PRIMACENES: novel non-cytotoxic primaquine-ferrocene conjugates with anti-*Pneumocystis carinii* activity

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ELECTRONIC SUPPLEMENTARY DATA

1. Chemistry

1.1. General materials and methods

N^{α}-Boc protected aminoacids, HBTU, HOBt and WSC.HCl were purchased to NovaBiochem (VWR International, Portugal). Ferrocenecarboxylic acid (FcCOOH) was from Fluka (Sigma-Aldrich, Spain). All solvents (*p.a.* quality) were from Merck (VWR International, Portugal) and dried over activated 4 Å molecular sieves prior to usage. All remaining chemicals were from Sigma-Aldrich (Spain). Silica-gel for column chromatography (ref. 60A, 35-70 μm; 550 m^2/g) was from SDS (France).

Ultrasound reactions were carried out in a Branson 2510 DTH ultrasound bath. NMR spectra were recorded on a Bruker Avance III 400 spectrometer and ESI-MS spectra were acquired on a Finnigan Surveyor LCQ DECA XP Max spectrometer.

Detailed synthetic procedures and spectroscopic data are only given for novel compounds. NMR data are abbreviated as follows: bs, broad singlet; d, doublet; dd, double doublet; δ, chemical shift (in ppm); m, unresolved multiplet; q, quartet; s, singlet; t, triplet; Q, hydrogen on quinoline ring; Q*C*, carbon on quinoline ring; Cp, hydrogen on cyclopentadiene ring; Cp_C, carbon on cyclopentadiene ring.

HPLC analyses were run on a Hitachi LaChrom Elite instrument equipped with a Diode Array Detector (DAD), using a Purospher ® STAR RP-18 endcapped (5 μm) column equipped with a LichroCART ® 125-4 pre-column. The solvents used on the mobile phase were water (A) and acetonitrile (B), each containing 0.05% (v/v) trifluoroacetic acid (TFA). Solvent gradient programmes employed are listed on Table S1.

Table S1. Solvent gradient programmes used in the HPLC analyses of primacenes.

Compound	Solvent gradient programme
2 **3a** **3b** **3c** **3d** **3e** **5**	0 to 10 min: 45-60% B 10 to 18 min: 60% B 18 to 19 min: 60-100% B 19 to 23 min: 100% B
4	0 to 8 min: 30% B 8 to 10 min: 30-50% B 10 to 12 min: 50% B 12 to 15 min: 50-100% B 15 to 20 min: 100% B
6	0 to 5 min: 70-100% B 5 to 15 min: 100% B

1.2. Synthetic Procedures

1.2.1. Synthesis of 2 by coupling ferrocenecarboxylic acid to primaquine

Ferrocenecarboxylic acid (FcCOOH, 0.9003 g, 3.913 mmol) was added to HBTU (1.8246 g, 4.8104 mmol) and DIEA (1.32 mL, 7.71 mmol) in 25 mL dry Dichloromethane (DCM). This solution was stirred in an ice bath for 30 minutes. A solution of PQ (1.2000 g, 4.6302 mmol) in 10 mL dry DCM was added. The mixture was kept in the ice bath for further 30 minutes, then kept under stirring at room temperature for 24 hours. The solution was washed three times with saturated aq. $NaHCO_3$, 1% aq. HCl and saturated aq. $NaHCO_3$. The organic layer was dried over

anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography on silica using DCM/acetone as eluent and obtained as a yellow-orange solid (m.p. 153-155 ºC) with correct spectroscopic and analytical data (section 2).

Compound **2** was also prepared by a slightly different methodology. In this case, FcCOOH (0.5718 g, 2.486 mmol) and a carbodiimide (DCC, DIC or EDC.HCl, for example, 0.5203 g, 2.714 mmol of EDC.HCl) were suspended in 20 mL DCM in a round-bottom flask that was immersed in an ultrasound bath (USB) for 10 minutes, at room temperature. A solution of PQ (0.7000 g, 2.700 mmol) in dry DCM (10 mL) was added to the flask and the reaction allowed proceeding for 2.5 h. The solution was washed three times with H_2O and the organic layer dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the crude product purified by column chromatography on silica using DCM/Me_2CO, yielding **2**. In the case of DCC-mediated coupling, isolation of pure **2** was impaired due to co-elution with DCU.

1.2.2. Synthesis of compounds 3a-e by coupling ferrocenecarboxylic acid to N-aminoacyl-PQ derivatives

Compounds **3a-e** (where R^1 is, respectively, H, Me, ^{i}Pr, ^{i}Bu and Bzl) were prepared by using HBTU to activate Fc-COOH for subsequent coupling with *N*-aminoacyl-PQs, as described in ***1.2.1***.. Compound **3b** was also prepared by *in situ* activation with DCC in an USB, following the procedure described in ***1.2.3.***.

