Supporting Information

For

Functionalized Naphthalimide-4-Aminoquinoline Conjugates as Promising Anti-plasmodials, with Mechanistic Insights

Shalini,[†] Jenny Legac,[‡] Adebayo A. Adeniyi,[§] Prishani Kisten,[‖] Philip J. Rosenthal,[‡] Parvesh Singh,*[‖] Vipan Kumar*[†]

[†] Department of Chemistry, Guru Nanak Dev University, Amritsar-143005, Punjab, India

[‡] Department of Medicine, University of California, San Francisco, CA, USA

[§] Department of Industrial Chemistry, Federal University of Oye-Ekiti, Nigeria

[‖] School of Chemistry and Physics, University of KwaZulu-Natal, Westville, Durban-4000, South Africa

Contents

1. Synthesis and spectral data of the synthesized hybrids (**3a-j** and **4a-o**)

General information:

Stuart Digital Melting Point apparatus (SMP10) and an open capillary was used to determine melting points and are uncorrected. BRUKER AVANCE II (500 and 125 MHz) and JEOL (400 and 100 MHz) NMR spectrometers were used in order to record ^{1}H and ^{13}C NMR spectra of the synthesized compounds dissolved in DMSO-d6 and/or $CDCl_3$ (Sigma-Aldrich). Tetramethylsilane (TMS) was kept as reference and chemical shift values are expressed as parts per million (ppm) downfield from TMS. While the coupling constants represented by *J* values are expressed in hertz (Hz). Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, dt: doublet of a triplet and br: broad peak. Mass spectra were recorded on a Bruker micrOTOF-QII high resolution mass spectrometer.

Chemical procedure for the synthesis of target hybrid compounds, 3a-j and 4a-o:

To a well stirred solution of 1,8-naphthalic anhydride (R=H/Br) (1 mmol) using anhydrous NMP as solvent, quinoline diamine (1 mmol) was added and the reaction mixture was heated at 150°C for 2h. The reaction was monitored using TLC and on its completion, ice cold water was added that resulted in the precipitation out of the desired hybrid. The reaction mixture was then filtered using suction pump and the compound obtained was washed with water, dried and recrystallized in ethanol to get first batch of desired hybrids **3a-j**.

In order to produce rest of the target compounds, a well stirred solution of **3** (for **3f-j**, when R=Br) (1mmol) in anhydrous NMP (1-2 mL), with added secondary amine (2 mmol) (piperidine/morpholine/hydroxyethyl piperazine), was allowed to heat at 180 °C for 1h. Again, ice cold water was added to the reaction mixture after its completion in order to get the solid compounds which were then filtered and washed with water. The recrystallization of the crude solid in ethanol afforded the final conjugates **4a-o**.

Spectral data of the compounds:

2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3a**)

Yield-86%, pale yellow solid, M.P=130-135 ºC, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.61-3.65 (m, 2H, CH_2), 4.30 (t, *J*= 6.8 Hz, 2H, CH_2), 6.83 (d, *J*= 5.1 Hz, 1H, H^2), 7.41 (dd, *J*= 1.4, 8.8 Hz, 1H, H^4), 7.70 (t, *J*= 5.6 Hz, 1H, NH-exchangeable with D_2O), 7.78 (s, 1H, H^5), 7.83-7.86

(m, 2H, H^7+H^{10}), 8.11 (d, *J*= 8.9 Hz, 1H, H^3), 8.42-8.53 (m, 5H, H^1+H^6+H^8+H^9+H^{11}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 164.15, 152.87, 145.45, 143.10, 136.45, 134.79, 131.71, 131.14, 127.63, 127.50, 125.95, 125.15, 122.85, 122.45, 117.50, 99.17, 40.85, 38.22. HRMS (ESI) calcd for $C_{23}H_{16}ClN_3O_2$ $[M+H]^+$ 402.1009, found 402.1024.

2-(3-((7-chloroquinolin-4-yl)amino)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(3b)***
Yield-90%, pale yellow solid, M.P=165-170 ºC, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.00-2.07 (m, 2H, CH_2), 3.42-3.46 (m, 2H, CH_2), 4.13 (t, *J*= 6.8 Hz, 2H, CH_2), 6.57 (d, *J*= 6.1 Hz, 1H, H^2), 7.41 (d, *J*= 2.1, 9.0 Hz, 1H, H^4), 7.74-7.78 (m, 3H, H^5+H^7+H^{10}), 8.17 (s, 1H, NH-exchangeable with D_2O), 8.24 (d, *J*= 9.1 Hz, 1H, H^3), 8.34-8.36 (m, 5H, H^1+H^6+H^8+H^9+H^{11}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.03, 152.63, 148.57, 145.06, 135.89, 134.75, 131.70, 131.15, 127.80, 127.64, 125.58, 125.06, 124.38, 122.50, 117.04, 99.16, 41.30, 38.34, 26.83. HRMS (ESI) calcd for $C_{24}H_{18}ClN_3O_2$ $[M+H]^+$ 416.1165, found 416.1183.

*2-(4-((7-chloroquinolin-4-yl)amino)butyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3c**)*
Yield-89%, grey solid, M.P=130-135 ºC, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.74-1.78 (m, 4H, $(CH_2)_2$), 3.45-3.46 (m, 2H, CH_2), 4.08 (t, *J*= 5.0 Hz, 2H, CH_2), 6.68 (d, *J*= 6.45 Hz, 1H, H^2), 7.53 (dd, *J*= 1.8, 8.9 Hz, 1H, H^4), 7.78-7.81 (m, 2H, H^7+H^{10}), 7.87 (d, *J*=1.6 Hz, 1H, H^5), 8.37-8.40 (m, 5H, H^1+H^6+H^8+H^9+H^{11}), 8.44 (d, *J*= 9.1 Hz, 1H, H^3), 8.76 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.87, 153.78, 146.25, 142.56, 136.62, 134.72, 131.61, 131.10, 127.65, 127.57, 126.07, 125.54, 122.33, 122.30, 116.47, 98.95, 43.07, 25.65, 25.62. HRMS (ESI) calcd for $C_{25}H_{20}ClN_3O_2$ $[M+H]^+$ 430.1322, found 430.1337.

*2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3d**)*
Yield-88%, light brown solid, M.P=185-190 ºC, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.38-1.44 (m, 4H, $(CH_2)_2$), 1.63-1.69 (m, 4H, $(CH_2)_2$), 3.41-3.45 (m, 2H, CH_2), 3.99-4.04 (m, 2H, CH_2), 6.73 (d, *J*= 6.75 Hz, 1H, H^2), 7.63 (dd, *J*= 1.7, 8.9 Hz, 1H, H^4), 7.81-7.85 (m, 2H, H^7+H^{10}), 7.95 (d, *J*= 1.6 Hz, 1H, H^5), 8.39-8.45 (m, 5H, H^1+H^6+H^8+H^9+H^{11}), 8.53 (d, *J*= 9.0 Hz, 1H, H^3), 9.02 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.80, 154.53, 145.15, 141.30, 137.26, 134.73, 131.69, 131.11, 127.73, 127.63, 126.50, 125.83,

122.42, 121.31, 116.29, 98.97, 43.41, 27.91, 27.87, 26.71, 26.59. HRMS (ESI) calcd for $C_{27}H_{24}ClN_3O_2$ $[M+H]^+$ 458.1635, found 458.1655.

*2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3e**)*

Yield-88%, grey solid, M.P=105-110 ºC, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.31 (s, 8H, $(CH_2)_4$), 1.61-1.62 (m, 4H, $(CH_2)_2$), 3.21-3.22 (m, 2H, CH_2), 4.00 (t, *J*= 7.2 Hz, 2H, CH_2), 6.42 (d, *J*= 5.4 Hz, 1H, H^2), 7.34 (s, 1H, NH- exchangeable with D_2O), 7.41 (dd, *J*= 1.7, 8.8 Hz, 1H, H^4), 7.75 (s, 1H, H^5), 7.80-7.83 (m, 2H, H^7+H^{10}), 8.26 (d, *J*= 9.0 Hz, 1H, H^3), 8.36 (d, *J*= 5.1 Hz, 1H, H^1), 8.39-8.45 (m, 4H, H^6+H^8+H^9+H^{11}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.77, 151.90, 150.75, 149.03, 134.68, 133.97, 131.70, 131.11, 127.74, 127.60, 127.50, 124.61, 124.48, 122.44, 117.79, 99.00, 42.90, 29.17, 29.15, 28.19, 27.89, 27.05, 26.94. HRMS (ESI) calcd for $C_{29}H_{28}ClN_3O_2$ $[M+H]^+$ 486.1948, found 486.1961.

*6-bromo-2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3f**)*

Yield-84%, pale yellow solid, M.P=240-245 ºC, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.56-3.58 (m, 2H, CH_2), 4.20 (t, *J*=6.5 Hz, 2H, CH_2), 6.62 (d, *J*= 6.4 Hz, 1H, H^2), 7.56 (dd, *J*= 1.9, 9.0 Hz, 1H, H^4), 7.89 (d, *J*=1.8 Hz, 1H, H^5), 7.92 (dd, *J*= 8.0, 7.9 Hz, 1H, H^9), 8.12 (d, *J*=7.6 Hz, 1H, H^7), 8.25 (d, *J*=7.5 Hz, 1H, H^6), 8.40 (d, *J*=6.3 Hz, 1H, H^1), 8.45-8.50 (m, 3H, H^3+H^8+H^{10}), 8.65 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.20, 163.15, 153.10, 146.40, 137.10, 133.10, 132.45, 132.10, 131.45, 131.20, 130.15, 129.24, 128.98, 128.65, 125.90, 125.35, 123.50, 122.03, 121.70, 116.23, 98.90, 40.75, 38.20. HRMS (ESI) calcd for $C_{23}H_{15}BrClN_3O_2$ $[M+H]^+$ 480.0114 found 480.0136 and $[M+3]^+$ 482.0036, found 482.0146.

*6-bromo-2-(3-((7-chloroquinolin-4-yl)amino)propyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3g**)*

Yield-83%, peach white solid, M.P=115-120 ºC, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.98-2.01 (m, 2H, CH_2), 3.40-3.43 (m, 2H, CH_2), 4.10 (t, *J*=6.7 Hz, 2H, CH_2), 6.61 (d, *J*= 6.5 Hz, 1H, H^2), 7.55 (dd, *J*= 2.0, 9.1 Hz, 1H, H^4), 7.88 (d, *J*=1.9 Hz, 1H, H^5), 7.91 (dd, *J*= 7.8, 8.0 Hz, 1H, H^9), 8.11 (d, *J*=7.5 Hz, 1H, H^7), 8.24 (d, *J*=7.4 Hz, 1H, H^6), 8.41 (d, *J*=6.4 Hz, 1H, H^1), 8.44-8.49 (m, 3H, H^3+H^8+H^{10}), 8.64 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.96, 163.90, 152.45, 145.10, 136.65, 133.31, 132.35, 132.01,

131.10, 131.35, 130.10, 129.15, 128.10, 127.98, 125.92, 125.45, 123.40, 122.15, 121.68, 115.85, 99.10, 40.92, 38.30, 25.99. HRMS (ESI) calcd for $C_{24}H_{17}BrClN_3O_2$ $[M+H]^+$ 494.0271, found 494.0343 and $[M+3]^+$ 496.0193, found 496.0353.

6-bromo-2-(4-((7-chloroquinolin-4-yl)amino)butyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(3h)***

Yield-85%, Off-white solid, M.P=240-245 ºC, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.70-1.75 (m, 4H, $(CH_2)_2$), 3.41-3.43 (m, 2H, CH_2), 4.05 (t, *J*=6.5 Hz, 2H, CH_2), 6.67 (d, *J*= 6.5 Hz, 1H, H^2), 7.60 (dd, *J*= 1.8, 9.0 Hz, 1H, H^4), 7.89 (d, *J*=1.9 Hz, 1H, H^5), 7.95 (dd, *J*= 8.0, 8.1 Hz, 1H, H^9), 8.17 (d, *J*=7.6 Hz, 1H, H^7), 8.27 (d, *J*=7.5 Hz, 1H, H^6), 8.40 (d, *J*=6.4 Hz, 1H, H^1), 8.45-8.50 (m, 3H, H^3+H^8+H^{10}), 8.68 (s, 1H, NH- exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.21, 163.10, 153.59, 146.26, 135.71, 133.20, 132.12, 131.42, 131.25, 131.10, 130.10, 129.72, 129.15, 127.74, 125.98, 125.62, 122.63, 121.65, 121.36, 116.42, 99.05, 43.06, 25.89, 25.21. HRMS (ESI) calcd for $C_{25}H_{19}BrClN_3O_2$ $[M+H]^+$ 508.0427, found 508.0459 and $[M+3]^+$ 510.0349, found 510.0449.

*6-bromo-2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (****3i****)*

Yield-84%, Off-white solid, M.P=220-225 ºC, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.41-1.45 (m, 4H, $(CH_2)_2$), 1.64-1.70 (m, 4H, $(CH_2)_2$), 3.39-3.43 (m, 2H, CH_2), 4.02 (t, *J*=7.3 Hz, 2H, CH_2), 6.69 (d, *J*= 6.45 Hz, 1H, H^2), 7.60 (dd, *J*= 1.7, 9.1 Hz, 1H, H^4), 7.90 (d, *J*=1.8 Hz, 1H, H^5), 7.96 (dd, *J*= 7.9, 7.9 Hz, 1H, H^9), 8.18 (d, *J*=7.8 Hz, 1H, H^7), 8.28 (d, *J*=7.8 Hz, 1H, H^6), 8.44 (d, *J*=6.2 Hz, 1H, H^1), 8.46-8.52 (m, 3H, H^3+H^8+H^{10}), 8.69 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.32, 163.27, 153.86, 146.44, 136.68, 133.04, 131.99, 131.80, 131.39, 131.13, 130.25, 129.55, 129.24, 128.74, 126.16, 125.57, 123.20, 122.46, 122.43, 116.57, 99.01, 43.33, 27.94, 27.80, 26.70, 26.62. HRMS (ESI) calcd for $C_{27}H_{23}BrClN_3O_2$ $[M+H]^+$ 536.0740, found 536.0802 and $[M+3]^+$ 538.0662, found 538.0782.

6-bromo-2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(3j)***

Yield-83%, light brown solid, M.P=100-105 ºC, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.33 (s, 8H, $(CH_2)_4$), 1.62-1.66 (m, 4H, $(CH_2)_2$), 3.39-3.40 (m, 2H, CH_2), 3.99 (t, *J*=7.0 Hz, 2H, CH_2),

6.69 (d, *J*= 6.4 Hz, 1H, H^2), 7.60 (d, *J*= 8.9 Hz, 1H, H^4), 7.90 (s, 1H, H^5), 7.96 (dd, *J*= 8.0, 7.6 Hz, 1H, H^9), 8.17 (d, *J*=7.8 Hz, 1H, H^7), 8.28 (d, *J*=7.8 Hz, 1H, H^6), 8.44-8.52 (m, 4H, H^1+H^3+H^8+H^{10}), 8.67 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.29, 163.24, 153.85, 146.48, 136.67, 133.02, 131.99, 131.80, 131.39, 131.13, 130.25, 129.54, 129.23, 128.73, 127.64, 126.14, 125.58, 123.19, 122.42, 116.58, 98.98, 43.37, 40.23, 29.10, 29.05, 28.07, 27.81, 26.89, 26.85. HRMS (ESI) calcd for $C_{29}H_{27}BrClN_3O_2$ $[M+H]^+$ 564.1053, found 564.1075 and $[M+3]^+$ 566.0975, found 566.1075.

