Supplementary Material (ESI) for Chemical Communications

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Metalloantimalarials: Synthesis and Characterization of a Novel Agent Possessing Activity Against *Plasmodium falciparum*

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EXPERIMENTAL SECTION

General Methods

Unless specified otherwise, all chemicals were purchased from Sigma-Aldrich chemical company, and used without further purification. Tetrahydrofuran, diethyl ether were dried by distillation from the sodium ketyl of benzophenone. Dichloromethane and dimethylformamide were dried by distillation from CaH_2. 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. Flash chromatography was performed on Merck silica gel 40 Å (70-230 mesh). Mass spectral data were either obtained on Varian Saturn 2000R GCMS spectrometer in our laboratory or from the Washington University Resource for Biomedical and Bioorganic Mass Spectrometry with 3-nitrobenzyl alcohol or thioglycerol as a matrix. Elemental analyses (C, H and N) were performed by Galbraith Laboratories, Knoxville, TN.

4-Bromo-2-hydroxymethyl-6-methoxy-phenol (1).

5-Bromo-2-hydroxy-3-methoxybenzaldehyde (1.0 g, 4.33 mmol) dissolved in anhydrous THF (50.0 ml) was treated with lithium aluminum hydride (0.20 g, 5.37 mmol). The reaction mixture was stirred at room temperature for 3h under argon and quenched with saturated solution of sodium sulfate. The excess solvent was removed and residue was treated with 10% HCl and extracted with ether (3 × 100 ml). The organic extract was dried over Na_2SO_4, filtered and the filtrate was evaporated to yield a brown solid **1** (0.92 g, 3.95 mmol, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.96 (s, 1H), 6.87 (s, 1H), 4.65 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 147.5, 143.1, 128.2, 123.6, 113.9, 111.7, 61.2, 56.5; Anal. Calcd for $C_8H_9BrO_3$: C, 41.22; H, 3.89. Found: C, 40.80; H, 3.96; MS (GCMS) Calcd for $C_8H_9BrO_3$: 232.0; Found: 232.0.

5-Bromo-1-methoxy-2-methoxymethoxy-3-methoxymethoxymethyl-benzene (2).

1 (0.12 g, 0.52 mmol) was dissolved in the dry THF (10.0 ml) and diisopropylethylamine (5.0 ml) was added to the solution. The reaction mixture was treated with dropwise addition of 1-chloro-1-methoxy-methane (0.2 ml, 2.63 mmol) and stirred overnight at room temperature. The excess solvent was removed; the residue was quenched with 10% HCl, and extracted with ether (3 × 100 ml). The organic extract was washed with 5M NaOH to remove unreacted starting precursor, dried over Na_2SO_4, filtered, and the filtrate was evaporated to yield pale brown oil. Flash chromatography of the crude oil using chloroform: ether (3:1) afforded pale brown oil **2** (0.14 g, 0.44 mmol, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.19 (s, 1H), 6.97 (s, 1H), 5.07 (s, 2H), 4.71 (s, 2H), 4.66 (s, 2H), 3.82 (s, 3H), 3.56 (s, 3H), 3.41 (s, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 152.6, 142.8, 134.0, 123.9, 116.6, 114.9, 98.8, 95.9, 63.5, 57.4, 55.8, 55.2.; Anal. Calcd for $C_{12}H_{17}BrO_5$: C, 44.88; H, 5.29. Found: C, 45.04; H, 5.53; MS; LRMS (FAB) Calcd for $C_{12}H_{17}BrO_5$: 320.0; Found: 320.0.

3-(3-Methoxy-4-methoxymethoxy-5-methoxymethoxymethyl-phenyl)-quinoline (3).

2 (200 mg; 0.62 mmol) was dissolved in anhydrous THF (10 ml), treated with n-butyl lithium (1.6M, 0.43ml, 0.69mmol) at –78°C, and stirred for 3h. Then, anhy. $ZnCl_2$ (93 mg; 0.69 mmol) was added to the stirred solution and the contents were stirred for 1h. The mixture was transferred via canula under argon to a mixture of 3-Bromoquinoline (86 mg, 0.42 mmol) and 7% $Pd(PPh_3)_4$ (33 mg, 0.03 mmol) in THF (3ml). The reaction mixture was heated at reflux overnight. The contents were cooled to RT and solvent was removed under reduced pressure. The residue was extracted with ether (3 × 100ml), combined organic extract was dried over anhydrous Na_2SO_4, filtered, evaporated, and the

