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

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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

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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,