*2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4a**)*

Yield-78%, green solid, M.P>250 ºC, ^1H NMR ($CDCl_3$, 400 MHz): δ 1.62-1.70 (m, 2H, CH_2-**CH_2**-CH_2), 1.78-1.88 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.15-3.18 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.58-3.60 (m, 2H, CH_2), 4.40 (t, *J*=7.1 Hz, 2H, CH_2), 5.90 (s, 1H, NH-exchangeable with D_2O), 6.30 (d, *J*=5.3 Hz, 1H, H^2), 7.05 (d, *J*=8.0 Hz, 1H, H^7), 7.21 (dd, *J*=1.8, 8.8 Hz, 1H, H^4), 7.58 (dd, *J*=7.8, 8.1 Hz, 1H, H^9), 7.80-7.84 (m, 2H, H^3+H^5), 8.30 (d, *J*=8.1 Hz, 1H, H^6), 8.35 (d, *J*=5.1 Hz, 1H, H^1), 8.40 (d, *J*=8.0 Hz, 1H, H^{10}), 8.45 (d, *J*=7.6 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.01, 164.79, 158.19, 150.70, 150.54, 135.36, 132.91, 131.33, 131.23, 130.85, 129.88, 127.52, 126.33, 125.56, 125.35, 122.80, 121.76, 117.10, 115.32, 114.72, 98.65, 54.45, 40.62, 38.16, 26.30, 24.27. HRMS (ESI) calcd for $C_{28}H_{25}ClN_4O_2$ $[M+H]^+$ 485.1744, found 485.1756.

*2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4b**)*

Yield-76%, pale yellow solid, M.P=190-195 ºC, ^1H NMR ($CDCl_3$, 500 MHz): δ 1.70-1.73 (m, 2H, CH_2-**CH_2**-CH_2), 1.85-1.91 (m, 4H, **CH_2**-CH_2-**CH_2**), 2.11-2.17 (m, 2H, CH_2), 3.23-3.25 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.35-3.40 (m, 2H, CH_2), 4.32 (t, *J*=7.7 Hz, 2H, CH_2), 6.35 (s, 1H, NH- exchangeable with D_2O), 6.40 (d, *J*=6.7 Hz, 1H, H^2), 7.16 (d, *J*=10.0 Hz, 1H, H^7), 7.37 (dd, *J*=2.7, 11.1 Hz, 1H, H^4), 7.68 (dd, *J*=9.1, 10.5 Hz, 1H, H^9), 7.90 (d, *J*=2.6 Hz, 1H, H^5), 7.97 (d, *J*=11.2 Hz, 1H, H^3), 8.39 (d, *J*=10.5 Hz, 1H, H^6), 8.47 (d, *J*=6.8 Hz, 1H, H^1), 8.51 (d, *J*=10.1 Hz, 1H, H^{10}), 8.59 (d, *J*=9.1 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.28, 164.79, 157.90, 151.98, 149.92, 134.87, 133.29, 131.53, 131.27, 130.11, 128.52, 127.17, 126.25, 125.49, 125.34, 122.73, 121.76, 117.56, 115.24, 114.82, 98.67, 54.64, 39.71,

37.39, 26.64, 26.27, 24.40. HRMS (ESI) calcd for $C_{29}H_{27}ClN_4O_2$ $[M+H]^+$ 499.1900, found 499.1840.

*2-(4-((7-chloroquinolin-4-yl)amino)butyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4c**)*

Yield-77%, pale yellow solid, M.P=125-130 ºC, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.65-1.68 (m, 2H, CH_2-**CH_2**-CH_2), 1.78-1.87 (m, 8H, **CH_2**-CH_2-**CH_2** + $(CH_2)_2$), 3.16-3.18 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.37-3.40 (m, 2H, CH_2), 4.20 (t, *J*=7.0 Hz, 2H, CH_2), 5.91 (s, 1H, NH-exchangeable with D_2O), 6.33 (d, *J*=5.5 Hz, 1H, H^2), 7.10 (d, *J*=8.1 Hz, 1H, H^7), 7.25 (dd, *J*=1.9, 8.9 Hz, 1H, H^4), 7.61 (dd, *J*=7.5, 8.1 Hz, 1H, H^9), 7.83-7.85 (m, 2H, H^3+H^5), 8.32 (d, *J*=8.3 Hz, 1H, H^6), 8.37 (d, *J*=5.5 Hz, 1H, H^1), 8.42 (d, *J*=8.1 Hz, 1H, H^{10}), 8.49 (d, *J*=7.1 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.83, 164.34, 157.61, 150.72, 150.54, 135.29, 132.90, 131.38, 131.19, 130.95, 129.96, 127.47, 126.21, 125.42, 125.38, 122.86, 121.81, 117.03, 115.48, 114.74, 98.80, 54.55, 42.94, 39.36, 26.21, 25.80, 25.35, 24.33. HRMS (ESI) calcd for $C_{30}H_{29}ClN_4O_2$ $[M+H]^+$ 513.2057, found 513.1991.

*2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4d**)*

Yield-76%, light brown solid, M.P=105-110 ºC, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40-1.44 (m, 4H, $(CH_2)_2$), 1.65-1.67 (m, 6H, CH_2-**CH_2**-CH_2 + $(CH_2)_2$), 1.85-1.90 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.23-3.25 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.30-3.32 (m, 2H, CH_2), 4.10 (t, *J*=7.4 Hz, 2H, CH_2), 4.48 (s, 1H, NH-exchangeable with D_2O), 6.38 (d, *J*=5.2 Hz, 1H, H^2), 7.12 (d, *J*=8.0 Hz, 1H, H^7), 7.31 (dd, *J*=1.9, 8.9 Hz, 1H, H^4), 7.61-7.68 (m, 2H, H^3+H^9), 7.90 (d, *J*=1.8 Hz, 1H, H^5), 8.46 (d, *J*=8.2 Hz, 1H, H^6), 8.49 (d, *J*=8.1 Hz, 1H, H^{10}), 8.52 (d, *J*=5.1 Hz, 1H, H^1), 8.56 (d, *J*=7.0 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.75, 164.10, 157.30, 152.15, 134.76, 132.52, 131.10, 130.90, 130.50, 129.90, 128.65, 126.93, 126.81, 125.42, 125.30, 123.15, 120.56, 117.25, 115.85, 114.69, 99.10, 54.60, 43.35, 40.65, 27.92, 27.76, 26.70, 26.50, 26.25, 24.50. HRMS (ESI) calcd for $C_{32}H_{33}ClN_4O_2$ $[M+H]^+$ 541.2370, found 541.2235.

*2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4e**)*

Yield-75%, dark green solid, M.P=95-100 ºC, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.44-1.48 (m, 8H, $(CH_2)_4$), 1.74-1.79 (m, 6H, CH_2-**CH_2**-CH_2 + $(CH_2)_2$), 1.89-1.91 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.24 (s, 4H, **CH_2**-CH_2-**CH_2**), 3.30-3.34 (m, 2H, CH_2), 4.18 (t, *J*=7.5 Hz, 2H, CH_2), 5.07 (s, 1H, NH- exchangeable with D_2O), 6.43 (d, *J*=5.3 Hz, 1H, H^2), 7.18 (d, *J*=8.1 Hz, 1H, H^7), 7.36 (dd, *J*=2.0, 8.8 Hz, 1H, H^4), 7.67-7.71 (m, 2H, H^3+H^9), 7.97 (d, *J*=1.7 Hz, 1H, H^5), 8.40 (d, *J*=8.4 Hz, 1H, H^6), 8.50 (d, *J*=8.1 Hz, 1H, H^{10}), 8.54 (d, *J*=5.3 Hz, 1H, H^1), 8.58 (d, *J*=7.1 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.63, 164.16, 157.32, 152.02, 134.79, 132.64, 131.17, 130.98, 130.60, 129.93, 128.77, 126.93, 126.29, 125.35, 125.22, 123.16, 120.88, 117.13, 115.95, 114.71, 99.08, 54.55, 43.23, 40.15, 29.01, 28.98, 28.76, 27.93, 26.95, 26.86, 26.23, 24.34. HRMS (ESI) calcd for $C_{34}H_{37}ClN_4O_2$ $[M+H]^+$ 569.2683, found 569.2698.

*2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4f**)*

Yield-75%, dark green solid, M.P=165-170 ºC, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.24-3.26 (m, 4H, CH_2-N-CH_2), 3.64-3.67 (m, 2H, CH_2), 3.98-4.00 (m, 4H, CH_2-O-CH_2), 4.64-4.67 (m, 2H, CH_2), 6.36 (d, *J*=5.4 Hz, 1H, H^2), 6.49 (s, 1H, NH-exchangeable with D_2O), 7.21 (d, *J*=8.0 Hz, 1H, H^7), 7.36 (dd, *J*=1.8, 8.9 Hz, 1H, H^4), 7.68-7.76 (m, 2H, H^3+H^9), 7.86 (s, 1H, H^5), 8.41 (d, *J*=8.4 Hz, 1H, H^6), 8.44 (d, *J*=5.5 Hz, 1H, H^1), 8.56 (d, *J*=8.1 Hz, 1H, H^{10}), 8.62 (d, *J*=7.3 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.58, 164.21, 156.10, 151.46, 149.95, 134.85, 133.10, 131.42, 130.40, 129.67, 128.30, 127.10, 126.23, 125.89, 125.15, 122.85, 121.64, 117.71, 116.25, 115.10, 98.52, 66.45, 52.98, 41.01, 37.90. HRMS (ESI) calcd for $C_{27}H_{23}ClN_4O_3$ $[M+H]^+$ 487.1537, found 487.1525.

*2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4g**)*

Yield-75%, light green solid, M.P=135-140 ºC, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.18-2.20 (m, 2H, CH_2), 3.31 (s, 4H, CH_2-N-CH_2), 3.42-3.43 (m, 2H, CH_2), 4.05 (s, 4H, CH_2-O-CH_2), 4.37 (t, *J*=6.0 Hz, 2H, CH_2), 6.33 (s, 1H, NH-exchangeable with D_2O), 6.43 (d, *J*=5.4 Hz, 1H, H^2), 7.26 (d, *J*=8.1 Hz, 1H, H^7), 7.39 (d, *J*=9.0 Hz, 1H, H^4), 7.75 (dd, *J*=7.9, 7.8 Hz, 1H, H^9), 7.92 (s, 1H, H^5), 7.97 (d, J=9.0 Hz, 1H, H^3), 8.46 (d, *J*=8.4 Hz, 1H, H^6), 8.50 (d, *J*=5.3 Hz, 1H, H^1), 8.59 (d, *J*=8.0 Hz, 1H, H^{10}), 8.64 (d, *J*=7.2 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.01, 164.53, 156.15, 151.75, 149.90, 134.89, 133.03, 131.58, 130.59, 129.95, 128.32, 127.08, 126.10, 125.92, 125.30, 122.90, 121.59, 117.42, 116.53, 115.01, 98.64, 66.93, 53.47,

39.80, 37.46, 26.60. HRMS (ESI) calcd for $C_{28}H_{25}ClN_4O_3$ $[M+H]^+$ 501.1693, found 501.4631.

*2-(4-((7-chloroquinolin-4-yl)amino)butyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4h**)*

Yield-74%, light green solid, M.P=130-135 ºC, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.67-1.71 (m, 4H, $(CH_2)_2$), 3.12 (s, 4H, CH_2-N-CH_2), 3.24-3.25 (m, 2H, CH_2), 3.84 (s, 4H, CH_2-O-CH_2), 4.10 (t, *J*=5.6 Hz, 2H, CH_2), 6.10 (s, 1H, NH- exchangeable with D_2O), 6.40 (d, *J*=5.6 Hz, 1H, H^2), 7.25 (d, *J*=8.2 Hz, 1H, H^7), 7.35 (d, *J*=8.9 Hz, 1H, H^4), 7.72 (dd, *J*=7.9, 8.0 Hz, 1H, H^9), 7.90 (s, 1H, H^5), 7.95 (d, *J*=9.0 Hz, 1H, H^3), 8.45 (d, *J*=8.3 Hz, 1H, H^6), 8.50 (d, *J*=5.5 Hz, 1H, H^1), 8.55 (d, *J*=8.1 Hz, 1H, H^{10}), 8.61 (d, *J*=7.8 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.06, 163.53, 155.85, 151.74, 150.83, 148.79, 134.09, 132.61, 131.11, 130.94, 129.48, 127.36, 126.50, 125.64, 124.61, 124.60, 122.94, 117.73, 116.21, 115.44, 98.08, 66.70, 53.51, 42.70, 25.84, 25.81. HRMS (ESI) calcd for $C_{29}H_{27}ClN_4O_3$ $[M+H]^+$ 515.1850, found 515.1872.

*2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4i**)*

Yield-74%, yellow solid, M.P=90-95 ºC, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40-1.44 (m, 4H, $(CH_2)_2$), 1.65-1.67 (m, 4H, $(CH_2)_2$), 3.21-3.25 (m, 6H, CH_2-N-CH_2 + CH_2), 3.91 (s, 4H, CH_2-O-CH_2), 4.05 (t, *J*=6.1 Hz, 2H, CH_2), 6.32 (s, 1H, NH-exchangeable with D_2O), 6.40 (d, *J*=5.5 Hz, 1H, H^2), 7.21 (d, *J*=8.1 Hz, 1H, H^7), 7.32 (d, *J*=8.8 Hz, 1H, H^4), 7.65-7.71 (m, 2H, H^3+H^9), 7.93 (s, 1H, H^5), 8.42 (d, *J*=8.2 Hz, 1H, H^6), 8.51-8.54 (m, 2H, H^1+H^{10}), 8.62 (d, *J*=7.3 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.16, 163.95, 155.60, 151.83, 150.61, 147.96, 134.45, 132.60, 131.15, 130.90, 129.45, 127.10, 126.17, 125.60, 124.75, 124.56, 122.83, 117.95, 116.18, 115.21, 99.10, 66.65, 53.50, 42.81, 39.85, 28.12, 28.07, 26.83, 26.80. HRMS (ESI) calcd for $C_{31}H_{31}ClN_4O_3$ $[M+H]^+$ 543.2163, found 543.2205.

*2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4j**)*

Yield-72%, light brown solid, M.P=85-90 ºC, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.44-1.47 (m, 8H, $(CH_2)_4$), 1.76-1.79 (m, 4H, $(CH_2)_2$), 3.27-3.29 (m, 4H, CH_2-N-CH_2), 3.30-3.34 (m, 2H, CH_2), 4.03-4.04 (m, 4H, CH_2-O-CH_2), 4.18 (t, *J*=7.6 Hz, 2H, CH_2), 5.08 (s, 1H, NH-

exchangeable with D_2O), 6.43 (d, *J*=5.3 Hz, 1H, H^2), 7.24 (d, *J*=8.0 Hz, 1H, H^7), 7.36 (d, *J*=8.9 Hz, 1H, H^4), 7.69-7.73 (m, 2H, H^3+H^9), 7.96 (s, 1H, H^5), 8.44 (d, *J*=8.3 Hz, 1H, H^6), 8.53-8.55 (m, 2H, H^1+H^{10}), 8.60 (d, *J*=7.3 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.44, 163.99, 155.63, 151.95, 134.83, 133.90, 132.49, 131.14, 130.04, 129.87, 128.72, 126.93, 126.16, 125.84, 125.24, 123.36, 120.89, 117.20, 117.11, 114.96, 99.07, 66.96, 53.45, 43.23, 40.22, 29.01, 28.99, 28.76, 27.92, 26.96, 26.86. HRMS (ESI) calcd for $C_{33}H_{35}ClN_4O_3$ $[M+H]^+$ 571.2476, found 571.2488.

*2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4k**)*

Yield-70%, dark green solid, M.P>250 ºC, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.54 (t, *J*=5.6 Hz, 2H, **CH_2**-CH_2-OH), 2.74 (s, 4H, CH_2-N-CH_2), 3.25 (s, 4H, CH_2-N-CH_2), 3.57 (t, *J*=5.8 Hz, 2H, CH_2-**CH_2**-OH), 3.60-3.62 (m, 2H, CH_2), 4.27 (t, *J*=6.5 Hz, 2H, CH_2), 4.50 (s, 1H, OH- exchangeable with D_2O), 6.35 (d, *J*=5.6 Hz, 1H, H^2), 7.20-7.25 (m, 2H, H^7 + NH- exchangeable with D_2O), 7.35 (dd, *J*=2.1, 9.0 Hz, 1H, H^4), 7.71 (d, *J*=2.0 Hz, 1H, H^5), 7.73 (dd, *J*=7.6, 8.1 Hz, 1H, H^9), 8.21 (d, *J*=8.9 Hz, 1H, H^3), 8.25-8.28 (m, 2H, H^1+H^6), 8.35 (d, *J*=8.2 Hz, 1H, H^{10}), 8.40 (d, *J*=7.3 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.81, 164.35, 156.12, 151.75, 150.20, 134.72, 132.65, 131.18, 130.46, 129.75, 128.24, 126.96, 126.10, 125.26, 124.90, 122.74, 121.85, 117.30, 116.30, 114.15, 99.10, 60.56, 59.15, 53.61, 53.10, 40.95, 37.80. HRMS (ESI) calcd for $C_{29}H_{28}ClN_5O_3$ $[M+H]^+$ 530.1959, found 530.1990.