residue was purified using flash chromatography with ether as an eluent to yield **3** (102.5 mg; 0.28 mmol, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.14 (s, 1H), 8.24 (s,1H), 8.11 (d, 1H), 7.82 (d, 1H), 7.66 (t, 1H), 7.52 (t, 1H), 7.37 (s, 1H), 7.14 (s, 1H), 5.16 (s, 2H), 4.77 (s, 2H), 4.75 (s, 2H), 3.90 (s, 3H), 3.59 (s, 3H), 3.41 (s, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 152.7, 149.9, 147.3, 144.2, 134.2, 133.7, 133.2, 133.1, 129.5, 129.2, 128.1, 127.2, 120.3, 111.0, 106.0, 99.2, 96.2, 64.4, 57.6, 56.1, 55.4; Anal. Calcd for $C_{21}H_{23}NO_5.H_2O$: C, 65.11; H, 6.50; N, 3.62. Found: C, 66.21; H, 6.45; N, 3.37; LRMS (FAB) Calcd for $C_{21}H_{23}NO_5$ $(M + H)^+$: 370.2; Found: 370.2.

2-Hydroxymethyl-6-methoxy-4-quinolin-3-yl-phenol (4).

3 (100 mg, 0.27 mmol) was dissolved in methanol (9 ml) and treated with conc. HCl (1 ml). The contents were refluxed for 1h. The reaction mixture was cooled, neutralized, and the solvent was removed under reduced pressure. The residue was extracted with CH_2Cl_2 (3 × 100ml), the organic extract was dried over anhydrous Na_2SO_4, and filtered. The filtrates were evaporated and the resulted residue was re-crystallized from $CHCl_3$ to yield a light yellow solid **4** (65 mg, 0.23 mmol, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.11 (s, 1H), 8.23 (s, 1H), 8.12 (d, 1H), 7.85 (d, 1H), 7.71 (t, 1H), 7.57 (t, 1H), 7.23 (s, 1H), 7.14 (s, 1H), 6.40 (bs, 1H), 4.85 (s, 2H), 3.97 (s, 3H), 2.16 (s, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 150.1, 147.5, 147.3, 144.4, 134.0, 132.9, 130.0, 129.5, 129.3, 128.4, 128.1, 127.5, 127.3, 120.2, 109.6, 61.8, 56.4; Anal. Calcd for $C_{17}H_{15}NO_3 \cdot 0.5H_2O$: C, 70.38; H, 5.91; N, 4.82. Found: C, 70.05; H, 6.24; N, 4.17; LRMS (FAB): Calcd for $C_{17}H_{15}NO_3$ $(M + H)^+$: 282.1; Found: 282.1.

2-Hydroxy-3-methoxy-5-quinolin-3-yl-benzaldehyde (5).

4 (70.0 mg, 0.25 mmol) was dissolved in dry THF (5.0 ml) and treated with an addition of ethylmagnesium bromide (3 M, 0.17 ml, 0.50 mmol). The contents were stirred, treated with HMPA (67.0 mg, 0.37 mmol) and paraformaldehyde (15 mg, 0.50 mmol) until bubbling ceased. The contents were refluxed for 5h, cooled, and acidified with 10% HCl. The residual acid was neutralized with addition of saturated solution of $NaHCO_3$. The excess solvent was removed, the residue was extracted CH_2Cl_2 (3 × 50 ml) and the organic fractions were combined, dried with Na_2SO_4, filtered, and the filtrate was evaporated. The residue was purified on silica using acetone:chloroform (1:3) as eluent to provide **5** (57 mg, 0.20 mmol, 83 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.06 (s, 1H), 9.16 (s, 1H), 8.28 (s,1H), 8.16 (d, 1H), 7.90 (d, 1H) 7.75 (t, 1H), 7.64 (t, 1H), 7.52 (s, 1H), 7.43 (s, 1H), 4.04 (s, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 196.8, 149.8, 149.6, 133.2, 132.6, 132.3, 132.0, 129.9, 129.5, 128.9, 128.7, 128.2, 127.6, 123.3, 122.6, 117.0, 56.7; HRMS (FAB) Calcd for $C_{17}H_{13}NO_3$: 279.0892; Found: 279.0890.

