Synthesis, Characterization, and Antimalarial Activity of Novel Schiff-Base-Phenol and Naphthalene-Amine Ligands

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Abstract: Emergence of chloroquine (CQ) resistant *Plasmodium falciparum* strains necessitates discovery of inexpensive antimalarial drugs capable of targeting CQ-resistant strains. Towards this objective, herein we have synthesized and characterized naphthalene-Schiff bases or naphthalene-amine phenols. Among these compounds, **7** demonstrated a significant bioactivity with a half-maximal inhibitory concentration (IC_{50}) of 1.7 μM against CQ-resistant Dd2 strains.

Key Words: Naphthalene-amine, Schiff-base phenol, antimalarials, chloroquine-resistance, *P. falciparum.*

INTRODUCTION

The efficacy, affordability, oral bioavailability, and low cytotoxicity profiles of quinoline-based antimalarials exemplified by chloroquine (CQ) have contributed to reduction in mortality and morbidity rates of malaria across Africa and Asia [1]. In spite of easy access of CQ and its wide distribution in the effected regions, CQ-resistant strains have emerged, creating new challenges to drug discovery [2, 3]. Thus, an urgent need exists for an inexpensive antimalarial drug possessing an ability to mimic the advantages and potency profiles of CQ yet able to bypass drug-resistant pathways, devised by the parasite [4, 5]. Newer drugs such as mefloquine [6-8] and halofantrine [9], as well as combination therapy using atovaquone-proguanil [10], and artemether-lumefantrine [11] are potent, but have several limitations including resistance, toxicity, and their high cost [12]. In particular, economic factors negatively impact access and distribution of these drugs in the underdeveloped regions of the world. Therefore alternative inexpensive drugs that are capable of overcoming resistance pathways are desired [13].

Recently, a novel class of metallo-antimalarials exemplified as Schiff-base phenol- and amine phenol-gallium(III) and -iron(III) complexes possessing activity against CQ-sensitive and -resistant strains has emerged [2, 5, 14-16]. These cationic and moderately hydrophobic compounds have been shown to target a novel biocrystallization process [13, 17], hemozoin formation [18]; thereby demonstrating putative mechanism(s) of action similar to that of CQ [14, 16]. Furthermore, it has been reasoned that cationic metallo-drugs permeate the membrane bilayers compared with their organic ligands thereby displaying significance of the delocalized positive charge on the outer surface of pharmacophore for biological activity [19]. To further evaluate, if this trend is applicable in other classes of Schiff-base phenol, amine phenol, Schiff-base naphthalene, and naphthalene amine ligands containing the side chain of CQ possessing both secondary and tertiary nitrogen atoms, we have synthesized and characterized a series of organic ligands. Herein, we describe synthesis and characterization of these organic ligands including evaluation of their cytotoxic potency as antimalarials in the CQ-sensitive (HB3) and CQ-resistant (Dd2) lines.

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RESULTS AND DISCUSSION

For evaluation, the targeted Schiff-base phenols or Schiff-base naphthalene ligands were obtained through condensation reactions of 2-Amino-5-diethylamino-pentane and appropriately substituted benzaldehyde or naphthaldehyde in methanol in equimolar ratio [19]. Further, substituted amine phenol or amine naphthalene ligands were also obtained by reduction of their corresponding Schiff-base analogues using KBH_4 at 80°C for 3h [20]. All compounds were purified and characterized through standard analytical techniques. The analytical and spectral data for **1-7** were consistent with the proposed formulation.