*2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4l**)*

Yield-70%, yellow solid, M.P=145-150 ºC, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.04-2.06 (m, 2H, CH_2), 2.53-2.54 (m, 2H, **CH_2**-CH_2-OH), 2.74 (s, 4H, CH_2-N-CH_2), 3.21 (s, 4H, CH_2-N-CH_2), 3.39-3.41 (m, 2H, CH_2), 3.58 (t, *J*=5.9 Hz, 2H, CH_2-**CH_2**-OH), 4.17 (t, *J*=6.8 Hz, 2H, CH_2), 4.50 (s, 1H, OH- exchangeable with D_2O), 6.44 (d, *J*=5.4 Hz, 1H, H^2), 7.25 (d, J=8.2 Hz, 1H, H^7), 7.28 (t, *J*=5.1 Hz, 1H, NH- exchangeable with D_2O), 7.35 (dd, *J*=2.0, 8.9 Hz, 1H, H^4), 7.73-7.77 (m, 2H, H^5+H^9), 8.13 (d, *J*=9.0 Hz, 1H, H^3), 8.31 (d, *J*=8.1 Hz, 1H, H^6), 8.35 (d, *J*=8.5 Hz, 1H, H^1), 8.38 (d, *J*=8.5 Hz, 1H, H^{10}), 8.41 (d, *J*=6.9 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 164.14, 163.61, 156.12, 152.27, 150.38, 149.42, 134.69, 133.74, 132.61, 131.02, 129.57, 127.83, 126.31, 125.67, 124.38, 124.29, 122.99, 117.88, 115.86,

115.31, 99.11, 60.66, 59.06, 53.60, 53.13, 40.85, 38.17, 26.98. HRMS (ESI) calcd for $C_{30}H_{30}ClN_5O_3$ $[M+H]^+$ 544.2115, found 544.1860.

*2-(4-((7-chloroquinolin-4-yl)amino)butyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4m**)*

Yield-68%, brown solid, M.P=140-145 ºC, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.68-1.72 (m, 4H, $(CH_2)_2$), 2.55 (t, *J*=5.5 Hz, 2H, **CH_2**-CH_2-OH), 2.75 (s, 4H, CH_2-N-CH_2), 3.20 (s, 4H, CH_2-N-CH_2), 3.30-3.35 (m, 2H, CH_2), 3.55 (t, *J*=5.8 Hz, 2H, CH_2-**CH_2**-OH), 4.10 (t, *J*=5.1 Hz, 2H, CH_2), 4.50 (s, 1H, OH- exchangeable with D_2O), 6.35 (d, *J*=5.6 Hz, 1H, H^2), 7.21-7.25 (m, 2H, H^7 + NH-exchangeable with D_2O), 7.35 (dd, *J*=2.1, 9.0 Hz, 1H, H^4), 7.71-7.75 (m, 2H, H^5+H^9), 8.21 (d, *J*=9.1 Hz, 1H, H^3), 8.25-8.30 (m, 2H, H^1+H^6), 8.35 (d, *J*=8.3 Hz, 1H, H^{10}), 8.40 (d, *J*=7.5 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.61, 164.12, 156.09, 151.69, 150.10, 134.67, 132.68, 131.17, 130.53, 129.81, 128.17, 126.95, 126.04, 125.65, 124.97, 122.94, 121.94, 117.29, 116.31, 114.91, 98.84, 59.56, 58.02, 53.21, 53.01, 42.80, 39.46, 25.73, 25.44. HRMS (ESI) calcd for $C_{31}H_{32}ClN_5O_3$ $[M+H]^+$ 558.2272, found 558.2113.

*2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4n**)*

Yield-68%, brown solid, M.P=85-90 ºC, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.36 (s, 4H, $(CH_2)_2$), 1.57-1.62 (m, 4H, $(CH_2)_2$), 2.48-2.49 (m, 2H, **CH_2**-CH_2-OH), 2.68 (s, 4H, CH_2-N-CH_2), 3.16 (s, 6H, CH_2-N-CH_2 + CH_2), 3.53 (t, *J*=6.0 Hz, 2H, CH_2-**CH_2**-OH), 3.97 (t, *J*=7.1 Hz, 2H, CH_2), 4.46 (s, 1H, OH- exchangeable with D_2O), 6.37 (d, *J*=5.4 Hz, 1H, H^2), 7.22-7.24 (m, 2H, H^7 + NH-exchangeable with D_2O), 7.36 (dd, *J*=2.2, 8.9 Hz, 1H, H^4), 7.70-7.74 (m, 2H, H^5+H^9), 8.20 (d, *J*=9.0 Hz, 1H, H^3), 8.27-8.31 (m, 2H, H^1+H^6), 8.34 (d, *J*=8.4 Hz, 1H, H^{10}), 8.38 (d, *J*=7.4 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.05, 163.52, 156.18, 152.40, 150.57, 149.57, 133.83, 132.70, 131.11, 131.10, 129.60, 127.94, 126.47, 125.75, 124.63, 124.45, 123.04, 117.94, 115.93, 115.44, 99.10, 60.73, 59.10, 53.67, 53.18, 42.85, 39.89 28.13, 28.02, 26.85, 26.83. HRMS (ESI) calcd for $C_{33}H_{36}ClN_5O_3$ $[M+H]^+$ 586.2585, found 586.2595.

*2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4o**)*

Yield-67%, dark green solid, M.P=85-90 ºC, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.35 (s, 8H, $(CH_2)_4$), 1.60-1.64 (m, 4H, $(CH_2)_2$), 2.46 (t, *J*=5.8 Hz, 2H, **CH_2**-CH_2-OH), 2.70 (s, 4H, CH_2-N-CH_2), 3.15 (s, 4H, CH_2-N-CH_2), 3.37-3.39 (m, 2H, CH_2), 3.55 (t, *J*=6.0 Hz, 2H, CH_2-**CH_2**-OH), 4.01 (t, *J*=6.9 Hz, 2H, CH_2), 4.52 (s, 1H, OH- exchangeable with D_2O), 6.36 (d, *J*=5.1 Hz, 1H, H^2), 7.21-7.25 (m, 2H, H^7 + NH-exchangeable with D_2O), 7.36 (dd, *J*=2.1, 8.8 Hz, 1H, H^4), 7.71-7.75 (m, 2H, H^5+H^9), 8.22 (d, *J*=8.5 Hz, 1H, H^3), 8.25-8.31 (m, 2H, H^1+H^6), 8.30 (d, *J*=8.2 Hz, 1H, H^{10}), 8.35 (d, *J*=7.1 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.94, 163.53, 155.76, 151.91, 150.15, 149.14, 133.72, 132.61, 131.45, 131.10, 129.15, 127.90, 126.45, 125.81, 125.01, 124.60, 123.10, 117.84, 115.90, 115.25, 98.91, 60.84, 59.15, 53.72, 53.20, 43.29, 40.25, 29.10, 28.96, 28.45, 27.96, 26.90, 26.56. HRMS (ESI) calcd for $C_{35}H_{40}ClN_5O_3$ $[M+H]^+$ 614.2898, found 614.2889.

2. *In vitro* anti-plasmodial assay against *P.falciparum*

The W2 strain of *P. falciparum* was cultured in RPMI-1640 medium with 0.5% Albumax I (Gibco) and using 5% D-sorbitol to synchronize parasites, by following the standard procedures. To initiate at the ring stage, microwell cultures were incubated with different concentrations of the compounds for 48 h. The compounds were added from DMSO stocks, not exceeding the concentration of DMSO by 0.1% (maximum value). Controls devoid of inhibitors included 0.1% DMSO. The culture medium was removed after 48 h when control cultures had developed new rings which were further incubated at RT for 48 h at pH 7.4 with 1% formaldehyde in PBS. Fixed parasites were then transferred to 0.1% Triton X-100 in PBS containing 1 nM YOYO-1 dye (Molecular Probes). Using Cell Quest software (Beckton Dickinson), parasitemia was determined from dot plots (forward scatter vs. fluorescence) acquired on a FACSort flow cytometer. The determination of IC_{50} values for growth inhibition was done from plots of percent control parasitemia over inhibitor concentration using Prism 5.0 program, (GraphPad Software), with data from duplicate experiments fitted by non-linear regression.

3. Cytotoxicity assay on Vero cell lines:

Vero kidney epithelial cells (ATCC® CCL-81™) were cultured in RPMI 1640 cell culture medium (Life technologies) supplemented with 10% foetal calf serum and incubated at 37°C with 5% CO_2. Once the desired confluency was achieved, cells were detached from the flask surface using trypsin and centrifuged at 220 x *g* for 5 min at room temperature to pellet cells.

The cell pellet was resuspended in 1 mL of culture medium and enumerated using a Malassez counting chamber. Cell density was adjusted to approximately 2 x 10^4 cells/well in 96-well plates and left to adhere overnight at 37°C and 5% CO_2. To determine cytotoxicity of synthetic compounds, serial dilutions were performed ranging from 100 µg to 3.1 µg/mL. Cell cultures were placed back in the incubator at 37°C and 5% CO_2 for 24 h. After incubation, 10% (vol/vol) resazurin was added to each well and left to incubate at 37°C and 5% CO_2 prior to data acquisition using a fluorescent plate reader (excitation 540 nm, emission 590 nm). DMSO was included as a negative control, while 20% SDS was included as a positive control at the pre-designated concentrations. Data was analysed using Graphpad Prism 5 (Graphphad software). The half maximal inhibitory concentration (IC_{50}) was calculated using a non-linear regression dose response curve. Selectivity Index (SI) was calculated as a function of the IC_{50}/MIC.

4. Binding studies with monomeric Heme:

The stock solution (1.2 mM) of hemin chloride was prepared by dissolving hemin chloride (7.8 mg) in DMSO (10 mL). Working solution (12 µM) of hemin chloride was prepared by diluting hemin stock solution (100 µL) to 1 mL 0.02M HEPES buffer (pH 7.4), 4mL DMSO and making final volume up to 10 mL with ultrapure, HPLC grade Hipersolv water. The resultant 40% DMSO solutions maintain the hemin solutions in monomeric state at concentrations used. Likewise, the stock solution (10 mM) of 4l was prepared in DMSO. Working solution (100 µM) of **4l** was prepared by diluting (100 µL) to 1mL 0.02M HEPES buffer (pH 7.4), 4mL DMSO and making final volume up to 10 mL. All the working solutions were kept in dark to avoid photo-sensitivity. Diluent solutions were prepared by dissolving 1 mL of 0.02 M HEPES and 4 mL of spectroscopic grade DMSO and made up to a final volume of 10 mL with ultrapure, HPLC grade Hipersolv water. Hemin chloride solution (12 µM, 2.5 mL) was titrated with increasing concentrations of 4l. Subsequent to each addition of aliquot of the compound in the solution of hemin chloride, absorbance was recorded at 401 nm. Likewise, solution of hemin chloride and 4l were titrated at pH 5.6 (2-[Nmorpholino] ethanesulphonate (MES, pH 5.4) buffer was used).

5. Computational studies:

a) Docking interaction:

The theoretical study involves the docking study of the interaction of the three ligands (3a, 4b and 4l) with three parasitic enzymes: (i) Peroxidase (pdb: 1h5a[1]), (ii) Plasmepsin-II (pdb: 1lf2) and Plasmepsin-IV (pdb: 2anl). The docking interaction with peroxidase which is an enzyme that host heme at its cofactor on the binding site was divided into two. First, all the residues on the binding site were preserved during the interaction of the three ligands (subsequently called limited-dock) and second the residues Arg38, Leu39, Phe41, and His42 were deleted in order for the ligands to have free access to whole surface of heme without the restriction of any binding site residues (will subsequently be referred to as free-dock). The limited-dock approach is similar to the method adopted by Córdoba *et al*. (2012) [2] while the free-dock is similar to the method used by Sakata *et al.* (2018) study of the interaction of the ligand heme [3]. Constraint was used for the docking interaction of the three ligands with peroxidase during both limited and free docking to enforce the interaction of any hydrogen bond acceptor or donor with heme iron atom. The molecular docking package used is Molegro Virtual Docker (MVD) software version 6.0 2013.6.0 by CLC bio Company[4].

Table S1: The docking score results of ligands interaction with peroxidase when the binding site residues were intact (Whole-Peroxidase) and when the binding site residues were deleted (Deleted-Peroxidase) and their respective interaction with heme surface as Limited-Heme and Free-Heme. Also the interaction of the ligands with Plasmepsin II and Plasmepsin IV. The docking scores are arbitrary unit.

Molecule	Whole-Peroxidase	Deleted-Peroxidase	Limited-Heme	Free-Heme	Plasmepsin II	Plasmepsin IV
3a	-72.085	-170.548	0.905938	-97.5041	-98.1559	-125.652
4b	-89.2104	-202.807	-30.4695	-92.5888	-117.018	-139.999
4l	-156.522	-201.064	-40.5702	-90.8678	-137.592	-148.483

The interaction of 4l with peroxidase is characterized four hydrophobic interaction (PHE 68, PHE 179, PHE 142 and ALA 136), one hydrogen bond acceptor with ASN135, one positive ionization with ALA 140 and two hydrogen bond donors with Heme. The interaction of 4l with plasmepsin II is characterized with four hydrophobic (ILE 32, TYR 77, ILE 123 and ILE 133), three hydrogen bond donor (PHE 16, SER 215 and ASP 303) and one hydrogen bond acceptor while interaction with plasmepsinIV is characterized mainly with more hydrophobic interactions (10 interactions: VAL 11, VAL 12, MET 15, ILE 32, TYR77, ALA 118, PHE 120, ILE 123, LEU 124, THR 217) and one positive ionization with ASP214. The better interaction of 4l with peroxidase over the two plasmepsin protein could be the results of its additional interactions with heme surface while the stronger interaction with plasmepsin IV compare to plasmepsin II could be as a results of increased number of hydrophobic interaction that are present in plasmepsin IV.

(b) Interaction energy with heme, hematin and dihematin

During the quantum study, the residue His170 that coordinate with Fe atom of heme on the non-reactive side was replaced with OH (called hematin model), Cl (hemin model). Another model considered isa dimer of hematin (called diHematin) as shown in **Figure S1**. The geometry of modeled hemin, hematin and dihematin were optimized but some of atoms of their interaction surface structures were constrained (the carbon atoms of the constrained region are in magenta color in **Figure S1**). The optimization was done using Gaussian 16 [5]with semi-empirical method PM7. The binding energies were computed as in the interaction of each ligand (fragment 2) with each of the heme models (fragment 1).

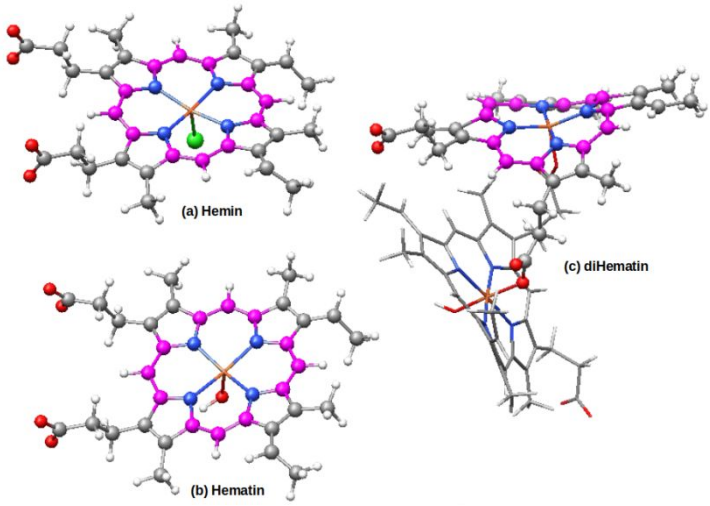


Figure S1: The three heme models: (a) hemin, (b) hematin and (c) diHematin showing the constrained regions during the optimization of the system (region of carbon atoms in magenta color).