1.2.3. Synthesis of 5 by coupling ferrocenecarboxylic acid to imidazoquines 7

Imidazoquines **7** were prepared by in-house procedures previously reported [15,18,21]. Compounds **5** were prepared by coupling Fc-COOH to **7** through carbodiimide

activation. Briefly, Fc-COOH (0.2073 g, 0.9011 mmol) and EDC.HCl (0.1746 g, 0.9108 mmol) were suspended in 10 mL of dry DCM in a round-bottom flask that was immersed in an USB. A solution of **7** (0.2913 g, 0.8172 mmol) in 20 mL of dry DCM was added to the reaction mixture and the reaction proceeded for 1,5 h at room temperature. The crude mixture was washed with 10% aq. $NaHCO_3$ (3×20 mL) and H_2O (3×20 mL). The product **5a** was purified through flash chromatography using DCM/Me_2CO and obtained as a yellow-orange oil in 41% yield, with correct spectroscopic and analytical data (section 2).

1.2.4. Synthesis of 4 by coupling ferrocenecarboxylic acid to PQ derivatives 8

A first attempt to prepare compounds **4** by using HBTU-mediated coupling as previously described [15] was not successful, as the ferrocenecarboxylic acid benzotriazolyl ester was obtained, as confirmed by ^{1}H-NMR (section 2). Activation of Fc-COOH with carbodiimides, as given in ***1.2.3.***, led to the successful synthesis of **4**.

1.2.5. Synthesis of 6 by coupling 6-bromohexylferrocene to 8-AQ

To a mixture of 6-methoxy-8-nitroquinoline (8-NQ, 0.2503 g; 1.226 mmol) and $SnCl_2$ (1.2024 g; 6.3418 mmol) were added 12.5 mL of concentrated HCl dropwise. This mixture was stirred for 4h at room temperature, and then neutralized by addition of 2M aqueous NaOH (30.0 mL) and stirring for 30 minutes. The aqueous mixture was washed three times with $CHCl_3$ (3×30.0 mL) and the organic layers pooled and dried over anhydrous Na_2SO_4. After removing the drying agent, the filtrate was evaporated to dryness on a rotary evaporator to obtain the product, 8-amino-6-methoxyquinoline (8-AQ), as a red oil (98 %).

8-AQ (0.2223 g; 1.276 mmol) was dissolved in 3.0 mL triethylamine (TEA). In a

separate flask, 6-bromohexylferrocene (0.2656 g; 7.608 mmol) was dissolved in 6.0 mL TEA and 3 mL of the resulting solution were then added to the 8-AQ. The reaction mixture was taken to 120 °C for 5h and, after 1h, another 3.0 mL-portion of 6-bromohexylferrocene in TEA was added. The reaction was allowed proceeding at 120 °C for more 4h. The reaction mixture was dissolved in 20.0 mL AcOEt and 50.0 mL of NaOH 2M was added. The aqueous phase was washed four times with AcOEt (4×30.0 mL) and the resulting organic layers were dried over anhydrous Na_2SO_4. After removal of the drying agent by filtration, and evaporation of the filtrate to dryness, the crude product was submitted to column chromatography on silica using AcOEt/n-hexane as eluent, yielding the desired product (**6**) as a green oil (23.7%) with correct spectroscopic and analytical data (section 2).

2. Spectroscopic Data

N-(4-(6-methoxyquinolin-8-ylamino)pentyl)ferrocenecarboxamide **(2)** δ_H (DMSO, 400 MHz) 8.52 (dd, J=4.20 Hz, J=1.65 Hz; 1H, Q2); 8.06 (dd, J=8.37 Hz, J=1.63 Hz; 1H, Q4); 7.77 (t, J=5.84 Hz; 1H, -CH_2-N*H*-CO-); 7.41 (dd, J=8.24 Hz, J=4.20 Hz; 1H, Q3); 6.64 (d, J=2.51 Hz; 1H, Q5); 6.29 (d, J=2.50 Hz; 1H, Q7); 6.14 (d, J=8.81 Hz; 1H, -N*H*-$CH(CH_3)$-); 4.77 to 4.75 (m; 2H, Cpa2 and Cpa5); 4.31 (t, J=1.90 Hz; 2H, Cpa3 and Cpa4); 4.11 (s; 5H, Cpb); 3.81 (s; 3H, C*H*$_3$-O-); 3.72 to 3.64 (m; 1H, -NH-C*H*(CH_3)-); 3.22 to 3.16 (m; 2H, -C*H*$_2$-NH-); 1.76 to 1.54 (m; 4H, -(C*H*$_2$)$_2$-CH_2-); 1.24 (d, J=6.13 Hz; 3H, -NH-CH(C*H*$_3$)-). δ_C (DMSO, 100 MHz) 186.6 (-NH-*C*O-); 158.9 (Q*C6*); 144.6 (Q*C2*); 144.2 (Q*C8*); 134.7 (Q*C4*); 134.5 (Q*C10*); 129.5 (Q*C*3); 122.0 (Q*C*9); 96.1 (Q*C*7); 91.6 (Q*C*5); 76.8 (Cpa$_{C1,2}$); 69.7 (Cpa$_{C3\text{-}5}$); 69.2 (Cpb); 68.0 and 67.9 (*C*H_3-O-);

54.9 (-NH-*C*H(CH$_3$)-); 46.9 (-*C*H$_2$-NH-); 33.5 (-*C*H$_2$-CH$_2$-CH$_2$-); 26.3 (-CH$_2$-*C*H$_2$-CH$_2$-); 20.2 (-CH(*C*H$_3$)-). ESI-MS: $[M+H]^+$, Found: 472.27, $C_{26}H_{29}FeN_3O_2$ requires 471.37. RP-HPLC: t_r = 7.35 min (% area = 99.0%).