We evaluated the ability of **1-7** to inhibit the growth of trophozoites in intraerythrocyte culture by assessing ^{3}H-hypoxanthine incorporation [21]. Hypoxanthine incorporation is a measure of parasite growth and its inhibition correlates well with direct blood smear counts but provides a more reliable quantification. Unfortunately, compounds **1**, **2**, **3**, **4**, and **6** did not demonstrate any significant efficacy against either the CQ-sensitive (HB3) or CQ-resistant (Dd2) strains. Among the active compounds, **5** and **7** demonstrated moderate half-maximal inhibitory concentration (IC_{50}) values of 5.53 and 6.38 μM, against CQ-sensitive lines, respectively. However, both **5** and **7** displayed slight preferential activity (IC_{50} 3.5 μM (**5**); 1.7 μM (**7**)) against CQ-resistant Dd2 lines, and the compound **7** was found to be twice more potent compared to **5**. The differential activities of **5** and **7** may likely be attributed to the structural variations of these compounds. While **5** possesses a simple substituted aromatic ring, the **7** contains a naphthalene ring which is structural variation of a quinoline ring of the CQ lacking chlorine at position 7 and the quinonyl basic nitrogen. Furthermore, it has been demonstrated previously that nitrogen atoms of side chain in chloroquine and aromatic ring assist in accumulation of the drug within the digestive vacuole of the parasite

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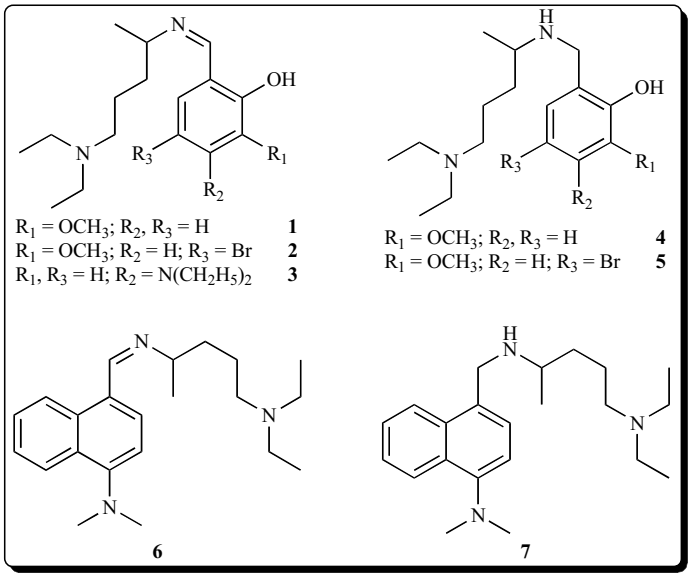

via pH trapping mechanism. In addition, aromatic rings were postulated to promote Pi-Pi interactions with ferriprotoporphyrin IX [Fe(III)PPIX] [22]. The dramatic decrease in activity of **7** against CQ-sensitive HB3 lines suggests that basicity of the quinonyl nitrogen may play a more predominant role in the CQ-selectivity and -permeation across bilayers into the digestive vacuole *via* pH trapping mechanism(s). Further, the substitution of the dimethylamino moiety into aromatic ring would likely further alter electronic environment of the naphthalene ring compared to quinoline thereby decreasing efficacy of **7** in CQ-sensitive lines and resulting into to differential cytotoxicity profiles.

In conclusion, organic molecules were synthesized through a rapid one step synthesis and characterized. Among these compounds, **7** showed the best activity against CQ-resistant lines. Further, **7** was found to be 3.7 times more active against CQ-resistant lines compared with their CQ-sensitive counterparts. Thus, it may be interesting to correlate these differential activities in terms of ability of **7** to inhibit hemozoin formation from both CQ-resistant and -sensitive lines. Additionally, substitutions on the side-chain of this molecule may reveal further relationships in terms of enhanced activity against CQ-resistant strains.

EXPERIMENTAL SECTION

Materials and Methods

Unless specified otherwise, all chemicals were purchased from Sigma-Aldrich chemical company, and used without further purification. Compounds were purified by flash chromatography on Merck silica gel 40 Å (70-230 mesh). The ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz spectrometer; chemical shifts are reported in δ (ppm) using TMS as an internal reference. Mass spectral data were obtained from the Washington University Resource for Biomedical and Bioorganic Mass Spectrometry using 3-nitrobenzyl alcohol-lithium (NBA-Li) matrix. Furthermore, the purity of compounds was ascertained on HPLC (Waters) system equipped with a dual λ detector using C4 column (Vydac) employing an eluent mixture of methanol and water (100% water for first 5 minutes, followed by a change to 50 : 50 (Water : MeOH) for the next 5-20 minutes, and finally 100% MeOH for the next 20-25 minutes). The retention times (Rt) are given for selected compounds.