The interaction energies were computed using two approaches: (i) The binding energies calculated from the sum values of steric and polar energy (referred to as ΔE_{bind}) using Gaussian 16[5] and (ii) calculated from the natural energy decomposition analysis (NEDA referred to as ΔE_{NEDA}) using Gamessversion R3 [6]coupled with NBO 6.0 [7]. The values of ΔE_{bind} were computed as expressed in the Multiwfn program [8,9] using DFT method B3LYP and basis set 6-31G(d).

$$\Delta E_{bind} = E^{complex} + \sum E_i^{frag} = (\Delta E_{ele} + \Delta E_{ex}) + \Delta E_{orb} = \Delta E_{steric} + \Delta E_{polar}$$

Detail expressions for calculating the steric and polar energies:

$$\Delta E_{polar} = \Delta E_{orb} = E_{SCF,last} - E_{SCF,1st}$$

$$\Delta E_{steric} = \Delta E_{ele} + \Delta E_{ex} = \Delta E_{tot} - \Delta E_{orb} = E_{SCF,1st} - \sum_i E_i^{frag}$$

The sum of electrostatic (E_{ele}) and exchange (E_{ex}) energies gives the steric energy (E_{steric}) while the orbital energy ($E_{orbital}$) is also called the polar energy (E_{polar}).

The typical expression for $\Delta E_{NEDA}$is shown below[10] using HF method and basis set 6-31G(d).

$\Delta E_{NEDA} = E\psi_{AB} - (E\psi_A + E\psi_B)$

Representing the wavefunction of complex AB (ψ_{AB}) and its individual fragment A (ψ_A) and B (ψ_B). The five partitions of the SCF interaction by Kitaura and Morokuma (KM) were used to compute the values of ΔE_{NEDA},which are electrostatic (ES), polarization (POL), charge transfer (CT), exchange (EX) and a coupling term (MIX) [10,11]:

ΔE_{NEDA} = EL + CORE + CT

EL = ES + POL + SE

CORE = EX + DEF – SE

Where EL is the electrical energies, DEF is deformation energy of the perturbed wavefunction and SE is self-energy correction.

The molecular rendering were achieved using chimera [12], Chemcraft[13] and LigandScout 4.0 [14].

Table S2: The interaction energy (ΔE_{bind}) of the molecule 3a, 4b and 4l with hemin, hematin and dihematin computed from steric and polar energy for the limited and free surfaces. The values are in kcal/mol.

Complex	ΔE_{steric}	ΔE_{polar}	ΔE_{bind}
Limited-surface			
hemin-3a	2661.41	-2602.94	58.47
hemin-4b	2594.26	-2567.78	26.47
hemin-4l	2520.73	-2498.61	**22.12**
hematin-3a	2525.88	-2467.63	58.25
hematin-4b	2499.94	-2494.34	5.60
hematin-4l	2482.64	-2460.58	22.06
dihematin-3a	4897.72	-4786.40	111.32
dihematin-4b	4887.77	-4836.36	51.41
dihematin-4l	4864.12	-4811.69	52.44
Free-surface			
hemin-3a	2824.58	-2817.00	7.57
hemin-4b	2565.25	-2556.61	8.64
hemin-4l	2586.84	-2578.36	8.48
hematin-3a	2369.65	-2361.27	8.38
hematin-4b	2478.42	-2488.50	-10.08

hematin-4l	2503.45	-2513.50	-10.05
dihematin-3a	4737.55	-4738.55	-1.01
dihematin-4b	4878.86	-4868.71	10.15
dihematin-4l	4889.42	-4883.49	5.93

Table S3: The interaction energy (ΔE_{NEDA}) of the molecule 3a, 4b and 4l with hemin and hematin and (excluding dihematin because they are computationally expensive) computed from electrical (EL = ES + POL + SE), CORE (CORE = EX + DEF – SE) and Charge transfer (CT) for the limited and free surfaces. The values are in kcal/mol.

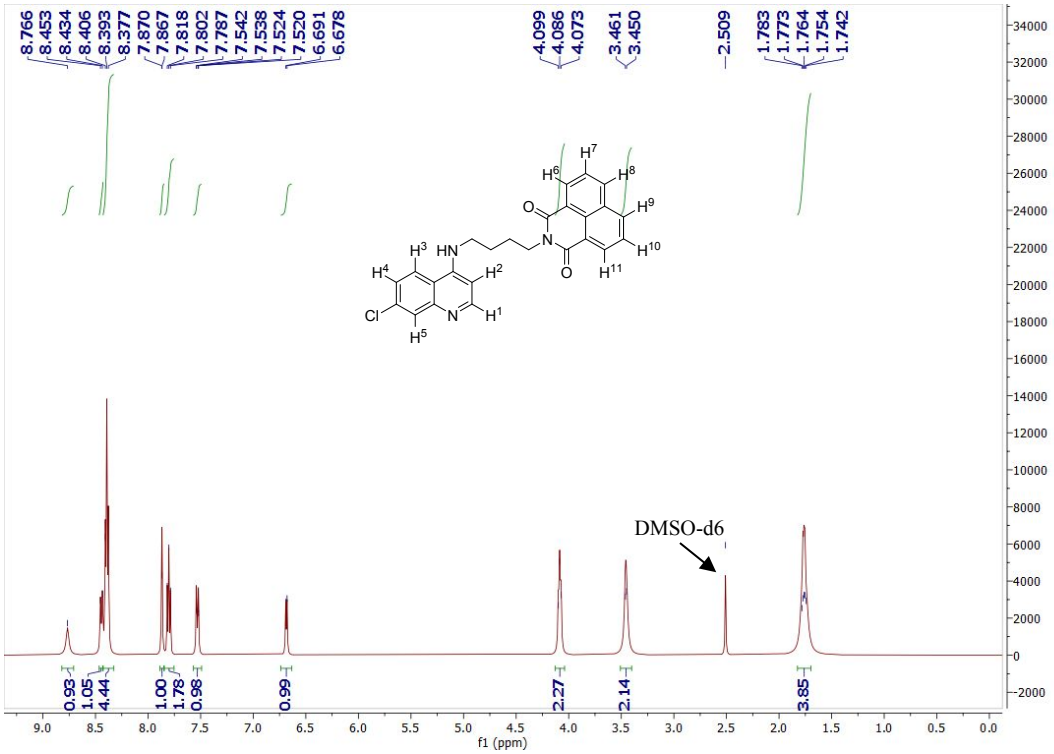
Complex	ES	POL	EL	CT	Ex	SE	DEF	CORE	ΔE_{NEDA}
Limited-surface									
hemin-3a	-48.51	-24.97	-60.61	-72.27	-28.23	12.87	262.10	221.01	88.12
hemin-4b	-14.24	-49.23	-39.16	-49.67	-21.01	24.31	181.74	136.42	47.59
hemin-4l	-35.03	-42.24	-56.04	-80.16	-21.41	21.23	211.15	168.51	**32.31**
hematin-3a	-47.75	-24.32	-59.67	-73.23	-28.22	12.39	250.32	209.71	76.80
hematin-4b	-13.73	-49.63	-38.89	-49.53	-21.01	24.48	187.48	141.99	53.57
hematin-4l	-29.29	-42.66	-50.59	-76.81	-20.58	21.37	205.81	163.87	**36.47**
Free-surface									
hemin-3a	-14.94	-55.49	-42.75	-39.64	-20.97	27.68	161.10	112.45	30.06

hemin-4b	-15.11	-58.57	-44.59	-42.87	-20.66	29.09	160.66	110.92	23.45
hemin-4l	-15.24	-55.10	-42.79	-45.64	-20.26	27.55	170.12	122.31	33.88
hematin-3a	-11.18	-54.82	-38.73	-39.75	-21.21	27.27	165.52	117.03	38.55
hematin-4b	-15.15	-58.92	-44.78	-42.34	-20.58	29.29	160.72	110.85	23.73
hematin-4l	-15.34	-55.45	-43.09	-45.64	-20.31	27.69	174.40	126.40	37.67

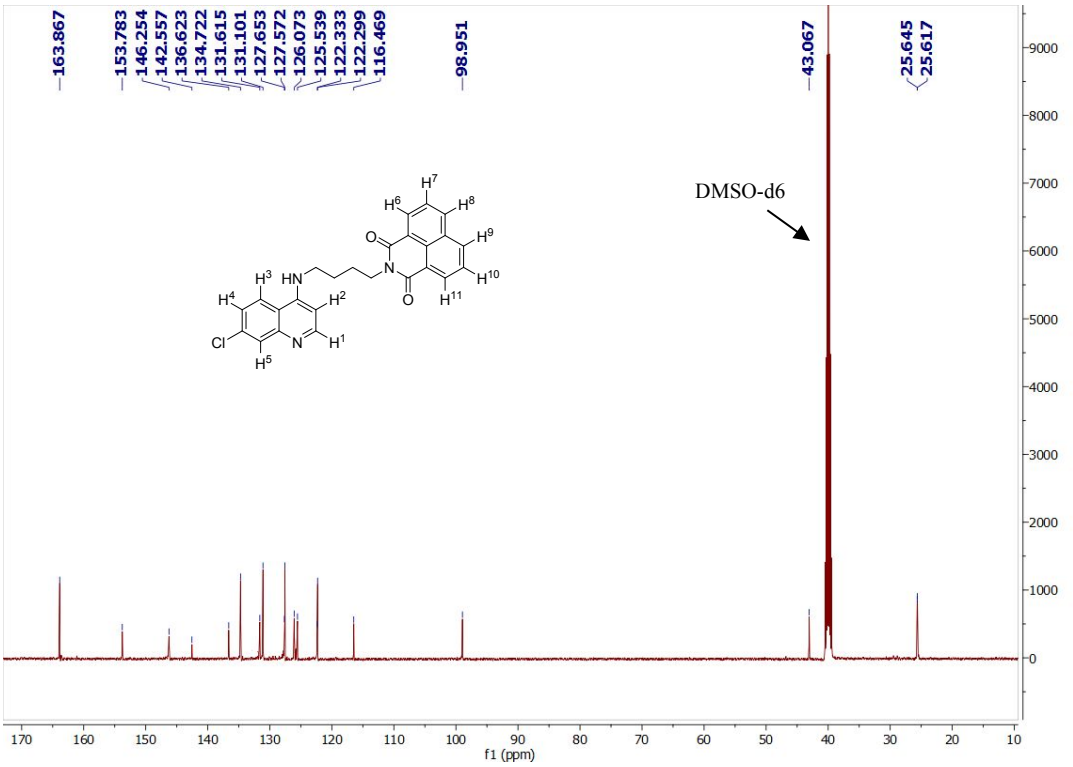
4. Scanned copies of ^{1}H and ^{13}C NMR spectra:

Scanned copies of ^{1}H and ^{13}C NMR spectra of representative compounds *viz.* **3c**, **3d**, **3e**, **3i**, **3j**, **4c**, **4e**, **4g**, **4j**, **4l**, **4n**.

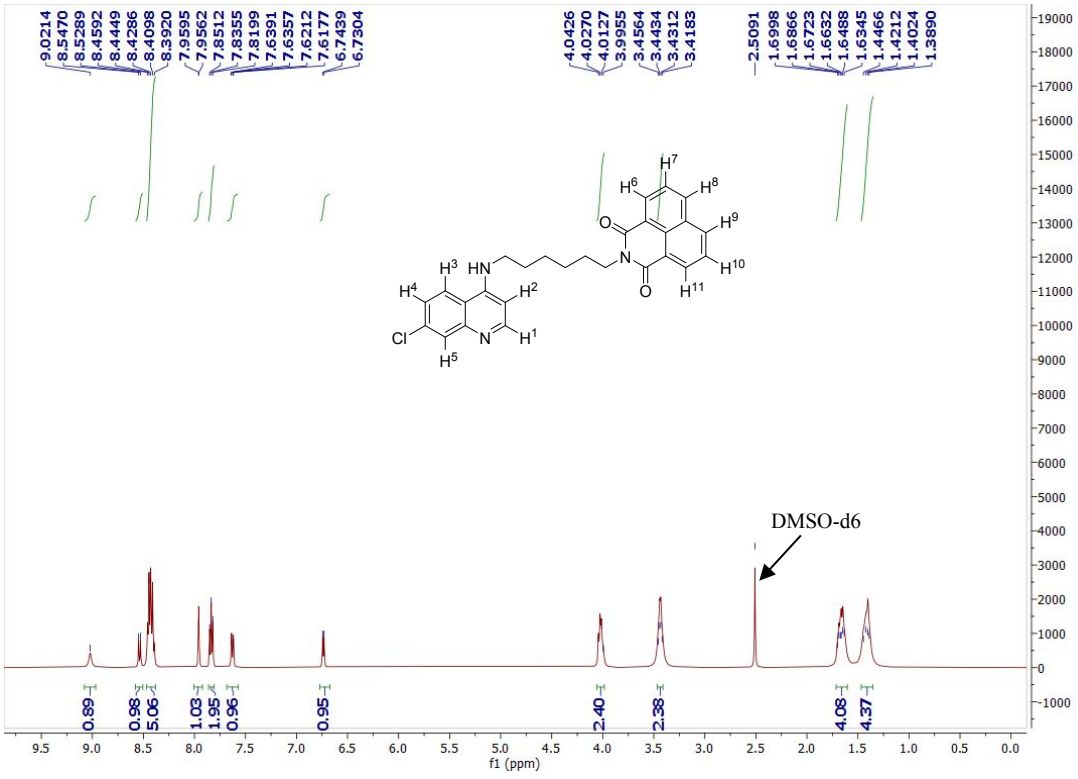
^{1}H NMR of 2-(4-((7-chloroquinolin-4-yl)amino)butyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3c):



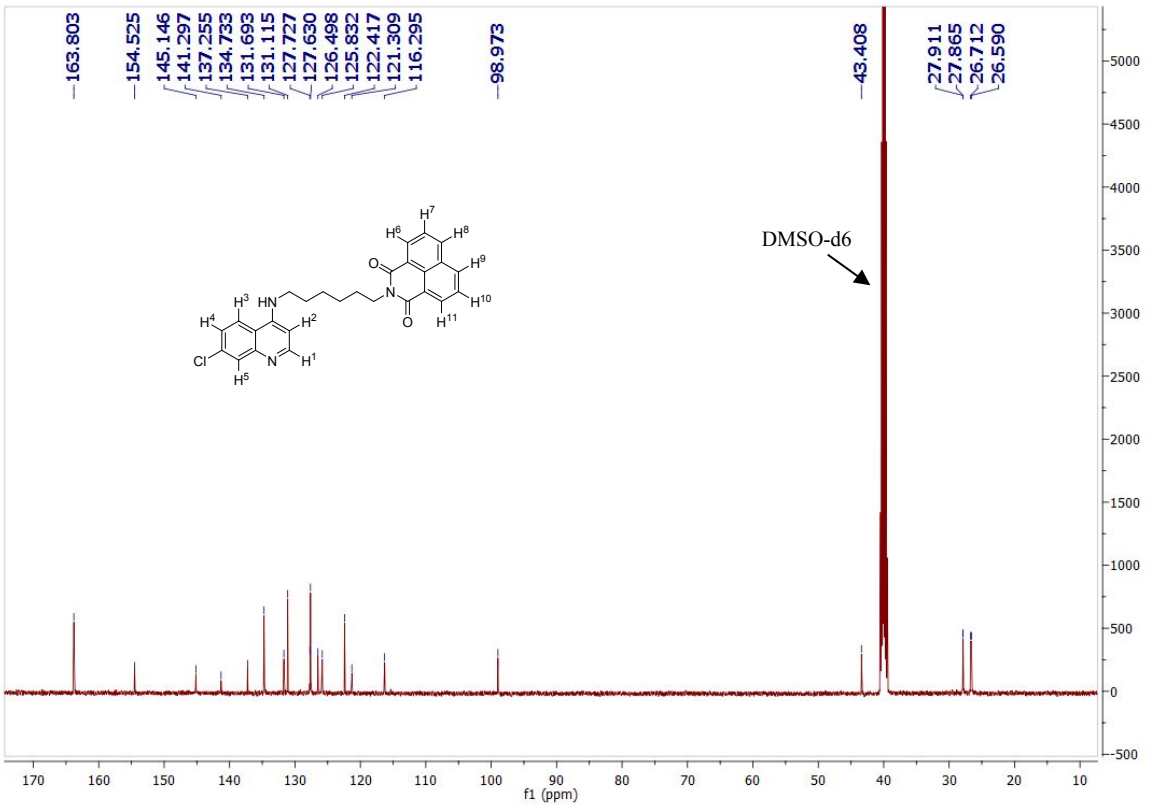
^{13}C NMR of 2-(4-((7-chloroquinolin-4-yl)amino)butyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3c):