N-(2-(4-(6-methoxyquinolin-8-ylamino)pentylamino)-2-oxoethyl)ferrocenecarboxamide **(3a)** The product was isolated as yellow-orange oil (70.5%) δ_H (DMSO, 400 MHz) 8.53 (dd, J=4.19 Hz, J=1.65 Hz; 1H, Q2); 8.07 (dd, J=8.38 Hz, J=1.63 Hz; 1H, Q4); 7.97 (t, J=5.95 Hz; 1H, -CH$_2$-N*H*-CO-); 7.83 (t, J=5.62 Hz; 1H, -(CH$_2$)$_3$-N*H*-); 7.42 (dd, J=8.24 Hz, J=4.20 Hz; 1H, Q3); 6.47 (d, J=2.52 Hz; 1H, Q5); 6.27 (d, J=2.50 Hz; 1H, Q7); 6.12 (d, J=8.79 Hz; 1H, -N*H*-CH(CH$_3$)-); 4.78 (t, J=1.88 Hz; 2H, Cpa2+5); 4.33 (t, J=1.92 Hz; 2H, Cpa3+4); 4.21 (s; 5H, Cpb); 3.82 (s; 3H, C*H$_3$*-O-); 3.75 (d, J=6.02 Hz; 2H, -CO-C*H$_2$*-NH-); 3.68 to 3.59 (m; 1H, -NH-C*H*(CH$_3$)-); 3.15 to 3.07 (m; 2H, -C*H*$_2$-NH-); 1.69 to 1.47 (m; 4H, -(C*H$_2$*)$_2$-CH$_2$-); 1.21 (d, J=6.30 Hz; 3H, -CH(C*H*$_3$)-). δ_C (DMSO, 100 MHz) 169.3 (-NH-*C*O-CH$_2$-); 168.9 (-NH-*C*O-Fc); 158.9 (Q*C6*); 144.6 (Q*C2*); 144.2 (Q*C8*); 134.7 (Q*C4*); 134.4 (Q*C10*); 129.5 (Q*C9*); 122.0 (Q*C3*); 96.0 (Q*C7*); 91.6 (Q*C5*); 76.1 (Cpa$_{C1}$); 69.8 (Cpa$_{C2+5}$); 69.3 (Cpb); 68.1 (Cpa$_{C3+4}$); 54.9 (*C*H$_3$-O-); 46.9 (-NH-*C*H(CH$_3$)-); 41.9 (-CO-*C*H$_2$-NH-); 38.5 (-*C*H$_2$-NH-CO-); 33.4 (-*C*H$_2$-CH$_2$-CH$_2$-); 25.9 (-CH$_2$-*C*H$_2$-CH$_2$-); 20.2 (-CH(*C*H$_3$)-). ESI-MS: $[M+H]^+$, Found: 529.31, $C_{28}H_{32}FeN_4O_3$ requires 528.42. RP-HPLC: t_r = 4.07 min (% area = 99.8%).

N-(1-(4-(6-methoxyquinolin-8-ylamino)pentylamino)-1-oxopropyl)ferrocenecarboxamide **(3b)** The product was isolated as yellow-orange oil (77.1%) δ_H ($CDCl_3$, 400 MHz) 8.52 (dd, J=4.22 Hz, J=1.62 Hz; 1H, Q2); 7.91 (dd, J=8.28 Hz, J=1.67 Hz; 1H, Q4); 7.30 (dd, J=8.24 Hz, J=4.23 Hz; 1H, Q3); 6.93 and 6.91 (t+t, J=5.82 Hz and J=5.80 Hz; 1H, -N*H*-CO-CH(CH$_3$)-); 6.60 and 6.59 (d+d, J=7.34 Hz and J=7.50 Hz; 1H, -N*H*-CO-

