 ± 48
CMP 18		11.53 ± 1.3	7.13 ± 0.55	64.8 ± 97	74 ± 36
CMP 20		11.49 ± 0.7	9.56 ± 0.54	92.9 ± 14.3	112.8 ± 38
CMP 21		35.75 ± 5.0	30.33 ± 13.2	83.7	151.2
CMP 22		26.5 ± 2.5	32.3 ± 7.8	85.3	228
CMP 23		66.2 ± 19.6	61.6 ± 15	115.7 ± 23	167
CMP 13		16.8 ± 3.5	10.96 ± 0.91	93.1 ± 17.8	143.3 ± 17.0
CMP 19		1.06 ± 0.03	0.78 ± 0.08	15.2 ± 2	1.9 ± 0.5
CMP 15		11.2 ± 4.1	2.03 ± 0.97	15.0 ± 0.1	7.8 ± 1.9
CMP 10		1.21 ± 0.03	1.36 ± 0.037	1.02 ± 0.05	2.02 ± 0.1
CQ		0.33 ± 0.044	0.40 ± 0.058	0.034 ± 0.002	0.044 ± 0.006

3. Results and discussion

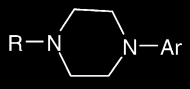
3.1. Chemistry

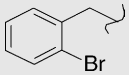
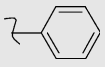
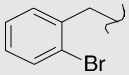
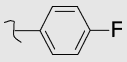
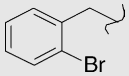
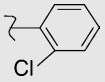
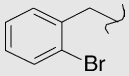
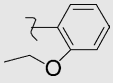
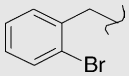
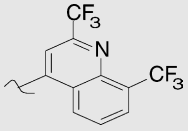
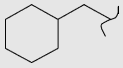
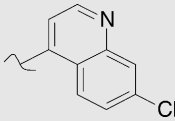
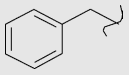
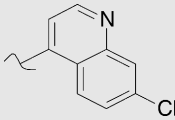
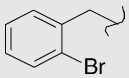
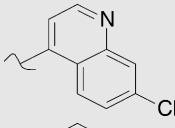
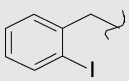
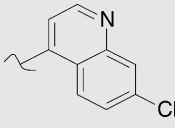
The 4-aminoquinoline-based heteroarylpiperazines (**CMP10** and **CMP15**) and substituted arylpiperazines **CMP–CMP** were synthesized in generally high yield using simple one-step chemistry (nucleophilic substitution and reductive amination) by way of Fig. 1. Compounds were characterized by spectroscopic and analytical techniques and the data obtained was found to be consistent with the expected structures. Purity of the target compounds was confirmed by the elemental analysis data.

3.2. Antiplasmodial activity

The abilities of a range of piperazines to inhibit the growth of chloroquine-sensitive (D10, NF54) and chloroquine-resistant (W2, FCR3) were determined and data are displayed in Tables 1 and 2. The different compounds (including chloroquine as a positive control) tested in this investigation displayed antiplasmodial activity in the IC_{50} range of 0.078–228 μM. Consistent with previous observations [3], unsubstituted arylpiperazines (**CMP11**, **CMP16**, **CMP12**, **CMP14**, **CMP17**, **CMP18**, **CMP20**, **CMP21**, **CMP22**, **CMP23**, **CMP13**) were significantly more potent against the chloroquine-resistant strains than against the chloroquine-sensitive strains (Table 1). The unsubstituted ferrocenic benzylpiperazine (**CMP19**) was also more active against the chloroquine-resistant strains. However, despite the superior activity of **CMP19** relative to the aforementioned unsubstituted arylpiperazines, the differences in activity between the resistant and sensitive strains is much less pronounced in the case of **CMP19** compared to the arylpiperazines. On the other hand, 4-aminoquinoline-based arylpiperazines (**CMP10** and **CMP15**) were equally active against the resistant and sensitive strains (Table 1).