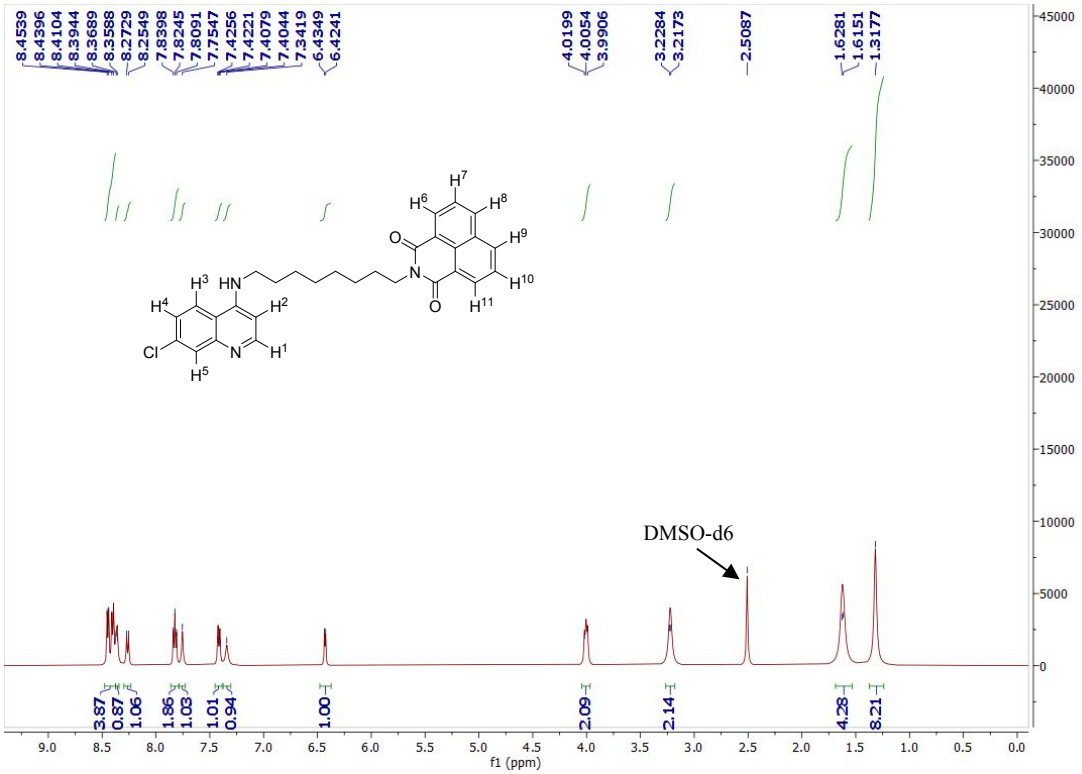
^{1}H NMR of 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3d):



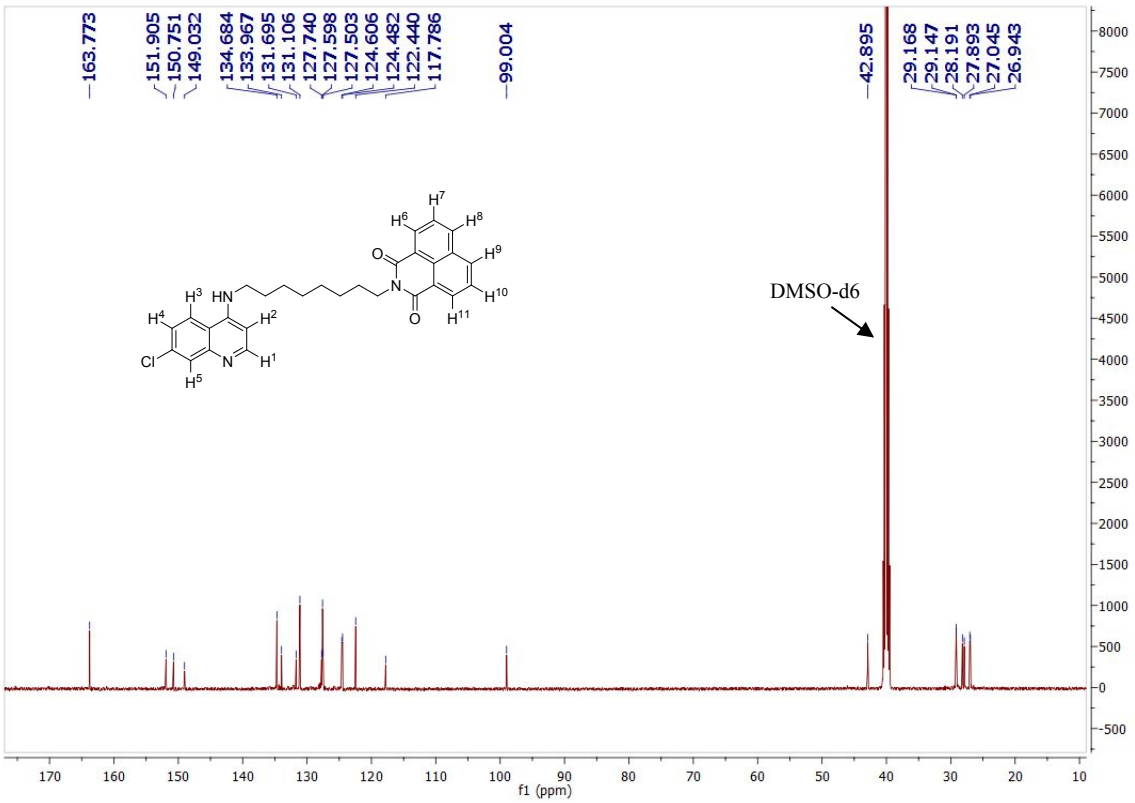
^{13}C NMR of 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3d):



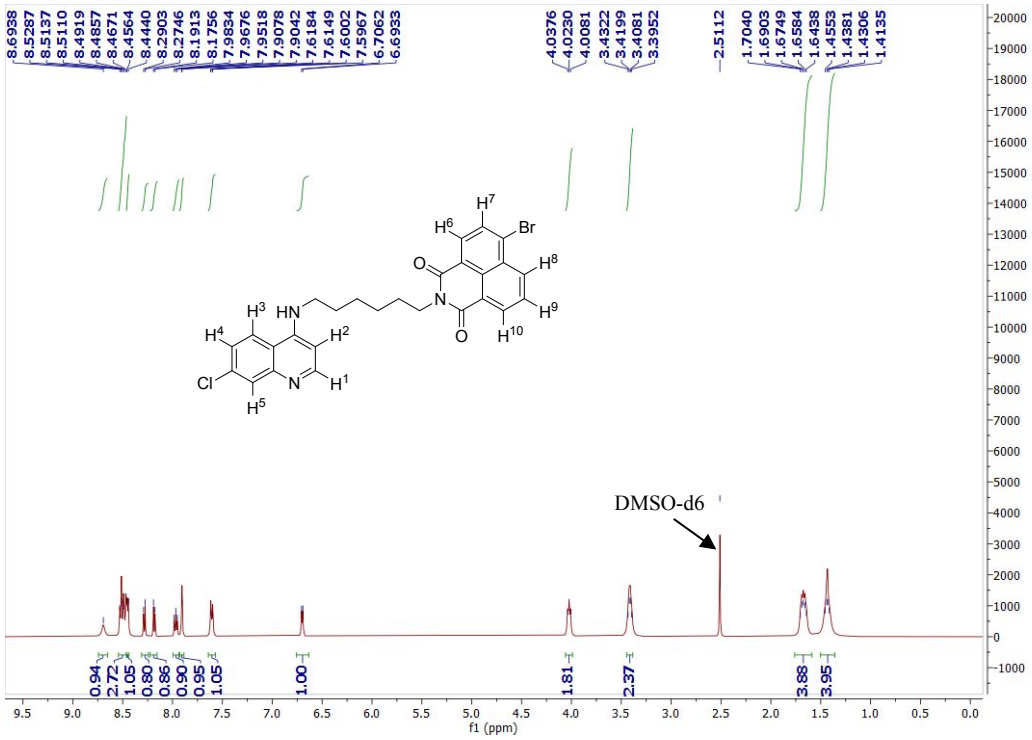
^{1}H NMR of 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3e):



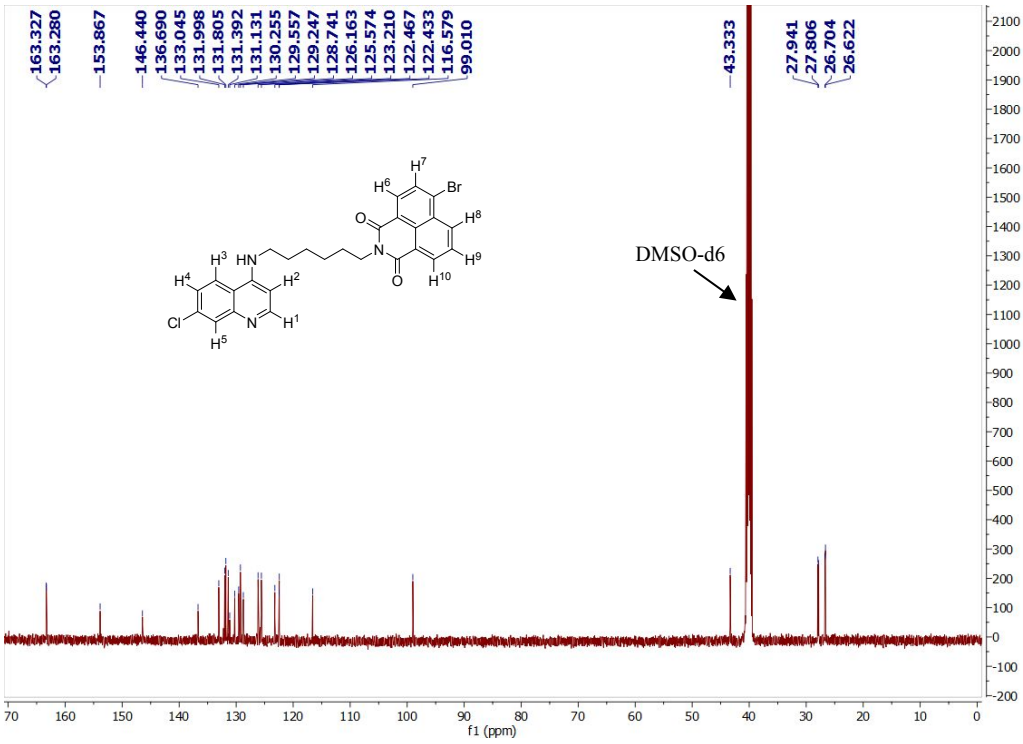
^{13}C NMR of 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3e):



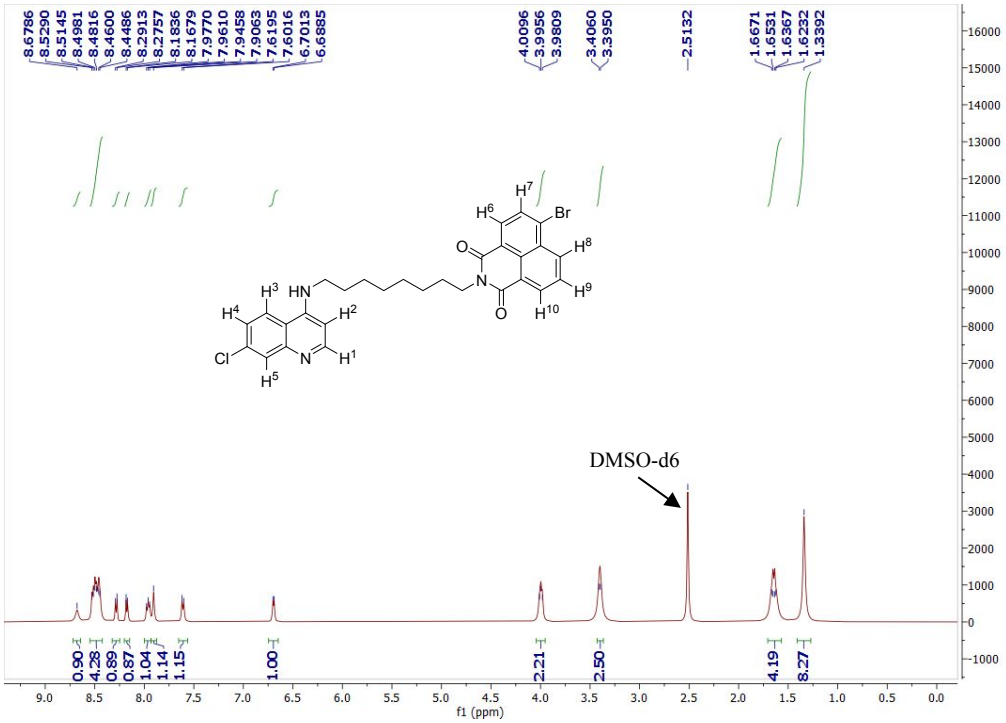
^{1}H NMR of 6-bromo-2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3i):



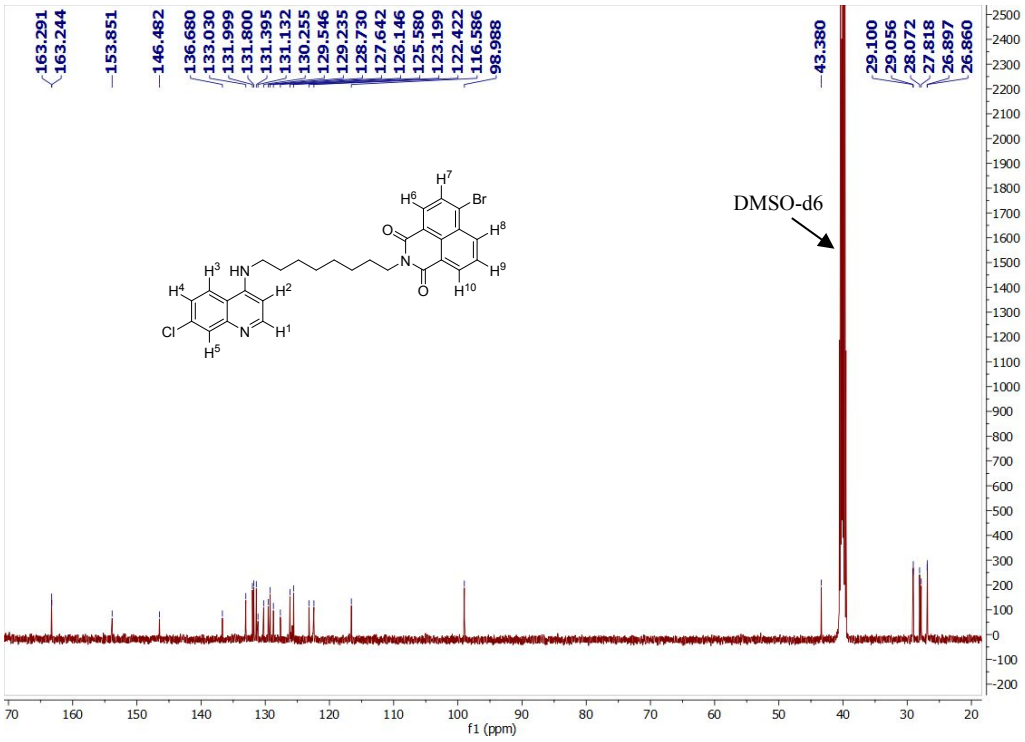
^{13}C NMR of 6-bromo-2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3i):