Fc); 6.33 and 6.32 (d+d, J=2.23 Hz and J=1.90 Hz; 1H, Q5); 6.27+6.26 (d+d, J=2.13 Hz and J=2.18 Hz; 1H, Q7); 6.00+5.99 (d+d, J=8.39 Hz and J=8.36 Hz; 1H, -N*H*-CH(CH_3)-); 4.70 to 4.66 (m; 3H, Cpa2+5 and -CO-C*H*(CH_3)-); 4.31 to 4.28 (m; 2H, Cpa3+4); 4.16 and 4.15 (s+s; 5H, Cpb); 3.88 and 3.87 (s+s; 3H, C*H*$_3$-O-); 3.66 to 3.56 (m; 1H, -NH-C*H*(CH_3)-); 3.40 to 3.19 (m; 2H, -C*H*$_2$-NH-CO-); 1.75 to 1.61 (m; 4H, -(C*H*$_2$)$_2$-CH_2-NH-); 1.45 and 1.44 (d+d, J=6.93 Hz and J=6.93 Hz; 3H, -CO-CH(C*H*$_3$)-); 1.27 (d, J=6.36 Hz; 3H, -NH-CH(C*H*$_3$)-). δ_C ($CDCl_3$, 100 MHz) 172.5 (-*C*O-CH(CH_3)-); 170.3 (Q*C6*); 159.3 (-*C*O-Fc); 144.8 (Q*C2*); 144.2 (Q*C8*); 135.3 (Q*C4*); 134.7 (Q*C10*); 129.8 (Q*C9*); 121.8 (Q*C3*); 96.7 (Q*C7*); 91.6 (Q*C5*); 75.2 (Cpa$_{C1}$); 70.6 (Cpa$_{C5}$); 69.7 (Cpb); 68.2 (Cpa$_{C2}$); 68.1 (Cpa$_{C3+4}$); 55.1 (*C*H_3-O-); 48.7 (-*C*H(CH_3)-); 47.8 and 47.7 (-NH-*C*H(CH_3)-); 39.5 and 39.4 (-*C*H_2-NH-CO-); 33.8 (-*C*H_2-CH_2-CH_2-); 26.2 and 26.1 (-CH_2-*C*H_2-CH_2-); 20.5 (-NH-CH(*C*H_3)-); 18.8 and 18.7 (-CO-CH(*C*H_3)-). ESI-MS: $[M+H]^+$, Found: 543.19, $C_{28}H_{32}FeN_4O_3$ requires 542.45. RP-HPLC: t_r = 4.83 min (% area = 99.9%).

N-(1-(4-(6-methoxyquinolin-8-ylamino)pentylamino)-3-methyl-1-oxobutyl)ferrocene-carboxamide **(3c)** The product was isolated as yellow-orange oil (81.1%) δ_H ($CDCl_3$, 400 MHz) 8.53 (dd, J=4.22 Hz, J=1.57 Hz; 1H, Q2); 7.92 and 7.91 (dd+dd, J=8.28 Hz, J=1.50 Hz and J=8.21 Hz, J=1.25 Hz; 1H, Q4); 7.30 and 7.29 (dd+dd, J=8.23 Hz, J=4.21 Hz and J=8.24 Hz, J=4.23 Hz; 1H, Q3); 6.57 and 6.55 (t+t, J=6.07 Hz and J=5.90 Hz; 1H, -CH_2-N*H*-CO-); 6.47 (d, J= 8.06 Hz; 1H, -N*H*-CO-Fc); 6.34 and 6.33 (d+d, J=2.41 Hz and J=2.34 Hz; 1H, Q5); 6.27 and 6.26 (d+d, J=2.87 Hz and J=2.80 Hz; 1H, Q7); 5.99 and 5.98 (d+d, J=8.27 Hz and J=8.25 Hz; 1H, -N*H*-CH(CH_3)-); 4.74 to 4.68 (m; 2H, Cpa5 and -C*H*(CH(CH_3)$_2$)-); 4.34 to 4.30 (m; 3H, Cpa2+3+4); 4.18 and 4.17 (s+s; 5H, Cpb); 3.88 and 3.87 (s+s; 3H, C*H*$_3$-O-); 3.64 to 3.57 (m; 1H, -C*H*(CH_3)-

); 3.42 to 3.14 (m; 2H, -C*H*$_2$-NH-CO-); 2.16 to 2.12 (m; 1H, -C*H*(CH_3)$_2$); 1.74 to 1.61 (m; 4H, -(C*H*$_2$)$_2$-CH_2-NH-); 1.27 and 1.26 (d+d, J=6.30 Hz and J=6.36 Hz; 3H, -CH(C*H*$_3$)-); 0.99 (d, J=6.77 Hz; 6H, -CH(C*H*$_3$)$_2$). δ_C ($CDCl_3$, 100 MHz) 171.4 (-*C*O-CH(CH(CH_3)$_2$)-); 170.6 (Q*C6*); 159.4 (-*C*O-Fc); 144.9 (Q*C2*); 144.3 (Q*C8*); 135.3 (Q*C4*); 134.8 (Q*C10*); 129.8 (Q*C9*); 121.8 (Q*C3*); 96.8 (Q*C7*); 91.7 (Q*C5*); 75.5 (Cpa$_{C1}$); 70.6 (Cpa$_{C5}$); 70.5 (Cpa$_{C2}$); 69.7 (Cpb); 68.5 (Cpa$_{C3}$); 68.0 (Cp$_{C4}$); 58.6 and 58.5 (-CO-*C*H(CH(CH_3)$_2$)-); 55.2 (*C*H_3-O-); 47.8 and 47.7 (-*C*H(CH_3)-); 39.5 and 39.4 (-*C*H_2-NH-); 34.0 and 33.9 (-*C*H_2-CH_2-CH_2-); 30.9 (-*C*H(CH_3)$_2$-); 26.2(4) and 26.2(1) (-CH_2-*C*H_2-CH_2-); 20.5 (-CH(*C*H_3)-); 19.4 (-CH(*C*H_3)(CH_3)); 18.5 (-CH(CH_3)(*C*H_3)). ESI-MS: $[M+H]^+$, Found: 571.33, $C_{28}H_{32}FeN_4O_3$ requires 570.50. RP-HPLC: t_r = 7.48 min (% area = 99.9%).