Method A

Synthesis of Schiff-Base Phenols or Schiff-Base Naphthalenes

2-Amino-5-diethylaminopentane dissolved in methanol was added to a stirred solution of either substituted benzaldehydes or naphthaldehydes in equimolar ratios. The contents were heated to reflux for 2 h. The volatiles were removed under reduced pressure, the residues were washed with pentane, and dried.

Method B

Synthesis of Amine-Phenols or Amine-Naphthalenes

2-Amino-5-diethylaminopentane dissolved in methanol was added to a stirred solution of either substituted benzaldehyde or naphthaldehyde in equimolar ratios. The contents were refluxed for 2 h, cooled, KBH_4 (2 equiv) was added, and reaction mixture was heated at 80°C for 3h. The contents were cooled, and volatiles were removed under reduced pressure. The residue was taken in water (70 ml), pH was adjusted to 1.0, and extracted with CH_2Cl_2 (3 ×70 ml). The organic extract was discarded, pH of aqueous extract was adjusted to 8.5, and extracted with CH_2Cl_2 (3 ×70 ml), combined, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure.

2-(5-(diethylamino)pentan-2-ylimino)methyl)-6-methoxyphenol (1)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.30 (s, 1H), 6.90-6.80 (dd, 2H), 6.78 (t, 1H), 3.90 (s, 3H), 3.40-3.30 (m, 1H); 2.50-2.30 (m, 6H), 1.65-1.50 (m, 2H), 1.50-1.38 (m, 2H), 1.25 (d, 3H), 0.98 (t, 6H); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 162.0, 151.7, 147.7, 122.0, 117.5, 116.7, 112.9, 63.3, 55.0, 51.8,

45.9, 34.9, 22.9, 21.6, 10.7; LRMS (FAB) Calcd for $C_{17}H_{28}N_2O_2$: 292.2; Found: $(M + Li)^+$, 299.2; HPLC: Rt = 16.8 min.

2-(5-(diethylamino)pentan-2-ylimino)methyl)-4-bromo-6-methoxyphenol (2)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.42 (s, 1H), 8.21 (s, 1H), 6.96 (d, 1H), 6.94 (d, 1H), 3.87 (s, 3H), 3.41-3.34 (m, 1H); 2.52-2.37 (m, 6H), 1.65-1.56 (m, 2H), 1.48-1.37 (m, 2H), 1.29 (d, 3H), 1.00 (t, 6H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 161.3, 152.5, 149.4, 124.3, 118.4, 116.3, 108.1, 63.6, 55.8, 52.3, 46.4, 35.3, 23.5, 22.0, 11.3; LRMS (FAB) Calcd for $C_{17}H_{27}BrN_2O_2$: 370.1; Found: $(M + Li)^+$ 377.1; HPLC: Rt = 19.1 min.

2-((5-(diethylamino)pentan-2-ylimino)methyl)-5-(diethylamino)phenol (3)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 6.96 (d, 1H), 6.12 (dd, 1H), 6.06 (d, 1H), 3.38-3.28 (m, 4H), 3.28-3.20 (m, 1H); 2.52-2.42 (m, 4H), 2.42-2.32 (m, 2H), 1.62-1.40 (m, 4H), 1.25 (d, 3H), 1.15 (t, 6H), 0.99 (t, 6H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 166.4, 160.3, 151.0, 132.3, 107.8, 102.4, 97.9, 62.0, 52.6, 46.3, 43.9, 35.4, 23.4, 22.2, 12.3, 11.2; LRMS (FAB) Calcd for $C_{20}H_{35}N_3O$: 333.3; Found: $(M + Li)^+$ 340.3.

2-((5-(diethylamino)pentan-2-ylamino)methyl)-6-methoxyphenol (4)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.78-6.62 (m, 2H), 6.60-6.50 (dd, 1H), 3.92 (d, 2H), 3.81 (s, 3H); 2.78-2.60 (m, 1H), 2.58-2.42 (m, 4H), 2.38 (m, 2H), 1.60-1.30 (m, 4H), 1.10 (d, 3H), 0.98 (t, 6H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 147.4, 146.9, 122.7, 119.6, 117.6, 110.1, 55.0, 52.1, 51.5, 48.8, 45.9, 33.7, 22.5, 18.8, 10.7; LRMS (FAB) Calcd for $C_{17}H_{30}N_2O_2$: 294.2; Found: $(M + Li)^+$, 301.2.