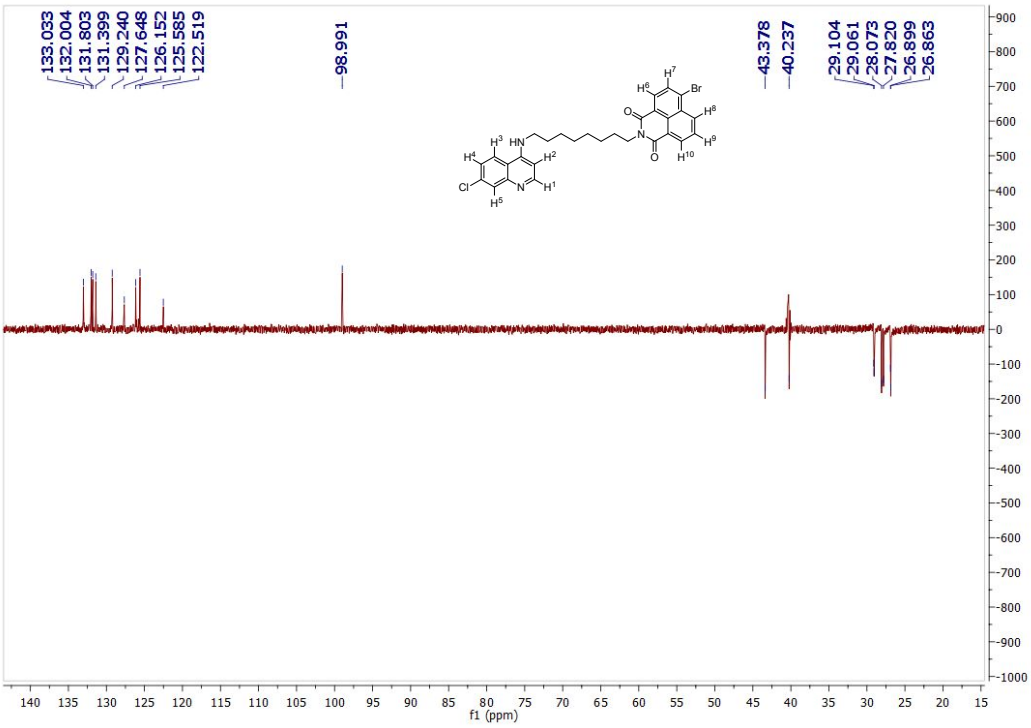
^{1}H NMR of 6-bromo-2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3j):



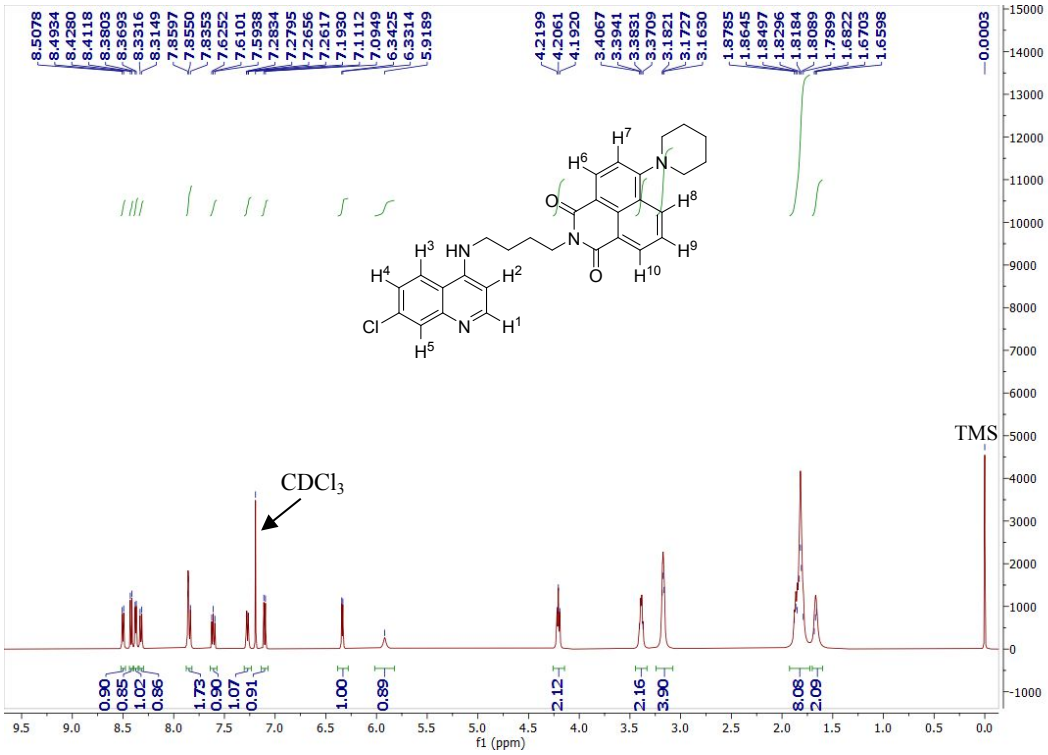
^{13}C NMR of 6-bromo-2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3j):



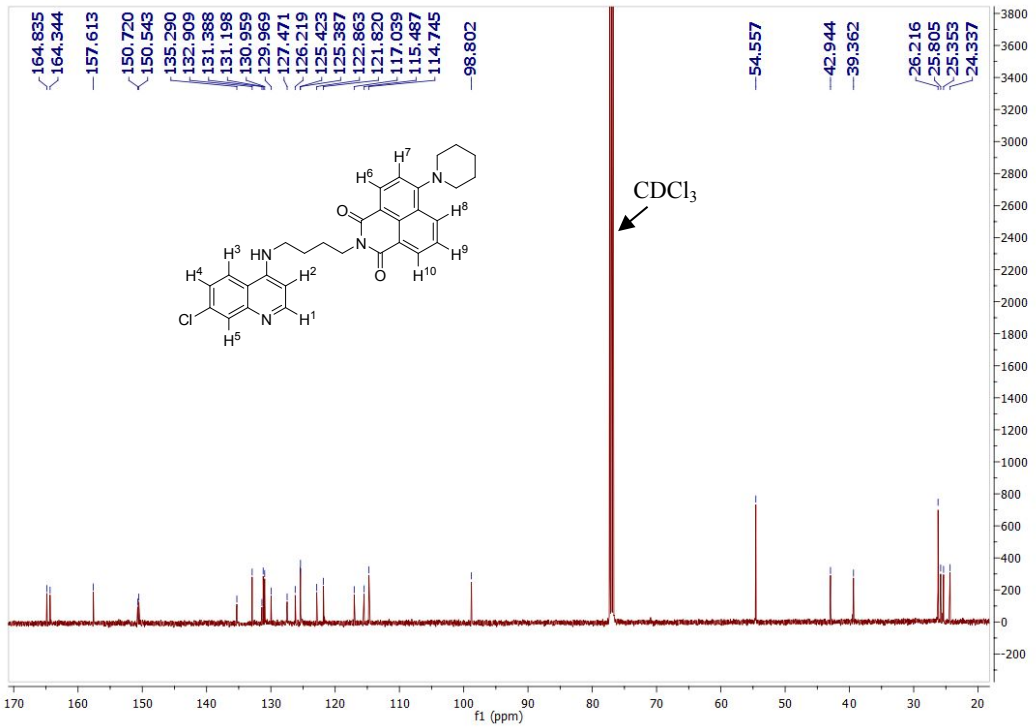
^{13}C-DEPT NMR of 6-bromo-2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3j):



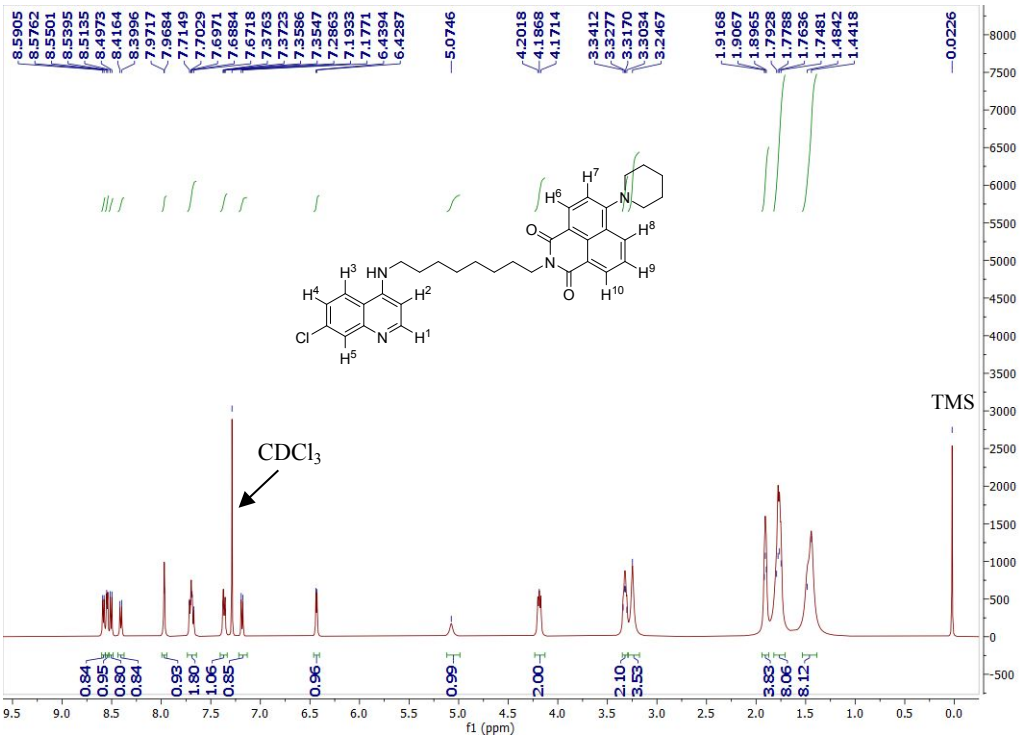
^{1}H NMR of 2-(4-((7-chloroquinolin-4-yl)amino)butyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4c):



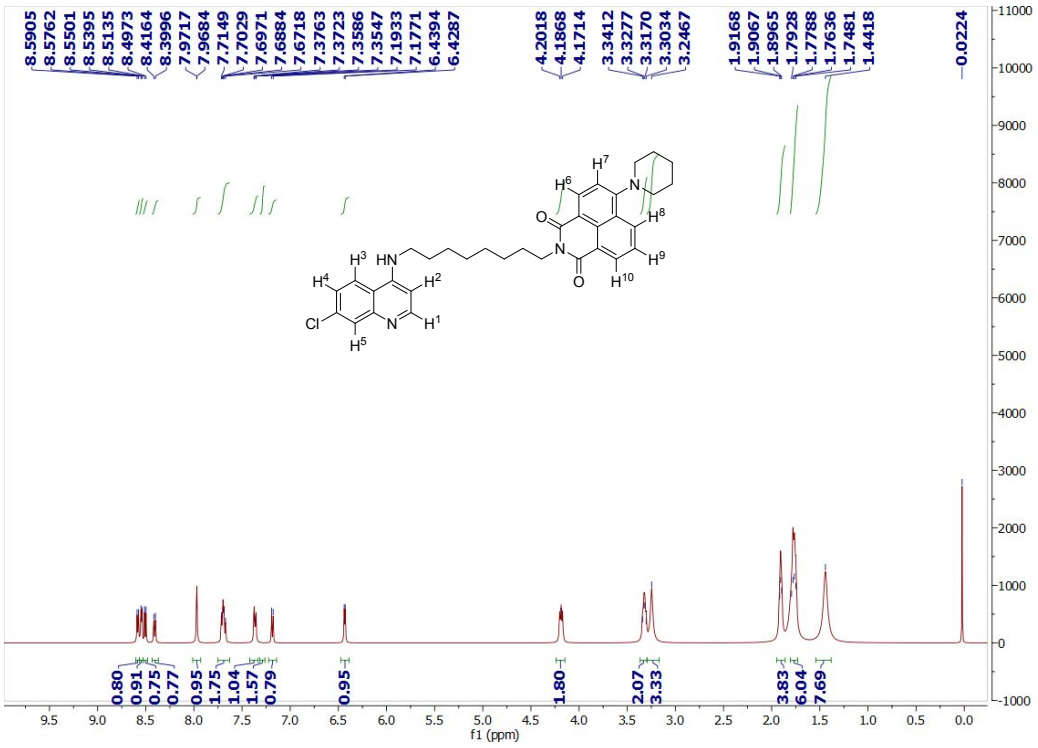
^{13}C NMR of 2-(4-((7-chloroquinolin-4-yl)amino)butyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4c):



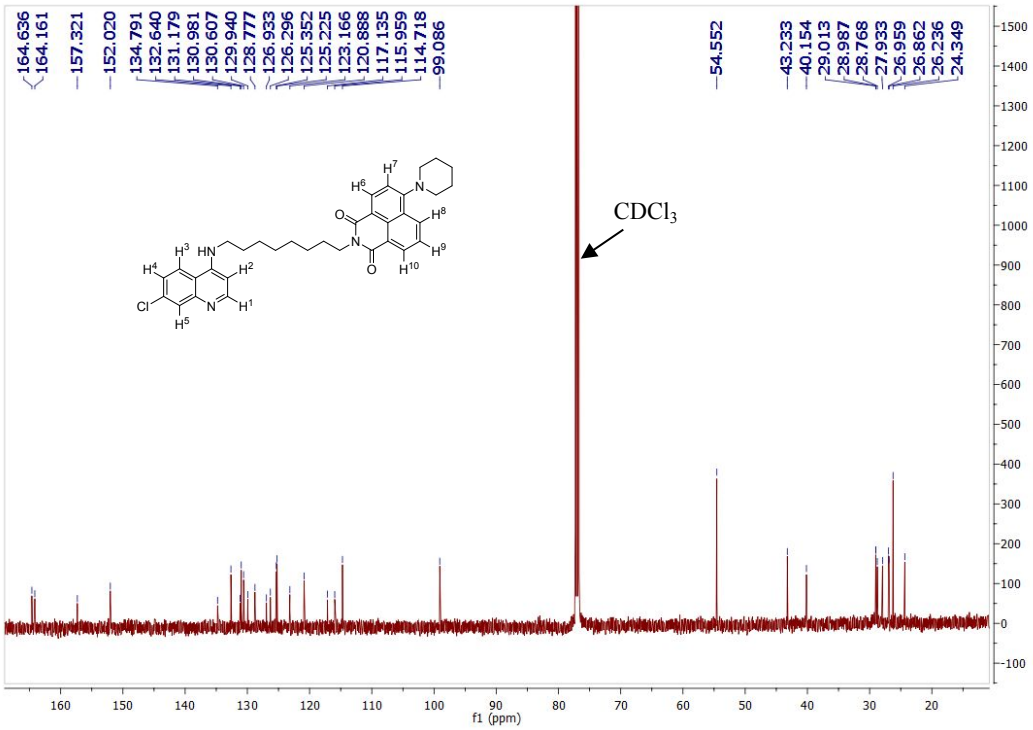
^{1}H NMR of 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4e):