N-(1-(4-(6-methoxyquinolin-8-ylamino)pentylamino)-4-methyl-1-oxopentyl)ferrocenecarboxamide **(3d)** The product was isolated as yellow-orange oil (47.8%). δ_H ($CDCl_3$, 400 MHz) 8.52+8.51 (dd+dd, J=4.23 Hz, J=1.86 Hz and J=4.12 Hz, J=1.81 Hz; 1H, Q2); 7.92 and 7.91 (dd+dd, J=8.27 Hz, J=1.81 Hz and J=8.27 Hz, J=1.77 Hz; 1H, Q4); 7.31 and 7.30 (dd+dd, J=8.22 Hz, J=4.22 Hz and J=8.24 Hz, J=4.22 Hz; 1H, Q3); 6.63 and 6.61 (t+t, J=5.84 Hz and J=5.88 Hz; 1H, -CH_2-N*H*-); 6.35 to 6.29 (m; 2H, -N*H*-CO-Fc and Q5); 6.27 and 6.26 (d+d, J=2.75 Hz and J=2.72 Hz; 1H, Q7); 5.99 and 5.97 (d+d, J=8.09 Hz and J=8.07 Hz; 1H, -N*H*-CH(CH_3)-); 4.72 to 4.65 (m; 2H, Cpa2+5); 4.61 to 4.54 (m; 1H, -C*H*(CH_2-CH(CH_3)$_2$)-); 4.33 to 4.31 (m; 2H, Cpa3+4); 4.18 and 4.16 (s+s; 5H, Cpb); 3.88 and 3.87 (s+s; 3H, C*H*$_3$-O-); 3.64 to 3.54 (m; 1H, -C*H*(CH_3)-); 3.37 to 3.17 (m; 2H, -C*H*$_2$-NH-); 1.75 to 1.58 (m; 7H, -(C*H*$_2$)$_2$-CH_2- and -C*H*$_2$-C*H*(CH_3)$_2$); 1.27 (d, J=6.36 Hz; 3H, -CH(C*H*$_3$)-); 1.00 to 0.93 (m; 6H, -CH(C*H*$_3$)$_2$). δ_C ($CDCl_3$, 100 MHz) 172.3 (-CH_2-NH-*C*O-); 170.6 (Q*C6*); 159.4 (-*C*O-

Fc); 144.9 (Q*C2*); 144.3 (Q*C8*); 135.3 (Q*C4*); 134.7 (Q*C10*); 129.9 (Q*C9*); 121.8 (Q*C3*); 96.7 (Q*C7*); 91.6 (Q*C5*); 75.2 (Cpa_{C1+C5}); 70.6 (Cpa_{C2}); 69.7 (Cpb); 68.3 (Cpa_{C3}); 68.1 (Cp_{C4}); 55.2 (*C*H_3-O-); 51.6 (-*C*H($CH_2CH(CH_3)_2$); 47.8 and 47.7 (-*C*H(CH_3)-); 41.1 and 41.0 (-C*H_2*-CH(CH_3)$_2$); 39.5 and 39.4 (-*C*H_2-NH-); 33.9 and 33.8 (-CH(CH_3)-*C*H_2-); 26.2 and 26.1 (-*C*H_2-CH_2-NH-); 24.9 (-*C*H-(CH_3)$_2$); 23.3 (-CH(*C*H_3)(CH_3)); 22.1 (-CH(CH_3)(*C*H_3)); 20.5 (-CH(*C*H_3)). ESI-MS: $[M+H]^+$, Found: 585.33, $C_{28}H_{32}FeN_4O_3$ requires 584.33. RP-HPLC: t_r = 9.39 min (% area = 91.4%).