2-((5-(diethylamino)pentan-2-ylamino)methyl)-4-bromo-6-methoxyphenol (5)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.83 (d, 1H), 6.69 (d, 1H), 3.90 (d, 2H), 3.80 (s, 3H); 2.80-2.60 (m, 1H), 2.60-2.42 (m, 4H), 2.40 (m, 2H), 1.60-1.30 (m, 4H), 1.10 (d, 3H), 0.99 (t, 6H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 148.2, 146.4, 124.1, 122.0, 113.3, 109.1, 55.3, 52.2, 51.8, 48.5, 46.0, 33.8, 22.7, 18.9, 10.9; LRMS (FAB) Calcd for $C_{17}H_{29}BrN_2O_2$: 372.1; Found: $(M + Li)^+$, 379.1.

4-((5-(diethylamino)pentan-2-ylimino)methyl)-N,N-dimethylnaphthalen-1-amine (6)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.93 (dd, 1H), 8.82 (s, 1H), 8.24 (dd,1H), 7.76 (d, 1H), 7.54 (dd, 1H), 7.49 (dd,1H), 7.01 (d, 1H), 3.36 (m, 1H), 2.89 (s, 6H); 2.54-2.40 (m, 6H), 1.78-1.60 (m, 2H), 1.60-1.40 (m, 2H), 1.32(d, 3H), 0.99 (t, 6H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 158.2, 152.9, 132.6, 129.1, 128.4, 126.6, 126.3, 124.9, 124.7, 124.6, 112.9, 67.4, 52.7, 46.7, 44.8, 35.9, 23.9, 22.8, 11.5; LRMS (FAB) Calcd for $C_{22}H_{33}N_3$: 339.3; Found: $(M + Li)^+$ 346.3; HPLC: Rt = 25.5 min.

4-((5-(diethylamino)pentan-2-ylamino)methyl)-N,N-dimethylnaphthalen-1-amine (7)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.30-8.24 (m, 1H), 8.12-8.04 (m, 1H), 7.52-7.42 (m, 2H), 7.34 (d, 1H), 6.98 (d, 1H), 4.21-4.05 (dd, 2H), 2.85 (s, 6H), 2.82-2.70 (m, 1H), 2.52-2.45 (q, 4H), 2.41-2.36 (m, 2H), 1.60-1.30 (m, 4H), 1.15 (d, 3H), 0.99 (t, 6H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 150.4, 132.9, 130.8, 129.1, 126.1, 125.8, 124.7, 124.6, 124.0, 113.3, 53.1, 52.9, 49.2, 46.7, 45.1, 34.9, 23.4, 20.4, 11.5; LRMS (FAB) Calcd for $C_{22}H_{36}N_3$: 341.3; Found: $(M + Li)^+$, 348.3; HPLC: Rt = 20.1 min.

BIOASSAY

Plasmodium culture

Plasmodium falciparum lines, chloroquine-sensitive (HB3) and chloroquine-resistant (Dd2) were grown in intraerythrocytic culture by the method of Trager and Jensen [23]. Cultures were maintained at 5% parasitemia, 2% hematocrit using human serum and erythrocytes, in a 3% oxygen/3% carbon dioxide atmosphere. Synchronization of developmental stage was achieved by sorbitol treatment [24]. Parasite growth inhibition and half-maximal inhibitory concentration values (IC_{50}) were determined by measuring ^{3}H-hypoxanthine incorporation [21]. Parasites were incubated with drug starting at the late ring stage and then ^{3}H-hypoxanthine added for 4 hours at the mid-trophozoite stage before harvesting parasites and assaying for incorporated radioactivity. Compounds **1-7** were added as 1:1000 dilutions of a 10 mM dimethylsulfoxide (DMSO) stock at increasing concentrations. Vehicle alone had no effect on ^{3}H -hypoxanthine incorporation.

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