(*N*-(2-Hydroxy-3-methoxy-5-quinolin-3-yl-benzyl)-*N'*-{2-[3-(2-hydroxy-3-methoxy-5-quinolin-3-yl-benzylamino)-propylamino]-ethyl}-propane-1,3-diamine (6).

Ligand (**6**) was obtained through methods described earlier.[1-3] Briefly, bis(*N*,*N*'-aminopropyl)ethylene-diamine (23.5 mg, 0.14 mmol) was dissolved in methanol (3 ml) and treated with dropwise addition of **7** (75.6 mg, 0.27 mmol) dissolved in methanol (5 ml). The contents were heated at 70°C for 3h, then treated with addition of potassium borohydride (29.2 mg, 0.54 mmol) and heated for an additional 3h. After cooling the mixture, excess solvent was removed to yield a white fluffy solid. NH_4OAc (83.2 mg, 1.08 mmol) dissolved in water (50 ml) was added to the residue and the resulted aqueous

solution was extracted with CH_2Cl_2 (3 × 50 ml). The organic layer was separated, washed with brine, dried with Na_2SO_4, filtered, evaporated and the residue dried under vacuum at 60°C to yield a colorless viscous oil (**6**; 62 mg, 0.09 mmol, 66.5%). ^{1}H NMR (300 MHz, CD_3OD) δ: 8.79 (d, 2H), 8.06 (d, 2H), 7.73 (dd, 2H), 7.59 (dd, 2H), 7.50-7.20 (m, 4H), 7.02 (d, 2H), 6.85 (d, 2H), 3.74 (s, 6H), 3.72-3.50 (m, 4H), 2.70-2.45 (m, 12H), 1.65-1.55 (m, 4H); ^{13}C NMR (75.4 MHz, CD_3OD) δ: 150.3, 147.1, 133.8, 133.5, 133.3, 133.1, 130.2, 130.0, 129.3, 128.8, 128.3, 126.2, 124.9, 122.0, 110.7, 56.6, 51.6, 50.5, 49.5, 49.0, 28.2.

[{1,12-bis(2-hydroxy-3-methoxy-5-(quinolin-3-yl)-benzyl)-1,5,8,12-tetraazadodecane}-gallium(III)]$^+$; [Ga-3-M-5-Quadd]$^+$[ClO_4]$^-$ (7).

Ligand (**6**) (62 mg, 0.09 mmol) was dissolved in methanol (3 ml) and treated with dropwise addition of gallium(III) acetylacetonate (32.5 mg, 0.09 mmol) dissolved in methanol (2 ml). The contents were refluxed for 1h. Potassium perchlorate (12.2 mg, 0.09 mmol) dissolved in hot water (1 ml) was added to the hot solution and refluxed for 15 min. On cooling to RT, slow evaporation of excess solvent resulted into a microcrystalline material, that was washed sequentially with water, methanol, and ether to yield **7** (32 mg, 0.04 mmol, 44.4%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.25 (d, 2H), 8.53 (d, 2H), 7.99 (dd, 2H), 7.80-7.50 (m, 8H), 7.43 (d, 2H), 7.31 (d, 2H), 4.7-4.6 (bm, 4H), 4.20 (m, 2H), 3.95 (m, 2H), 3.89 (s, 6H), 3.85-3.70 (m, 3H), 3.55-3.20 (m, 3H), 3.0-2.65 (m, 6H), 1.95-1.85 (m, 1H), 1.60 (m, 1H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ: 152.6, 150.8, 149.3, 133.3, 132.1, 131.5, 130.6, 128.8, 128.7, 128.1, 126.9, 126.2, 124.0, 120.4., 110.7, 56.6, 51.6, 50.5, 49.5, 49.0, 27.0; LRMS (FAB) Calcd for $C_{42}H_{46}N_6O_4Ga$: 767.2826; Found: $(M + H)^+$ 768.2808.

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Caution. Metal perchlorates are potentially explosive and should be handled with care!

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.[4] 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.[5] Parasite growth inhibition and half-maximal inhibitory concentration values (IC_{50}) were determined by measuring ^{3}H-hypoxanthine incorporation.[6] 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. Metal complex was added as 1:1000 dilutions of a 10 mM dimethylsulfoxide (DMSO) stock. Vehicle alone had no effect on ^{3}H -hypoxanthine incorporation.

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