N-(1-(4-(6-methoxyquinolin-8-ylamino)pentylamino)-2-oxo-1-phenylethyl)ferrocene-carboxamide **(3e)** The product was isolated as yellow-orange solid (71.9%, m.p. 123-125 ºC). δ_H (DMSO, 400 MHz) 8.53 (dd, J=4.19 Hz, J=1.61 Hz; 1H, Q2); 8.06 (dd, J=8.30 Hz, J=1.59 Hz; 1H, Q4); 8.03 (t, J=5.71 Hz; 1H, -N*H*-CO-Fc); 7.80 (dd, J=8.52 Hz, J=5.78 Hz; 1H, -CH_2-N*H*-); 7.42 (dd, J=8.24 Hz, J=4.20 Hz; 1H, Q3); 7.38 to 7.36 (m; 2H, Ph_{3+5}); 7.29 to 7.26 (m; 2H, Ph_{2+6}); 7.16 to 7.12 (m; 1H, Ph_4); 6.46 and 6.45 (d+d, J=2.58 Hz and J=2.57 Hz; 1H, Q5); 6.26 and 6.25 (d+d, J=2.72 Hz and J=2.67 Hz; 1H, Q7); 6.11 (d, J=8.76 Hz; 1H, -N*H*-CH(CH_3)-); 4.84 to 4.83 (m; 1H, Cpa2); 4.75 to 4.73 (m; 1H, Cpa5); 4.68 to 4.62 (m; 1H, -C*H*(Bzl)-); 4.28 to 4.27 (m; 2H, Cpa3+4); 3.91 and 3.90 (s+s; 5H, Cpb); 3.79 and 3.77 (s+s; 3H, C*H_3*-O-); 3.66 to 3.57 (m; 1H, -C*H*(CH_3)-); 3.20 to 2.85 (m; 4H, -C*H_2*-Ph- and -C*H_2*-NH-); 1.65 to 1.43 (m; 4H, -(C*H_2*)$_2$-CH_2-); 1.19 and 1.18 (d+d, J=6.28 Hz and J=6.27Hz; 3H, -CH(C*H_3*)-). δ_C (DMSO, 100 MHz) 171.5 (-CH_2-NH-*C*O-); 168.1 (-*C*O-Fc); 158.9 (Q*C6*); 144.5 (Q*C2*); 144.2 (Q*C8*); 138.6 (Ph_{C1}); 134.8 (Q*C4*); 134.5 (Q*C10*); 129.5 (Q*C9*); 129.1 (Ph_{C3+5}); 128.0 (Ph_{C2+6}); 126.1 (Ph_{C4}); 122.1 (Q*C3*); 96.1 (Q*C7*); 91.5 (Q*C5*); 75.8 (Cpa_{C1+C5}); 69.8 (Cpa_{C2}); 69.2 (Cpb); 68.6 (Cpa_{C4}); 67.8 (Cp_{C3}); 54.9 and 54.8 (*C*H_3-O-); 54.3 (-*C*H(Bzl)-); 47.0 and 46.9 (-*C*H(CH_3)-); 38.2 (-*C*H_2-NH-); 37.3 (-*C*H_2-Ph); 33.3 (-*C*H_2-

CH_2-CH_2-); 25.9 and 25.8 (-*C*H_2-CH_2-NH-); 20.2 (-CH(*C*H_3)). ESI-MS: $[M+H]^+$, Found: 619.39, $C_{28}H_{32}FeN_4O_3$ requires 618.55. RP-HPLC: t_r = 9.97 min (% area = 99.9%).

N-(2-(2-(4-(6-methoxyquinolin-8-ylamino)pentylamino)-2-oxoethylamino)-2-oxo-ethyl)ferrocenecarboxamide **(4)** δ_H (DMSO, 400 MHz) 8.54 (dd, J=4.20 Hz, J=1.66 Hz; 1H, Q2); 8.20 (t, J=5.79 Hz; 1H, -N*H*-CO-CH_2); 8.18 (t, J=5.89 Hz; 1H, -N*H*-CO-Fc); 8.09 (dd, J=8.38 Hz, J=1.63 Hz; 1H, Q4); 7.82 (t, J=5.56 Hz; 1H, -$(CH_2)_3$-N*H*-); 7.43 (dd, J=8.24 Hz, J=4.20 Hz; 1H, Q3); 6.49 (d, J=2.50 Hz; 1H, Q5); 6.28 (d, J=2.50 Hz; 1H, Q7); 6.14 (d, J=8.85 Hz; 1H, -N*H*-CH(CH_3)-); 4.76 (t, J=1.93 Hz; 2H, Cpa2+5); 4.29 to 4.23 (m; 2H, Cpa3+4); 4.17 (s; 5H, Cpb); 3.83 (s; 3H, C*H*$_3$-O-); 3.78 (d, J=5.77 Hz; 2H, -CO-C*H*$_2$-NH-); 3.69 (d, J=5.84 Hz; 2H, -C*H*$_2$-NH-CO-Fc); 3.66 to 3.61 (m; 1H, -C*H*(CH_3)-); 3.11 (td, J=5.92 Hz, J=5.92 Hz; 2H, -CH_2-C*H*$_2$-NH-); 1.75 to 1.48 (m; 4H, -(C*H*$_2$)$_2$-CH_2-); 1.20 (d, J=6.29 Hz; 3H, -CH(C*H*$_3$)-). δ_C (DMSO, 100 MHz) 171.5 (-NH-*C*O-CH_2-); 171.0 (-$(CH_2)_3$-NH-*C*O-); 169.9 (Q*C*6); 160.4 (-NH-*C*O-Fc); 146.1 (Q*C*2); 145.6 (Q*C*8); 136.2 (Q*C*4); 136.0 (Q*C*10); 131.0 (Q*C*9); 123.5 (Q*C*3); 97.5 (Q*C*7); 93.0 (Q*C*5); 77.1 (Cpa$_{C1}$); 71.4 (Cpa$_{C2+C5}$); 70.8 (Cpb); 69.6 (Cpa$_{C3+C4}$); 56.4 (*C*H_3-O-); 48.4 (-*C*H(CH_3)-); 44.1 (-*C*H_2-NH-CO-Fc); 43.5 (-CO-*C*H_2-NH-); 40.1 (-$(CH_2)_2$-*C*H_2-); 34.9 (-CH(CH_3)-*C*H_2-); 27.4 (-CH_2-*C*H_2-CH_2-); 21.6 (-CH(*C*H_3)-). ESI-MS: $[M+H]^+$, Found: 586.33, $C_{28}H_{32}FeN_4O_3$ requires 585.48. RP-HPLC: t_r = 12.6 min (% area = 99.7%).