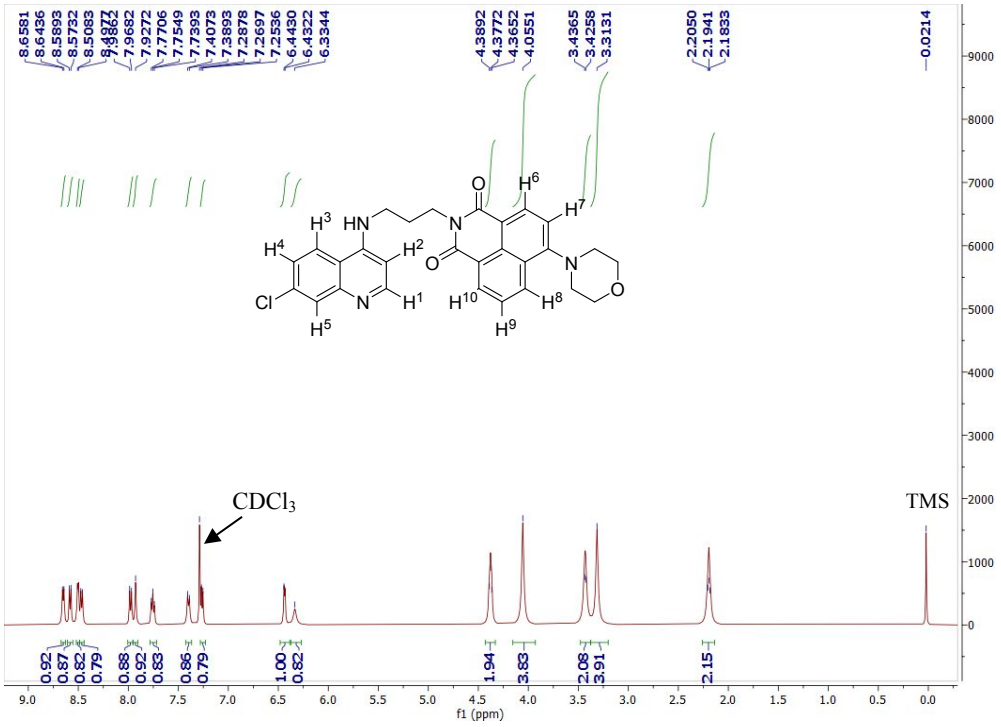
^{1}H (D_2O-shake) NMR of 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4e):



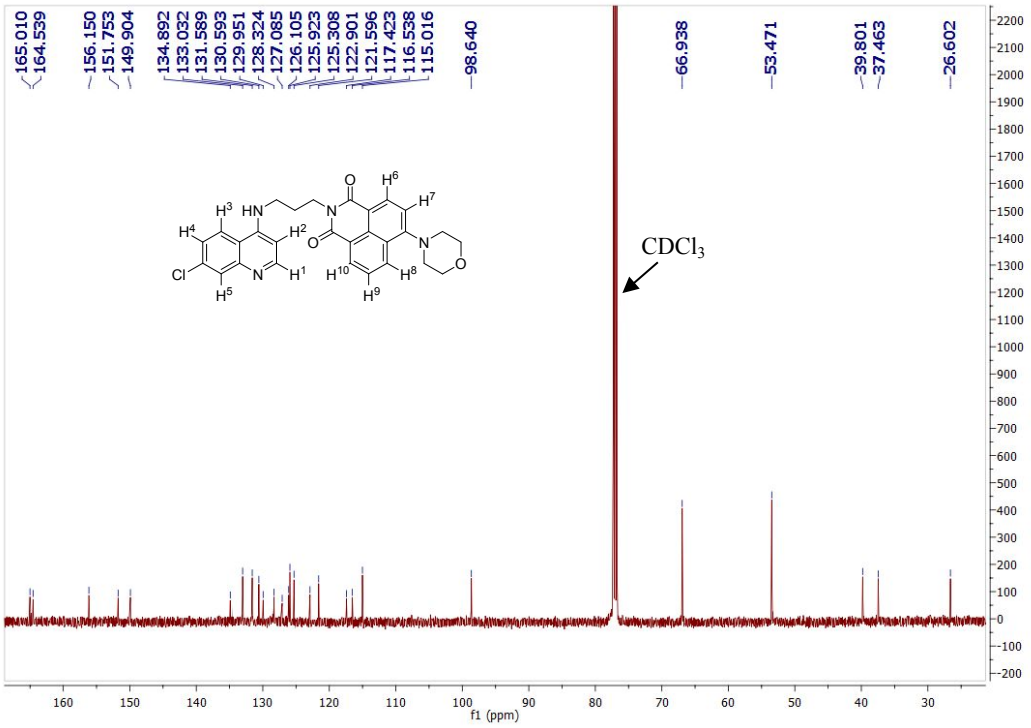
^{13}C NMR of 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4e):



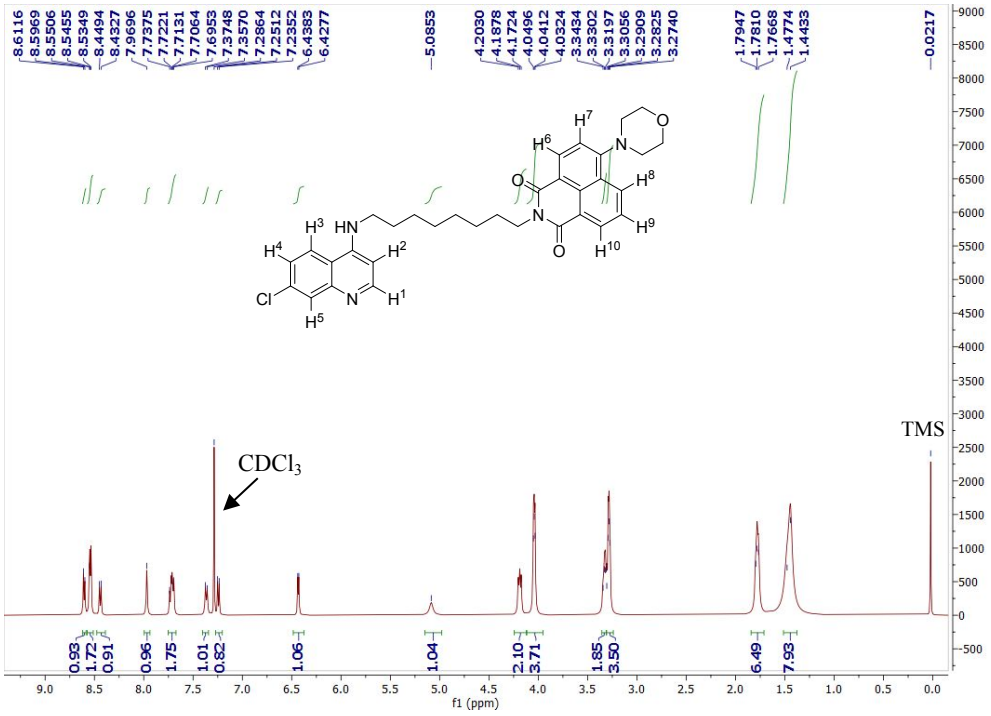
^{1}H NMR of 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (4g):



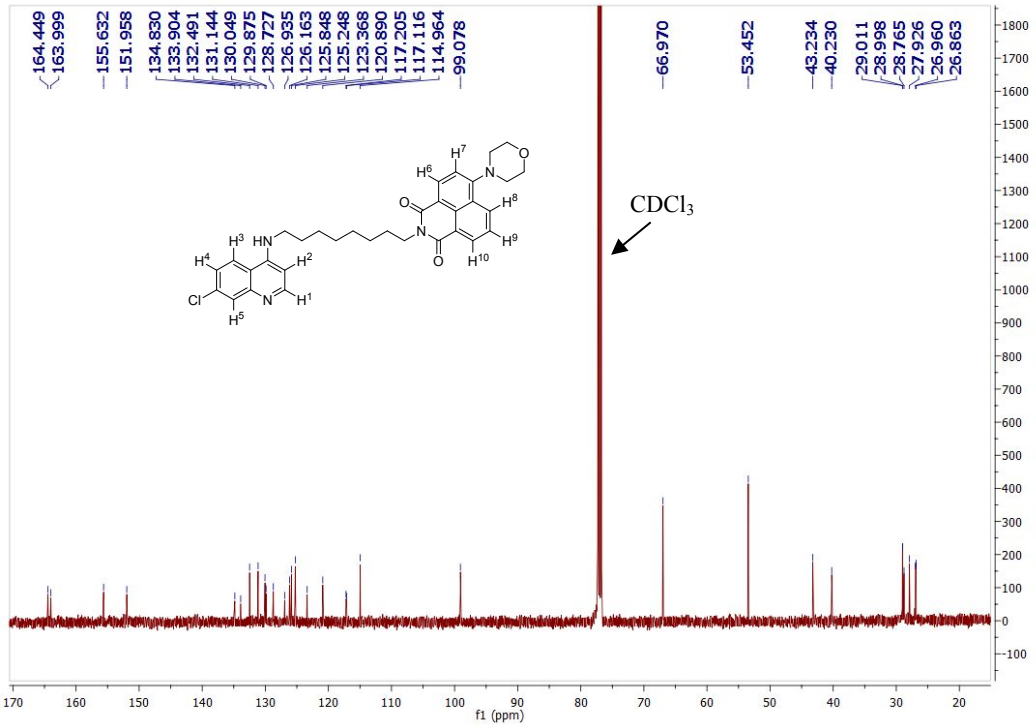
^{13}C NMR of 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (4g):



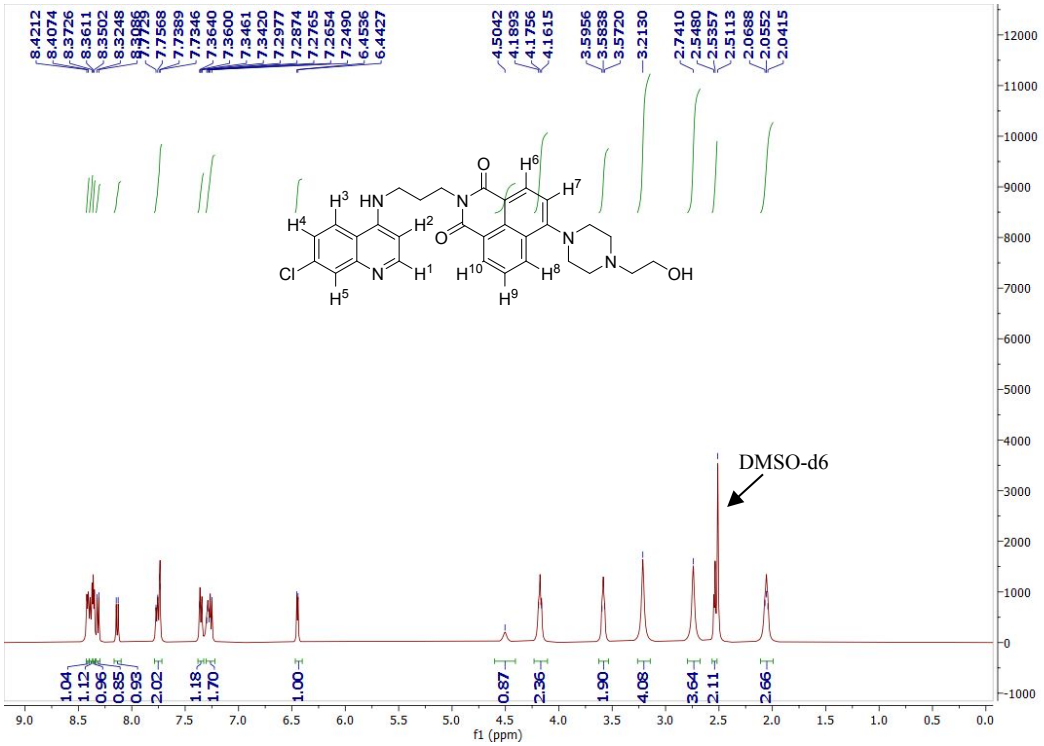
^{1}H NMR of 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (4j):



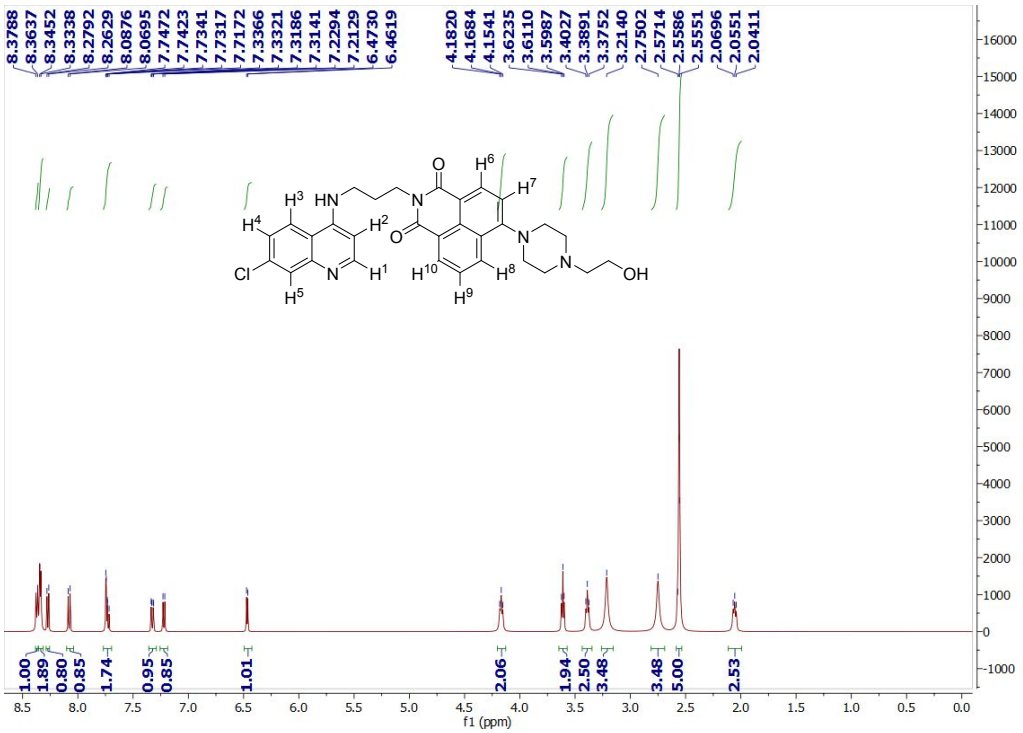
^{13}C NMR of 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (4j):



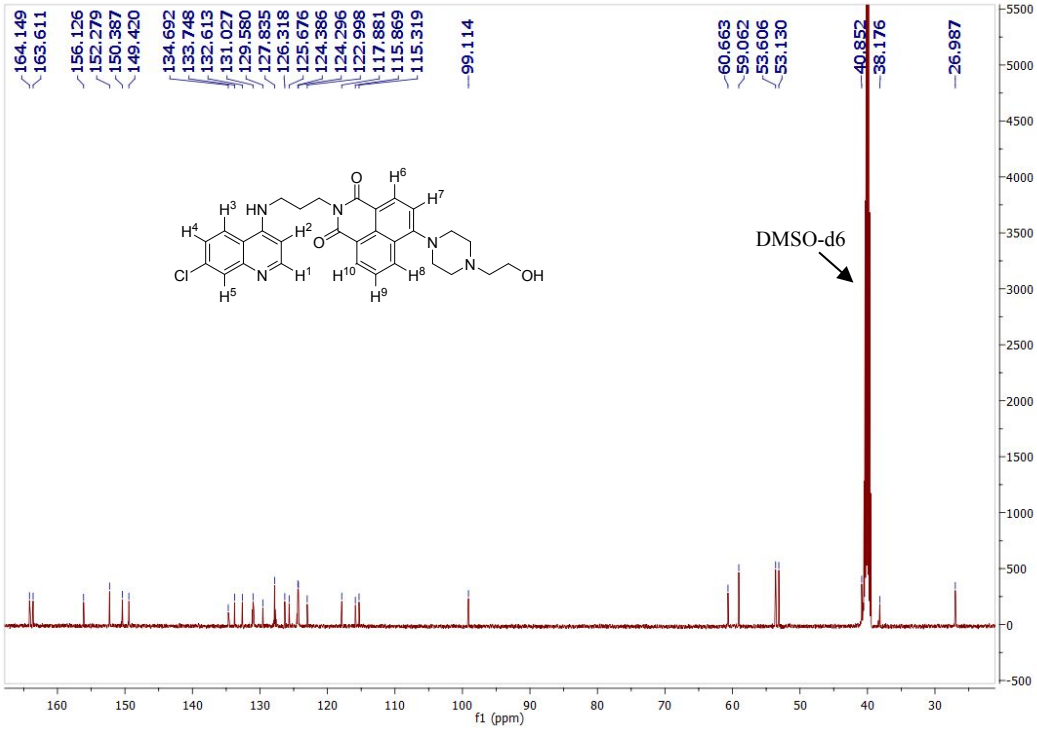
^{1}H NMR of 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4l):