Table 2 – Antiplasmodial activities of substituted piperazine derivatives



Compound	R	Ar	W2 IC_{50} (μM)	FCR3 IC_{50} (μM)	NF54 IC_{50} (μM)	D10 IC_{50} (μM)
CMP 6			69.6 ± 14.5	62.1 ± 10.9	58 ± 13	101.3 ± 15.4
CMP 7			41.5 ± 5.7	62.9 ± 8.29	63.1 ± 12.3	103.1 ± 27.1
CMP 9			56.0 ± 3.7	75.1 ± 28.8	52.4 ± 7.6	104.1 ± 18.2
CMP 8			52.6 ± 8.9	54.2 ± 4.74	46.9 ± 8.3	79.7 ± 13.3
CMP 5			43.0 ± 6.7	36.1 ± 7.49	23.6 ± 4.6	47.3 ± 1.8
CMP 1			15.6 ± 1.8	4.12 ± 0.24	8.06 ± 0.42	19.2 ± 2.3
CMP 2			16.9 ± 3.7	13.5 ± 0.42	11.62 ± 0.69	9.04 ± 0.44
CMP 3			58.1 ± 9.2	20.1 ± 1.73	11.46 ± 0.86	18.3 ± 0.99
CMP 4			13.0 ± 2.1	6.49 ± 0.34	3.9 ± 0.89	12.3 ± 1.8
CQ			0.33 ± 0.044	0.40 ± 0.058	0.034 ± 0.002	0.044 ± 0.006

Within the unsubstituted arylpiperazine series, the most favorable position for substitution appears generally to be the *meta* position, at least on the basis of the data obtained for the chloro (**CMP14**, **CMP17**, **CMP18**) and the methoxy (**CMP21**, **CMP22**, **CMP23**) series of compounds. Indeed, compound **CMP17** with a chloro substituent in the *meta* position of the aryl ring was the most active (IC_{50} = 4.68 μM) amongst all aryl substituents irrespective of parasite strain. As can be seen in Table 2, upon *N*-substitution of the arylpiperazine (NH) nitrogen, there are two notable outcomes: (a) there was a dramatic drop in activity across all strains and (b) the preferential and/or selective potency against resistant strains observed for the unsubstituted counterparts (Table 1) is completely abolished. Some compounds such as **CMP1**, **CMP2**, **CMP4**, **CMP5** and **CMP8** were equally active against the sensitive and resistant strains. Table 3 gives a clearer picture of the reduction in the resistance index (RI) on moving from unsubstituted to substituted arylpiperazine.

A number of chloroquine resistance mechanisms have been proposed. These include essentially reduced accumulation of the drug [6] and higher levels of cellular glutathione [7]. Although some details on the precise mechanism of chloroquine resistance remains to be elucidated, and the mode of action of unsubsituted arylpiperazines is yet to be determined, based on our data it is quite clear that simple unsubstituted arylpiperazines are displaying a mefloquine (Fig. 2)-type behaviour in being more active against chloroquine-resistant strains than against chloroquine-sensitive strains such as D10 [8]. By implication, unsubstituted arylpiperazines appear not to be sufficiently well-recognized by the chloroquine resistance mechanism. This may imply that the unsubstituted arylpiperazines mechanistically act differently from chloroquine. The data obtained for the two 4-aminoquinoline-based unsubstituted piperazines (**CMP10** and **CMP15**), which were found to be equally active against the resistant and sensitive strains, suggests that chloroquine cross-resistance is not observed with these two 4-aminoquinolines. However, relative to the simple arylpiperazines, there is increased recognition of **CMP10** and **CMP15** by the chloroquine resistance mechanism. The lower antiplasmodial activity of **CMP10** and **CMP15** relative to chloroquine may be due to the absence of a more basic terminal nitrogen and lipophilic alkyl side chain in these two compounds. The more basic tertiary nitrogen in chloroquine, which is absent in **CMP10** and **CMP15**, is critical for accumulation in the acidic compartment of the parasite food vacuole via pH trapping [9]. The increased basicity and lipophilicity of the side chain in chloroquine may also be important for uptake of the compound or increased toxicities of drug–ferriprotoporphrin IX complexes.