N-ferrocenecarbonyl-3-(4-(6-methoxyquinolin-8-ylamino)pentyl)-2,2-dimethylimida-zolidin-4-one **(5)** δ_H (DMSO, 400 MHz) 8.54 (dd, J=4.20 Hz, J=1.61 Hz; 1H, Q2); 8.08 (dd, J=8.30 Hz, J=1.55 Hz; 1H, Q4); 7.42 (dd, J=8.25 Hz, J=4.20 Hz; 1H, Q3); 6.48 (d,

J=2.46 Hz; 1H, Q5); 6.30 (d, J=2.42 Hz; 1H, Q7); 6.15 (d, J=8.91 Hz; 1H, -N*H*-CH(CH_3)-); 4.76 (t, J=1.94 Hz; 2H, Cpa2+5); 4.41 to 4.40 (m; 2H, Cpa3+4); 4.31 (s; 2H, -CO-CH_2-); 4.20 (s; 5H, Cpb); 3.81 (s; 3H, CH_3-O-); 3.72 to 3.63 (m; 1H, -NH-C*H*(CH_3)-); 3.29 to 3.18 (m; 2H, -CH_2-CH_2-CH_2-); 1.80 to 1.55 (m; 10H, -(CH_2)$_2$-CH_2- and -C(CH_3)$_2$-); 1.22 (d, J=6.29 Hz; 3H, -CH(CH_3)-). δ_C (DMSO, 100 MHz) 166.8 (-*C*O-CH2-); 166.0 (Q*C6*); 159.0 (-*C*O-Fc); 144.6 (Q*C2*); 144.2 (Q*C8*); 134.8 (Q*C4*); 134.5 (Q*C10*); 129.5 (Q*C9*); 122.1 (Q*C3*); 96.2 (Q*C5*); 91.6 (Q*C7*); 80.8 (-*C*(CH_3)$_2$-); 76.8 (Cpa$_{C1}$); 70.4 (Cpa$_{C2+C5}$); 70.3 (Cpa$_{C3+C4}$); 69.3 (Cpb); 55.0 (*C*H_3-O-); 54.9 (-*C*H(CH_3)-); 48.6 (-CO-*C*H_2-); 47.0 (-(CH_2)$_2$-*C*H_2-); 33.4 (-*C*H_2-CH_2-CH_2-); 25.5 (-CH_2-*C*H_2-CH_2-); 24.0 (-C(*C*H_3)$_2$-); 20.2 (-CH(*C*H_3)-). ESI-MS: $[M+H]^+$, Found: 569.33, $C_{28}H_{32}FeN_4O_3$ requires 568.39. RP-HPLC: t_r = 9.13 min (% area = 99.9%).