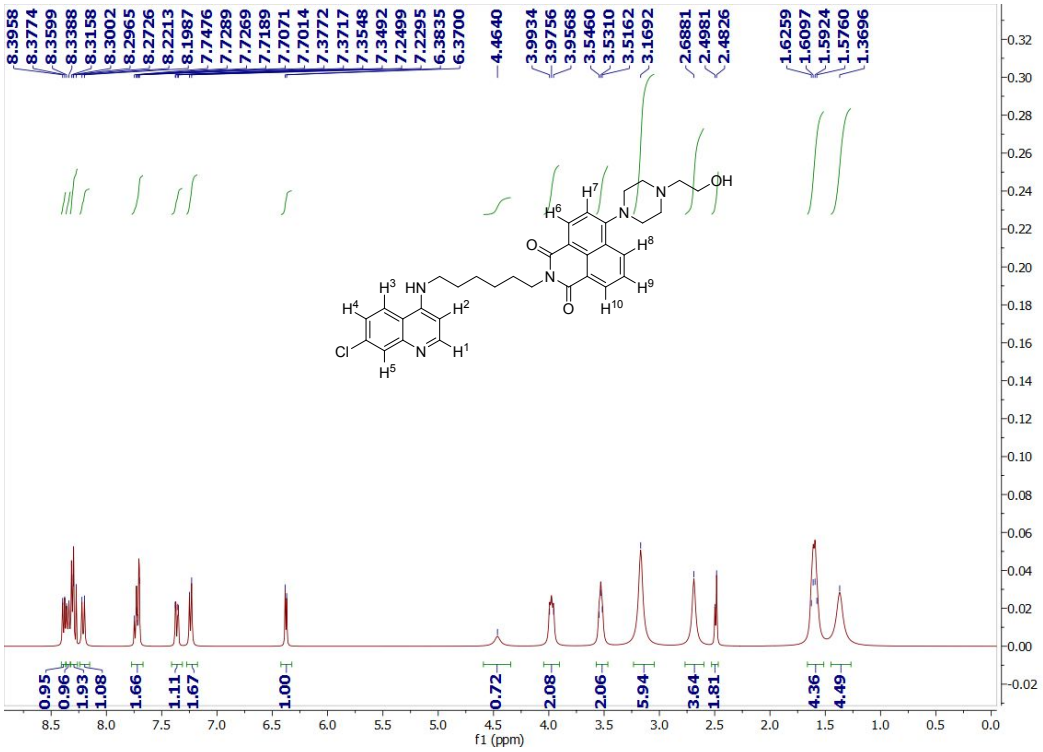
^{1}H (D_2O) NMR of 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4l):



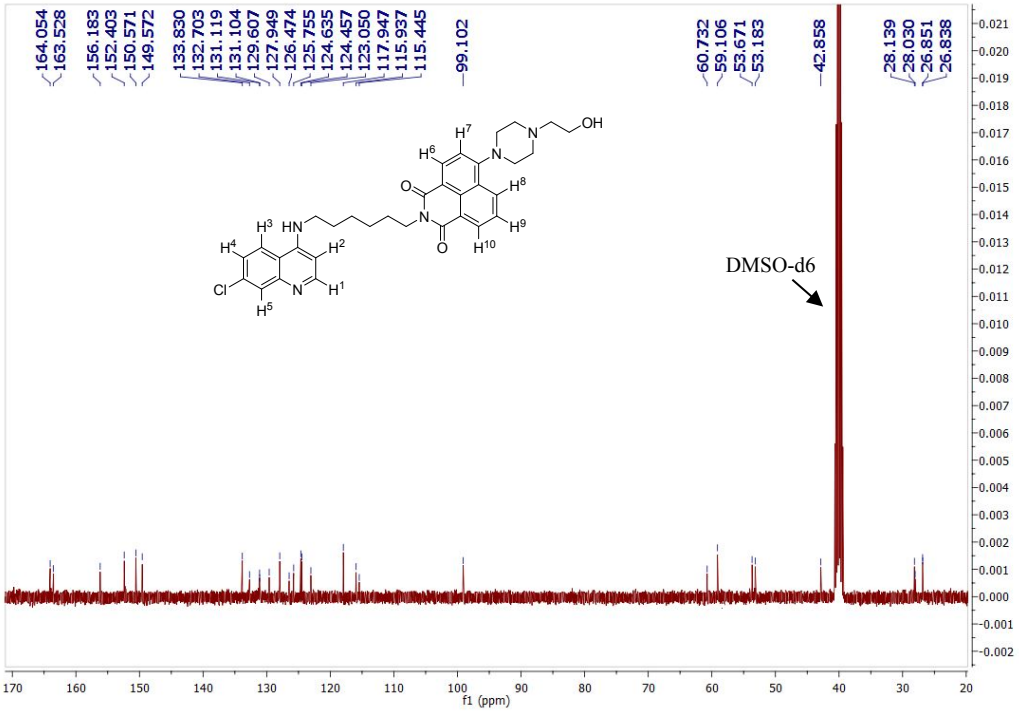
^{13}C NMR of 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4l):



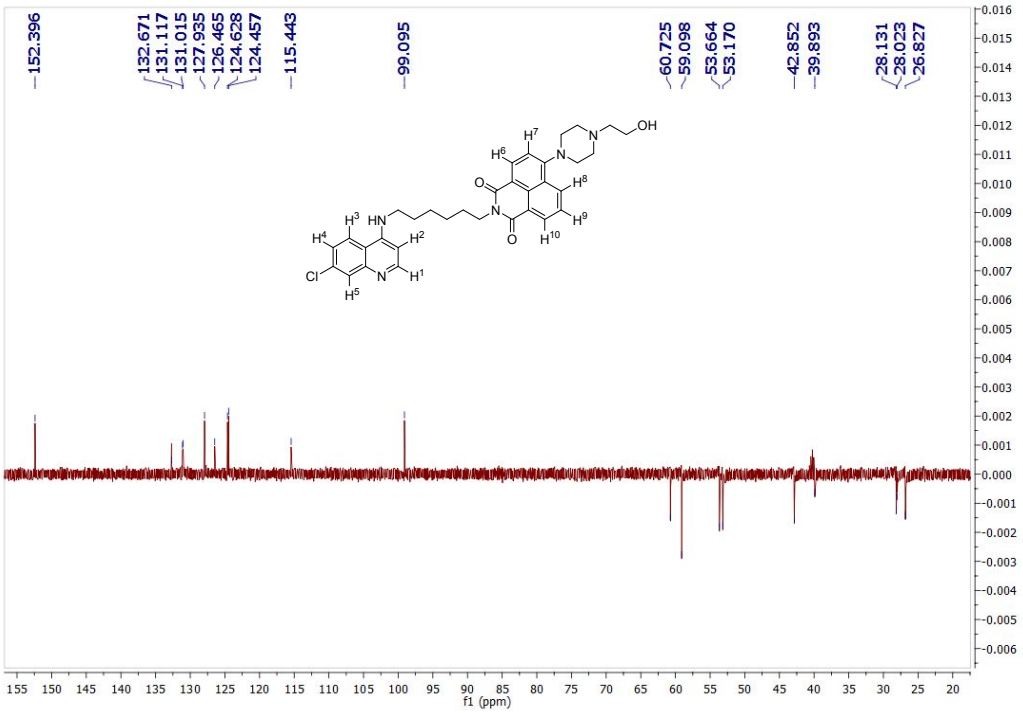
^{1}H NMR of 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4n):



^{13}C NMR of 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4n):



^{13}C (DEPT) NMR of 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4n):



7. UV-Vis spectra titration curve for chloroquine and diluent:

Figure S2: Titration of monomeric heme (12 µM) at pH 7.4 (0.02 M HEPES buffer in aqueous DMSO solution) with increasing concentration of chloroquine (0.02 M HEPES buffer in aqueous DMSO solution).

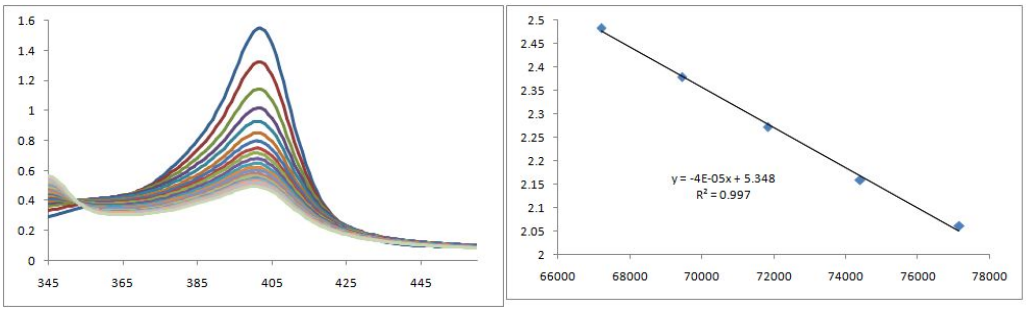


Figure S3: Titration of monomeric heme (12 µM) at pH 5.6 (0.02 M MES buffer in aqueous DMSO solution) with increasing concentration of chloroquine (0.02 M MES buffer in aqueous DMSO solution).

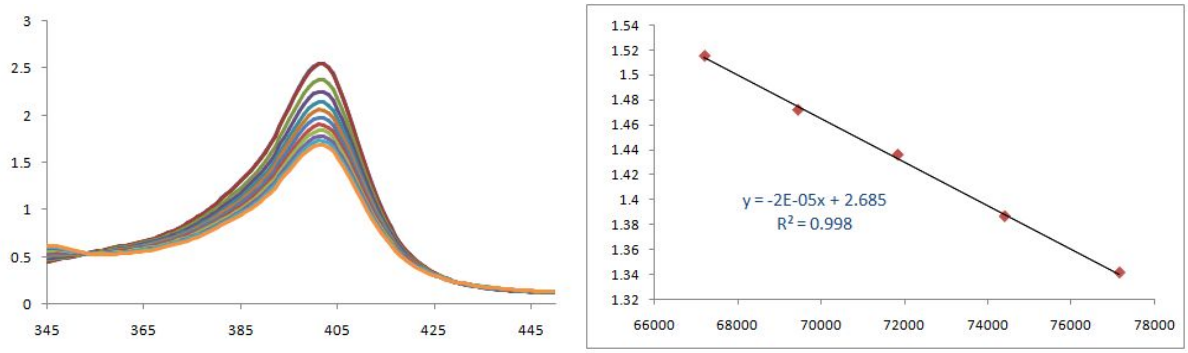
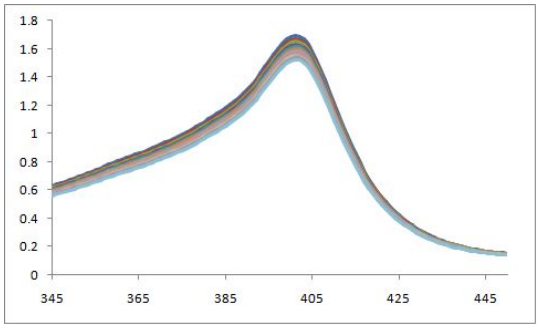


Figure S4: Titration of monomeric heme (12 µM) at pH 5.6 (0.02 M HEPES buffer in aqueous DMSO solution) with increasing concentration of diluent (0.02 M HEPES buffer in aqueous DMSO solution).



8. References:

[1] W. Xac, G.I. Berglund, G.H. Carlsson, A.T. Smith, A. Henriksen, J. Hajdu, H. Szo, The catalytic pathway of horseradish peroxidase at high resolution, Nature. 417 (2002) 463–468. doi:PDB ID:1H5A: 10.2210/pdb1H5A/pdb.

[2] A. Córdoba, I. Magario, M. Luján, Experimental design and MM2 – PM6 molecular modelling of hematin as a peroxidase-like catalyst in Alizarin Red S degradation, "Journal Mol. Catal. A, Chem. 355 (2012) 44–60. doi:10.1016/j.molcata.2011.12.011.

[3] Y. Sakata, K. Yabunaka, Y. Kobayashi, H. Omiya, N. Umezawa, T. Satoh, K. Kato, H. Ishikawa, T. Higuchi, Potent Antimalarial Activity of Two Arenes Linked with Triamine Designed To Have Multiple Interactions with Heme, ACS Med. Chem. Lett. 9 (2018) 980–985. doi:10.1021/acsmedchemlett.8b00222.

[4] CLC-bio, Molegro virtual docker, (2013).

[5] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, G.A. Petersson, X. Nakatsuji, H.; Li, M. Caricato, A. V. Marenich, J. Bloino, B.G. Janesko, R. Gomperts, B. Mennucci, H.P. Hratchian, J. V. Ortiz, A.F. Izmaylov, J.L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V.G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. Montgomery, J. A., J.E. Peralta, F. Ogliaro, M.J. Bearpark, J.J. Heyd, E.N. Brothers, K.N. Kudin, V.N. Staroverov, T.A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A.P. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, J.M. Millam, M. Klene, C. Adamo, R. Cammi, J.W. Ochterski, R.L. Martin, K. Morokuma, O. Farkas, J.B. Foresman, D.J. Fox, Gaussian 16, Revision B.01, (2016).

[6] M.W. Schmidt, K.K. Baldridge, J.A. Boatz, S.T. Elbert, M.S. Gordon, J.H. Jensen, S. Koseki, N. Matsunaga, K.A. Nguyen, S.U. Shyjun, M. Dupuis, J.A. Montgomery, General Atomic and Molecular Electronic Structure System, J. Comput. Chem. 14 (1993) 1347–1363.

[7] E.D. Glendening, J.K. Badenhoop, A.E. Reed, J.E. Carpenter, J.A. Bohmann, C.M. Morales, C.R. Landis, F. Weinhold, NBO 6.0., (2013). doi:http://nbo6.chem.wisc.edu.

[8] T. Lu, F. Chen, Multiwfn: a multifunctional wavefunction analyzer., J. Comput. Chem. 33 (2012) 580–592. doi:10.1002/jcc.22885.

[9] T. Lu, F. Chen, Quantitative analysis of molecular surface based on improved Marching Tetrahedra algorithm, J. Mol. Graph. Model. 38 (2012) 314–323. doi:https://doi.org/10.1016/j.jmgm.2012.07.004.

[10] E.D. Glendening, A. Streitwieser, Natural energy decomposition analysis : An energy partitioning procedure for molecular interactions with application to weak hydrogen

bonding , strong ionic , and moderate donor – acceptor interactions Natural energy decomposition analysis : An energy par, J. Chem. Phys. 100 (1994) 2900–2909. doi:10.1063/1.466432.

[11] K. Kazuo, M. Keiji, A new energy decomposition scheme for molecular interactions within the Hartree-Fock approximation, Int. J. Quantum Chem. 10 (2004) 325–340. doi:10.1002/qua.560100211.

[12] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera--a visualization system for exploratory research and analysis., J. Comput. Chem. 25 (2004) 1605–1612. doi:10.1002/jcc.20084.

[13] G.A. Andrienko, Chemcraft - graphical software for visualization of quantum chemistry computations., (2017). https://www.chemcraftprog.com.

[14] G. Wolber, T. Langer, LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters, J. Chem. Inf. Model. 45 (2005) 160–169. doi:10.1021/ci049885e.