On the other hand, the weaker antiplasmodial activity displayed by the simple arylpiperazines compared to

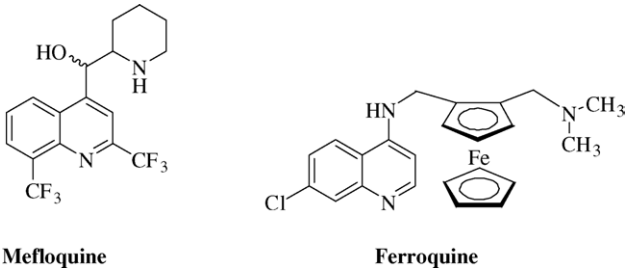
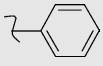
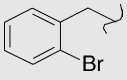
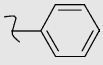
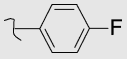
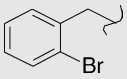
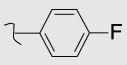
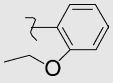
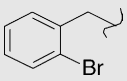
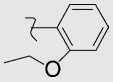
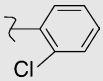
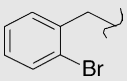
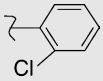


Fig. 2 – Chemical structures for mefloquine and ferroquine.

Table 3 – Resistance indices of unsubstituted and substituted piperazines

Compound	R	Ar	Resistance index			
			W2/NF54	W2/D10	FCR3/NF54	FCR3/D10
CMP 11	H		0.27	0.09	0.15	0.09
CMP6			1.20	0.69	1.07	0.61
CMP 12	H		0.26	0.18	0.21	0.15
CMP7			0.66	0.40	0.997	0.61
CMP13	H		0.18	0.12	0.12	0.08
CMP8			1.12	0.66	1.16	0.68
CMP14	H		0.23	0.12	0.15	0.08
CMP9			1.07	0.54	1.43	0.72

CMP10 and **CMP15** may be due to the absence of the quinoline nitrogen in the arylpiperazines. The quinoline nitrogen in 4-aminoquinolines is also important for uptake and accumulation [10]. This fact may also account for the comparable activity of ferrocenic benzylpiperazine **CMP19**, which has a second protonatable nitrogen, relative to **CMP10** and **CMP15**. With the exception of the NF54 strain, the data against W2, FCR3 and D10 in respect of **CMP19** are comparable to those of the 7-chloro-4-aminoquinoline piperazine (**CMP10**). In fact apart from chloroquine, **CMP19** and **CMP10** are the most active compounds in the series. The major structural difference between **CMP10** and **CMP19** is the presence of the 7-chloroquinolyl group in **CMP10** and the ferrocenyl moiety in **CM19**. The 7-chloroquinolinyl moiety is a well-known heme binding template while the ferrocenyl moiety is not. However, the ferrocenyl moiety is a well-known hydrophobic and cytotoxic group [11]. Incorporation of a ferrocenyl moiety into the side chain of chloroquine has led to the discovery of ferroquine, which has excellent activity particularly against chloroquine-resistant parasites [12]. Ferroquine is currently under phase I clinical trials [13]. Although the precise mechanism of action of ferroquine is unknown, a probable mechanism has recently been shown to be in part similar to that of chloroquine in as far as hematin as the drug target and inhibition of hemozoin formation are concerned. Since ferrocene itself does not inhibit β-hematin formation [14], the activity of **CMP19** may in part be due to the liphophilic ($\log P_{\text{octanol/water}} = 3.28$) and/or cytotoxic nature of the ferrocene unit. The lipophilicity imparted by the ferrocenyl moiety presumably allows **CMP19** to traverse parasite membranes. The dramatic drop in activity upon *N*-substitution of the arylpiperazine (NH) nitrogen, as for compounds depicted in Table 2, may suggest that the free NH in the unsubstiuted arylpiperazines is involved in binding to an (as yet) unknown target. Substitution of the NH may prevent effective binding or interaction with the target. The abolition of the significant potency against resistant strains (relative to sensitive strains) upon *N*-substitution may also suggest a change in the mechanism of action and/or increased recognition of substituted arylpiperazines by the chloroquine resistance mechanism.

In conclusion, we have tested a broader range of substituted and unsubstituted aryl- and heteroarylpiperazines. The results obtained have allowed us to partly confirm our earlier data obtained with the unsubstituted arylpiperazines and partly allow establishment of preliminary structure–activity relationships. Despite the modest antiplasmodial data obtained at this stage, the unsubstituted arylpiperazine nucleus shows promise as a scaffold for the assembly of novel antimalarial agents particularly active against chloroquine-resistant parasites and potentially with novel mechanisms of action. Detailed meaningful structure–activity relationship studies need to be delineated clearly for these arylpiperazines to warrant mechanistic studies and further development as novel antimalarials.

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