N-(6-ferrocenhexyl)-8-amino-6-methoxyquinolin **(6)** δ_H (DMSO, 400 MHz) 8.54 (dd, J=4.22 Hz, J=1.66 Hz; 1H, Q2); 7.92 (dd, J=8.27 Hz, J=1.64 Hz; 1H, Q4); 7.30 (dd, J=8.24 Hz, J=4.23 Hz; 1H, Q3); 6.34 (d, J=2.54 Hz; 1H, Q5); 6.29 (d, J=2.53 Hz; 1H Q7); 6.09 (t, J=5.25 Hz; 1H, -N*H*-CH_2-); 4.08 (s; 5H, Cpb); 4.05 to 4.04(m; 2H, Cpa2+5); 4.03 to 4.02 (m; 2H, Cpa3+4); 3.89 (s; 3H, CH_3-O-); 3.29 to 3.22 (m; 2H, -NH-CH_2-); 2.35 to 2.28 (m; 2H –CH_2-Cpa-); 1,81 to 1.72 (m; 2H, -NH-CH_2-CH_2-); 1.57 to 1.44 (m; 6H, -(CH_2)$_3$-CH_2-Cpa). δ_C (DMSO, 100 MHz) 159.4 (Q*C6*); 145.9 (Q*C2*); 144.3 (Q*C8*); 135.3 (Q*C4*); 134.7 (Q*C10*); 129.7 (Q*C9*); 121.8 (Q*C3*); 96.5 (Q*C7*); 91.7 (Q*C5*); 89.3 (Cpa$_{C1}$); 68.4 (Cpb); 68.0 (Cpa$_{C2+C5}$); 67.0 (Cpa$_{C3+C4}$); 55.2 (*C*H_3-O-); 43.3 (-NH-*C*H_2-); 31.1 (-NH-CH_2-*C*H_2-); 29.5 (-*C*H_2-Cpa-); 29.4 (-*C*H_2-CH_2-CH_2-Cpa-); 29.1 (-*C*H_2-CH_2-Cpa-); 27.2 (-NH-(CH_2)$_2$-*C*H_2-). ESI-MS: $[M+H]^+$, Found: 443.33, $C_{28}H_{32}FeN_4O_3$ requires 442.37. RP-HPLC: t_r = 8.17 min (% area = 98.9%).

3. *In vitro* biological assays

3.1. Blood-schizontocidal activity against *Plasmodium falciparum*

These assays have been conducted as reported elsewhere [23]. Briefly, synchronized ring-stage W2 strain *P. falciparum* parasites were cultured with multiple concentrations of test compounds (added from 1,000× stocks in DMSO) in RPMI 1640 medium with 10% human serum. After a 48 h incubation, when control cultures contained new rings, parasites were fixed with 1% formaldehyde in PBS, pH 7.4, for 48 h at room temperature and then labeled with YOYO-1 (1 nM; Molecular Probes) in 0.1% Triton X-100 in PBS. Parasitemias were determined from dot plots (forward scatter vs. fluorescence) acquired on a FACSort flow cytometer using CELLQUEST software (Becton Dickinson). IC_{50}s for growth inhibition were determined with GraphPad Prism software from plots of percentages of the level of parasitemia of the control relative to inhibitor concentration. In each case, goodness of curve fit was documented by R^2 values of > 0.95.

3.2. Anti-*Pneumocystis carinii* activity

***P. carinii* organisms.** Organisms for the ATP assays were obtained from chronically immunosuppressed Long Evans and Brown Norway rats housed under barrier conditions at the Cincinnati VA Medical Center (VAMC) and inoculated intratracheally with *P. carinii*. These were extracted and purified from the lungs of rats

after 8-12 weeks of immunosuppression, enumerated, cryopreserved, and stored in liquid nitrogen. Typically, infected rat lungs yield up to 2×10^{10} organism nuclei with the vast majority (about 95%) of the life cycle forms present as trophic forms with the remainder (about 5%) being composed of cysts. *P. carinii* preparations were evaluated for microbial contamination, ATP content, karyotype, and host cell content prior to use in the ATP assay.

ATP assay. Isolated organisms used for ATP analyses were suspended in a supplemented RPMI 1640 medium containing 20% calf serum and other additives, pH 7.5 to 8.0, 380 mOsm, as previously described [23]. Drugs were added to the culture medium in DMSO (the final concentration of DMSO was <0.2%, vol/vol), and 10^8 organisms (as total nuclei) per ml were added to 1 to 2 ml of the culture medium in multiwell plates. For every assay, each drug concentration was assayed in triplicate using different organism isolation batches. The final ATP content was expressed as the average relative light units of nine values (three readings per well). To assess the effects of extended exposure to PQ and imidazoquines, the ATP contents of cultures sampled after 24, 48, and 72 h of incubation at 35°C in a 10% CO_2 humidified atmosphere were measured. The media of all wells were changed on a daily basis after centrifugation of the multiwell plates at 2,400×*g* and removal of the previous medium. The ATP content was determined by the luciferin-luciferase assay as described previously [23] and was expressed as relative light units. The effects of the compounds on the *P. carinii* ATP content were compared with the ATP contents of *P. carinii* populations that did not receive experimental compounds and expressed as percentages of these control values. In addition, other controls for each assay included quench controls to evaluate the effects of the highest drug concentrations used on the luciferase-luciferin reaction; vehicle controls to evaluate the effects of any solvent on the same reaction and on the

organism ATP content.

3.3. Cytotoxicity

Preparations with less than 85% viability were not used in the cytoxicity assay. After enumeration, organisms were added to 24-well plates containing 1 to 2 mL of RPMI supplemented with 20% FBS, vitamins, minerals, and other additives at a standard density of 10^8/mL with or without a test compound. At least three concentrations of each compound were evaluated (10, 1.0, and 0.1 μg/mL) by incorporation into the medium. Triplicate wells were used for each concentration of test compound evaluated and for control groups [23].